(-)-SCLAREOL CONVERSION IN THE RITTER'S REACTION CONDITIONS

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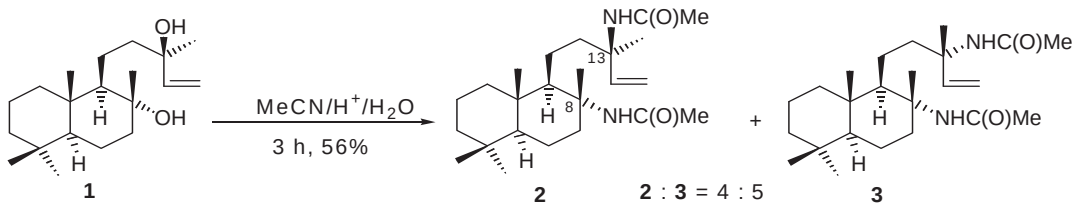
Abstract. The main products of sclareol (**1**) Ritter's reaction in mild conditions are (8*R*,13*R*)-Labd-14(15)-en-8,13-diacetamide (**2**) (8*R*,13*S*)-Labd-14(15)-en-8,13-diacetamide (**3**) stereoisomeric on C13 atom and having unrearranged native diol skeleton.

Keywords: diterpenoids, Ritter's reaction, diamide, sclareol.

Labdanoids represent one of the numerous and important diterpenoids sub-class and a lot of them posess a broad biological activity spectrum [1-3]. Different natural sources provided an array of nitrogen-containing labdanoids with different biological activities [4-9]. Due to this properties, an encreased interest towards nitrogen-containing labdanoids synthesis has been wittnessed in recent years [10-16].

We present in the current communication the results of sclareol converting (**1**) into nitrogen-containing labdanes in the Ritter's reaction conditions.



Scheme 1

Our first expectations have been connected to the posibility of obtaining chiral amides of labdane series, with a potential biological activity. Provided the fact that sclareol (**1**) (Scheme 1) molecule contains three potential reaction centers, namely two hydroxyl groups and an allylic double bond, a non-selective course of Ritter reaction was expected. Accordingly, investigation of the corresponding reaction products has revealed formation of two labdanic diamides (**2**) and (**3**), diastereomeric on C-13 chiral center, along with a minor amount of polymeric material. The double bond integrity is kept in the products and no allylic rearrangements occur. It is noteworthy mentioning that obtaining of amides from tertiarry alcohols by Ritter reaction has been recently described [17].

The structure of the synthesized diamides (**2**) and (**3**) was revealed on the basis of spectral data (IR, ^{1}H and ^{13}C NMR). The IR spectra of both compounds contain NH-characteristic bands (~3270 and 1550 cm^{-1}) and those corresponding to amidic carbonyl (1650 cm^{-1}). The ^{1}H NMR spectrum of both diamides (**2**) [18] and (**3**) [19] contains three signals of tertiarry methyl groups (δ = 0.78 - 0.90 ppm), as well as the signals of two methyl groups, geminally disposed versus amidic groups (δ = 1.20 ppm) along with two acetyl groups (δ = 1.80 ppm). There are also signals of three olefinic protons, corresponding to the terminal double bond. The position and shape of other signals are similar to that of sclareol (**1**). The ^{13}C NMR data are in full agreament with the suggested structures for diamides (**2**) and (**3**). Each diamide spectrum shows the presence of three shelded methyls (δ = 16, 18 and 21 ppm), two methyls of the acetyl group (δ = 23 ppm) and two low shelded methyl groups (δ = 33 ppm) corresponding to methyls geminally disposed versus amide group. The signals corresponding to quaternary carbons at C-8 and C-13 both linked to amide groups are detected at δ = 60 ppm and δ = 61 ppm, while the signals of terminal double bond apear at δ = 108 ppm and δ = 131 ppm. The carbonyl signals are detected at δ = 169 ppm. Besides, the ^{13}C NMR spectra contain other two signals of quaternary carbons, two CH- signals and seven CH_2- signals with chemical shifts being quite similar to those of sclareol (**1**), showing conservation of the bicyclic framework during the reaction. In such a way the Ritter reaction performed with sclareol (**1**) occurs stereoselectively at C-8 atom with less stereocontrol at C-13 (**2** and **3** isomers ratio is ~4:5) and without allylic rearrangement.

The high stereoselectivity at C-8 center is due to the concerted actions of both steric and thermodinamic factors: formation of equatorial amide isomer is preponderent, which takes place via adition of the acetonitrile molecule to a *trans*- stereochemistry versus the bulky hydroxyalkenyl (or amidoalkenyl) at C-9. The moderate reaction selectivity

at C-13 seems to be logic, provided the advanced flexibility of the lateral chain and its less interaction with the rigid bicyclic framework. In the same time, the fact that Ritter reaction of allylic alcohol **1** is not acompanied by a allylic rearrangement is quite surprising, since interaction of the tertiarry hydroxyl with a week nucleophile (acetonitrile) shall lead to elimination and the resulting carbonium ion would be inevitably acompanied by an allyl rearrangement, providing the isomeric allylic cation more accessible at C-15.

In conclusion, it was realized a single step synthesis of diterpenic diamides epimeric at C-13 chiral center from commercially available sclareol. The obtained products represent interest as substances with potential biological activity.

Acknowledgment

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[18]. Data of (8*R*,13*R*)-Labd-14(15)-en-8,13-diacetamide (**2**) as an oil: IR (liquid film): 3270, 3080, 2925, 2865, 2845, 1650, 1550 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, selected): δ_H = 0.74 (s, 3H, 10-CH_3), 0.77 (s, 3H, 4-CH_3), 0.82 (s, 3H, 4-CH_3-*e*), 0.92 (m, 2H), 1.08 (m, 2H), 1.19 (dt, 1H, $^2J = {}^3J_{a,a}$ = 12 Hz, $^3J_{a,e}$ = 3 Hz, 1-H*a*), 1.22 (s, 3H, 13-CH_3), 1.28 (s, 3H, 8-CH_3), 1.35 (m, 4H), 1.61 (m, 6H), 1.78 (s, 3H, $COCH_3$), 1.84 (s, 3H, $COCH_3$), 1.87 (dd, (1H, ^1J = 12 Hz, and ^2J = 2 Hz, 9-H), 4.88 (d, 1H, $^3J_{cis}$ = 11 Hz, 15-H-*cis*), 5.17 (d, 1H, $^3J_{trans}$ = 17 Hz, 15-H-*trans*), 5.81 (dd, 1H, $^3J_{trans}$ 17, $^3J_{cis}$ = 11 Hz, 14-H), 6.35 (br s, 1H, NH), 6.50 (br s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 15.7 (q, 10-CH_3), 18.4 (t, C-3), 18.7 (t, C-2), 20.4 (q, 4-CH_3-*a*), 22.8 (t, C-1), 23.1 (q, 4-CH_3-*e*), 24.4 (q, CO$\underline{CH_3}$), 24.5 (q, CO$\underline{CH_3}$), 28.1 (q, 13-CH_3), 33.0 (s, C-4), 33.3 (q, 8-CH_3), 38.9 (s, C-10), 39.6 (t, C-6), 41.6 (t, C-7), 42.1 (t, C-11), 43.2 (t, C-12), 53.7 (t, C-5), 60.8 (d, C-9), 63.8 (s, C-13), 64.7 (s, C-8), 109.3 (t, C-15), 146.0 (d, C-14), 169.3 (s, C=O), 169.8 (s, C=O).
[19]. Data of (8*R*,13*S*)-Labd-14(15)-en-8,13-diacetamide (**3**) as an oil: IR (liquid film): 3270, 3080, 2925, 2865, 2845, 1650, 1550 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$, selected): δ_H = 0.74 (s, 3H, 10-CH_3), 0.76 (s, 3H, 4-CH_3), 0.83 (s, 3H, 4-CH_3-*e*), 0.91 (m, 2H), 1.10 (m, 2H), 1.17 (dt, 1H, $^2J = {}^3J_{a,a}$ = 12 Hz, $^3J_{a,e}$ = 3 Hz, 1-H*a*), 1.21 (s, 3H, 13-CH_3), 1.26 (s, 3H, 8-CH_3), 1.37 (m, 4H), 1.64 (m, 6H), 1.81 (s, 3H, $COCH_3$), 1.83 (s, 3H, $COCH_3$), 1.91 (dd, (1H, ^1J = 12 Hz, and ^2J = 2 Hz, 9-H), 4.87 (d, 1H, $^3J_{cis}$ = 15 Hz, 15-H-*cis*), 5.19 (d, 1H, $^3J_{trans}$ = 17 Hz, 15-H-*trans*), 5.84 (dd, 1H, $^3J_{trans}$ 17 Hz, $^3J_{cis}$ = 11 Hz, 14-H), 6.40 (br s, 1H, NH), 6.60 (br s, 1H, NH). ^{13}C NMR (125 MHz, $CDCl_3$): δ_C = 15.6 (q, 10-CH_3), 18.5 (t, C-3), 18.8 (t, C-2), 20.7 (q, 4-CH_3-*a*), 22.4 (t, C-1), 22.9 (q, 4-CH_3-*e*), 24.2 (q, CO$\underline{CH_3}$), 24.4 (q, CO$\underline{CH_3}$), 31.2 (q, 13-CH_3), 32.9 (s, C-4), 33.3 (q, 8-CH_3), 38.7 (s, C-10), 39.5 (t, C-6), 41.6 (t, C-7), 42.0 (t, C-11), 43.4 (t, C-12), 53.4 (t, C-5), 60.9 (d, C-9), 64.1 (s, C-13), 64.4 (s, C-8), 109.9 (t, C-15), 145.1 (d, C-14), 169.6 (s, C=O), 169.9 (s, C=O).

INTERACTION OF ALBUMIN AND IMMUNOGLOBULIN G WITH SYNTHETIC HYDROXYAPATITE

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Abstract. It was shown by X-ray phase analysis, IR spectra analysis and MALDI-ToF mass spectrometry methods that interaction of synthetic hydroxyapatite with a solution of immunoglobulin G leads to its partial dissolution due to leaching from the surface of calcium triphosphate which, in our opinion, forms complexes with immunoglobulin G.

Keywords: MALDI-ToF, X-ray phase analysis, albumin, immunoglobulin G, hydroxyapatite, interaction.

Introduction

Hydroxyapatite (HA, $Ca_{10}(PO_4)_6(OH)_2$) is a synthetic analogue of the mineral component of bone and is widely used in catalysis, adsorption, laser technology and other fields [1]. Its most promising application is as implant in biological systems, due to its good biocompatibility and chemical affinity to the mineral basis of bone, that is, to the calcified tissues. On the other hand, synthetic calcium phosphates do not fully possess biological properties of the bone tissues [2]. This is due to some differences in chemical composition as bone contains calcium phosphates, which have also carbonates, phosphate, trace elements, etc. In turn, the chemical differences contribute to making changes in the texture of the material [3]. At the same time, the mineral component of bone tissue in the body fluids is susceptible to partial dissolution, but to a lesser extent than synthetic HA, which is associated with the presence of some trace elements in natural materials [4-5].

The latter considerations justify the need to study synthetic HA in contact with body fluids to determine possible ways of its dissolution.

As a model system we have chosen HA−immunoglobulin G (IgG) and HA−human serum albumin (HSA). We studied the interaction of these components.

Materials and methods

HA synthesis [14-15]

HA was synthesized using commercially available $Ca(NO_3)_2$ (99% ACS, Aldrich) and $(NH_4)_2HPO_4$ (Aldrich) as starting precursors. The reaction was conducted using de-ionized water. The method consisted of the following steps. Stoichiometric amount of $Ca(NO_3)_2$ (0.1 M) was first dissolved in 100 ml water and 25% ammonia in water was added until pH=10 (solution #1). At the same time stoichiometric amount of $(NH_4)_2HPO_4$ (0.1 M) was dissolved in 100 ml water and 25% ammonia in water was added until pH=10 (solution #2). Then solution #2 dropwise was added to solution #1 with continuous stirring. Upon completion of addition the resulting solution was heated on boiling for 10 minutes. Solution was left at room temperature for 24h. The resulting precipitate was filtered and dried in air at 110°C.

Chemical precipitation route can be represented by the following reaction:

$$10\ Ca(NO_3)_2 + 6\ (NH_4)_2HPO_4 + 2\ H_2O \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 12\ NH_4NO_3 + 8\ HNO_3$$

Preparations of IgG and HSA

IgG and HSA were dissolved in water to a concentration of 1 mg / ml. To 1 ml of IgG or HSA 20 or 100 mg of HA were added. After incubation at room temperature, the solution of HA with proteins was centrifuged at 10000/min for 15 minutes. The supernatant was investigated by MALDI. The sediments were examined by XRD HA and IR.

MALDI-ToF

Matrix for the mass spectrometric studies were prepared by standard procedures: 12 mg of SA (Sinapic Acid Fluka (SA)) (Sigma) was dissolved in 1 ml water-acetonitrile mixture in the ratio 1:1. To the solution trifluoroacetic acid was added in 1mkl aliquots [6].

The studies were conducted in the range of 19 kDa -300 kDa. The resulting spectrum is the result of the accumulation of 600 single spectra.

IR spectra

IR spectra were recorded on a spectrophotometer “Perkin Elmer” (Model 1720H) in the range of 400-4000 cm^{-1}.

X-ray phase analysis

DRON-UM1 using Kα radiation anode x-ray focusing Bragg-Brentano, Co K (λ = 0,179021 nm) and Fe - in the filter the reflected rays.

Results and discussion

MALDI mass spectrum of IgG is represented by a series of peaks which include the molecular ion IgG + (150 kDa) and the various possible combinations of singly-charged fragments of IgG formed by cleavage of its light (23 kDa) and heavy (50 kDa) chains. The obtained mass spectrum and its interpretation matches previously published data [7].

As a control, HA samples of two different concentrations (20 and 100 mg\ml) were incubated in distillates for 2 and 24 hours. After centrifugation, the supernatant was treated with IgG and albumin (separately) and kept for 2h and 24 h. Then the solution was investigated by MALDI. No changes in the mass spectra of proteins have been recorded. Consequently, all subsequent changes in the mass spectra of proteins are the result of their interactions with HA.

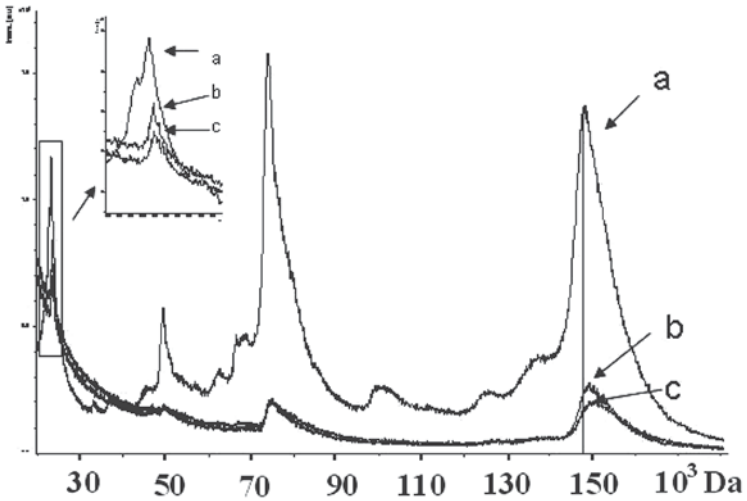

Fig. 1. MALDI-TOF mass spectrum of IgG (a), after one hour of incubation (b), and 4-hour incubation (d) in the presence of 100 mg / ml of HA in positive mode.

After exposure of the solution with HA IgG (20 mg/ml) changes have been observed in the mass spectrum of positive ions, namely an increase in the mass values of the corresponding peaks. Since the molecular ion of IgG + shifted by about 520 amu and 1500 amu after 2 and 4-hour incubations, respectively. With increasing concentration of HA to 100 mg/ml more critical changes of mass values were observed (Figure 1). The increase in mass of the ion IgG was 1000 amu and 2200 amu in 2 and 4-hour incubations, respectively. The mass of heavy IgG fragment increased by 700 amu after 2 h incubation and remained so after 4 hours of incubation. The change in the mass of light fragment of IgG at 220 amu in 2 and 4 hours of incubation was noted. This indicates that the light chains of IgG are involved in interaction with the inorganic surface, which is fundamentally different from the mechanism of the interaction of IgG with salts of heavy metals, which have been recorded by their interaction with light chain protein [8]. A similar exhibition of HA in solution of HSA did not lead to a change in mass of the protein even at 24 h incubation. The general trend for the HSA and IgG represents a decrease in the relative intensities of the ions in the mass spectrum with increasing concentration of HA and the exposure time, which is related to processes of proteins adsorption on HA.

To assess the state of GA, after interaction with IgG, we used the method of IR spectroscopy, which is able to detect the presence of such a local change of symmetry, and phosphate anions that make up the architecture and surface properties of HA .

Figure 2 shows the IR spectra of HA, HA with adsorbed IgG and HA with adsorbed IgG after repetitive washing with water.

It is noteworthy, that in the original HA characteristic bands in the region 400-800 cm $^{-1}$ are due mainly to deformation vibrations of -PO_4 tetrahedrons and the bands in the region 900-1200 cm $^{-1}$ - stretching vibrations of the phosphate anion (P-O bonds). The bands in the range 1300-1650 cm-1 and about 873 cm^{-1} correspond to the v3 and v2 modes of vibrations of carbonate groups. Deformation vibrations of water molecules are located at 1620 cm^{-1}. The broad absorption band in the range 2500-3700 cm^{-1} refers to water adsorption.

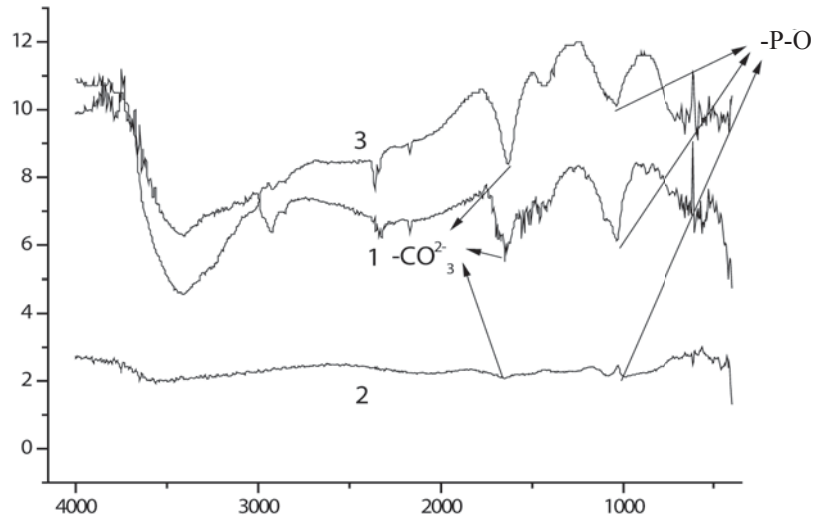

Fig. 2. IR spectra of HA (1), with adsorbed IgG (2) and after washing (3)

After interaction with a solution of IgG following changes are observed in the IR spectrum. The intensity of the characteristic absorption bands changes, as well as the observed shift of their maxima. An increase of the intensity of the bands at 601cm^{-1} for the HA protein with adsorbed IgG is detected, along with a simultaneous decrease in the intensity of the absorption band at 562 cm^{-1} compared to the original HA indicating the involvement of P-OH bonds in the process of protein adsorption. The structure of the absorption band at 1700-1600 cm^{-1} the largest contribution corresponds to the C ═ O group in the peptide bond (80%) with a small contribution of plane bending vibrations of NH groups (10%) and valence vibrations of C-N bond (10%). Peptide groups form hydrogen bonds that define secondary structure of proteins. According to literature data, in aqueous solution it is dominated by the structure of β-sheet (the peak of 1638 cm^{-1}, which is clearly visible in the spectrum of HA and protein), which is changed or even destroyed after the interaction in the HA. The absorption bands of carbonate groups are becoming more distinct and separate too, which can be explained by immunoglobuline "nanogrinding" the IgG surface and by the involvement of the HA surface acid centers on that process. Considering the analysis of the IR spectra suggests that the interaction of IgG molecules with the HA surface, assumes involvement of -PO_4 tetrahedrons, which leads to their partial polarization, change of geometry and as a consequence - changing the bond lengths. After washing the protein from the surface of HA a partial restoration of the original form of the spectrum is observed, indicating a partial restoration of HA structure after washing.

Information about changes in the structure of HA after interaction with the protein was supplemented by X-ray phase analysis (Figure 3). It is noted that the ratio of the intensity of the reflection (28 deg) decreases with respect to the reflex in the (29 deg.). However, the protein is partially returned to its original position after desorption. It should be noted that in the synthetic HA there is always about 5% of tricalcium phosphate (TCP) [12], whose lines are just in the 28-29 deg and their intensity changes after washing the protein.

The degree of conversion of TCP to HA was determined by the formula [9]:

$$R_n = \frac{I_{HA}}{I_{HA} + I_{TCP}} \cdot$$

For pure HA it was obtained the value Rn = 0,79, for the HA with immune globulin after washing Rn = 0,8 indicating the decrease of TCP share in HA.

According to the XRD average particle size constitutes 36 nm [11]. Amount of β-TCP impurity, according to the standard JCPDS 9-169, which is always present in the synthetic HA is ~ 5% (within the sensitivity of XRD). If we assume that the particles have a spherical shape, we can determine the surface area of nanoparticles and, therefore, estimate the area occupied by the TCP. We have identified the surface area of sphere particles representing 2826 nm^2, of them 148 nm^2 falls on the surface occupied by the TCP. Obviously, the percentage contribution of TCP structure, which in our opinion determines the characteristic changes in all the spectra related to IgG experiments, is negligible as compared to other parts of the surface and particle volume. This shows little change in the intensities of reflections of X-ray diffraction and transmission lines in the IR spectra. There are also data in the literature to explain the change in the diffraction patterns. According to the authors, the abrupt change in the diffraction patterns shows the transition from mono-crystalline to poly-crystalline HA with 2θ in the range of 31.5 and 33.2 deg [13].

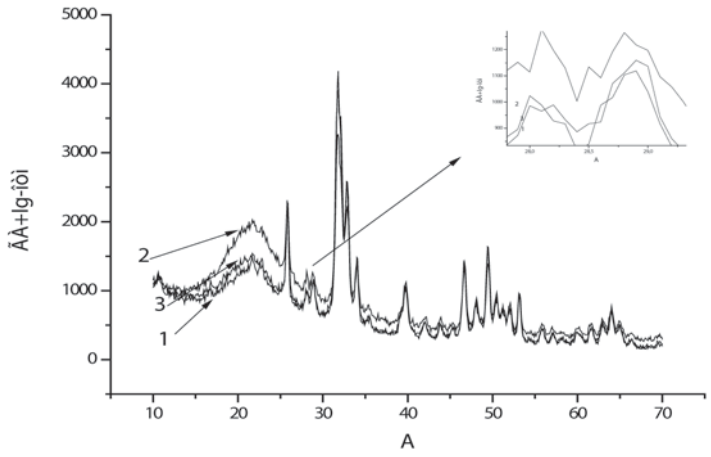

Fig. 3. XRD for the HA (3), IgG (2) and after washing the adsorbed protein (1)

It was previously shown the possibility to influence the structure of inorganic phosphate with amino acids, namely, that some of them are able to transform amorphous calcium phosphates in monocrystalline HA and participate in the structuring of the surface [9] and asparagine can produce changes in the structure of calcium hydrogen phosphate surface in 72 hours [10]. Theoretical studies have shown that the interaction of aminoacids with HA surface is associated with changes in the geometry of the surface groups with a change in bond lengths [9].

Conclusions

In connection with the above, we conclude that the HA interactions with a solution of IgG leads to its partial dissolution due to leaching from the surface of calcium triphosphate which, in our opinion, forms complexes with IgG. The interaction of HA and IgG acquire new qualities: HA alters the chemistry and structure of the surface and the IgG in complex with fragments of the surface of HA, which may lead to a change in its biological activity. To answer the question why such changes occur in the case of IgG but not of HAS, further investigations must be conducted.

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SYNTHESIS OF MONO-SUBSTITUTED AND SIMMETRICALLY 2,5-DISUBSTITUTED 1,3,4-OXADIAZOLES¶

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Abstract. In recent years 1,3,4-oxadiazoles have received considerable attention due to their wide range of biological activities and practical importance. They form an important class of five-member heterocyclic compounds with a variety of derivatives. This review will focus on several methods of synthesis for mono-substituted and symmetrically 2,5-disubstituted 1,3,4-oxadiazoles.

Keywords: mono-substituted 1,3,4-oxadiazoles, symmetrically 2,5-disubstituted 1,3,4-oxadiazoles.

1. Introduction

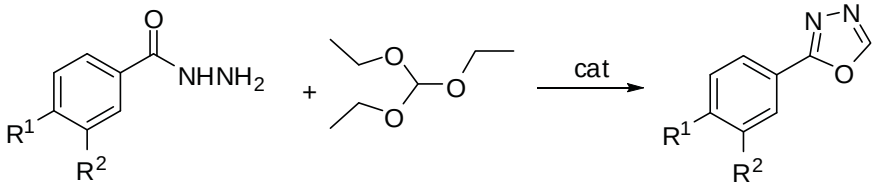
In the last decade a large amount of experimental data on synthesis and study of substituted 1,3,4-oxadiazoles has been accumulated in literature. Available data on the synthesis of substituted 1,3,4-oxadiazoles was previously summarized in a series of reviews [1-28]. This review will focus on the studies published over the last 10 years, as well as those unmarked in publications [1-28].

The specificity of 1,3,4-oxadiazole ring determines its high lability. According to this, the presented group of compounds can be transformed into substances with different cycle sizes, chemical nature of substituents, and number of heteroatoms. It should be noted that substituted 1,3,4-oxadiazoles have been used in medicinal chemistry [29-33], chemistry of pesticides [34], polymer chemistry [35-37], and material sciences [38-40].

2. Synthesis of mono-substituted 1,3,4-oxadiazoles

In earlier articles it was reported that sulfuric acid impregnated with silica gel catalyzes synthesis of 5-aryl-1,3,4-oxadiazoles **2a-e** [41].

Scheme 1



1a R^1=R^2=H
1b R^1=Cl, R^2=H
1c R^1=H, R^2=NO_2
1d R^1=OMe, R^2=H
1e R^1=F, R^2=H

2a R^1=R^2=H 85-91%
2b R^1=Cl, R^2=H 92%
2c R^1=H, R^2=NO_2 88%
2d R^1=OMe, R^2=H 90%
2e R^1=F, R^2=H 90%

It's been shown that heterocyclization of hydrazides **1a-c** with triethyl orthoester lasts 10 minutes, at room temperature without solvent, giving 5-aryl-1,3,4-oxadiazoles **2a-c** in 91%, 92% and 88%, yields respectively.

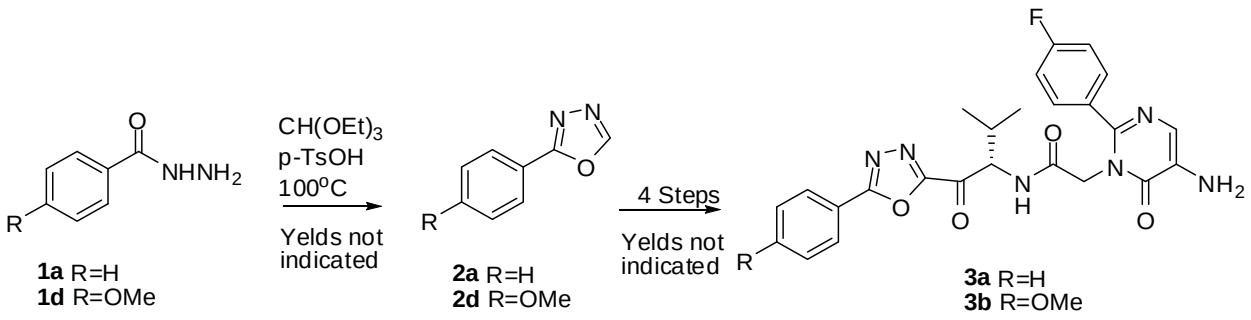
Another group of researchers reported the synthesis of 1,3,4-oxadiazole **2a**, under microwave irradiation of the mixture of hydrazide **1a** and triethyl orthoester on the surface of Nafion-NR50 (P_4S_{10}/Al_2O_3), however yield didn't exceed 85% [42]. A higher yield was observed (up to 90%) for target products **2d**, **2e** in presence of substituents (OMe and F) in the 4-position of the aromatic ring in the initial acyl hydrazides **1d**, **1e**.

The synthesis of 5-aryl-1,3,4-oxadiazoles **2a-c** catalyzed by 40 mol-% of $KAl(SO_4)_2 12H_2O$ is also known in literature [43]. Formation of 1,3,4-oxadiazoles **2a**, **2b** and **2c** in 89%, 95% and 93% yields, respectively, was observed upon heating the reaction mixture at 100°C for 6 h.

¶ This article is an extended abstract of a communication presented at the Conference Ecological Chemistry 2012

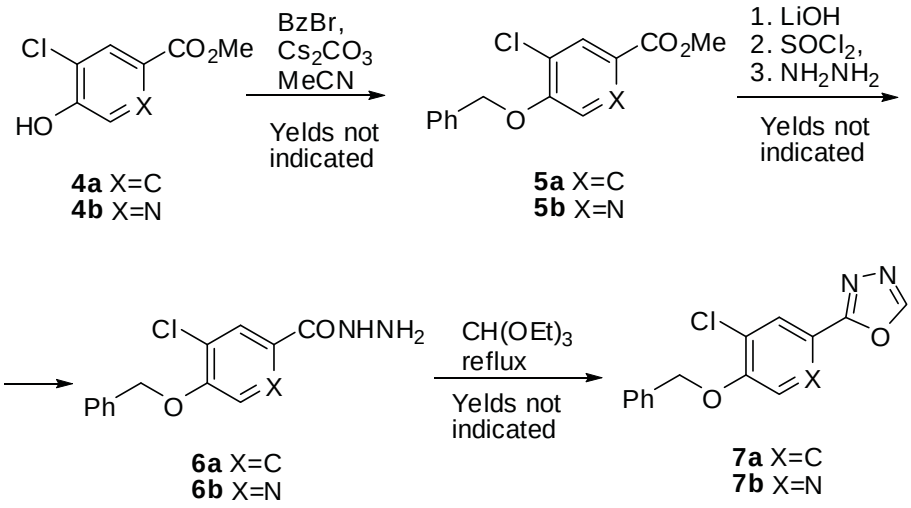
1,3,4-oxadiazoles **2a**, **2d** were obtained by heating the corresponding hydrazides **1a**, **1d** with triethyl orthoester up to 100°C in the presence of catalytic amounts of p-TsOH. Authors [44], used products **2a**, **2d** for the synthesis of the not peptide type inhibitors for human pancreatic elastase illustrated in structures **3a**, **3b** (Scheme 2).

Scheme 2



New inhibitors of biosynthesis of pathogenic bacteria were obtained by initial benzylation of hydroxy esters **4a**, **4b** in 2009 by the authors [45] (Scheme 3).

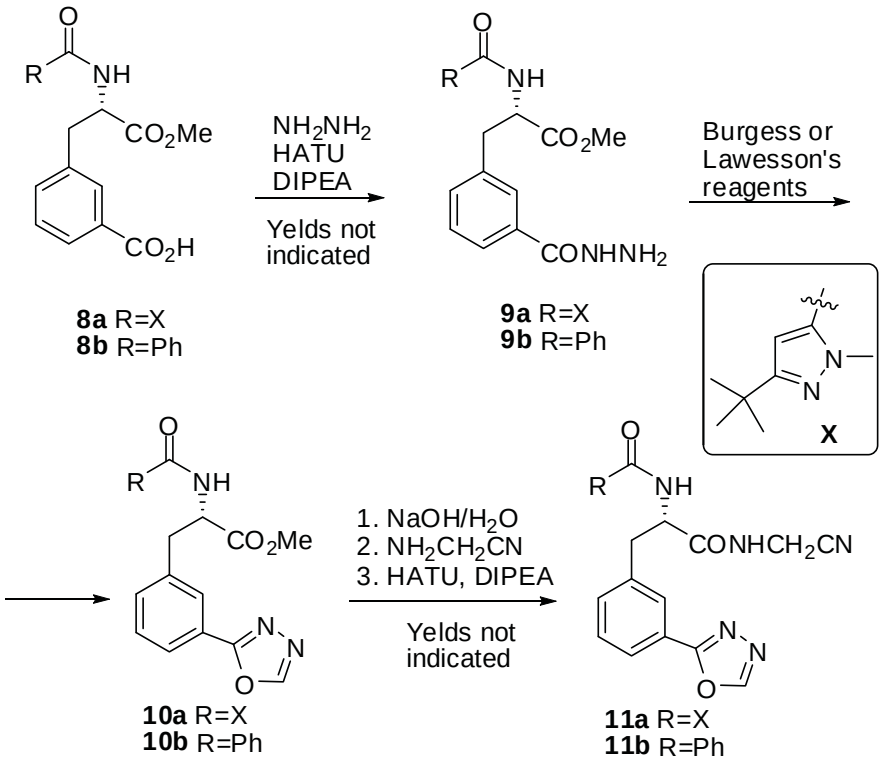
Scheme 3



Hydrazinolysis of esters **5a**, **5b** has led to the products **6a**, **6b**. It's been shown that heating of hydrazides **6a**, **6b** reflux in the solution of triethyl or trimethyl orthoester with distillation of the formed alcohol, facilitate the reaction of heterocyclization with the 1,3,4-oxadiazoles **7a**, **7b** formation. It was established that the product **7a** has a higher bioactivity in comparison with the bioactivity of the nitrogen-containing analogue **7b**.

Another group of researchers reported [46] about synthesis of cathepsin K inhibitors (Cathepsin K), which provokes human breast cancer (Scheme 4).

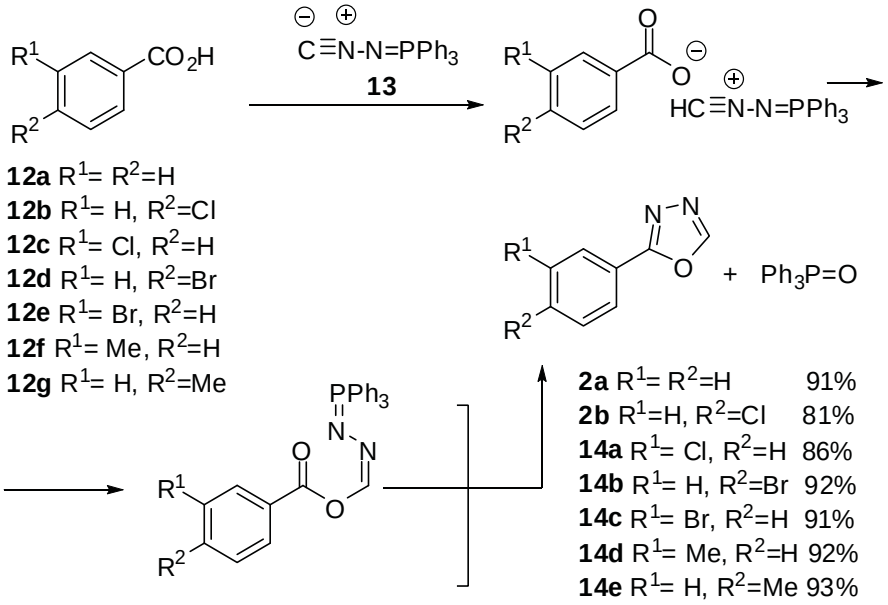
Scheme 4



Regioselective synthesis of hydrazides **9a**, **9b** from the acids **8a**, **8b** was the precursor of heterocyclization. It was found that the latter reaction is realized in presence of 1-methoxy-N-trimethylammoniosulfonyl-methanimidate (Burgess reagent) under microwave irradiation, or in THF solution at 40°C in presence of 2,4-bis(methoxyphenyl)-1,3,2,4-dithiaphosphetane-2,4-disulfide (Lavenson reagent). 1,3,4-Oxadiazoles **10a**, **10b** were further transformed into the target amides **11a**, **11b**.

In conclusion it should be noted a number of publications on the synthesis of 2-aryl-1,3,4-oxadiazoles involving benzoic acids **12a-12g** and (N-isocyananimino) triphenylphosphorane **13** [47-50] according to the scheme 5.

Scheme 5



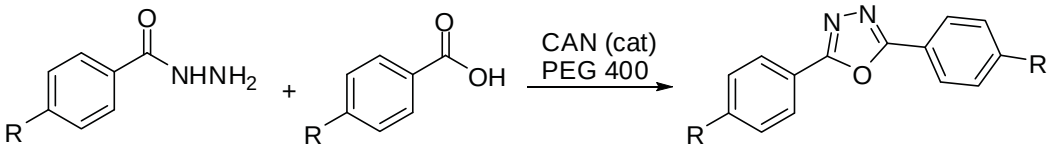
The reaction is carried out in methylene chloride at room temperature with ratio of reagents 1:1. The yields of final products **2a**, **2b**, **14a-14e** practically don't depend on the position or chemical nature of the substituent in initial benzoic acids **12a-12g**. This approach avoids transformation of the carboxyl group into hydrazide one and its cyclization into target 2-aryl-1,3,4-oxadiazoles.

Thus, two alternative approaches to monosubstituted 1,3,4-oxadiazoles have been reviewed, one of them can be carried out directly from benzoic acids and (N-isocyananimino) triphenylphosphorane, or by cyclization of benzoic acid hydrazide in presence of orthoesters.

3. Synthesis of symmetrically 2,5-disubstituted 1,3,4-oxadiazoles

One-pot synthesis of 2,5-diphenyl-1,3,4-oxadiazole **17a** has been realized by heating of benzoic acid hydrazide **1a** and benzoic acid **12a** in polyethylene glycol [51] (Scheme 6).

Scheme 6

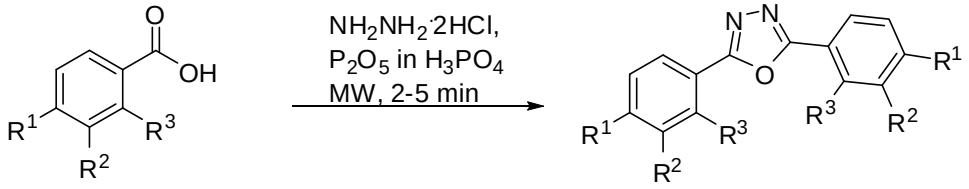


1a R=H	**12a** R=H	**17a** R=H 98%
1b R=Cl	**12b** R=Cl	**17b** R=Cl 97%
1d R=OMe	**12g** R=Me	**17c** R=Me 95%
15a R=OH	**16a** R=OH	**17d** R=OH 91%
15b R=Me	**16b** R=OMe	**17e** R=OMe 98%
15c R=NO_2	**16c** R=NO_2	**17f** R=NO_2 95%

Full conversion of the initial **1a**, **12a** catalized by 5 mol% $InCl_3$, CuO, $NbCl_5$, $FeCl_3$, I_2, $CuSO_4$ and CAN for 11, 8.5, 9, 9.5, 7, 8 and 5 hours, gave 2,5-diphenyl-1,3,4-oxadiazole **17a** with yields 87%, 79 %, 82%, 73%, 76%, 83% and 98% respectively. In the similar conditions 2,5-diaryl-1,3,4-oxadiazoles **17b-17f** have been synthesized in high yields using 5 mol% CAN, acids **12b**, **12g**, **16a-16c**, and hydrazides **1b**, **1d**, **15a-15c**. Another, less efficient synthesis of 2,5-diphenyl-1,3,4-oxadiazole **17a**, involved the consequent addition of 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and then 1-methoxy-N-trimethylammoniosulfonyl-methanimidate (Burgess reagent) to the mixture of hydrazide of benzoic acid **1a** and benzoic acid **12a** [52, 53]. The reaction is carried out at room temperature in THF solution, and the yield of **17a** does not exceed 88%.

Microwave irradiation of the mixture containing benzoic acid **12a**, $NH_2NH_2 \cdot 2HCl$ and P_2O_5 in H_3PO_4 make it possible to synthesize symmetrical 1,3,4-oxadiazole **17a** in 2 minutes [54] (Scheme 7).

Scheme 7

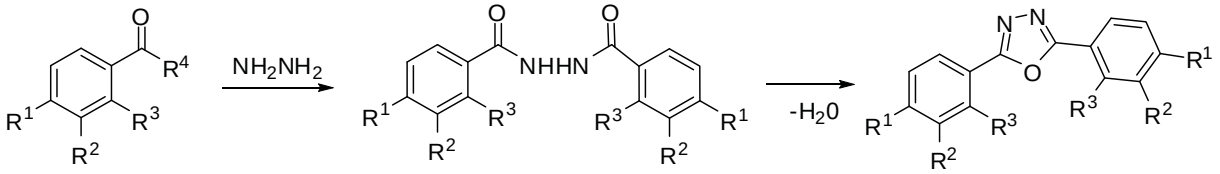


12a $R^1=R^2=R^3=H$	**17a** $R^1=R^2=R^3=H$ 80%
12b $R^1=Cl$, $R^2=R^3=H$	**17b** $R^1=Cl$, $R^2=R^3=H$ 95%
12g $R^1=Me$, $R^2=R^3=H$	**17c** $R^1=Me$, $R^2=R^3=H$ 98%
12f $R^1=R^3=H$, $R^2=Me$	**17d** $R^1=OH$, $R^2=R^3=H$ 30%
16a $R^1=OH$, $R^2=R^3=H$	**17e** $R^1=OMe$, $R^2=R^3=H$ 92%
16b $R^1=OMe$, $R^2=R^3=H$	**19a** $R^1=R^3=H$, $R^2=Me$ 97%
18a $R^1=R^3=H$, $R^2=OMe$	**19b** $R^1=R^3=H$, $R^2=OMe$ 91%
18b $R^1=R^3=H$, $R^3=OH$	**19c** $R^1=R^3=H$, $R^3=OH$ 64%

For the transformation of substituted benzoic acids **12b-12f**, **16a**, **16b**, **18a, 18b** to the corresponding 2,5-diaryl-1,3,4-oxadiazoles **17b-17e**, **19a-19c,** it's necessary to increase the reaction time up to 5 minutes. In most cases, the products of heterocyclization were obtained in high yields (91% - 98%), except the hydroxy-derivatives **17d**, **19c**, which were obtained in 30% and 64% yields respectively.

Alternative approach to the method discussed above, is a procedure of preliminary obtaining diarylhydrazines **22a-22h** from acids **12a**, **12g**, **16b**, **20a** or from chloranhydrides **21a-21f** [55-58] scheme 8.

Scheme 8



12a R^1=R^2=R^3=H, R^4=OH
12g R^1=Me, R^2=R^3=H, R^4=OH
16b R^1=OMe, R^2=R^3=H, R^4=OH
20a R^1=F, R^2=R^3=H, R^4=OH
21a R^1=R^2=R^3=H, R^4=Cl
21b R^1=R^3=H, R^2=R^4=Cl
21c R^1=R^2=H, R^3=OH, R^4=Cl
21d R^1=R^2=H, R^3=F, R^4=Cl
21e R^1=R^3=H, R^2=NO_2, R^4=Cl
21f R^1=Me, R^2=R^3=H, R^4=Cl

22a R^1=R^2=R^3=H
22b R^1=Me, R^2=R^3=H
22c R^1=OMe, R^2=R^3=H
22d R^1=F, R^2=R^3=H
22e R^1=R^3=H, R^2=Cl
22f R^1=R^2=H, R^3=OH
22g R^1=R^2=H, R^3=F
22h R^1=R^3=H, R^2=NO_2

17a R^1=R^2=R^3=H 71-98%
17c R^1=Me, R^2=R^3=H 65-100%
17e R^1=OMe, R^2=R^3=H 75%
19c R^1=R^3=H, R^3=OH 98%
23a R^1=F, R^2=R^3=H 78-100%
23b R^1=R^3=H, R^2=Cl 91%
23c R^1=R^2=H, R^3=F 93%
23d R^1=R^3=H, R^2=NO_2 94%

The limiting stage of the transformation: acid (anhydride) → hydrazide → 2,5-bis-(aryl)-1,3,4-oxadiazole, in most cases is the reaction of dehydration. When heating hydrazide **22b** in $POCl_3$ reflux for 8 hours, the yield of 2,5-bis-(4-methylphenyl)-1,3,4-oxadiazole **17c** did not exceed 65% [55]. The quantitative yield of the latter compound was obtained by using the ratio of 1 eq. of 2-chloro-1,3-dimethylimidazolinium chloride (DMC) to 2 eq. of Et_3N in the solution of CH_2Cl_2 at room temperature [56]. In the latter case, the reaction time was increased up to 21 hours. This method has been used for the synthesis of compounds **17a**, **23a**. The yield and time of the reaction was 86% (16 hours) and 100% (20 hours), respectively. It was also found that $ZrCl_4$ catalyzes diarylhydrazines cyclization in CH_2Cl_2 at room temperature [57]. 1,3,4-oxadiazoles **17a**, **17e**, **23a** have been synthesized in 3 hours in 71%, 75% and 78% yields respectively.

It should be mentioned that the synthesis of symmetrical 2,5-disubstituted 1,3,4-oxadiazoles from chloranhydrides **21a-21f** without isolation of intermediate diarylhydrazines **22a, 22b, 22e, 22f, 22g, 22h** [58]. Cyclization is realized with $BF_3 \cdot Et_2O$ in 1,4-dioxane reflux for 1-2 hours giving products **17a, 17c, 19c, 23b, 23c, 23d** in 98%, 97%, 98%, 91%, 93% and 94% yields, respectively.

4. Conclusions

Hereby, the various ways of synthesis for mono-substituted and symmetrically 2,5-disubstituted 1,3,4-oxadiazoles have been analyzed. The important structural characteristic of 2,5-disubstituted 1,3,4-oxadiazoles is the presence of two aromatic rings with a diversity of substituents spaced by a heterocycle. This fact opens up new opportunities for a wide variety of derivatives, which can serve as new useful substances for medicinal chemistry, polymer chemistry and material sciences. It should also be noted that the literature publications contain data on the synthesis of asymmetrical disubstituted 1,3,4-oxadiazoles, discussion of which will be presented in a separate review.

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ZNCL$_2$/ALCL$_3$/SILICA (ZAS) AS AN EFFECTIVE HETEROGENEOUS CATALYST FOR THE SYNTHESIS OF 5-ARYLOXY TETRAZOLES

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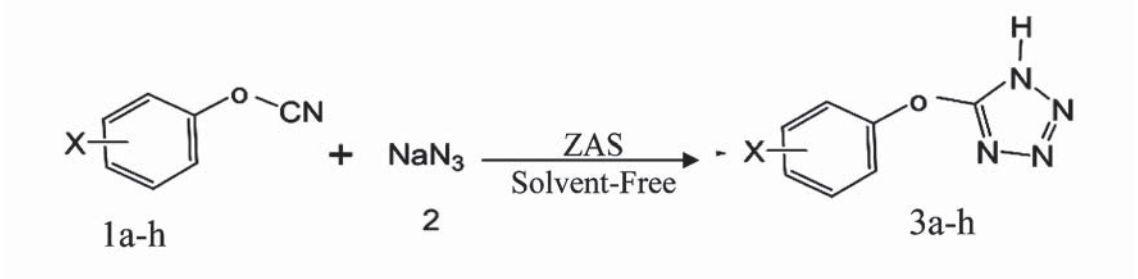
Abstract. A simple and efficient method for preparation of 5-aryloxy tetrazoles (**3a-h**) from arylcyanates (**1a-h**) with using $ZnCl_2/AlCl_3$/silica (ZAS) as an effective heterogeneous catalyst in solvent free is described with excellent yields and high purity. The rate of product formation was enhanced by introduction of electron donating substituents. The ^1H NMR and chemical shifts and multiplicities are also discussed.

Keywords: 5-Aryloxy tetrazoles, arylcyanates, guanyl azides, $ZnCl_2/AlCl_3$/silica (ZAS).

1. Introduction

The tetrazole ring system has attracted considerable attention in recent years [1-8], especially among medicinal chemists, as a potential surrogate for *cis*-peptide linkage [1,9-12], and carboxylic acids [1,13,14]. Indeed, the number of patent claims and publications related to medicinal uses of tetrazoles continue to grow rapidly and cover a wide range of applications: Tetrazoles have been found to exhibit antihypertensive, antiallergic and antibiotic activities [15-22], and they are currently used, for example, as activator [23-25], anticonvulsants [1,26], also in cancer [27-29], and AIDS treatment [1,30,31]. Furthermore aminotetrazoles derivatives have been patented for muscle relaxation, anti-inflammatory anti-arthritic, analgesic, ulcer therapeutic and coccidiostatic properties. Tetrazoles are also applied in agriculture, as plant growth regulators, herbicides and fungicides [32,33], stabilizers in photography and photoimaging [32,34], and explosives in rocket propellants [35-37]. Another important application of tetrazoles is the preparation of imidoylazides [38-43]. The addition of azide anion to nitriles, cyanates and cyanamides is the most common direction for preparing 5-substituted tetrazoles and 5-aryl/alkyl oxytetrazoles [1-7,38-45]. In most cases, reaction actually proceeds in solutions of hydrazoic acid in solvents such as benzene, toluene, xylene and chloroform. When hydrazoic acid is used, care must be taken by monitoring the concentration of hydrazoic acid in the reaction mixture to avoid an explosion [1-7,32,45,46,73]. A substitute for hydrazoic acid is a mixture of sodium azide and ammonium chloride with dimethylformamide as the solvent [1-7,17,32,45,47,72,73,74]. In dimethylformamide, the reaction mixture must be heated at ~150°C from several hours to several days. Additional disadvantage of dimethylformamide is the solubility in both organic solvents and water. Thus, removing the DMF from tetrazole is difficult. To resolve this problem, the reaction was carried out in several solvents, which allowed the temperature to be elevated to the necessary degree to enhance the reaction [32,45,47]. Another possible method of obtaining the 5-alkyltetrazoles was an adaption of the von Braun degradation of tertiary amines with cyanogens bromide. In this way it might be possible to eliminate an alkyl group from a 5-dialkyltetrazoles [48,52]. These methods suffer from one or more disadvantages such as low yield, long reaction times, harsh reaction conditions, lack of easy availability/preparation of the starting materials, difficulty of workup due to the application of homogeneous catalyst, use of expensive and toxic reagents and the in situ generated hydrazoic acid is highly toxic and explosive. Because of the safety considerations, we required a method that did not use hydrazoic acid or apply an azide source because of the in situ production of hydrazoic acid. Thus, a convenient and efficient method was required for preparation of the aryloxytetrazoles. From the standpoint of 'green chemistry', significant efforts have been made to find an alternative to organic solvents. A very attractive substitute for these solvents is a solvent–free reaction (industrially important due to reduced pollution, low cost, and simplicity in process and handling). In view of the importance of aryloxy tetrazoles and aryloxy imidoylazides [38-43], we want to report a facile, effective and less hazard method for synthesis the 5-aryloxy tetrazoles (**3a-h**) from arylcyanates (**1a-h**) in quantitative yields by using $ZnCl_2/AlCl_3$/Silica (ZAS) as an effective catalyst in solvent free (Scheme 1).



Scheme 1

2. Results and discussion

The cyanates **1a-h** were prepared according to literature [9,54,55]. In order to gain insight to the electronic effects. The nature of the substituent appears to play an important role for directing the course of the reaction. As shown in Table 1, among the various cyanates tested, electron-rich aromatic cyanates reach completion after 6-9 h, whereas electron-poor aromatic species require little higher times (compare entries 1-4 with 5-6 in Table 1). In addition, there is an excellent correlation between the effect of substitution on the benzene ring and the time of reaction. Scheme 1 and Table 1.

Table 1

The preparation of 5-aryloxy tetrazoles (3a-h) from arylcyanates by using ZAS at r.t.

Entry	Cyanate	Ar	Product (tetrazole)	Reaction time (h)	Yield (%)[a]	Mp °C	Mp(lit, ref) °C [8,16]
1	**1a**	4-$CH_3C_6H_4$	**3a**	8	89	139-140	140-142
2	**1b**	2,6-$(CH_3)_2C_6H_3$	**3b**	9	90	171-173	173-174
3	**1c**	4-$CH_3OC_6H_4$	**3c**	6	97	150-151	149-150
4	**1d**	C_6H_5	**3d**	9	87	136-138	137-138
5	**1e**	4-ClC_6H_4	**3e**	17	78	156-158	166-167
6	**1f**	4-$NO_2C_6H_4$	**3f**	20	53	161-163	162-163
7	**1g**	α-naphthyl	**3g**	11	80	174-176	-
8	**1h**	β-naphthyl	**3h**	10	81	152-154	-

[A] Yields refer to the pure isolated products.

The following important results is extracted from data in Table 1 and compared to those reported in literature [50-52]. In general, when the substitution is electron-donating group reaction is completed at shorter reaction time (starting cyanate **1a-c** consumes faster) than when the substitution is electron-withdrawing (compare entries 5 and 6 with 1, 2, 3 and 4 in Table 1). The entries 5 (17 h) and 6 (20 h) confirms this result. The rate of reaction was found to decrease with increasing the electronegativity of a substituent on the aryl group along. These results are the reverse of what was reported for the nitriles [45,65,66]. When the substitution on aryl ring is electron-donating in arylcyanates **1** the oxygen attached to aryl ring has more power basicity. On the other hand, the electron-donating group acts to increase the electron density on the oxygen attached to aryl group, and thus assists in the cyclisation of guanyl azides to give 5-aryloxy tetrazoles. Furthermore, it is worthy to mention that reaction of α– and β–naphthol (entries 7 and 8) did not proceed completely in the recently reported method [9]. Indeed, considerable amounts of starting material remained even after long times, and/or at high temperatures. However, the time to complete reaction in solvent free and room temperature (Table 1) is a good indication that, the first step, addition of hydrogen ion to arylcyanate **1a-h** the most important step or the rate determining step of reaction, because, when the substitution is electron-donating, reaction is completed at shorter reaction period the products were characterized by ^{1}H NMR,^{13}C NMR, IR, elemental analyses (CHN).

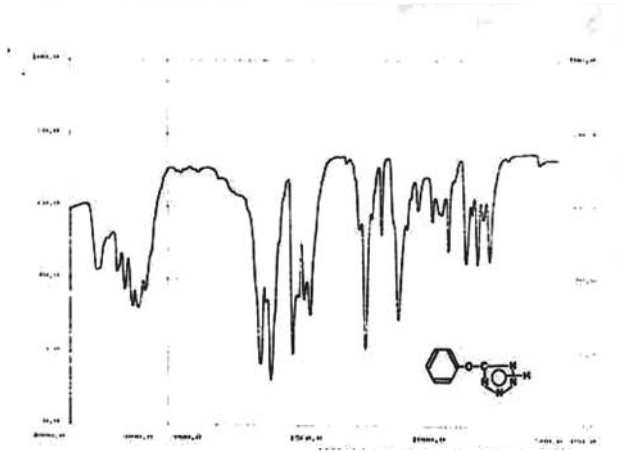


Fig. 1. IR spectrum (KBr) 5-phenoxy tetrazole (3d)

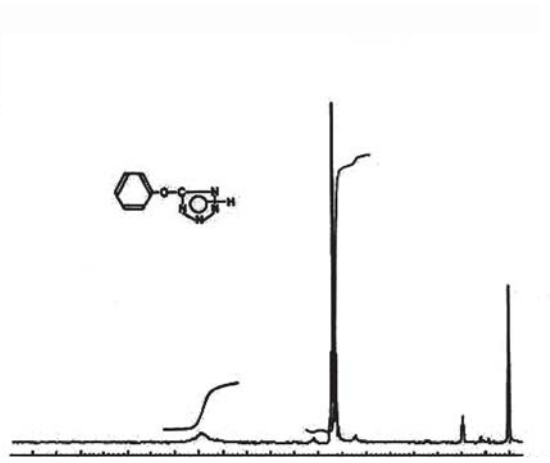


Fig. 2. ^{1}HNMR spectrum 5-phenoxy tetrazole (3d)

The disappearance of one strong and sharp absorption band (CN stretching band) and the appearance of a NH stretching band and CO stretching band in the IR spectra provided clear evidence for the formation of aryloxytetrazoles (Fig. 2). ^{13}C NMR spectra displayed signals at δ=154–157.5 ppm, indicative of C5 in the tetrazole ring. The free N–H bond of tetrazoles (NH) makes them acidic molecules, and not surprisingly it has been shown that both the aliphatic and aromatic heterocycles have pKa values that are similar to the corresponding carboxylic acids, due to the ability of the moiety to stabilize a negative charge by electron delocalization. In general, tetrazolic acids exhibit physical characteristics similar those of to carboxylic acids. Thus, the signal of the NH proton of the tetrazole ring (NH) shifted downfield (see Fig. 2 and ^{1}H NMR data of **3a–h**).

3. Conclusions

A facile, convenient and less hazard synthetic method for 5-aryloxy tetrazoles **3** from arylcyanates in solvent-free was achieved with quantitative yields high purity without involvement of expensive reagents or the formation of undesirable side products. To show the advantages of $ZnCl_2/AlCl_3$/Silica (ZAS) as a catalyst in comparison with other materials, we compared the reaction of $ZnCl_2/AlCl_3$/Silica (ZAS) with $ZnCl_2$, glacial acetic acid, PPh_3, HCl [9], $SnCl_4$ and LiCl in the synthesis of 5-(4-chlorophenoxy)-tetrazole (**3e**) (Table 1, Entry 5). As shown in Table 2, $ZnCl_2/AlCl_3$/ Silica (ZAS) is a better catalyst in the synthesis of 5-(4-Chloro phenoxy)- tetrazole (**3e**).

Table 2

Comparison effect of different catalysts in the synthesis of 5-(4-Chlorophenoxy)-tetrazole (3e)

Entry	Catalyst	Solvent	Time (min)	Temperature (ºC)	Yield%
1	PPh_3	DMF	120	120	57
2	HCl[b]	CH_3COCH_3	80	65	46
3	LiCl	DMF	110	120	55
4	CH_3COOH[a]	CH_3COOH	35h	25	59
5	$SnCl_4$	DMF	120	120	58
5	ZnCl2	DMF	100	120	71
6	ZAS	-	17h	25	76

[A] Glacial acetic acid as both solvent and proton donor source. [a]Added on work up. [9]

4. Experimental Section

CAUTION: Although aryloxytetrazoles are kinetically stable and in most cases are insensitive to electrostatic discharge, friction, and impact, they are nonetheless energetic materials and appropriate safety precautions should be taken, especially when these compounds are prepared on a larger scale. Hydrazoic acid is an unstable component which may decompose violently, forming nitrogen and hydrogen. Depending on the literature source, an explosive gas mixture can be formed with air or nitrogen above a concentration of more than 8-15% [56].

All reagents were purchased from the Merck and Aldrich chemical companies and used without further purification. Products were characterized by spectroscopic data [infrared (IR), fourier transform (FT)–IR,^{1}H NMR, and ^{13}CNMR spectra, elemental analyses (CHN), and melting points. The NMR spectra were recorded in chloroform and acetone.^{1}H NMR spectra were recorded on Bruker Avance DRX 500-MHz instruments. The chemical shifts (d) are reported in parts per million (ppm) relative to the tetrametleysilene (TMS) as internal standard. J values are hertz given in (Hz).^{13}C NMR spectra were recorded on Bruker Avance DRX 500-MHz instruments. IR (KBr) and FT-IR (KBr) spectra were recorded on Shimadzu 470 and Perkin-Elmer 781 spectrophotometers, respectively. Melting points were taken in open capillary tubes with a Buchi 510 melting-point apparatus and were uncorrected. The elemental analysis was performed using Heraeus CHN-O-Rapid analyzer.Thin-layer chromatography (TLC) was performed on silica gel polygram SIL G=UV 254 plates. All products are known compounds and are identified by comparison of some their spectral data (IR and ^{1}H-NMR) and physical properties with those of authentic samples [48,49,53,57]. All starting materials and solvents were purified with the proper purification techniques before use, when necessary [71]. The cyanates **1a-h** were prepared according to literature [9,54,55]. $ZnCl_2/AlCl_3$/Silica (ZAS) was prepared from silica gel and perchloric acid according to literature [75].

Typical Experimental Procedure for the Preparation of 5-Aryloxy tetrazoles 3 Using $ZnCl_2/AlCl_3$/Silica (ZAS)

The cyanate (1 mmol), (ZAS) (0.1 gr) and sodium azide (3 mmol) were added. the mixture was pulverized in a mortar (or the mixture was stirred by a magnet in a test tube) at room temperature for an appropriate time (Table 1). The reaction was monitored in TLC. After completion of the reaction, $CHCl_3$ was added and the mixture was filtered for separating the reagent. The solvent ($CHCl_3$) evaporated to give the product. Pure products were obtained at high yields, as summarized in Table 1. The desired pure products were then filtered and characterized by ^{1}H NMR, IR and melting points. This method did not require any further purification. No side product was observed under the reaction conditions. The spectral data of 5-aryloxy tetrazoles are given below.

Melting Point, IR and ^{1}H -NMR chemical shifts of 5-aryloxy tetrazoles (3a-h):

5-(4-Methylphenoxy)-tetrazole(3a): M.p. = 139-140°C. lit [9], 140-142°C. IR (KBr, cm^{-1}): 3005 (m), 2900 (m), 2705 (m), 2550 (m), 2455 (m), 2350 (m), 1620 (s), 1590 (s), 1500 (s), 1440 (m), 1190 (s), 1120 (m), 1050 (m), 820 (s). cm^{-1}. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$), δ ppm; 2.20 (S, 6H), 7.12 (S, 3H), 10.3 (S, 1H).

5-(2,6-Dimethylphenoxy)-tetrazole(3b): M.p. = 171-173 °C. Lit [9], 173-174 °C. IR (KBr, cm^{-1}): 3010 (m), 2905 (s), 2855 (s), 2705 (s), 2605 (s), 2450 (s), 1605 (s), 1570 (s), 1470 (s), 1440 (s), 1410 (s), 1160 (s), 1045 (s), 785 (s), 775 (s). cm^{-1}. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$), δ ppm; 2.38 (S, 3H), 7.30 (S, 4H), 9.8 (S, br, 1H).

5-(4-Methoxyphenoxy)-tetrazole(3c): M.p. = 150-151 °C. Lit [9], 149-150 °C. IR (KBr, cm^{-1}): 3060 (m), 2950 (m), 2900 (m), 2850 (m), 2750 (m), 2600 (m), 1620 (m), 1600 (m), 1590 (m), 1500 (s), 1470 (m), 1440 (s), 1190 (m), 1180 (m), 1040 (m), 1030 (m), 820 (s). cm^{-1}. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$), δ ppm; 3.88 (S, 3H), 7.00 (d , J = 10.5 Hz, 2H), 7.4 (d, J = 10.5 Hz, 2H), 8.4 (S, 1H).

5-(phenoxy)-tetrazole(3d): M.p. = 136-138°C. Lit [9], 137-138°C. IR (KBr, cm^{-1}): 2450-3000 (m), 1615 (s), 1575 (s), 1485 (s), 1440 (s), 1185 (s), 1050 (s),680- 820 (m). cm^{-1}. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$), δ ppm; 7.20 (S, 5H), 13.00 (br, 1H).

5-(4-Chlorophenoxy)-tetrazole(3e): M.p. = 156-158°C. Lit [9], 166-167 °C. IR (KBr, cm^{-1}): 3010 (m), 3000 (m), 2850 (m), 2700 (s), 2600 (m), 2450 (s), 1610 (s), 1590 (s), 1480 (s), 1410 (m), 1190 (s), 1175 (m), 1080 (m), 1050 (s), 825 (s). cm^{-1}. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$), δ ppm; 7.35 (S, 4H), 8.66 (br, 1H).

5-(4-Nitrophenoxy)-tetrazole(3f): M.p. = 161-163 °C. Lit [9], 162-163 °C. IR (KBr, cm^{-1}): 3120 (m), 3100 (s), 3000(m), 2750 (s), 2600 (s), 2450 (s), 1650 (vs), 1600 (s), 1570 (vs), 1540 (vs), 1480 (vs), 1450 (s), 1410 (s), 1350 (vs), 1310 (s), 1200 (vs), 1190 (s), 1120 (s), 1100 (s), 1060 (vs),860 (s), 850 (s). cm^{-1}. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$), δ ppm; 7.55 (d, j=9Hz ,2H), 8.33 (d, j=9Hz, 2H), 14.1 (S, 1H).

5-(Naphthalen-1-yloxy)-tetrazole(3g): M.p. = 174-176°C. IR (KBr, cm^{-1}): 2750-3060 (m), 1630 (s), 1600 (s), 1500 (m), 1485 (m), 1390 (m), 1280 (m), 1170 (s), 1105 (m), 1050 (w), 680- 950 (m). cm^{-1}. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$), δ ppm; 7.20 (d, J = 7.5 Hz, 1H), 7.35-7.45 (m, 3H), 7.63 (d, J = 8.2 Hz, 1H), 7.78 (dd, J = 9.3 Hz, J = 2.1Hz, 1H), 7.92 (dd, J = 8.8 Hz, J = 2.1 Hz, 1H), 11.1 (S , 1H). ^{13}CNMR(500 MHz, $(CD_3)_2CO$), δ ppm; 117.7, 120.8, 124.8,124.9, 125.6, 125.7, 126.9, 127.3, 134.0, 146.2, 156.7. Analysis Calcd. For $C_{11}H_8N_4O$: C, 62.26; H, 3.77; N, 26.42;% Found; C, 62.46; H, 3.66; N, 26.53%.

5-(Naphthalen-2-yloxy)-tetrazole(3h): M.p. = 152-154 °C. IR (KBr, cm^{-1}):): 2800-3070 (m), 1625(s), 1590(s), 1510 (m), 1475 (m), 1400 (m), 1275 (m), 1170(s), 1110 (m), 1070 (w), 660- 970 (m). cm^{-1}. ^{1}H-NMR (500 MHz, $(CD_3)_2CO$), δ ppm; 7.20 (dd, J = 8.7 Hz,J = 2.1 Hz, 1H), 7.34-7.41 (m, 2H), 7.49 (d, J = 2.1 Hz, 1H), 7.71 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 8.7Hz, 2H)., 11.3 (S, 1H). ^{13}CNMR(500 MHz, $(CD_3)_2CO$), δ ppm; 117.9, 121.2, 124.8, 125.8, 126.9, 127.1, 128.5, 130.5, 133.1, 148.3, 156.5. Analysis Calcd. For $C_{11}H_8N_4O$: C, 62.26; H, 3.77; N, 26.42;% Found; C, 61.87; H, 3.61; N, 26.50%.

5. Acknowledgements

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6. References

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A REACTION OF 4,5-DIPHENYLIMIDAZOLE NITRATION IN THE PRESENCE OF SOME 3D-METALS NITRATES

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Abstract. Three new coordination compounds of Cobalt(II), Nickel(II) and Zinc(II) with 4,5-diphenyl-2-nitroimidazole ligand of general formulae $[M(C_3N_2(C_6H_5)_2NO_2)_2(CH_3OH)_2]$ have been synthesized and characterised by elemental, thermogravimetric, X-ray diffraction analysis, IR, UV-Vis and NMR spectroscopies.

Keywords: solvothermal synthesis; Cobalt(II), Nickel(II), Zinc(II) complexes; 4,5-diphenyl-2-nitroimidazole, physico-chemical properties.

Introduction

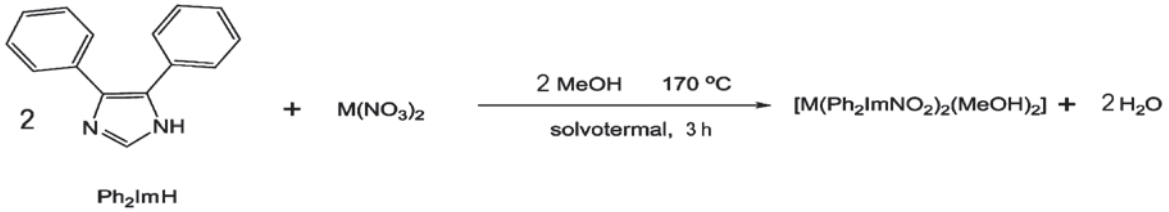
Imidazole's fragment is a part of histidine molecule, which is an important component of many proteins. It plays an important role in enzymes activity [1-4], and can also form various supramolecular structures with metal ions [5-6]. From this point of view coordination compounds of imidazole are of particular interest to be investigated. Of interest are compounds of 4,5-diphenylimidazole which have specific steric regulations and electronic structure.

It is known that nitro-imidazole compounds have a high microbial activity and are widely used in medicine [1]. Direct nitration reaction of imidazole and its derivatives at C2 position is difficult to realise - it can be done in several stages. On the other hand there is known the natural compound *azomicin* which contain nitro-group in position C2 of imidazole and has strong antibiotic properties [2].

In this paper the synthesis and physico-chemical properties of 4,5-diphenyl-2-nitroimidazole and three new coordination compounds of $[M(C_3N_2(C_6H_5)_2NO_2)_2(CH_3OH)_2]$ composition are presented.

Results and discussion

During solvothermal synthesis an unexpected reaction of 4,5-diphenylimidazole nitration in the presence of some 3d metals nitrates takes place. Thus, under solvothermal conditions (170°C, 3 h) in methanol the cobalt, nickel or zinc nitrate react with 4,5-diphenyilimidazole and the 4,5-diphenyl-2-nitroimidazole and its complexes of $[M(C_3N_2(C_6H_5)_2NO_2)_2(CH_3OH)_2]$ composition, where M = Co(II) (**I**), Ni(II) (**II**) and Zn(II) (**III**), have been obtained (Scheme 1).



Scheme 1. The synthesis of complexes I-III

The analytical data analysis is in good agreement with the presented compositions.

Infrared spectra

IR spectra of complexes **I-III,** free ligands 4,5-diphenylimidazole and 4,5-diphenyl-2-nitroimidazole (**IV**) were studied (experimantal part). Comparing these spectra one can see that complexes contain 4,5-diphenyl-2-nitroimidazole. In addition new bands for coordinated methanol (for ν(OH) – 3650 - 3676 cm^{-1}, $\nu_{as,s}$ (CH)(CH_3) -– 2989 - 2772, δ(CH_3) -1437 - 1438 and 1386 - 1400 cm^{-1}, ν(C-OH) – 1014 - 1025 cm^{-1}) were observed. They correspond to the valence vibrations of OH group, stretching and deformation vibrations of CH_3 group, and C-OH valence vibration, respectively.

Asymmetric valence vibration of NO_2 group is designated by the appearance of strong bands in the spectra of complexes between 1499 - 1504 cm^{-1}, shifted to the lower energy than the signal of NO_2 group in free ligand 4,5-diphenyl-2-nitroimidazole (1539 cm^{-1}) indicating its coordination to metal ions. Appearance of absorption bands at 480-540 cm^{-1} in the spectra of the complexes is due to valence vibrations of the coordination bonds M-O and M-N [7-8]. In conclusion, IR spectra points in favor of methanol and 4,5-diphenyl-2-nitroimidazole coordination to metal ion.

NMR studies

^{1}H NMR spectrum of the diamagnetic complex **III** was analyzed and compared with that of the starting ligand (Figure 1). The spectra allow elucidation of the hyperfine structure. In the ligand's spectrum, the signals in the form of multiplets (δ = 7,30 - 7,61 ppm (multiplet, 10H, CH_{Ph})) corresponding to three types of protons of the phenyl ring are well separated, while in complex these bands overlap forming a broadband. This fact may be explained by the presence of the reflection plane, passing through the nitrogen atom of the nitro- group and C2 carbon atom in the molecule of ligand and the absence of it in the complex molecule. In the first case two phenyl groups of 4,5-diphenylimidazole appear as an entity, but in complex each of 4 phenyl groups appear with their unique spectrum. Quite small differences in chemical displacement values of proton signals lead to an overlap in the spectrum.

In ^{1}H NMR spectrum of complex **III** signals at 3,85 ppm, corresponding to protons of the CH_3 groups of coordinated methanol molecules, were observed. As a result of coordination of methanol molecules to metal ion, there is an additional deshielding of their methyl protons, with shifting of corresponding signals to higher ppm values, than signals known for free methanol (3,31 ppm). May be supposed that the signal of hydroxylic proton of methanol in the spectrum of complex **III** is partially overlapped by the signals of water protons at 3 ppm, which was present in deuterated acetone as impurity.

It should be noted that broadband at 11,67 ppm, corresponding to iminic proton of imidazole's ring, disappears in the spectrum of Zn(II) complex. It shows that by coordination to metal the ligand is deprotonated.

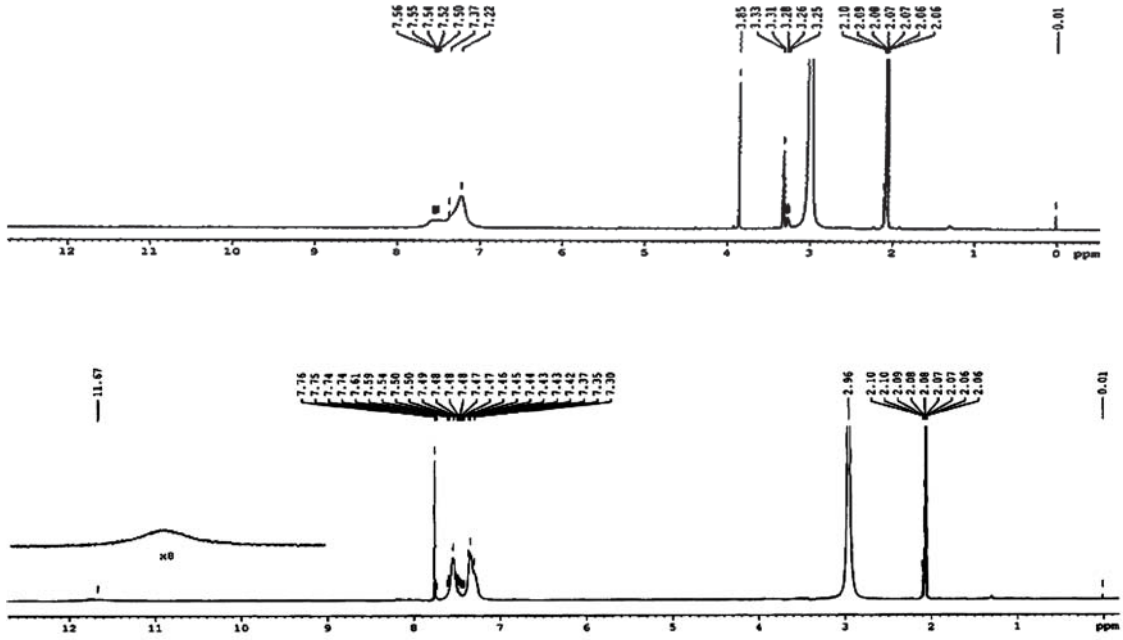


Figure l. ^{1}HNMR spectra of complex $[Zn(4,5\text{-}Ph_2ImNO_2)_2(CH_3OH)_2]$ (up) and 4,5-diphenylimidazole (down)

Magnetic properties

Magnetic susceptibilities of the samples were determined at room temperature (287 K - 283 K) by Gouy method [9]. The following values of the effective molar magnetic moment of complexes **I** and **II** were obtained: $(\mu_{ef})_M$ (**I**) = 5,00 BM; $(\mu_{ef})_M$ (**II**) = 3,10 BM. These values of magnetic moment are in the early observed limits of this parameter for cobalt(II) and nickel(II) complexes [10]. According to obtained experimental data, it can be argued that the metal ions in complexes are in high spin state and have a distorted octahedral neighborhood, which demonstrates that the ligand field is weak.

Thermogravimetric analysis

The results of thermal analysis in air conditions revealed that complexes are stable up to 120 - 150°C. At higher temperatures the complexes decompose and there are three steps of decomposition processes. At the first stage (120 - 220°C) it was observed a weight loss of 11 - 12 % which corresponds to elimination of two molecules of coordinated methanol. The second stage (200 - 300°C) ends with elimination of NO_2-groups, followed by slow decomposition of the residue to form corresponding metal oxides (Scheme 2).

$$[M(Ph_2ImNO_2)_2(MeOH)_2] \xrightarrow[-2MeOH]{} \{M(Ph_2ImNO_2)_2\} \xrightarrow[-2\ NO_2]{} \{M(Ph_2Im)_2\} \longrightarrow MO$$

Scheme 2. The scheme of thermal decomposition of I-III compounds

Electronic spectra

The UV-Vis spectra of complexes **I-III** and starting ligand 4,5-diphenylimidazole are presented in Figure 2 and values of their parameters – in Table 1.

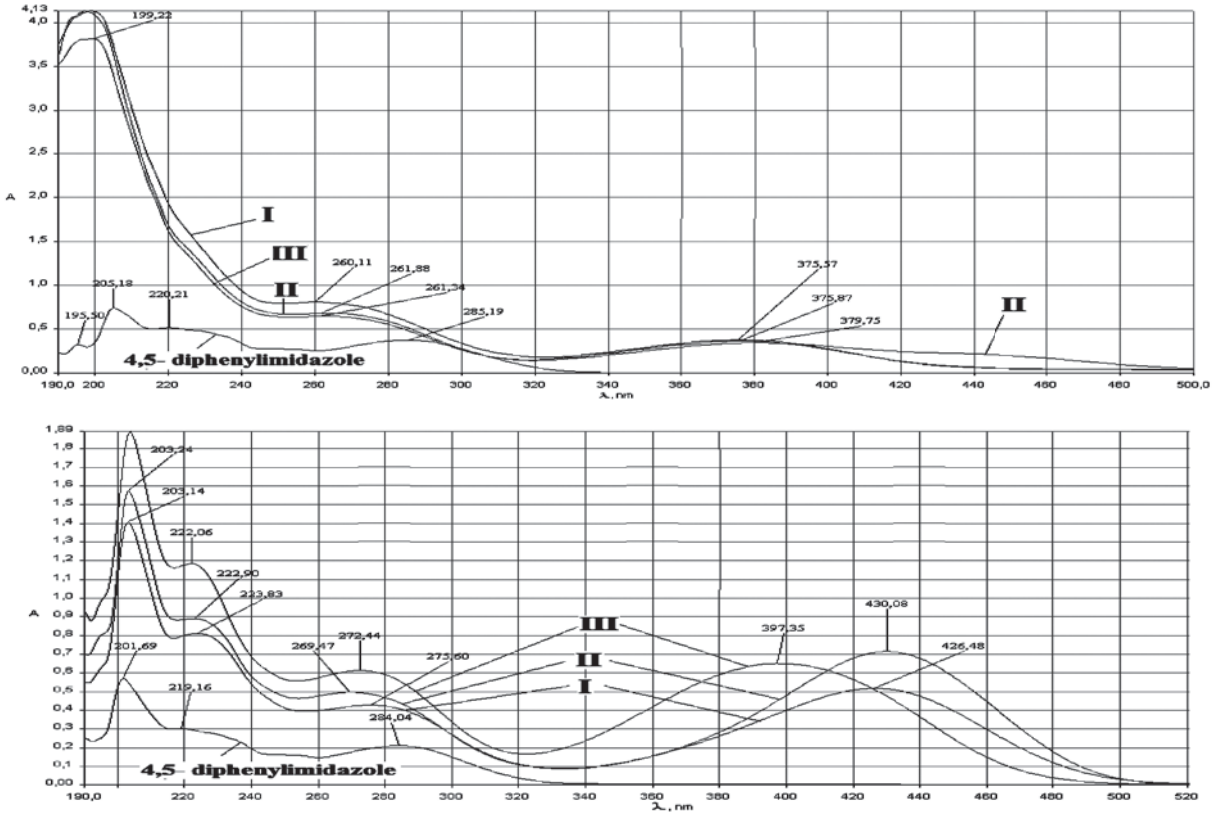


Figure 2. Electronic spectra of complexes I-III and initial ligand 4,5-diphenylimidazole in isopropanol (up) and in ethanol (down)

Table 1

UV-Vis data for investigated substances in different solvents

Compound / Solvent	λ_{max}(ε, ×10^{-3} M^{-1}·cm^{-1})			
	(I)	(II)	(III)	4,5-diphenylimidazole
Isopropanol	200 (276,993)	200 (186,288)	199 (301,772)	205 (17,566)
	260 (53,401)	262 (39,891)	261 (51,398)	220 (17,370)
	376 (27,093)	379 (20,264)	376 (29,501)	285 (12,606)

Ethanol	203 (41,630)	203 (50,022)	203 (52,758)	202 (17,635)
	224(24,210)	223 (28,177)	222 (33,218)	219 (9,281)
	276(12,467)	270 (15,424)	273 (17,487)	284 (6,274)
	426 (15,106)	430 (21,961)	397 (18,463)	

In the starting ligand spectrum there are three absorption bands only in ultraviolet domain (190 - 320 nm), but in the spectra of complexes the absorption bands appear both in the ultraviolet, and in the visible (between 320 and 520 nm) domains. Bands in the visible domain are due to metal - ligand charge transfer.

This electron transfer occurs with approximately the same energy in all three studied complexes when isopropanol was used as a solvent – 316 - 319 kJ/mol and showed different values of energy for ethanol: **I** - 281 kJ/mol, **II** – 279 kJ/mol, and **III** - 302 kJ/mol.

Analyzing electronic spectra of complexes in two solvents with different polarity was observed dipole-dipole interaction of solvent with dissolved molecules. The spectrum of starting ligand shows only a slight shift of the absorption $\Delta \sim 1$ nm), so the solvent does not influence the nature of the electronic state of the ligand. In the spectra of the complexes appear displacement of the absorption peaks (in ethanol - to higher wavelength values than in the isopropanol) for **I** $\Delta =$ 50 nm, for **II** $\Delta = 51$ nm, and for **III** $\Delta = 21$ nm.

A specific feature of complex **II** spectra is the appearance of an additional band in the visible domain (450 nm) when using isopropanol. This is probably due to the changes in the symmetry of the complex to a lower one, an effect caused by the nature of the solvent.

When using less polar solvent (isopropanol) the extinction coefficients have two times higher values than for more polar solvent (ethanol), hence for the complexes **I** and **III**, the difference being 11 987 and 11 038 $M^{-1}\cdot cm^{-1}$ respectively and the complex **II** the extinction coefficient decreases with 1697 $M^{-1}\cdot cm^{-1}$.

UV-Vis spectra of the complexes showed high values of extinction coefficients (15106-29501 $M^{-1}\cdot cm^{-1}$) which potentially allow their use in analytical chemistry.

Structure

Single crystals of compounds **I-III** were investigated by X-ray method and their detailed structural study is the subject of separate work [11]. Compounds **I-III** are isostructural, here is presented only schematic structure of the compounds **I-III** (Figure 3).

In complexes metal atom coordinates with two deprotonated bidentate ligands L_1 (where L_1 = 4,5-diphenyl-2-nitroimidazolyl) and two molecules of non-deprotonated CH_3OH. The coordination polyhedron of the metal is a square bi-pyramide (4+1+1), which is determined by oxygen and nitrogen atoms of the deprotonated ligand L_1. In all complexes bipyramide's axial positions are occupied by oxygen atoms from nitro-groups of the ligand L_1.

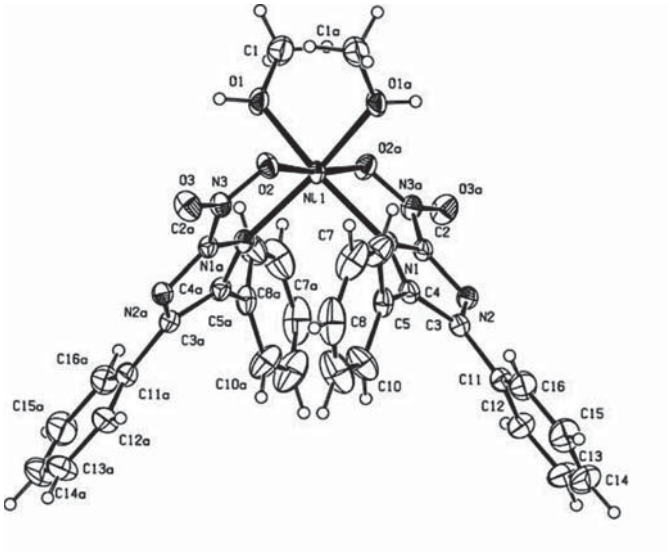


Figure 3. Structure of the complex II

Preliminary research of biological activity performed by colleagues from the Microbiology Institute of ASM showed that adding a solution of Co complex described in this paper in the nutrient composition of *Porphyridium cruentum* red microalgae increases substantially the content of different useful substances in biomass.

Conclusions

By the action of nitrates of 3d metals (Ni, Zn, Co) under solvothermal conditions was carried out the nitration at the C2 atom of the imidazole ring of 4,5-diphenylimidazole and nitro-group is involved in complexation of the corresponding metal ions.

The ligand is deprotonated and the nitrogen atom from imidazole ring coordinates the metal. Two molecules of the solvent used in the reaction solvent - methanol – also coordinate the metal through the oxygen atom without deprotonation. Final complexes are neutral, charge compensation of M^{2+} being performed by ligand deprotonation to form $Ph_2ImNO_2^-$. Two residues of $Ph_2ImNO_2^-$ are bidentatly involved in complex formation. Due to this mode of coordination in the structure there are two metalocycles with five atoms each.

Experimental

General

Starting materials ($Co(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, 4,5-diphenylimidazole, CH_3OH) were purchased from commercial sources and used without further purification (reagent grade).

Elemental analysis (C, H, N) were carried out by standard methods [12], analysis of metals (Zn, Co, Ni) were done on spectrophotometer AAS-3N (Karl Zeiss Jena) in the Atomic absorption spectroscopy laboratory (Institute of Chemistry, ASM). Melting points of synthesized compounds were determined on Boetius apparatus.

Thermogravimetric studies of synthesized combinations were performed on Paulik-Paulik-Erdei derivatograph, in the temperature range 20 - 500°C in air. Heating speed was 5°C/min and Al_2O_3 was used as a standard. Samples weighing 50 mg were placed in platinum crucible. DTA measurement sensitivity - 1/5 and DTG - 1/5, TG - 100/100.

IR spectra were recorded on FT-IR Perkin Elmer "Spectrum 100" spectrometer (4000 ÷ 650 cm^{-1}) and Specord M 80 (4000 ÷ 250 cm^{-1}).

UV-Vis spectra were obtained using Perkin-Elmer "Lambda 25" spectrophotometer. The solutions of complexes **I-III** and the starting ligand 4,5-diphenylimidazole in two solvents with different polarity (isopropanol and ethanol). Electronic spectra were obtained at different concentrations of the solutions to calculate the extinction coefficient in each case.

NMR spectra were obtained on Bruker Avance spectrometer (400 MHz). Both ^1H NMR spectra and ^{13}C NMR were recorded in CD_3COCD_3 and reported to the reference signal of TMS taken as internal standard.

Magnetic measurements were done at room temperature (287 K - 283 K) by Gouy method on an installation from Institute of Chemistry. The experimental magnetic susceptibility data values were corrected by Pascal constants [9]. As a standard sample was used doubly distilled water.

Synthesis of $[Co(C_3N_2(C_6H_5)_2NO_2)_2(CH_3OH)_2]$*(I) - *bis(4,5-diphenyl-2-nitroimidazolyl)bis(methanol) cobalt(II)

A mixture of 0,093 g (0,32 mmol) $Co(NO_3)_2 \cdot 6H_2O$ and 0,141 g (0,64 mmol) of 4,5-diphenylimidazole in 10 ml CH_3OH was heated at 170°C for 3 hours in a Teflon-lined autoclave, which were cooled to 30°C at a rate of 0,06°C per minute. Red-brown needle-shaped crystals were washed three times with methanol and air dried. Yield – 0,07 g (33,6%).

The results of elemental analysis, %: Found: C 58,67; H 4,47; N 12,40; Co 9,03. Calculated for $C_{32}H_{28}N_6O_6Co$: C 58,99; H 4,33; N 12,90; Co 9,05. Melting point: mp > 355°C.

IR spectrum (cm^{-1}): 3657(w), 2989(m), 2902(m), 2772(m), 2542(m), 1605(w), 1577(w), 1523(w), 1499(s), 1468(m), 1437(vs), 1388(vs), 1314(m), 1286(m), 1269(s), 1241(m), 1150(vs), 1099(s), 1073(m), 1032(m), 1018(vs), 1009(vs), 976(s), 919(m), 833(m), 784(m), 772(m), 740(w), 734(m), 696(s), 680(m), 661(m), 535(w), 512(w), 487(w).

Synthesis of $[Ni(C_3N_2(C_6H_5)_2NO_2)_2(CH_3OH)_2]$*(II) - *bis(4,5-diphenyl-2-nitroimidazolyl)bis(methanol) nickel(II)

Compound **II** was obtained by the same procedure as **I**, using 0,093 g (0,32 mmol) of $Ni(NO_3)_2 \cdot 6H_2O$ and 0,141 g (0,64 mmol) of 4,5-diphenylimidazole. Yellow-brown needle-shaped crystals were washed three times with methanol and air dried. Yield - 0,11 g (52,8%).

The results of elemental analysis, %: Found: C 58,78; H 4,50; N 12,65; Ni 9,00. Calculated for $C_{32}H_{28}N_6O_6Ni$: C 59,01; H 4,33; N 12,90; Ni 9,01. Melting point: mp > 355°C.

IR spectrum (cm^{-1}): 3676(w), 2988(m), 2901(m), 2796(m), 1604(w), 1576(w), 1500(m), 1467(m), 1438(s), 1387(vs), 1314(m), 1285(m), 1265(s), 1241(m), 1150(vs), 1099(s), 1073(s), 1015(vs), 976(s), 918(m), 835(m), 783(m), 772(m), 740(m), 733(m), 696(s), 679(m), 662(m), 537(w), 511(w), 488(w).

Synthesis of $[Zn(C_3N_2(C_6H_5)_2NO_2)_2(CH_3OH)_2]$*(III) - *bis(4,5-diphenyl-2-nitroimidazolyl)bis(methanol) zinc(II)

Compound **III** was obtained by the same procedure as **I**, using 0,095 g (0,32 mmol) of $Zn(NO_3)_2 \cdot 6H_2O$ and 0,141 g (0,64 mmol) of 4,5-diphenylimidazole. Orange needle crystals were washed three times with methanol and air dried. Yield - 0,14 g (67,0%).

The results of elemental analysis, %: Found: C 58,13; H 4,42; N 12,95; Zn 9,54. Calculated for $C_{32}H_{28}N_6O_6Zn$: C 58,41; H 4,27; N 12,77; Zn 9,94. Melting point: mp = 291 – 293°C.

IR spectrum (cm^{-1}): 3649(m), 2988(m), 2901(m), 2796(w), 1603(m), 1577(w), 1502(m), 1471(m), 1457(m), 1438(s), 1401(vs), 1293(s), 1265(s), 1233(m), 1157(vs), 1099(s), 1076(m), 1045(s), 1025(s), 975(s), 920(s), 833(m), 782(m), 770(s), 733(m), 695(s), 680(m), 657(w), 534(w), 510(w), 486(w).

^{1}H RMN spectrum (in CD_3COCD_3): δ = 3,00 ppm (s, 1H, OH); 3,31 şi 3,33 ppm (d, 3H, CH_3); 3,85 ppm (s, 3H, CH_3); 7,22-7,56 ppm (m, CH_{Ph}).

Complexes **I-III** are soluble in ethanol, DMF, acetone, acetonitrile, slightly soluble in water, methanol, chloroform and toluene, insoluble in hexane and THF. The same compounds were obtained when the reactions were carried out in a mixture of methanol and water (4:1).

Synthesis of $C_3N_2H(C_6H_5)_2NO_2$* (IV) - *4,5-diphenyl-2-nitroimidazole

200 mg (0,30 mmol) of complex **III** was boiled 30 min in reflux with an excess of H_2SO_4 (2 ml) and water (10 ml). After the demetallation of complex, the pH of solution was adjusted to 3-4 using a saturated Na_2CO_3 aqueous solution, then organic components of aqueous phase were extracted in chloroform (3×10 ml). The extract was dried over anhydrous Na_2SO_4, concentrated to 15 ml and allowed to evaporate. After two days were obtained yellow aciforme crystals, which were filtered and dried in air. Yield - 142 mg (88 %). The compound is soluble in toluene, chloroform, acetone, and methanol and insoluble in water.

The results of elemental analysis, %: Found: C 81,76; H 4,28; N 15,36; Calculated for $C_{15}H_{11}N_3O_2$: C 82,19; H 4,15; N 15,85. Melting point: mp = 216 – 218°C.

IR spectrum (cm^{-1}): 3055(m), 2923(m), 2693(m), 1605(w), 1577(w), 1539(m), 1483(m), 1436(s), 1408(vs), 1349(vs), 1319(m), 1287(m), 1262(m), 1193(m), 1159(s), 1092(m), 1075(m), 1021(s), 969(m), 924(m), 842(m), 826(s), 783(m), 765(s), 734(m), 727(m), 694(vs), 675(m).

^{1}H RMN spectrum (CD_3COCD_3): δ = 7,14 - 7,58 ppm (multiplet, 10H, CH_{Ph}). ^{13}C RMN spectrum (CD_3COCD_3): δ = 125,23 ppm (singlet, C2 Im); 128,15 - 128,87 ppm (multiplet, C_{Ph}).

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IVESTIGATION OF THE PROCESS OF TARTARIC ACID SEPARATION ON AMBERLITE XAD2 IMPREGNATED WITH AMBERLITE LA-2¶

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Abstract. Tartaric acid is one of the most valuable compounds that can be obtained from secondary wine products. The last are accumulating in big quantities at winemaking factories from Moldova. This work describes an investigation of the process of reactive ion-exchange separation of tartaric acid from model systems with macroreticular resin Amberlite XAD2 impregnated with liquid ion-exchanger Amberlite LA-2 in batch equipment. The condition of Amberlite XAD2 impregnation process was investigated. Freudlich and Langmuir equations were verified and values of enthalpy, entropy and Gibbs energy were calculated.

Key words: reactive sorption, tartaric acid, Freudlich equation, Langmuir equation, thermodynamic constants.

Introduction

Classical technologies of organic compounds separation from aqueous streams possess several difficulties, which can be solved by application of reactive sorption. This procedure is a combination of reactive extraction and adsorption [1-5]. Impregnated ion-exchangers are obtained by physical incorporation of a selective extraction reagent into a porous adsorbent. The impregnated extractants manifest strong affinity towards polymeric resins, but, also, maintain its extractive properties (capacity and selectivity) as in liquid state [6-8]. The separation of the polar organic substance is performed during the contact between aqueous solution and impregnated ionites. It is obtained the complex between the solute and extractant. The reaction takes place in the pores of the resin, where the products remain [1-5]. In comparison to the reactive extraction, this procedure involves simpler technological equipment and operations, allows reduction of the total process time, does not need intensive agitation for increase the contact surface, avoids the emulsion formation, and can be used in case of small concentrations of the solute. Moreover, it is overcame basic disadvantage – low selectivity [5].

Impregnated resins with selective extractants were initially used for the separation of the metals from waste waters [2, 7, 9-10]. Later, this procedure was applied in water purification from organic compounds, and, finally, for their separation from other aqueous solutions [5, 11-14]. Besides, studies of organic acids recovery are of strong interest [3, 4, 15-17].

This work presents the study of the impregnation procedure of nonionic macroreticular resin Amberlite XAD2 with the solution of the secondary amine Amberlite LA-2 in organic solvent, and thermodynamic study of the reactive sorption of the tartaric acid on the obtained impregnated anionite.

Experimental Section

Materials

The aqueous phase was prepared by dissolving tartaric acid (*Reahim*, *Russia*) in distilled water. Several solvents were used as inert diluents for amine organic phase – chloroform, butyl acetate, hexane (all from *Reahim*, *Russia*). The organic phase for impregnation was prepared by dissolving amine: Amberlite LA-2, tri-n-octylamine, tri-iso-octylamine (*Sigma-Aldrich, Germany*) and synthesized amines (N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpirazyl)dodecylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylen-1,2,4-triazyl)dodecylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(4-methylenmorpholyl)dodecylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpyrrolidyl)dodecylamine, N-(N,N-diethylamino-N-methylen)-N-(2,2,4,4,6,6-hexamethylhexanyl) dodecylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpyridyl)-dodecylamine, N-(N,N-dimethylamino-N-methylen)-N-(2,2,4,4,6,6-hexamethylhexanyl)dodecylamine, N,N,N-tri-iso-octylamine chloride, N-hydroxyethyl-N,N,N-trioctylammonium chloride and N,N,N,N-tetramethylheptylammonium bromide) in diluent. The synthesized tertiary amine and ammonium salts were obtained from commercial ones in laboratory of Organic Synthesize of Institute of Chemistry of ASM. Glacial acetic acid (*Reactivul, Romania*) and sodium metavadanate (*Reahim, Russia*) were used for determination of tartaric acid concentration.

Methods

Concentration determination of tartaric acid was realized by photometric method on spectrophotometer PD-303 (*APEL, Japan*). For measurements 5 cm^3 of analyzed solution were passed into 50 cm^3 flask, 4 cm^3 of sodium

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metavanadate solution (5%) and 1 cm^3 of glacial acetic acid were added, then flask was fitted with distilled water. Obtained solution is left for 15-20 min at dark for the formation of the stable color during 24 h. Measurements were done at 525 nm wave length (green filter) against a blank similarly prepared without tested solution [18].

Procedure

The experimental procedure of the impregnation of the Amberlite XAD2 with Amberlite LA-2 consists of several stages. Initially, solid anionite is washed with acetone and distilled water to eliminate organic and inorganic impurities. Liquid amines are used without additional purification. Impregnation consists in immersion of the solid resin in solution of the liquid anionite in organic solvent. After 24 h, necessary to fulfill impregnation process, the excess of the organic substance is removed by addition of the distilled water to the obtained anionite and its exposition to the ultrasound with the frequency 35 kHz in ultrasound bath Sonica 1200M (*Soltec, Italy*) during 5 min. Another possibility is to pour out remained amine solution by washing of the impregnated anionite with the same solvent, used for the preparation of the amine solution. Anionites obtained by both methods do not differ by their properties. For further experiments dry ion-exchanger was used.

Determination of the reactive sorption performance of tartaric acid from model solution on obtained impregnated anionite was done as follows: 1 g of impregnated anionite is stirred with universal stirrer WU-4 (*Premed, Poland*) or agitator AVU-6s (*Russia*) with 50 cm^3 of tartaric acid solution till the equilibrium is reached, and concentration of the tartaric acid is determined in the final solution [18].

Conditions of the impregnation process were determined from investigation of the next parameters: type of the amine (Amberlite LA-2, tri-n-octylamine, tri-iso-octylamine and synthesized amines), type of the solvent (hexane, chloroform, butylacetate), amine concentration in the impregnation solution, the volume of the solvent used in preparation of the impregnation solution and the time of impregnation. The impregnation performance was appreciated by determination of reactive sorption of the tartaric acid from model solution on impregnated anionite.

In order to estimate equilibrium constants for the reactive sorption process of the tartaric acid on anionite Amberlite XAD2 impregnated with Amberlite LA-2, 0.5 g of the anionite were stirred with 50 cm^3 of tartaric acid solution with the concentration between 0.33 g/dm^3 and 6.99 g/dm^3, with the magnetic stirrer for an hour (time enough to create equilibrium state). The contact between the phases was ensured in thermostat during the whole process till the equilibrium state. In this way there were estimated the isotherms of the sorption at 296 K, 300 K, 305 K and 313 K.

Definition of Characteristic Parameters

Reactive sorption at equilibrium q_e (g/g) of the impregnated anionite is calculated by the formula:

$$q_e = \frac{(c_o - c_e)\cdot V}{m}, \quad (1)$$

where: m – mass of the dry ion-exchanger, g; V – tartaric acid solution volume, dm^3; c_o – initial concentration of the tartaric acid in model solution, g/dm^3; c_e – concentration of the tartaric acid in model solution at equilibrium, g/dm^3.

The constants of equilibrium were determined applying Freundlich and Langmuir equations.

For the determination of the constants K_F and n, the data were used the Freundlich isotherm in logarithmic form:

$$\ln q_e = \ln K_F + \frac{1}{n}\ln c_e \quad (2)$$

where: K_F – Freundlich equilibrium constant; n – empiric parameter of heterogeneity of the surface [19-24].

The reverse of the Langmuir equation takes linear dependency:

$$\frac{c_e}{q_e} = \frac{c_e}{q_m} + \frac{1}{q_m \cdot K_L} \quad (3)$$

where: K_L – Langmuir constant; q_m – maximal specific adsorption (mg/g), correspondent to the space occupied by a monomolecular layer on the adsorbent surface [19-25].

The precision of the correlation was appreciated by correlation coefficient R^2:

$$R_{yx}^2 = r_{yx^2}^2 = \frac{\left(\sum_i (y_i - \bar{y})(x_i - \bar{x})\right)^2}{\sum_i (y_i - \bar{y})^2 \sum_i (x_i - \bar{x})^2} \quad (4)$$

For the determination of the entropy and enthalpy through the graphic method was applied next form of the Van′t Hoff equation:

$$\ln K_L = -\frac{\Delta H^o}{RT} + \frac{\Delta S^o}{R} \quad (5)$$

where: ΔH^o – enthalpy, J/mol; ΔS^o – entropy, J/(mol·K); R – universal constant of the ideal gas (8,314 J/(mol·K)), and T – temperature of the process realization [24, 26, 27].

Results and discussion

The first stage of the determination of the conditions of impregnation process was amine selection. For this, there were prepared several impregnation solutions by dissolving 0.8 cm^3 of amine in 3 cm^3 of hexane. Next amines were used: Amberlite LA-2, tri-n-octylamine, tri-iso-octylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpirazyl)dodecylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylen-1,2,4-triazyl)dodecylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(4-methylenmorpholyl)dodecylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpyrrolidyl)dodecylamine, N-(N,N-diethylamino-N-methylen)-N-(2,2,4,4,6,6-hexamethylhexanyl) dodecylamine, N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpyridyl)dodecylamine, N-(N,N-dimethylamino-N-methylen)-N-(2,2,4,4,6,6-hexamethylhexanyl)dodecylamine, N,N,N-tri-iso-octylamine chloride, N-hydroxyethyl-N,N,N-trioctylammonium chloride and N,N,N,N-tetramethylheptylammonium bromide. One gram of Amberlite XAD2 were added to the amine solution, stirred and left for 24 h.

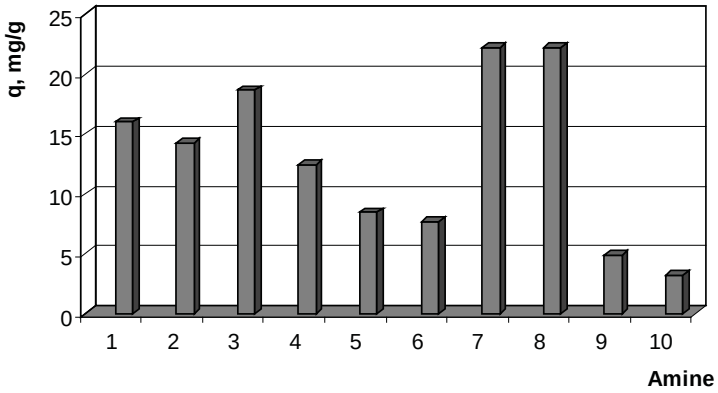

Fig. 1. The effect of the nature of the amine used for the impregnation of Amberlite XAD2 on reactive sorption of the tartaric acid from aqueous solution

(m(anionite) = 0.4 g; d_p = 0.75 mm; V(AT) = 40 cm^3; c_o(AT) = 3.46 g/dm^3; τ_{agit} = 60 min; t = 23°C; n = 200 min^{-1})

1 – N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpirazyl)dodecylamine, 2 – N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylen-1,2,4-triazyl) dodecylamine, 3 – N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(4-methylenmorpholyl)dodecylamine, 4 – N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpyrrolidyl) dodecylamine, 5 – N-(N,N-diethylamino-N-methylen)-N-(2,2,4,4,6,6-hexamethylhexanyl) dodecylamine, 6 – N-(2,2,4,4,6,6-hexamethylhexanyl)-N-(1-methylenpyridyl) dodecylamine, 7 – N-(N,N-dimethylamino-N-methylen)-N-(2,2,4,4,6,6-hexamethylhexanyl)dodecylamine, 8 – N-(2,2,4,4,6,6-hexamethylhexanyl) dodecylamine (Amberlite LA-2), 9 – N,N,N-tri-iso-octylamine chloride, 10 – N-hydroxyethyl-N,N,N-trioctylammonium chloride

Tests of the obtained anionites revealed that maximal performance of reactive sorption of the tartaric acid was received for anionites impregnated with Amberlite LA-2 and (N,N-dimethylamino-N-methylen)-N-(2,2,4,4,6,6-hexamethylhexanyl)dodecylamine. During separation with anionites impregnated with tri-n-octylamine, tri-iso-octylamine, and N,N,N,N-tetramethylheptylammonium bromide was detected formation of the emulsion during stirring, caused by transfer of the amines from resin to aqueous solution, so they were excluded from following investigations. Thus, it was proposed to use Amberlite LA-2 amine for the impregnation of Amberlite XAD2, because the synthesis of (N,N-dimethylamino-N-methylen)-N-(2,2,4,4,6,6-hexamethylhexanyl)dodecylamine involves extra expenses, though process performance was unchangeable.

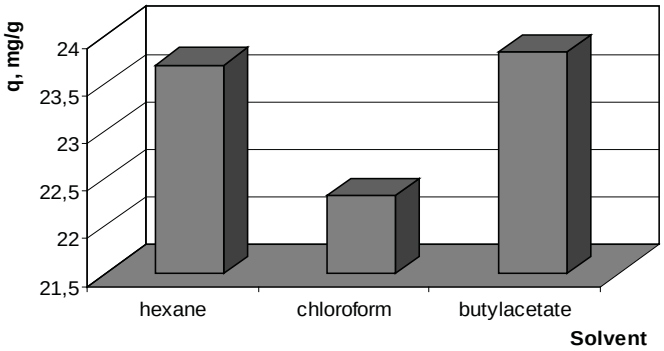

Fig. 2. The effect of amine solution solvent on reactive sorption of the tartaric acid from aqueous solution (m(anionite) = 1.0 g; d_p = 0.75 mm; V(AT) = 50 cm^3; c_o(AT) = 3.48 g/dm^3; τ_{agit} = 60 min; t = 28°C; n = 200 min^{-1})

As solvents for impregnation were used: butylacetate, chloroform and hexane. According to the figure 2, amine solution preparation using butylacetate allows obtaining of the impregnated anionite with maximal sorption. Efficiency of the tartaric acid separation process with ion-exchanger prepared on basis of Amberlite LA-2 solution in chloroform was lower than in the case of other solvents. In addition, the separation process of the excess of impregnation solution becomes difficult, because chloroform has a higher density than water. Thus, hexane was used as solvent in the next experiments because the impregnated resins obtained from amine solution in hexane and from solution in butylacetate had nearly similar reactive sorption, but the separation of hexane was easier and faster, due to low boiling point (69°C).

Amine concentration in impregnation solution varied from 5% to 60%. After, processing and testing of the impregnated anionite, there were obtained the results, presented in figure 3.

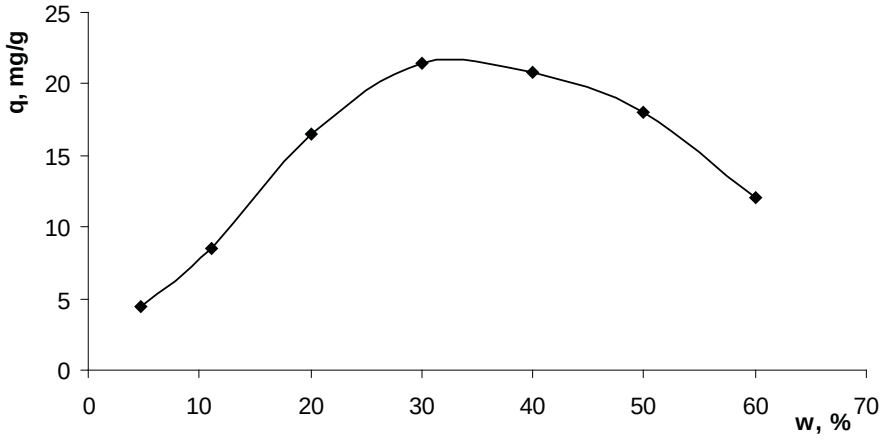

Fig. 3. The influence of the amine solution concentration used for impregnation on reactive sorption of tartaric acid by impregnated anionites
(m(anionite) = 0.5 g; d_p = 0.75 mm; V(AT) = 50 cm^3; c_o(AT) = 0.8 g/dm^3; τ_{agit} = 60 min; t = 28°C; n = 150 min^{-1})

The increase of the amine solution concentration used for impregnation influenced positively anionite reactive sorption up to the concentration 30%, and then decreased it. The explication is that the quantity of the fixed amine closes the access of the aqueous solution of tartaric acid to the resin pores.

The quantity of the solvent necessary for preparation of the amine solution also influenced the efficiency of the impregnation process and, further, of the tartaric acid separation. It was taken into account that the ration between amine content and resin weight must be constant, varying only solvent's volume. The organic solution with following volume ratio between Amberlite LA-2 and hexane were prepared: 1:1, 1:2, 1:3, 1:4 and 1:5. They corresponded to the amine solutions with concentration 56.23%; 39.11%; 29.99%; 24.31% and 20.44%, respectively. The quantities of the solutions used for impregnation were different (0.754 g; 1.084 g; 1.414 g; 1.744 g and 2.074 g).

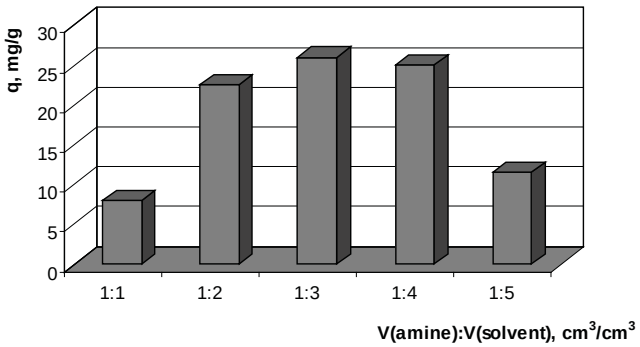

Fig. 4. The effect of the volume ratio between Amberlite LA-2 and hexane in impregnation solution on reactive sorption of the tartaric acid
(m(anionite) = 0.5 g; d_p = 0.75 mm; V(AT) = 50 cm^3; c_o(AT) = 0.95 g/dm^3; τ_{agit} = 15 min; t = 27°C; n = 150 min^{-1})

From the analysis of the figure 4 it is observed that the most adequate volume ratio between the amine and the solvent in impregnation solution was 1:3, corresponding to the solution of Amberlite LA-2 with concentration 30%. This fact confirms once more experimental data received during determination of the impregnation solution concentration.

Amberlite XAD2 was impregnated with 30% solution of Amberlite LA-2 for 1, 2, 4, 6, 8, 14, 16, 20 and 24 h. It was shown that for the finalization of the impregnation was necessary 2 h. The extension of procedure up to 12 h did not modify properties of the obtained anionite. Impregnation over 16 h led to insignificant decrease of obtained ion exchangers sorption, that can be explained by accumulation of the amine excess in resin pores (fig. 5). The anionite resulted after 24 h of Amberlite XAD2 impregnation did not differ by their properties from that obtained after 4 days of impregnation.

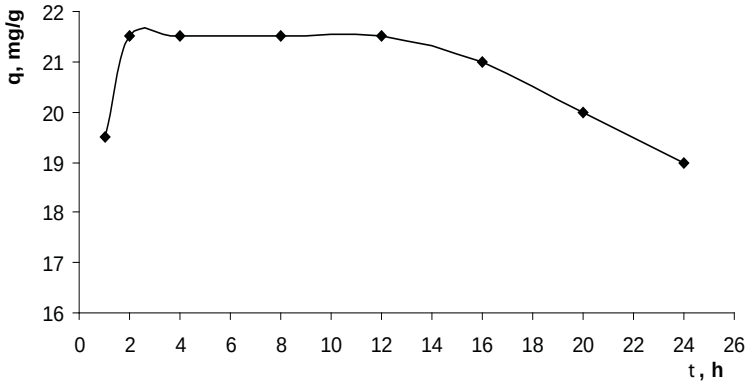

Fig. 5. The influence of the time of impregnation process on tartaric acid sorption on impregnated anionite
(m(anionite) = 0.5 g; d_p = 0.75 mm; V(AT) = 50 cm^3; c_o(AT) = 0.92 g/dm^3; τ_{agit} = 60 min; t = 28°C; n = 150 min^{-1})

Then, Freundlich and Langmuir isotherms for obtained anionite were verified. Isotherms of the reactive sorption of tartaric acid on Amberlite XAD2 impregnated with Amberlite LA-2 are present in figure 6.

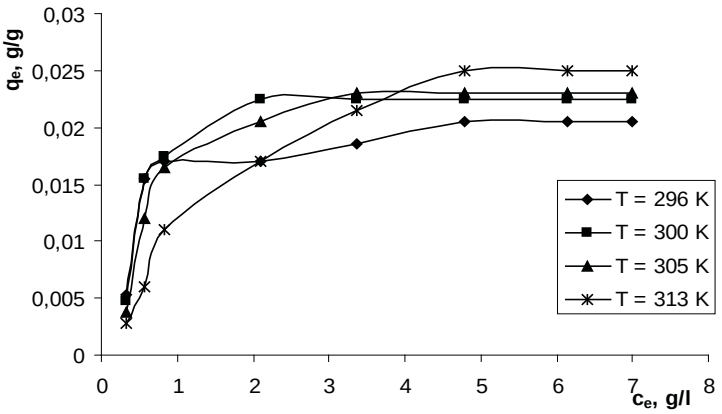

Fig. 6. Isotherms of reactive sorption of tartaric acid on Amberlite XAD2 impregnated with Amberlite LA-2

Experimental data were processed according to Freundlich and Langmuir equations, and the results were presented in table 1. In figures 7 and 9 was illustrated experimental validation of the Freundlich and Langmuir equations for the reactive sorption of tartaric acid on Amberlite XAD2 impregnated with Amberlite LA-2 at 296 K. In the same way were analyzed all isotherms.

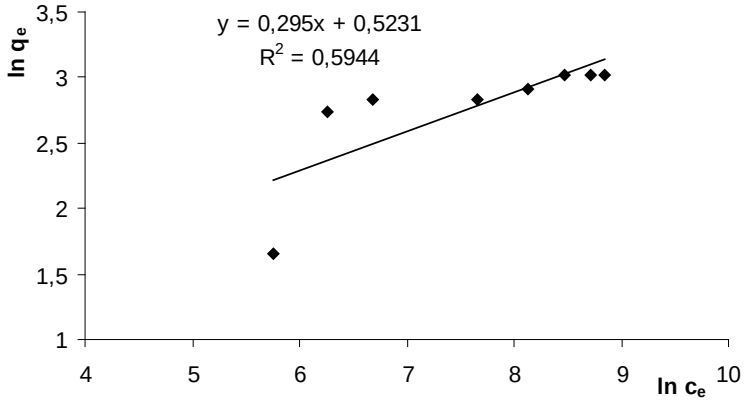

Fig. 7. Experimental validation of the Freundlich equation for the reactive sorption of the tartaric acid on Amberlite XAD2 impregnated with Amberlite LA-2 at 296 K

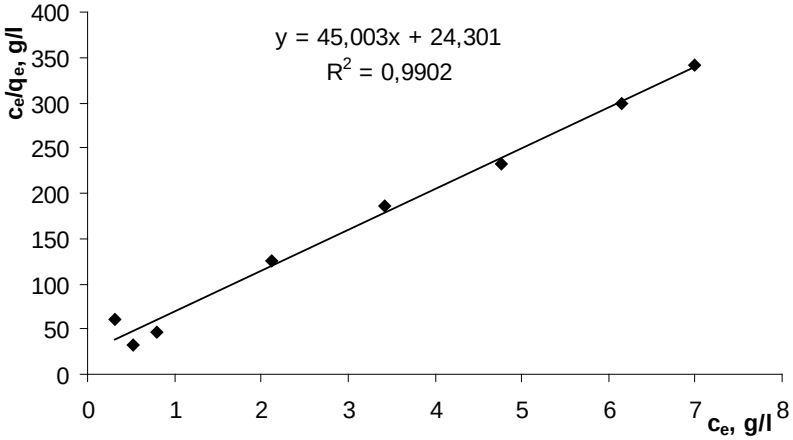

Fig. 8. Experimental validation of the Langmuir equation for the reactive sorption of the tartaric acid on Amberlite XAD2 impregnated with Amberlite LA-2 at 296 K

Table 1

Constants of Freundlich and Langmuir isotherms

T, K	Freundlich			Langmuir		
	n	K_F	R^2	K_L, L/g	q_m, g/g	R^2
296	3,3898	1,6872	0,5944	1,8536	0,0222	0,9902
300	2,7809	1,1264	0,6207	1,8079	0,0248	0,9807
305	2,1925	0,5044	0,7096	1,0484	0,0270	0,9637
313	1,4575	0,0727	0,9143	0,3253	0,0383	0,9259

Comparison of the values of the correlation coefficients demonstrated that Langmuir equation verify experimental data in a better way. The values of the maximal specific reactive sorption, calculated with the same equation, increased at the same time with the temperature, which meant the interaction between tartaric ions and impregnated resin was an endothermic process.

Langmuir isotherm is mostly adequate for adsorption on energetic homogeneous surfaces, accompanied with the reaction [19-23]. Due to the established hypotheses, it allowed, after verification, determination of thermodynamic values ΔG^o, ΔH^o, ΔS^o (table 2).

Table 2

Calculated thermodynamic constants

T, K	ΔGº, J/mol	ΔHº, J/mol	ΔSº, J/(mol·K)
296	-1518,83	-78850,18	-261,26
300	-1477,09		-257,91
305	-119,86		-258,13
313	2922,59		-261,25

Graphic dependency between the constant of equilibrium and the temperature allows estimating enthalpy and entropy of the tartaric acid separation process on Amberlite XAD2 impregnated with Amberlite LA-2.

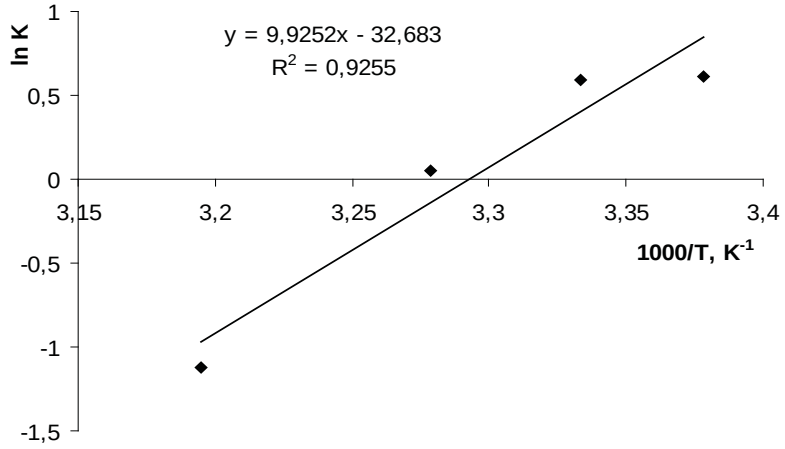

Fig. 9. The dependency of the natural logarithm of equilibrium constant (ln K) of the tartaric acid reactive sorption process on Amberlite XAD2 resin impregnated with Amberlite LA-2 on temperature (1000/T)

Enthalpy value determined by graphic method was -82524.07 J/mol, while entropy value -271.75 J/(mol·K). Differences between the calculated and graphically determined results were 4.45 %.

ΔG^o represents modification of the free energy in standard state during the ion-exchange and sorption or desorption of the solvent and dissolved electrolyte [26, 27]. Negative value of Gibbs free energy modification denote spontaneous process of ion-exchange. But, increase of ΔG^o along with temperature shows its negative effect on the stability of the bonds between tartaric acid and ion-exchanger, which was confirmed by the decrease of equilibrium constant. Negative value of the enthalpy variation indicates an exothermic process, which is an additional argument. The selectivity of the sorption process characterized by negative value of the thermodynamic potential, is determined by the significant decrease of the entropy. This process represents the increasing of the energy of interaction between selectively fixed ions on the ionite, in comparison to the interaction energy of ionite with concurrent substituted ions, thus the complex between tartaric acid ion and anionite became more ordered.

System enthalpy variation in case of ion-exchange for diluted solutions was caused by the variation of the energy of interactions between contra-ions and ionite. Generally, bond energy between the ions of organic substances and ion-exchangers is lower than between simple monovalent ions of hydrogen, chloride or metal ions (the influence of ion radius). Thus, the values of the thermodynamic potential, enthalpy and entropy support the existence of supplementary interactions, e.g., van der Waals bonds or hydrophobic interactions [27].

Conclusions

The investigation revealed that for the impregnation of macroporous resin Amberlite XAD2 should be used Amberlite LA-2 amine, in following conditions: solvent – hexane, ratio between amine volume and solvent volume was 1:3, the concentration of amine solution in hexane 30% and impregnation time at least 2 h.

Tartaric acid reactive sorption process on the obtained impregnated anionite verifies Langmuir equation with correlation higher than 0.92, and thermodynamic constants denotes the presence of exothermal process, associated with chemical reaction.

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WATER ACTIVITY CONCEPT AND ITS ROLE IN STRAWBERRIES FOOD

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Abstract. This paper presents the study information related to the processing of fruit by combined methods. Is presented on the concept of water activity and its role in product quality; mathematical modeling methods (GAB, BET et al.) in estimate the activity coefficients of non-electrolyte and electrolyte mixture. Have been carried experimental research to study the evolution of the biologically active substances in frozen strawberries with and without sugar addition. It proved that frozen strawberries with sugar, during storage are more stable regarding antioxidants content that strawberry frozen without sugar.

Keywords: water activity, frozen strawerries, sugar addition, processing, osmotic treatment, stability.

Introduction

Quality and food security depends on pH and water activity (a_w) in food environment. Foods with water activity are perishable. Water activity, pH, temperature, and other parameters, have a direct impact on the growth of microorganisms, thus a_w and pH are two the most important parameters. Free water that is available to molds, yeasts, and bacteria is responsible for their growth and even toxin production. Or it may participate in chemical/biochemical reactions (e.g. Maillard reactions), which might deteriorate: texture, flavor, color, taste, nutritional value of a product, and its stability → shelf-life time [4, 11, 22, 33].

Water activity (a_w) has its most useful application in predicting the growth of bacteria, yeasts and moulds. For a food to have a useful shelf life without relying on refrigerated storage, it is necessary to control either its acidity level (pH) or the level of water activity (a_w) or a suitable combination of the two. This can effectively increase the product's stability and make it possible to predict its shelf life under known ambient storage conditions [22, 34, 45].

Strawberries are delicious fruits, with a good smell and taste, nice appearance.

Strawberries are one of the most important seasonal fruit crops. Most of its production is destined for the fresh market, but because of the short shelf life and seasonal nature of this fruit, part of its production is processed. In this way, it is used as a food ingredient in yogurts, pies, milk shakes, jams, ice creams, etc. because of its interesting sensory attributes. The types of strawberry processing most commonly used to increase product shelf life are freezing, partial or total dehydration and other combined methods. In these cases, the processed fruit undergoes changes in sensory attributes such as texture, color [5, 25, 48] and changes in the profile of volatile compounds [9, 45], making the product different from nontreated products. Other quality attributes, such as product taste or flavor related to fruit composition on major sugars and acidity, may also be altered during such processes [25-27, 53].

Strawberries are perishable and under the action of peroxidase enzymes that contribute to the appearance of brown compounds and the loss of smell, they support permanent changes of phenol substances (their oxidation) [40, 41]. During storage and processing, the content of these substances is reduced according to their involvement in the process of oxido - reduction. Their evolution depends on several factors, as: the chemical composition of the fruits, parameters of the technological process, storage conditions, etc. [38, 41 and 47]. Different approaches have been explored for obtaining shelf-stability and freshness in fruit products. Commercial, minimally processed fruits are fresh (with high moisture), and are prepared for convenient consumption and distribution to the consumer in a fresh state. Minimum processing includes minimum preparation procedures like washing, peeling and/or cutting, packing etc., after which the fruit product is usually placed in refrigerated storage where its stability varies depending on the type of product, processing, and storage conditions.

As a preservation method, freezing combines low temperature and a water activity (a_w) reduction associated with the cry concentration of the fruit liquid phase during ice crystal formation. However, because of the highly freezable water content of strawberries, freezing implies important cellular damage and losses in product quality [23]. Water content reduction by dehydration treatments applied before freezing (dehydrofreezing) have been reported as a tool in fruit cryopreservation, mainly because of the reduction of freezable water content [5, 26].

In this sense, osmotic dehydration (OD) or air drying (AD) and combined treatments (OD–AD) can be used to reduce ice crystal injuries during frozen storage. Dehydration treatments have been used to obtain strawberry products in the form of ingredients [11, 25]. These treatments affect the cells of the plant tissue as a result of ruptures in cellular

bonds and induced deformations (shrinkage/swelling) both in cells and intercellular spaces taking place throughout the drying process. During AD treatment, elimination of water involves phase changes and so, although low temperatures are used during the process, a loss of cell functionality may occur and consequently, considerable changes in sensory and nutritional quality [44].

OD of fruit into an osmotic solution implies an opposite flux of different components: water and some natural soluble substances such as sugars, vitamins, pigments, organicacids, mineral salts, etc. [10] flow out from the fruits the osmotic solution, and in the opposite direction, soluble solutes may be transferred from the solution to the fruit, which may change product taste and acceptability. The penetration of sugar into the fruit is quite a slow process, particularly if high fruit particle integrity is desired in the final product [53]. This method has received considerable attention because of the low energy requirements and fruit quality improvement [13, 14]. As high temperatures are not normally used in OD processes, and no water phase changes occur, changes in sensory attributes, such as color, aroma, flavor and texture are minimized [14, 30].

Strawberry is an excellent source of food ingredients, although compositional changes might occur in improperly controlled processing, affecting product quality. In this sense, OD has been recommended to improve fruit quality in fruits sensitive to AD such as kiwi fruits and strawberries [48]. The operation has been used as an initial step in other processes such as AD [25] and freezing [5]. However, when partial dehydration of the product is used to obtain slightly processed fruits, a section of the tissue remains alive so that cellular respiration still occurs depending on the dehydration level [37]. Osmotic stress causes physiological and biochemical changes that will lead to modifications in the fruit composition, such as changes in sugars, acids or reserve substances [3, 7].

Acellular biochemical response to the osmotic stress has also been observed, resulting in the accumulation of osmolites (compatible solutes) in the cytoplasm that is induced by enzyme action [9].

In this work, the influence of OD (osmotic dehydration of sugar) applied to partially reduce moisture content and a_w on major sugars and strawberry acidity was analyzed. Likewise, the effect due to subsequent sample freezing and frozen storage was also studied.

1. Materials and methods

Chemicals

Folin-Ciocalteu phenol reagent, gallic acid monohydrate, glacial acetic acid, L-ascorbic acid, sodium carbonate was obtained from EM Science, alcohol, hydrochloric acid, sucrose.

Raw material

Strawberries (var. Victoria) were purchased in a local market in Chisinau (Republic of Moldova). Eight, different batches were used in the study, two for each treatment subsequently described. Strawberry samples were frozen and kept at the temperature of minus 18°C for 6 months. The study was conducted both for fresh and frozen strawberries. The estimation of content SH, antioxidants and the reducing state of frozen strawberries was carried out in samples stored 0, 3 to 6 months. The samples studied were calculated theoretically and modification of water activity (a_w). It was evaluated the dependency between the reducing state of frozen strawberries on bio antioxidants content and the influence of the technological process on the quality of strawberries.

Sample freezing

The samples were congealed in "Ghiocel" freezer at the temperature of -18°C. Before congelation preventive operations were made: sorting, washing, top water –drying. The samples were packed in polisterol bags or Al/bald with weight 30.0 – 50.0g. Before being hermetically closed, the samples were treated with N_2 for reducing the O_2 strength. For freezing the samples it was performed the following scheme: reception of raw material → washing → Inspection → removing water from the surface (through vibrating and air blowing) → weighting the samples (g) → Packaging, polystyrene packages, aluminium foil. Sample mass 30–50g → Vacuuming and nitrogen treatment (Pressure value P =1.5 atm, for 2-3 minutes) → Tightening the packages → Freezing at -18°C, freezer → Storage. Depositing the frozen samples (t = -18°C, for 3 and 6 months). Nitrogen (N_2) was used to remove the air from the package, in order to have anaerobic conditions (prevents aerobic spoilage and oxidative degradation of bioantioxidants).

Osmotic treatments

In some lots, strawberries were packed whole, in other once were congealed whole strawberries or with sugar syrup (syrup strength 39%, syrup: fruits ratio -1:3).

Thawing of samples

Rapid thawing (temperature of 98°C, for 1-2 minutes), for the samples in which was determined the content ascorbic acid, polyphenols and anthocyanins. Slow thowning (room temperature, for 30 minutes) for the samples in which was investigated the loss of juice during thawing. In fresh samples and in the congealed one, was estimated the antioxidant capacity, acid ascorbic through method potentiometric; total pholyphenol content through Folin-Ciocalteu

method; total anthocyanins content through physical-chemical method [39] and property of redox state, expressed with K-index, mg AA/ g HS [46].

Tested indicators

There were determined the following indicators in the samples: content of soluble substances, the active acidity, pH, the maturity and the content of bio antioxidants: ascorbic acid by potentiometric method, total polyphenols by Folin-Ciocalteau method, and total anthocyanins by the standard physicochemical method. Also, it was assessed the oxido-reducing state of strawberries by potentiometric method, expressed by the oxido-reducing index (K) [46, 47]. Optical density of samples was evaluated at spectophotometer DR-5000, at a wavelenght of 190-1100 nm.

1.1. Combining control of water activity with other preservation techniques

The most important techniques commonly used in food preservation act by inhibiting the growth of microorganisms rather than by inactivating them [12]. Among the inhibitory techniques are: Water activity reduction (curing, conserving, drying, evaporation); Temperature (high or low); Acidity or pH reduction by addition of inorganic and organic acids; Redox potential (Eh), preservatives (e.g., nitrite, sorbets, and sulphites); Competitive microorganisms (e.g., lactic acid bacteria); Modified atmosphere packaging (vacuum, nitrogen, carbon dioxide, oxygen). As stated by Leistner [21, 22] it soon became apparent that in most foods for which a_w is important for quality and stability, other factors, referred to by Leistner as ”Hurdles”, contribute to the desired product, and the interest taken initially in a_w for food manufacturers was extended to other factors (e.g., Eh, pH, temperature, incorporation of additives, etc.). The goal was to obtain stable products based on an intelligent combination of factors by combination preservation technology or hurdle technology [2, 27].

This paper presents the influence of freezing and osmotic treatment (use sucrose solution) on the quality of frozen strawberries.

2.1. Water activity (a_w) concept

Water activity (aw) is defined as the ratio of the equilibrium water vapour pressure of a foodstuff (pw, kPa) to the saturated vapour pressure (p_{wo}, kPa) at the same temperature. It is an important concept in the food industry since it is related to microbiological stability and physico-chemical deterioration reactions. Indeed, water is often the main component of a foodstuff, which also contains carbohydrates, proteins, fats and mineral salts. At particular conditions of temperature and moisture, the interactions between these constituents can cause browning and lipid oxidation, among other reactions, and can provide the appropriate conditions for microbiological growth [17].

Sorption isotherms, which describe water activity at a given temperature at different moisture contents, are therefore of special interest in the design of food preservation processes such as drying, freeze-drying, mixing, packaging, storage, etc., since they are required for the prediction of food stability, shelf life, and glass transitions, for estimating drying times, etc. The sorption behavior of various types of foods and the influence of temperature on equilibrium moisture content have been studied and modelled extensively during the past 50 years [31, 32, and 48]. The numerous mathematical expressions reported in the literature may be classified as theoretical, semi-empirical, or empirical models [17].

Each of these models had relative success in reproducing equilibrium moisture content, depending on the water activity range or the type of foodstuff. Some of the better known correlations of water activity as a function of moisture content and temperature are the modification of the correlation [17]. However, none of these are applicable to both high and low moisture products. A very popular general correlation, which is recommended by the European project COST90 on physical properties of foods [17], is the Guggenheim-Anderson-de Boer (GAB) equation. Other semi-empirical correlations based on thermodynamic considerations are those of [17, 34].

2.2. Prediction of water activity in practical applications

Water activity **(**a_w) can be influenced in at least three ways during the preparation of dried, intermediate and high moisture foods [6, 21-25, 48]:

1. Water can be removed by a dehydration, evaporation or concentration process.
2. Additional solute can be added. The impregnation of solute can be preformed by moist infusion or dry infusion. Moist infusion consists in soaking the food pieces in a water-solute solution of lower a_w while dry infusion involves direct mixing of food pieces and solutes in required proportions. When water-rich solid products, such as fruit, are subjected to moist or dry infusion, three flows arise: a water outflow, from product to the environment; a solute flow, from the environment to product, and an outflow of the product’s own solutes.

This process is called “osmotic dehydration” and allows the infusion of not only the solute used to control a_w but also the desired quantities of antimicrobial and ant browning agents or any solute for improving sensory and nutritional quality. By controlling these above complex exchanges it is possible to conceive different combinations of water loss and solid gain, from a simple dewatering process (with substantial water removal and only marginal sugar pickup) to a

candying or salting process (in which solute penetration is favored and water removal limited) [48]. For porous foods, moist infusion can be also performed under vacuum, as previously mentioned. The internal gas or liquid occluded in the open pores is exchanged for an external liquid phase (of controlled composition) due to pressure changes.

3. Combining 1. & 2. When the food pieces are infused with the solutes and additives and then partially dried. The advantages obtained with this combination as compared to only drying are an increase in the stability of the pigments responsible for the colour, an enhancement of the natural flavour, a better texture and a greater loading of the dryer.

Whatever the procedure used to reduce a_w, it is necessary to know the water activity-moisture content relationship in the food. Important contributions have been made in the field of a_w prediction over the past 50 years and comprehensive analysis of the procedures traditionally employed to calculate a_w have been performed by Chirife [6, 12]. In each case, the applicability of various theoretical and empirical equations was analyzed, presenting some descriptive exemples.

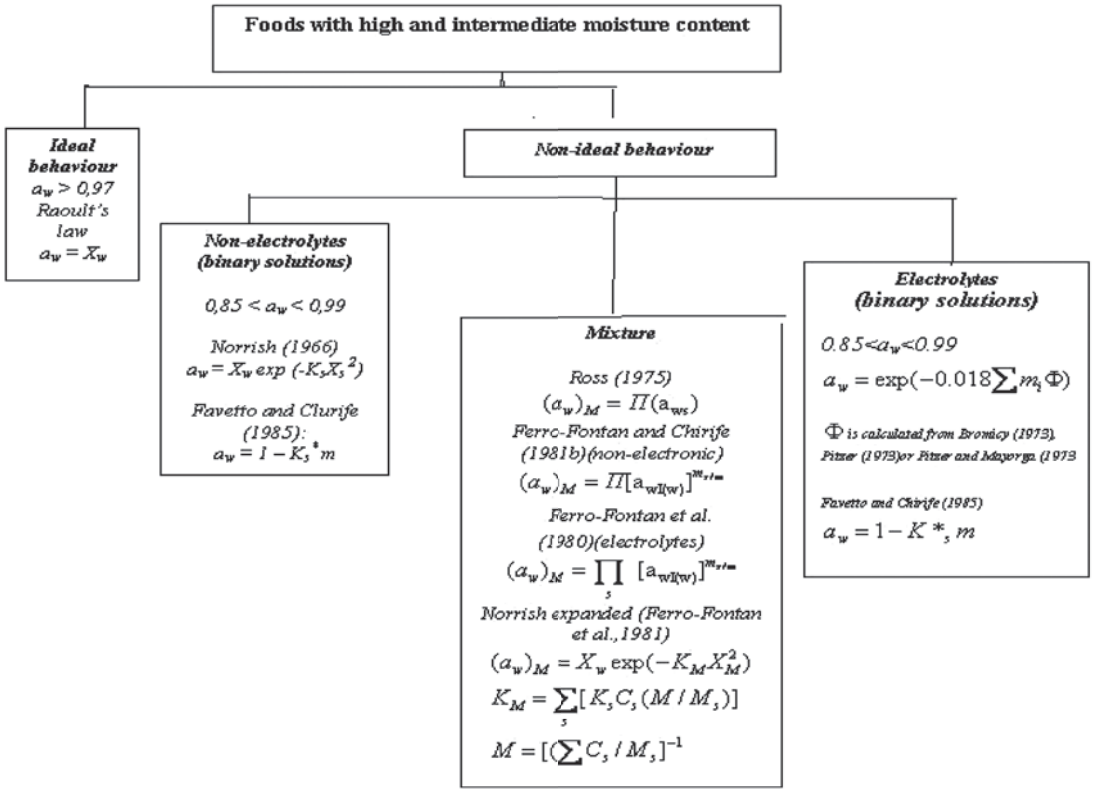

Figure 1. Scheme and selected models for partical prediction of water activity in moist and semi-moist foods

where: X_w: molar fraction of water; X_s: molar fraction of solute; K_s: Norrish 'content for a non-electrolyte s; K_s^*: constant for each solute (electrolyte or non-electrolyte); m: molality; $m_i = \nu m$, here ν is the number of ionic species per mol the solute i: Φ:osmotic coefficient; $(a_w)_M$: water activity of a complex solution; (a_{ws}): water activity of each s component when measured at the same molality as in the compex solution; m_s: molality of the s component in the mixture; $m_{s(I)}$: total molality (dissociated) of the solute that would produce an ionic force equal to the one of the mixture, $a_{ws(I)}$: water activity of solute s in a binary solution at a molality $m_{s(I)}$; C_s: weight of solute s/weight of total solids; M_s molecular weight of solute s.

A number of equations, based on the thermodynamic properties of binary and multicomponent electrolyte and non-electrolyte solutions, have been studied theoretically and experimentally for calculating or predicting the a_w of these foods. Figure 1 summarizes several of theoretical and empirical models suggested for the calculation of a_w in semi-moist and moist food [6, 12, 16].

In low-moisture foods, adsorption of water in surfaces is responsible for a_w reduction [6, I5]. Although the physical chemistry of surfaces has provided the food scientists with a large number of the theoretical equations, the relationship of water sorption -a_w cannot be predicted but must be experimentally determined due to many reasons. As food sorbs water, it can undergo changes of constitution, dimensions and other properties and sugars contained in the food may experience phase transformations. Although the theoretical assumptions are incorrect for heterogeneous food surface interactions, for practical purposes this equation has been found very useful in determining the optimum moisture content (i.e., that coresond to the monolayer water) for storage chemical stability of dehydrated foods [29].

The Guggenheim, Anderson and Boer (GAB) equation [50, 54] (applicability range $0 < a_a < 0,9$), is now recognized as the most versatile sorption model and recommended as such by the European COST 90 Project. This, it was found to be suitable for analysing more then 50% of fruits, meat and vegetables. It also allows calculation of the monolayer water (Maroulis et al., 1988) [1]. The thermodynamic description of these osmotic solutions has been the object of intense research all along the last century, particularly those involving sugars and/or salts. Excellent review soft such efforts have been written [29-35]. Most thermodynamic models used to describe vapors-liquid equilibrium of osmotic solutions are based on relations involving Gibbs free energy of the system. Of particular interest is the excess Gibbs energy (G^E) and for each of the components the partial molar excess Gibbs energy (g_i^E), both allowing a convenient way to quantify the deviations from ideal behavior [1, 19, 44].

From G^E, a large number of physical parameters may be calculated such as water and solute activities, partial equilibrium properties (solubility, relative volatility, etc.) and others. The activity of water in aqueous solutions is defined as [44]:

$$a_a(T,P,x) = \gamma_W(T,P,x) = \frac{f_w(T,P,x)}{f_w^o(T,P,x)} \tag{1}$$

where:

a_w is the water activity, x_w is the mole fraction of water,

γ_w is the activity coefficient for water,

f_w, f_w^o are the water fugacity in the system and at reference conditions, respectively.

It is usually assumed that under normal working conditions of ambient temperature and atmospheric pressure, gas phases behave ideally and so the ratio fugacity can be taken as the ratio of partial pressures [44]:

$$\frac{f_w}{f_w^o} = \frac{p_w}{p_w^o}, \tag{2}$$

Where:

$\boldsymbol{p}_w$ and $\boldsymbol{p^o}_w$ are the vapor pressures of water in the system and of pure liquid water at the same temperature, respectively.

Under this assumption, from eq.(1):

$$a_w = \frac{p_w}{p_w^0} = \frac{RH}{100}, \tag{3}$$

Where:

RH is the percent relative humidity of the air layer in equilibrium with the sample.

The activity coefficient γ_w may be calculated directly from the partial molar excess Gibbs energy of water, g_w^E [44, 54]:

$$g_w^E = RT \ln \gamma_w, \tag{4}$$

And for the total molar excess Gibbs energy, g^E:

$$g^E = RT \sum_i x_x \ln \gamma_i, \tag{5}$$

For an ideal solution all the activity coefficients, $\gamma_i(T,P,x)$, are equal to one, corresponding to an excess, Gibbs energy equal to zero.

Dependence of activity coefficient on temperature may be expressed in terms of the excess partial molar enthalpy or the partial molar excess heat of mixing as [52]:

$$\left[\frac{d\ln\gamma_i}{dT}\right]_p = \frac{h_i^E}{RT^2}, \quad (6)$$

After integration between two different temperatures, a form of the well-known Clausius-Clapeyron [49] equation is obtained:

$$\ln\frac{(a_w)_{T1}}{(a_w)_{T2}} = \frac{h_W^E}{R}\left(\frac{1}{T_1}-\frac{1}{T_2}\right), \quad (7)$$

When h^E_w is constant along the path T_1 to T_2.

In spite of this elegant way of expressing temperature dependence, some empirical equations have been proposed [44, 52], often involving a considerable number system specific parameters. Analysis of the comparative performance of different predictive models for water activity has revealed that good estimations may be obtained, not only from empirical equations with parameters fitted to experimental data but also from theoretical models derived from expressions of the excess Gibbs free energy and from equations of state [42] It is somehow surprising how good the predictions made from group contribution models are. These truly predictive models use group interaction parameters not necessarily calculated from data involving the components of interest. Due to these capabilities, further effort and testing should be devoted to this group of techniques [52].

For practical applications of osmotic treatments, the most widely used equations for prediction of water activity in binary are the ones of Norrish [28] and Ross [36]. Parameters calculated by Chirife bet al., 1980 should be preferred for use with Norris equation. For solution containing electrolytes [40] equation are recommended. For water activity of multi-component mixtures good results are obtained using Ross [36] equation with any of the already mentioned single solute models. Recently Roa and Tapia [35] have proposed a simple equation, based on the first-order sum of molalities, with a reasonable predictive accuracy.

The use of UNIFAC and ASOG models as well as equations of state, although constituting a good and accurate tool to estimate the activity coefficients of non-electrolyte and electrolyte mixture, are not yet widely used in everyday practical application of osmotic treatments [55, 44].

2.3. Mathematical modeling

The GAB and Ratti et al. [1, 34] models of sorption equilibrium were tested against the experimental data. The GAB equation is usually presented in the form [17]:

$$\frac{X}{X_m} = \frac{CKa_w}{(1-Ka_w)(1-Ka_w+CKa_w)} \quad (8)$$

Where:
X = water content (dry basis, kg water/kg dry solids),
a_w = water activity,
Xm = monolayer water content, a constant of the GAB equation, and
C, K = constants related to temperature (defined by Eqs. 9 and 10).

$$C = C_o \exp(\frac{\Delta H_1}{RT}) \quad (9)$$

$$K = K_o \exp(\frac{\Delta H_2}{RT}) \quad (10)$$

Where C_o, K_o, ΔH_1, ΔH_2 = fitting constants, which can be obtained through non-linear regression.

The mathematical model developed by Ratti et al. [34] is an equilibrium equation based on thermodynamic concepts [17]:

$$\ln a_w = M(X) + N(X)\ln p_{wo} \quad (11)$$

Where: the effect of temperature is included in the vapour pressure of pure water, p_{wo}.

For high moisture foods, such as fruits, mushrooms, and vegetables, the parameters M(X) and N(X) are functions of water content, expressed as:

$$M(X) = -k_1 X^{k_2} \tag{12}$$

$$N(X) = k_3 \exp(-k_4 X) X^{k_3} \tag{13}$$

The parameters k_1, k_2, k_3, k_4, and k_5 of Eqs. 5 and 6 can be obtained from experimental data through non-linear regression.

When fitting the models to the experimental data, constants were obtained using the Levenberg-Marquardt procedure for non-linear least squares problems as implemented Sigma Plot (1992) in [17] In this method, the stopping criterion that determines when the least squares minimum has been attained is based on the tolerance, which is set to 0.0001 by default. The parameter Norm, used by the method, represents the closeness of the most recent iteration. Numerically, it is the square root of the sum of the residuals [17, 44]:

$$Norm = \sqrt{\sum_{i=1}^{n} (yp_i - y_i)^2} \tag{14}$$

Where: y_{pi}, y_i = predicted and experimental values, respectively.

The comparison to find the best correlation to represent the experimental data was based on the percentage standard error, E, of experimental versus predicted water activity [34]:

$$E = 100 \sqrt{\sum_{i=1}^{n} (yp_i - y_i)^2 / n} \tag{15}$$

Where: n = number of observations.

Strawberries are one of the most important seasonal fruit crops. Most of its production is destined for the fresh market, but because of the short shelf life and seasonal nature of this fruit, part of its production is processed. In this way, it is used as a food ingredient in yogurts, pies, milk shakes, jams, ice creams, etc. because of its interesting sensory attributes. The types of strawberry processing most commonly used to increase product shelf life are freezing, partial or total dehydration and other combined methods. In these cases, the processed fruit undergoes changes in sensory attributes such as texture, color [5, 26, 47] and changes in the profile of volatile compounds [7, 45], making the product different from nontreated products. Other quality attributes, such as product taste or flavor related to fruit composition on major sugars and acidity, may also be altered during such processes [25, 53].

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3. Results and Discussion

The objective of the present work was to evaluate storage stability of frozen strawberry processed by a previous osmotic dehydration at atmospheric pressure (760 mmHg). The stability was assessed monthly by means of physico-

chemical and sensorial methods, during 6 months of storage at congelend temperature. Table 1 shows Aw and pH values, concentrations of water, soluble solids, sucrose and citric acid of each strawberry fresh, before and after dehydration treatment (osmotic dehydration OD).

Table 1

Physico-chemical characteristics of strawberry

Sample	HS, %	W,%	pH	Total acidity (AC), %	Total Sucrose (S), %	$a_{w,}$ calculated theoretically
Fresh fruits	7.5 ± 0.5	92,4 ± 0,6	3.5 ± 0.05	0.85 ± 0,06	6.4 ± 0,6	0.990 ± 0.001
OD	8.3 ± 0,3	90.2 ± 0,5	3.75 ± 0.08	0.82 ±0.05	7.0 ± 0,3	0.982 ±0.002

OD, osmotic dehydration

Table 2

Changes the physical and chemical indicators in frozen fruit

Sample	Maintenance time in frozen decrease, months	W,%	SH, %	S, %	AC, %
FS	BF	92.4 ± 0,6	7.5 ± 0.5	6.4 ± 0,6	0.85 ± 0,06
	3 months	92.2 ±0.3	7.6 ± 0.3	6.8 ± 0.5	0,86 ± 0.04
	6 months	92.1 ± 0.5	7.8 ± 0.6	6,7 ± 0.5	0.88 ± 0.03
OD	BF	90.2 ± 0,5	8.3 ± 0,3	7.0 ± 0,3	0.82 ± 0.05
	3 months	90.2	8.5 ± 0.0	6.8 ± 0.4	0,82 ± 0.04
	6 months	90.1	8.3 ± 0.1	6.9 ± 0.5	0.81 ±0,04

Sensorial analyze of frozen strawberry appearance showed that the fruit color remained almost the same compared to the fresh fruits. We observed just a weak appearance of brown color. Analyzing the contents of L-hidroascorbic acid, pholyphenols and whole anthocyanins it was observed a diminution of these indexes on depositing way. Medium values are presented in Table 2. Higher shown index diminution was different: depending by the staple primary quality, staple procession previous freezing, keeping term and pack quality. The antioxidants activity consists the biological value of the fruits. These indicators are directly proportional [46, 47]. The biological value of the product is higher with the increasing of the reducing activity. The basic indexes that characterize the reducing activity of the antioxidants are oxido-reducing condition [47]. As the chemical composition of strawberries, including content and antioxidant activity was very variable, the experimental data were analyzed by statistical methods, using the probability theory [40, 42].

Table 3

The capacity modification of antioxidants in congealed strawberry

Maintenance time, months	Antioxidants content, mg/100g			Redox state decrease K, mg AA/gHS
	L-hidro-ascorbic acid	Total polyphenols	Total anthocyanins	
Entier strawberry without sugar				
Primary	21.7 ± 0.39	157.0 ± 1.93	35.2 ± 0.37	15.0 ± 0.38

After 3 months	19.6 ± 0.49	130.8 ± 1.68	30.6 ± 0.36	8.9 ± 0.43
After 6 months	19.2 ± 0.54	96.5 ± 1.60	28.9 ± 0.42	3.5 ± 0.45
Entier strawberry with sugar				
Primary	21.7 ± 0.39	157.0 ± 1.93	35.2 ± 0.37	15.0 ± 0.38
After 3 months	19.8 ± 0.30	145.5 ± 0.85	34.6 ± 0.25	12.5 ± 0.30
After 6 months	19.2 ± 0.28	115.3 ± 1.05	32.5 ± 0.33	7.3 ± 0.25

It was found that there is a slow degradation of antioxidants in the frozen strawberries in the first three months of storage. At a longer storage – the oxidative degradation of antioxidants is faster. The study reveals a decrease in: polyphenol content within 157.0 ± 1.93.... 96.5 ± 1.60 mg/100g; anthocyanin content within 35.2 ± 0.37… 28.9 ± 0.42 mg/100g , and L-Hydroascorbic acid content 21.7 ± 0.39 … 19.2 ± 0.54 mg/100g (full strawberry's without sugar). The content of antioxidants in congealed fruits, before and after dehydration treatment (osmotic dehydration OD) is less reduced (Table 3).

Table 4

Rate value antioxidants contents in congealed strawberry diminutions

Maintenance time in frozen decrease, months	Medium value of antioxidants content diminution, %			
	L-hidroascorbic acid	Total polyphenols	Total anthocyanins	Redox state decreasing K, mg AA/gHS
Entier strawberry without sugar				
3	9.68 ± 0.44	16.69 ± 1.81	13.8 ± 0.37	40.67 ± 0.41
6	11.52 ± 0.47	38.54 ± 1.76	17.9 ± 0.40	76.87 ± 0.42
Entier strawberry with sugar				
3	8.76 ± 0.35	7.32 ± 1.39	1.70 ± 0.31	16.67 ±0.34
6	11.52 ± 0.34	26.56 ± 1.49	7.6 ± 0.55	51.33 ±0.32

Therefore, it was found that during the storage of frozen strawberries there are changes in the content of antioxidants and their oxido-reducing. Reduction of these features is a function of shelf life and of freezing temperature. Contents diminution in polyphenols after 6 months at frozen strawberry without sugar was medium estimated at 38.54 ± 1.76 %, contents diminutions in anthocyanins were medium estimated at 17.9 ± 0.40%, but contents diminution in ascorbic acid were estimated at 11.52 ± 0.47 (Table 4). This study showed the lower reduction of all investigated parameters within processing strawberries OD: contents in anthocyanins were medium of 7.65, being of about 2.36 times less. Probably this is due to the osmosis.

It was found that in congealed and stored strawberries at the temperature of -18^{o} C there is a slow antioxidant degradation in first months. At a longer depositing period (10 months, t=-18°C) biologically active substances are oxidative degraded at an accelerated rate. The degree of degradations occurs in the following sequence: polyphenols, ascorbic acid, anthocyanins, and the content of biologically active substances in dependency of raw material, conditions of storage, processing, freezing and storage [37-39]. Probably through the water contents in unfrozen decrease we can explain a very big diminution of polyphenols, ascorbic acid and anthocyanins in investigation samples. The activity and enzymatic reaction speed almost touches the maximal values of water stratums in frozen strawberry. Probably this phenomenon leads at modification the chemical compounds, plus antioxidants and redox state decrease in food environment.

In the frozen products the enzymatic reactions are slower, but not completely blocked. In general, the enzyme activity in frozen strawberries is related to the presence of unfrozen water. At a temperature of minus 18°C the water content in frozen strawberries is about 89% of total water content. The unfrozen water will be 11%. At a temperature of minus 30°C the frozen water content will be 91% of the total water content and 9% of unfrozen water [8].

One of the majore changes that arise in foods during storage is constituted by Maillard reactions, in which gives rise to a series of final products which contribute particularly to modifying the taste, other chemical changes that can take place are the degradation of vitamin C and other nutrients, state that both carotenoids such as vitamin C may gradually degrade during storage, the stability of temperature being affected [26]. During storage of concentrated apple juice, [3] we can observe that although the amino acids involved in the Maillard reactions, reaction rates being very different. Thus, for example, glutamic acid, asparagine and aspartic acid, were affected by this process. The biochemical changes that occur in food are also the origin of other variations in quality parameters, eg., flavor, aroma and color [18].

Analyzing the color changes in grapefruit juice during storage, [20] found a clear negative linear relationship with other parameters, such as sugars and ascorbic acid, so that as the browning increases, the acid concentrations ascorbic acid and sugars decrease, also saw a clear relationship with the accumulation of furfural and 5-hydroxymethyl furfural. In this regard, [33] consider that the analysis of the variation in the content of 5-hydroxymethyl furfural is a good indicator of the occurence of non-enzymatic browning processes.

It was proved that depreciation of the final products exterior aspect and the appearance of a brown colour is due to antocyanins and polyphenols degradation and formation of brown composites identified in the spectral UV area of 220…270 nm. Spectral analysis of strawberries samples demonstrated that appearance impairment of strawberries and occurrence of brown color is determined by degradation of anthocyanins, polyphenols and creation of brown compounds. In figure 2 are presented Optic density spectrums for frozen strawberries samples after six months of depositing.

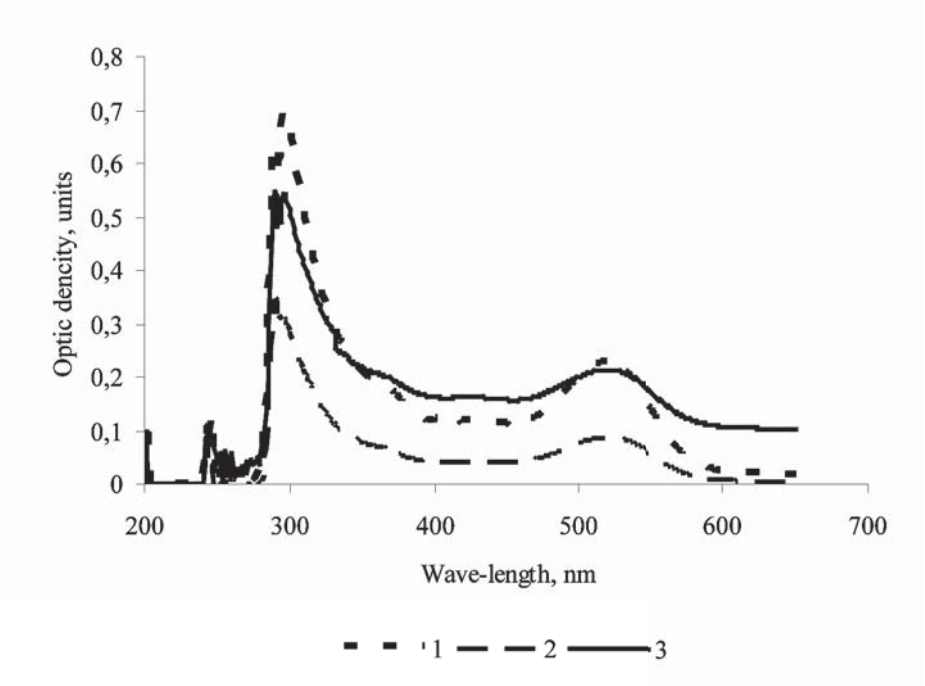

Figure 2. Optic density spectrums for frozen strawberries after six months of depositing

In freezing case when using sugar, we can explain the sugar action, by the reduced content of water in fruits. Analyzing examined samples we see optic density spectrum: frozen strawberries without sugar (1), frozen strawberries with sugar syrup (2), and frozen strawberries with sugar (3) after 6 months of depositing at the temperature -18°C we can see the sugar influence on strawberry color stability. We observe in the case of samples 1 and 2 that optic density extension on wave-light interval λ=500-540 nm, associated with anthocyanins presents in samples, it was more relevant compared with 3-rd sample.$D_{\lambda 519}$=0.228-for frozen strawberries without sugar (1). $D_{\lambda 519}$=0.088- for frozen strawberries with sugar syrup (2). $D_{\lambda 519}$=0.214- for frozen strawberries with sugar (3).

Conclusions

The oxido-reducing state of strawberries depends on the content of polyphenols, anthocyanins and ascorbic acid. The oxido-reducing state is strongly influenced by the content of polyphenols.

In frozen strawberries, during storage there are nutritional and sensory properties changes, resulting in loss of quality. Quality can be determined by analyzing the kinetics of the changing of the bio antioxidants activity.

It was found that the frozen strawberries stored at a temperature of -18°C in the first three months show a slow degradation of the biologically active substances. We discover that in congealed strawberry of keeping at the temperature of -18°C in first months, we see slow antioxidant degradation, but at a longer depositing way we see a higher speed oxidative degradation. Sugar has a significant effect on water activity and stability of the antioxidants in frozen strawberries.

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THE STUDY OF PHTHALATES MIGRATION IN WINE PRODUCTS BY GC-MS METHOD¶

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Abstract. A number of studies have shown phthalates' potential impact on human health due to their carcinogenic and endocrine-disrupting effects. More than 2000 analyses for determination of phthalates' rests in alcoholic beverages were done in the laboratory of National Center for Quality Testing of the Alcoholic Beverages (Republic of Moldova) using modern method of analysis like GC-MS.

Keywords: phthalates, wine, gas-chromatography, mass-spectrometry, migration.

1. Introduction

Today, in modern, industrialized society people can hardly imagine life without home appliances, communication systems, a convenient plastic packaging, fragrance and cosmetics. Most of these and many other chemical products have their properties such as strength, ductility, durability, incombustibility, etc., owing to a number of synthetic organic chemicals. Phthalates are among the members of this series. Phthalates (esters of phthalic acid) are included in the compositions of almost all types of plastics, rubber, paints and varnishes, giving them elasticity and strength [1]. Almost 90% of produced phthalates are used as plasticizers. Phthalates act as solvents and flavor fixatives especially in perfumes and cosmetics.

Humans always are surrounded by materials containing phthalates, such as linoleum, insulation of wires, pipes, plastic housings of domestic appliances, toys, varnishes and paints.

It is supposed that phthalates accumulate in the human body, which negatively affects its hormones, liver and kidneys may also become the causes of allergies, asthma and cancer, neurodevelopmental disorders and abnormalities in the development of children. Molecules of phthalates are not structural elements of the polymer chains and therefore easily stand out in the environment, getting into the human body through food, skin or by inhalation.

In a number of investigated wine-products released by vendors the presence of phthalates was detected. Particular attention was given to the dibutylphthalate.

2. Experimental

2.1. Material and methods

Measuring the concentration of phthalates in wine and base-wine relied on its elimination by chloroform extraction, chromatographic separation on a capillary column, identify the retention time and mass spectrum, and quantify with the characteristic ions m/z . Measuring the concentration of phthalates in alcoholic beverages such as vodka, brandy, cognac alcohol, rectified ethyl alcohol was based on chromatographic separation of the sample on a capillary column using Aldrin with a purity above 99.3% and supplied by SUPELCO as an internal standard, the identification was made by retention times and mass spectrum, quantification of characteristic ions m/z for phthalates and for Aldrin. Tab.1.

Table 1

Measured m/z ratios characteristic for phthalates and IS

Compound	Abbreviation	m/z
Dimethylphthalate	DMP	163, 164, 194
Diethylphthalate	DEP	149, 177
Dibutylphthalate	DBP	149
Bis(2-ethylhexyl)phthalate	DEHP	149, 167, 279
Dioctylphthalate	DOP	149, 279
Didecylphthalate	DDP	149, 307
Aldrin (IS)		250, 261, 263, 265, 291, 293, 298

¶ This article is an extended abstract of a communication presented at the Conference Ecological Chemistry 2012

The background solution (synthetic wine) was used to prepare the calibration solutions. It consisted of aqueous solution of 15% ethanol and tartaric acid (6g/dm^3) (tartaric acid, supplied by Fluka, puriss. p.a. for ion chromatography) and carried to the pH to 3.5 with 5M sodium hydroxide. Synthetic wine was used for calibration standard solutions with concentrations of phthalates: 0 - 1,00 mg/dm^3 (DMP - 99.6%, DEP - 99.6%, DBP - 99.8%, DEHP - 99.7%, DNOP - 99.7%, DNDP - 99.8% Pestanal from Sigma-Aldrich). For the extraction of phthalates, 100 ml of sample (calibration solution) was placed in a separatoring funnel of 250 cm^3 with addition of 10 cm^3 of chloroform (Chloroform, LGC Promochem, for HPLC). Extraction was implemented in 10 min with continuous shaking. After separating the organic layer the bottom layer of chloroform was drained through a paper filter with anhydrous sodium sulfate (sodium sulfate anhydrous, Stanchem, Spain). Collected 10 ml of the chloroform extract was transferred into a gas chromatography vial, from which was selected 1,0 µl of extract by microsyringe directly for analysis using gas chromatography with mass-spectrometer.

2.2. Instruments

SHIMADZU GCMS-QP-2010S (IS) with a COMBI PAL autosampler (CTC ANALYTICS, Zwingen, Switzerland) equipped with fused silica column RESTEK-Rtx-5MS (30m/0.25mm/0.25µm 5% diphenyl / 95% dimethylpolisiloxane phase) was used to perform injections and gas chromatographic analyses in an automated way.

2.3. Gas chromatography–mass-spectrometry

The oven program started at an initial temperature of 160°C for 1 min. Temperature was then increased at a rate of 10°C/min to 200°C, maintained for 1 min, then increased at a rate of 20°C/min to 320°C and maintained for 10 min. The carrier gas was helium at 1.0 ml/min (99.9990%), split 5. Ionisation was performed by electron impact (EI). The temperatures used were 320°C for the injector, 320°C for the transfer line, and 200°C for the ion source. The compounds were quantified in selected ion monitoring (SIM) mode[3]. The analyte to internal standard peak area ratio was used as analytical signal for constructing the calibration graphs.

Duration of gas chromatography-mass spectrometric analysis for phthalates constituted 30 minutes.

For the analysis of strong alcoholic beverages calibration solutions of phthalates were prepared on the basis of 50% water-alcohol mixture with the addition of a solution of aldrin as internal standard (IS).

3. Results and discussion

The studies conducted in the laboratory of National Center for Quality Testing of the Alcoholic Beverages (Republic of Moldova) included more than 2000 samples of the bottled wine, base-wine and strong alcoholic beverages for the presence of phthalates.

For determination of optimal conditions of extraction there were done a number of studies described below.

There was made comparative analysis (investigation) of extraction grade obtained with different organic solvents, such as chloroform, diethyl ether, hexane, carbon tetrachloride, benzene and isoamyl alcohol. Conditions: ratio wine/ organic solvent = 100mL/10mL; extraction time = 10min. The results are shown in fig.1. and fig. 4. (average between four extractions).

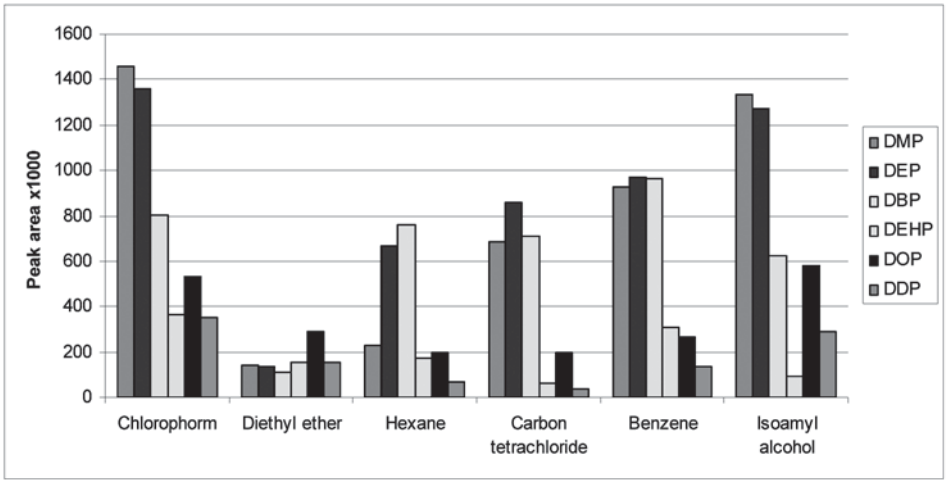

Fig. 1. Signals intensity of phtalates in various extractants

In fig.1 there are shown signal intensities which correspond to extraction of phthalates from the same model contaminated solution by various solvents. RSD% (relative standard deviation) was calculated from the results of four parallel extractions, what is shown in fig. 2 with prices for 1L of solvent grade *p.a.* or similar in Euro.

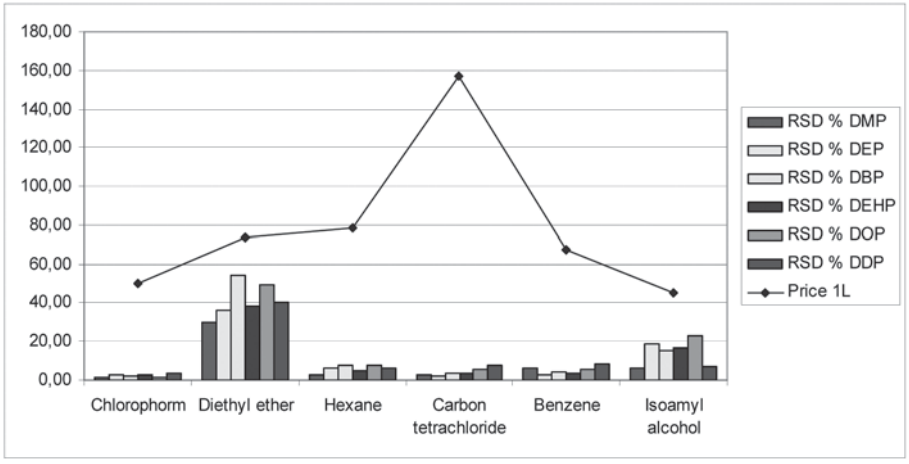

Fig. 2. Relative standard deviation of four extractions. Prices of organic solvents for 1L/€*

** - Prices of organic solvents according to the catalogue (SIGMA-ALDRICH 2011) for 1L grade p.a. or similar*

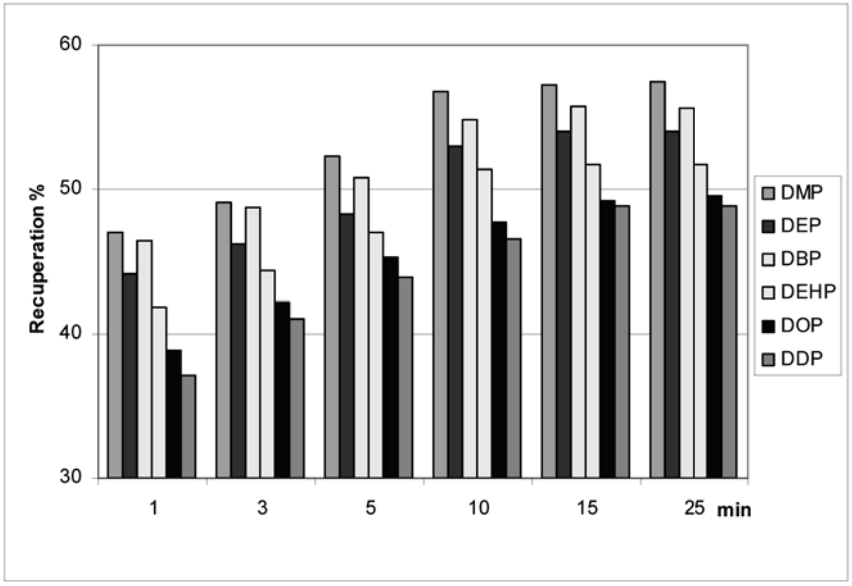

Fig. 3. Determination of optimum phthalates extraction time from wine with chloroform

For the purpose of improvement of analytical characteristics the comparative analysis of two technics of injection has been carried out: method of direct injection of liquid samples into the capillary column and the solid phase microextraction (SPME) – method was recommended by Carrillo J. D. at al. [2]. The results are shown in tab.1.

Table 1

Comparative analysis of injection methods - direct injection and SPME

Methods	Liquid injection	SPME
Sample preparation	Extraction: chloroform/ wine sample 10ml/100ml	Solid Phase Micro Extraction with CW-DVB fiber.
RSD %	2,18±0.51	3,11±0.72
The cost of presampling of one sample	~1.65€*	~9.55€*

*- According to the catalogue prices (SIGMA-ALDRICH)

In order to optimize the extraction process of phtalates during presampling were investigated some dependencies:

a) Effect of pH on the level of recovery was established. Samples of synthetic wine with different values of pH (3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0) were contaminated by DBP. The level of recuperation for a solution with pH = 7.0 was taken as 100%. The results are expressed in fig. 4.

b) Similarly, the influence of sugar content on the extractability of DBP was investigated with Synthetic wine (2.1). Sugar concentration in the samples was formed using concentrated must (C (DBP) <0.01mg/dm^3). DBP was added to the obtained model solutions with concentrations of sugars: 0, 30, 50, 100 and 150 g/dm^3. Chloroform extracts of these samples were analyzed. The results are expressed in fig. 5.

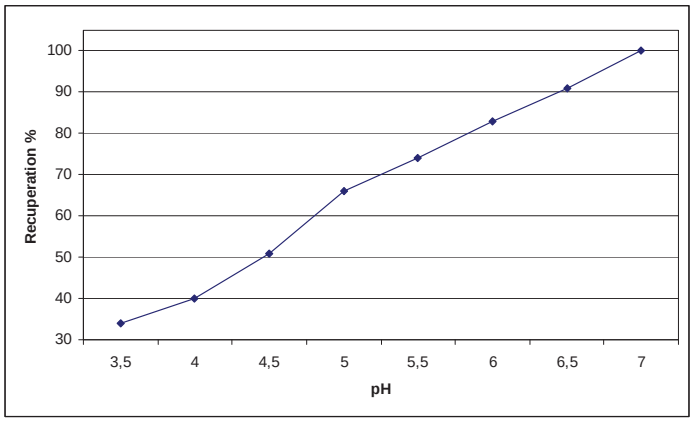

Fig. 4. Dependence: Recuperation level % =f(pH)

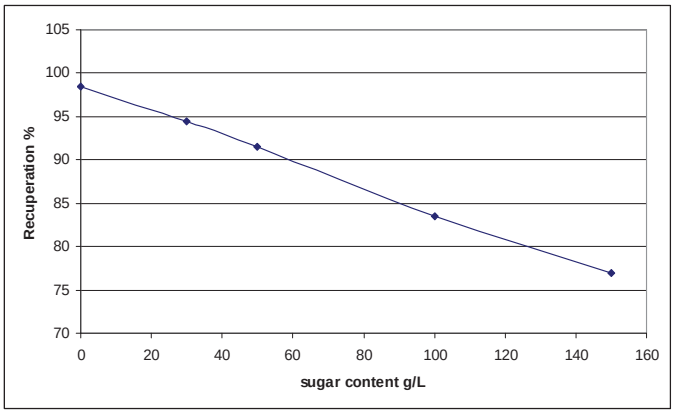

Fig. 5. Dependence: Recuperation level % =f(sugar content g/L)

c) Effect of alcohol content on the extractability of DBP was also investigated using synthetic wine (2.1). Alcohol content in the samples was formed by ethyl alcohol (C (DBP) <0.01mg/dm^3). DBP was added to the obtained model solutions with concentrations of alcohol: 6, 9, 12, 15, 18 and 21% v/v. Chloroform extracts of these samples were tested. As it follows from the results of investigation alcohol content doesn't influence significantly on the level of recovery. (Fig. 6).

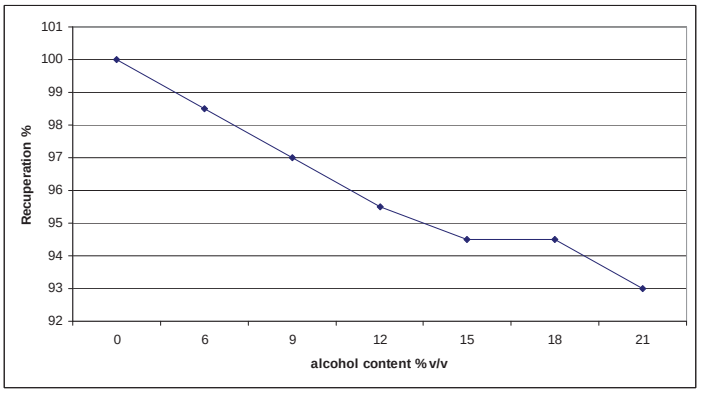

Fig. 6. Effect of alcohol content on the recuperation level of DBP

To establish the sources of DBP pollution in wines there were studied samples of sulfitated and concentrated must: <0.01-0.15ppm of DBP was detected. The lowest concentration level of DBP was characteristic for sulfitated

must, then concentrated - 0.05-0.15ppm. The results of investigations of 15 grapes samples were negative or represent traces. In addition, at the five wineries was investigated water used in wine production. It was found that concentration of DBP in natural water is lower than LOQ, while content in flushing water is 0.04-0.05ppm, and 0.09-0.15 ppm of DBP in softened water.

Therefore contamination with phthalates has mainly a technogenic character, and it is the result of contact with polymeric materials. In the sequel, we studied samples of different materials, which were in contact with wine production during the winemaking process and storage, such as paints, varnishes, primers, pipes, rubber seals. All these tests were conducted according the Directive 2007/19/EG. Also was investigated migration of DBP to a model solution - 15% aqueous ethanol solution, acidified with tartaric acid. Migration of phthalates from materials, which are in contact with wine, is a continuous process that can continue throughout the period of production or storage. The rate of migration was determined basing on these investigations. Studies have been conducted on materials submitted by Moldovan winemakers and distributors. In addition to fresh paint (intended for contact with food) were analyzed paints, which were in contact with wine during a certain period of time. Fresh (liquid) paint was applied to the flask's inner surface, dried on air in 2-3 days, and then a model solution was placed into the flask. Content of DBP was determined in the model solution, which was in contact with the dry polymer within 1 day. Ratio of polymer and model was 1:100. Migration took place at the room temperature (20-22°C).

36 different samples of cork stoppers (for wine and brandy), 6 samples of polymer stoppers for sealing wines and more than 20 samples of caps, seals, dispensers bottle and other polymer elements, which can contact with bottled alcoholic beverages have been studied as a potential source of contamination. All the samples were crushed to accelerate the potential migration of phthalates in the model solution, in which further was determined the content of phthalates. In some cases, migration of phthalates was determined from the surface of the products. As the model solutions were used water-alcohol solution and acidic water-alcohol mixtures simulating wine. As a result, it should be noted that DBP was detected in trace amounts only in four of the investigated samples. In these cases, the observed DBP was on the surface of cork, what is probably due to a violation of the conditions of capping material storage. Quantities of phthalates sufficient for essential change of them in beverages were found in no one of the studied samples capping materials.

4. Conclusions

In the context of studies conducted in the laboratory of National Center for Quality Testing of the Alcoholic Beverages (Republic of Moldova) were included more than 2000 samples of the bottled wine, base-wine and some types of strong alcoholic beverages for the presence of most widespread and toxic phthalate – dibutylphthalate. Results display presences DBP in 85% of studied samples of wines, i.e. a content of DBP more than LOQ (0.01mg/dm^3). Samples of sulfitated and concentrated must, natural and softened water and grapes samples were studied to establish the sources of DBP pollution in wines. Has been determined that contamination of phthalates has a technogenic character, and it is the result of contact with polymeric materials. Optimum conditions of extraction DBP from liquid samples were obtained. Also has been established, that significant influence on extractability is performed by pH value and sugars content value, the alcohol contents in synthetic wine has not displayed significant effect. In addition migration DBP from polymeric materials has been learnt. In the nearest future we plan to research plugs and other materials used in winemaking process on presence of DBP and its migration.

Acknowledgments

Authors would like to acknowledge Moldavian wineries and distributors for the represented samples and the required information.

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STUDY OF PROCESSES OF ACTIVATED CARBON OBTAINING FROM WOOD CHARCOAL

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Abstract. This paper presents the results of scientific research aimed at studying processes of obtaining activated carbon from wood charcoal. We presented methods for determining the structure parameters and specific surface of carbonic adsorbents. Scientific research results allow concluding that wood charcoal is a cheap and effective material for the synthesis of activated charcoal.

Keywords: wood charcoal, physico-chemical activation, activated carbons, pore volume, specific surface area.

Introduction

Active coal is a chemical of certain value, widely used in various industries: pharmaceutical, food, mining, chemical and petrochemical industries, for individual and collective protective equipment in industry and military, for the purification of wastewater, surface water and groundwater etc. A specific area where active coal is used is medicine. Carbon adsorbents are used in this area as enterosorbents and haemosorbents to detoxify the body. These utilizations of activated charcoal characterize it as a product of prime importance in economy and the production of a wide range of activated carbons is of major interest for various industries, due to their special needs.

Activated carbons are adsorbents obtained by different methods of activating the carbonized and un-carbonized raw material as a result of chemical treatment, physico-chemical and mixed treatment of raw materials. Obtained activated carbons fix and retain on their surface organic and inorganic substances they contact. They are composed of a carbon skeleton with very fine pores and channels of varying depths and diameters; on these surfaces takes place the concentration of adsorbed substances [1].

The most commonly used is the physico-chemical activation process, which is based on the interaction of oxygen, carbon dioxide or water vapor with heavy hydrocarbons that fill the pores of carbonized material subjected to activation and/or amorphous carbon atoms in charcoal skeleton. The mentioned procedure shall apply only in the case of carbonized vegetal substances. As an example can serve the wood charcoal from fruit stones, nut shells, activated earth coal, synthetic organic polymers etc, regarded as a mixture of amorphous carbon and hydrocarbons. The activation process is carried out at temperatures of 800-1100° C in special ovens.

As raw materials for obtaining activated carbon are used different carbon-rich materials, for example - wood, peat, coal land [2]. To obtain activated carbon used in anti-gas masks and other specific uses, which should have increased strength and volume of micro-and super-micropores, coconut shell is used as raw material. Should be mentioned here the use of metal carbides, carbon black, lignin, used tires, waste from polyvinyl chloride production and waste from other synthetic polymers production [3].

Due to its physical and chemical properties of activated carbons are unique and ideal sorption materials that address wide range of issues of chemical and biological safety and the environment. Activated carbon is a highly porous carbon materials with highly developed internal surface. In the pore structure of activated carbon (volume of micropores and mesopores) is absorbed by all types of organic trace contaminants by adsorption forces [4].

It was established that with increasing ratio H:C and O:C, the raw material activation is enhanced. However, a significant ratio requires the elimination from the raw material of a large proportion of volatile substances. The charcoal burning procedure is used for such purposes [5].

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Experimental Part

As the raw material for obtaining of activated carbon, it was used the wood charcoal from the manufactured in Străşeni (MS) and Călăraşi (MC), that is quite widespread and more accessible than other materials.

Structural parameters and adsorption capacity of activated carbon depends on many factors, among which the most important are: activity time, activation temperature, steam flow, quality and origin of charcoal [6]. Structural parameters and geometric surface of carbonic adsorbents were determined from the adsorption - desorption isotherms of nitrogen at a temperature of 77 K. Research has been conducted using an Autosorb 1 MP instrument. Analogical research was carried out for all obtained activated carbon samples. An example of such adsorption isotherms are shown in Figure 1.

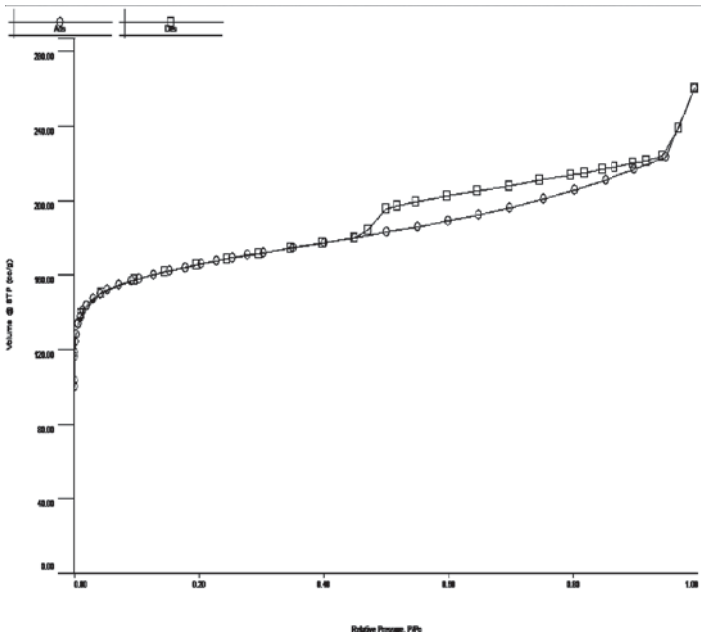

Fig. 1. Adsorption-desorption isotherms of nitrogen (77 K) on the activated carbon obtained from wood from (MS), activated during 30 min at 850°C

Derived activated carbon pore sizes were determined from adsorption-desorption isotherms. An example of pore distribution curve of the dimensions is shown in Figure 2.

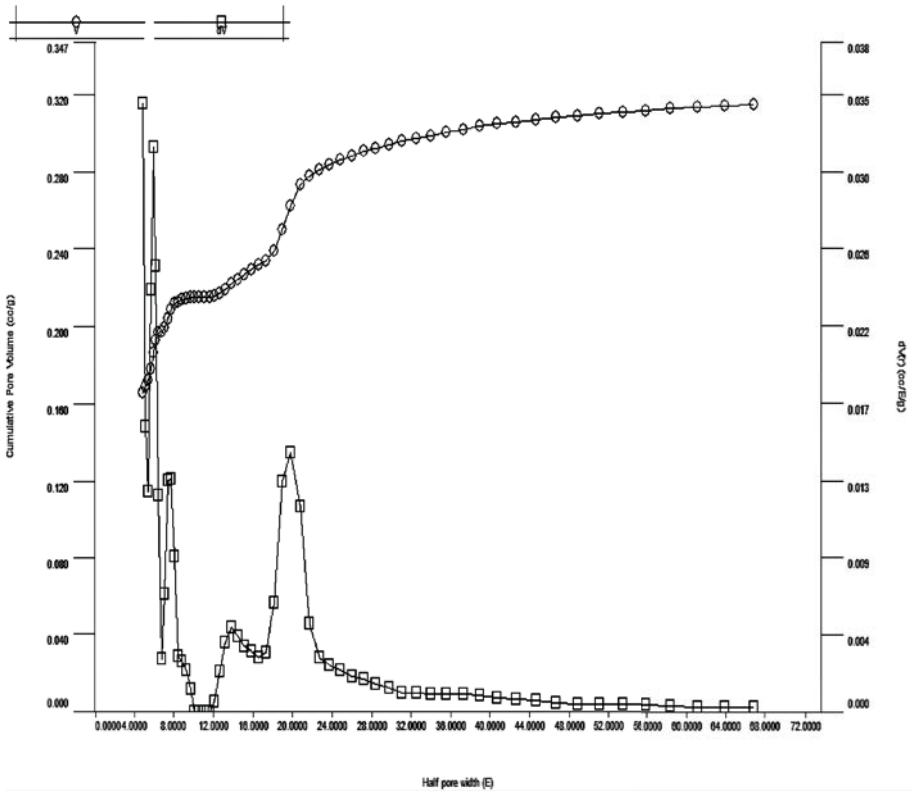

Fig. 2. The pore distribution curve on the sample sizes of activated charcoal obtained from wood from (MS), activated during 30 min at 850°C

The geometric surface of new activated carbons was calculated using the BET equation in its linear form [7]:

$$\frac{P/P_s}{a(1-P/P_s)} = \frac{1}{a_m c} + \frac{c-1}{a_m c} \cdot P/P_s \quad (1)$$

Where a is the adsorption at relative pressure P/P_s; a_m – adsorption at pressure $P/P_s = 1$; C – constant that depends on the heat of adsorption and condensation of adsorbent.

In fact, the following correlation is set:

$$\frac{P/P_s}{a(1-P/P_s)} \quad \text{from } P/P_s \quad (2)$$

As seen in Fig.3 it is a linear correlation.

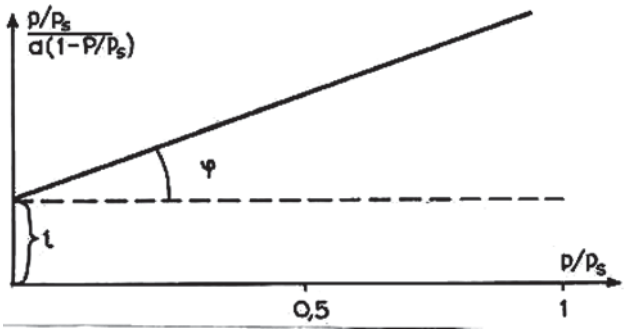

Fig. 3. Adsorption isotherm in the coordinates of the linearized BET equation.

The values a_m and C from the presented equations are determined using this figure:

$$\text{tg}\,\varphi = \frac{c-1}{a_m c} \quad (3)$$

$$l = \frac{1}{a_m c} \quad (4)$$

The geometric surface (S) of the activated carbons was calculated using the following correlation [8]:

$$S = a_m \cdot \omega \cdot N \quad (5)$$

where ω is the area occupied by an adsorption molecule in the compact monomolecular adsorption layer; N is the Avogadro number.

Sorption volume (V_s) of the pores is calculated from the relation [9]:

$$V_s = a_m \cdot V^* \quad (6)$$

where $V^*$is the molar volume of the adsorbate.

Results and discussions

Results regarding geometrical surfaces, process efficiency, sorption pore volume as function of the activation temperature, the flow of water vapor during activation are presented in Tables 1-4.

Analysis of the results presented in the tables below allow to conclude that the geometric surface of activated charcoal depends on the time of activation, the water vapor flow, temperature of chemical process. Thus, increasing the duration of the activation temperature of 850°C from 30 to 90 minutes increases the geometric surface of the activated carbon from 625 to 947 m^2/g, for a water flow of 6 mL/min (Table 1).

Table 1

Quality indices of activated carbons from wood charcoal (MS) obtained by physico-chemical activation at 850°C as function of activation time at a vapor flow of 6 mL/min

Type of AC	Temperature, °C	Time, min	Vapor flow, mL/min	Rate, %	Specific surface, m^2/g	Sorption volume, cm^3/g
S-1	850	30	6	78,1	625	0,402
S-2	850	50	6	69,2	633	0,416
S-3	850	70	6	43,4	833	0,574
S-4	850	90	6	21,3	947	0,631
S-5	850	120	6	-	-	-

Table 2

Quality indices of activated carbons from wood charcoal (MS) obtained by physico-chemical activation at 750°C and 950°C as function of activation time at a vapor flow of 6 mL/min

Type of AC	Temperature, °C	Time, min	Vapor flow, mL/min	Rate, %	Specific surface, m^2/g	Sorption volume, cm^3/g
T-1	950	30	6	20,0	1249	0,825
T-2	950	50	6	-	-	-
T-3	750	30	6	69,0	563	0,340
T-4	750	50	6	65,3	644	0,381
T-5	750	70	6	59,3	660	0,490
T-6	750	90	6	56,0	695	0,515
T-7	750	120	6	52,3	716	0,556
T-8	750	180	6	36,9	889	0,679

Geometric surface value of carbonic adsorbents increases under similar activation conditions from 643 to 1126 m^2/g increasing the flow of water vapor from 6 mL/min to 12 mL/min (Table 3).

Table 3

Quality indices of activated carbons from wood charcoal (MS) obtained by physico-chemical activation at 850°C as function of activation time at a vapor flow of 12 mL/min

Type of AC	Temperature, °C	Time, min	Vapor flow, mL/min	Rate, %	Specific surface, m^2/g	Sorption volume, cm^3/g
D-1	850	30	12	51,3	643	0,406
D-2	850	50	12	44,4	892	0,683
D-3	850	70	12	30,0	963	0,755
D-4	850	90	12	18,8	1216	0,837
D-5	850	120	12	6,0	1007	0,783

This is explained by the fact that increasing water flow leads to higher vapor mass which reacts more intensely with amorphous carbon, volatile substances from charcoal shifting chemical equilibrium of reactions to right.

$$C(\text{amorphous carbon}) + H_2O \xrightarrow{t°C} CO + H_2 + \Delta H$$

$$C_x H_y O_z\,(\text{volatile substances}) + H_2O \xrightarrow{t°C} XCO + YH_2 + \Delta H$$

Table 4

Quality indices of activated carbons from wood charcoal (MC) obtained by physico-chemical activation at 850 °C as function of activation time at a vapor flow of 6 mL/min

Type of AC	Temperature, °C	Time, min	Vapor flow, mL/min	Rate, %	Specific surface, m^2/g	Sorption volume, cm^3/g
C-1	850	30	6	70,6	580	0,322
C-2	850	50	6	69,0	698	0,425
C-3	850	70	6	54,4	807	0,612
C-4	850	90	6	44,4	794	0,592
C-5	850	120	6	30,3	649	0,624

Increasing geometric surface of activated carbons is synchronized by the increase of pores sorption volume. Thus, sorption pore volume increases from 0.402 to 0.631 cm^3/g for a water flow of 6 mL/min (Table 1) and from 0.406 to 0.837 cm^3/g for a water flow of 12 mL/min (Table 3). A special role in the process of obtaining activated carbon is played by the activation temperature. Thus, the results presented in tables 1 and 2 clearly demonstrates that increasing activation temperature under the same conditions leads to increased quality indicators of activated carbons. Increasing the activation temperature from 750°C to 950°C, leads to the increase of activated carbon geometric surface from 563 to 1249 m^2/g, for 30 min of activation and a vapor flow of 6 mL/min. Under the same conditions, sorption volume of carbonic adsorbents increases from 0.340 to 0.825 cm^3/g. This is explained by the fact that increasing the activation temperature increases the diffusion coefficient of water vapor in the porous structure of charcoal, which interacts more intensely with amorphous carbon atoms and volatile substances of charred wood.

Analysis of the results presented in Tables 1-4 reveals that increase of the geometric surface and pore volume of activated carbons is proportional to the increase in temperature and activation time up to certain values and then these indices decrease. This is explained by the fact that under such conditions there is an intense oxidation of carbon atoms in the graphite structure of charcoal. Another quality index that influences the efficiency of carbonic adsorbents production is the rate of activated carbon production process. This parameter decreases proportionally with increasing time, activation temperature and water vapor flow. The rate of activated carbon production process decreases with increasing specific surface area and pore volume of carbonic adsorbents.

Conclusions

1. Quality indices of carbonic adsorbents can be programmed depending on the application, by varying the temperature and activation time and the flow of water vapor.

2. Quality of activated carbons, determined and presented in the current paper, shows that the charcoal obtained from Străşeni wood and that obtained from Călăraşi wood represents a good and cheap source of obtaining carbonic adsorbents.

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THE STUDY OF CHEMICAL COMPOSITION AND EVALUATION OF GROUNDWATER QUALITY IN SOME REGIONS OF MOLDOVA

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Abstract. The paper presents the results of groundwater study from different sources, selected in v. Myndreshty, Telenesti district, in various villages of Cahul district and in artesian wells in and around Chisinau. Large differences were evidenced in water chemistry in different villages of one district, as well as within the same village. Was assessed thee compliance of analyzed water to quality requirements of drinking water.

Keywords: drinking water, wells, chemical composition, maximum allowable concentration.

Introduction

Drinking water must be chemically harmless, safe in epidemiological and radiation meanings, and have favorable organoleptic properties. Water quality is determined by a number of indicators, limiting concentrations of which are given by the relevant regulations. In Moldova, the quality requirements for drinking water are established by the Governmental Decision №.934 from 15.08.2007. This document is valid as an alternative to GOST 2874-82.

However, in our work to assess water quality by analyzing its chemical composition, we used both documents. This is due to the fact that some of the parameters are not reflected in the new document, or their maximum allowable concentrations are increased. For example, the content of allowable solids has increased from 1000 mg/L to 1,500 mg/L, although the standard of physiological usefulness of water is 100-1000 mg/L.

The world's first standard of the dry residue in water was adopted by the Brussels Commission in 1853. It was set (500 mg/L) on the basis of the average solids in the water reservoirs of Saxony-Weimar Duchy, which was considered benign in terms of organoleptic properties and did not cause disease in population. Since then, the idea of water quality has extended, but the standard of solids is still determined on the basis of organoleptic properties of water and the impact on public health.

When the content of dry residue exceeds 1000 mg/L the organoleptic properties of water perceived by the consumers are deteriorated. Mineral salts in great amounts give water a salty or bitter taste. Sodium and calcium chlorides are the main species that confer a salty taste, sulphates and chlorides of magnesium make it bitter. In addition, studies show that the use of highly mineralized water is harmful for the body: it can lead to frustration of many metabolic and biochemical processes and to development of various functional, as well as morphological disorders.

The content of strontium in the water is not regulated by the Governmental Decision № .934 from 15.08.2007, while according to GOST 2874-82 the strontium content must not exceed 7 mg/L. The necessity of a strontium standard in the Country is due to the fact that its content in some areas of Moldova (Chisinau, Orhei district) is much higher than 7 mg/L, and the presence of this element in the water is not safe for human health. Strontium excess affects bones, liver and brain. Being close to calcium by its chemical properties, strontium is very different from it in its biological action. An excess of this element in soil, water and food induces "the urov disease" in humans and animals (named after the river Urov In East Trans-Baikal region) - failure and deformation of the joints, growth retardation and other abnormalities. This pathology is a reflection of the competitive relationship of strontium and calcium in their distribution in the body.

As for the content of hardness inducing salts in water, according to GOST 2874-82, it must not exceed 7 mgEq/L (or 7 mol/m^3), while the Governmental Decision № .934 from 15.08.2007 established only the lower limit of hardness -5 German degrees, which is equal to 1.79 mgEq/L or 1.79 mol/m^3, and the upper limit is not specified. The ratio of the physiological usefulness of water hardness ranges from 1.5 to 7 mgEq/L.

The main source of centralized water supply in Moldova is the surface water of the rivers Dniester and Prut, and artesian wells. The rural population mainly uses groundwater as drinking water - wells and springs. River water resources consist of large rivers - the Prut and the Dniester - and small rivers. Chemical composition of river water is affected by anthropogenic factors; it varies significantly and may change depending on the season.

Groundwater, presented mainly in springs and wells, is characterized by a widely varied chemical composition, which depends on the geological and physical-chemical factors, as well as on anthropogenic factors. Groundwater in Moldova often doesn't match the requirements for drinking water regarding the content of hardness salts, sulfates, chlorides, sodium, ammonia, nitrite, nitrate, total soluble solids.

With regard to groundwater, its composition largely depends on the geological and physical-chemical factors. This

explains the presence of high content of selenium, fluoride, strontium, hydrogen sulfide and ammonia in the groundwater of Moldova. Anthropogenic effects on underlying aquifers are minimal, and substances such as nitrates, pesticides and heavy metals can be hardly found there.

Results and discussions

This paper presents the results of a study of groundwater from different sources (wells and artesian wells) sampled in different regions of Moldova.

We studied 30 wells in v. Myndreshty, Telenesti district (figure 1), seven wells in various villages of Cahul district (table 1), and six artesian wells in and around Chisinau (table 2).

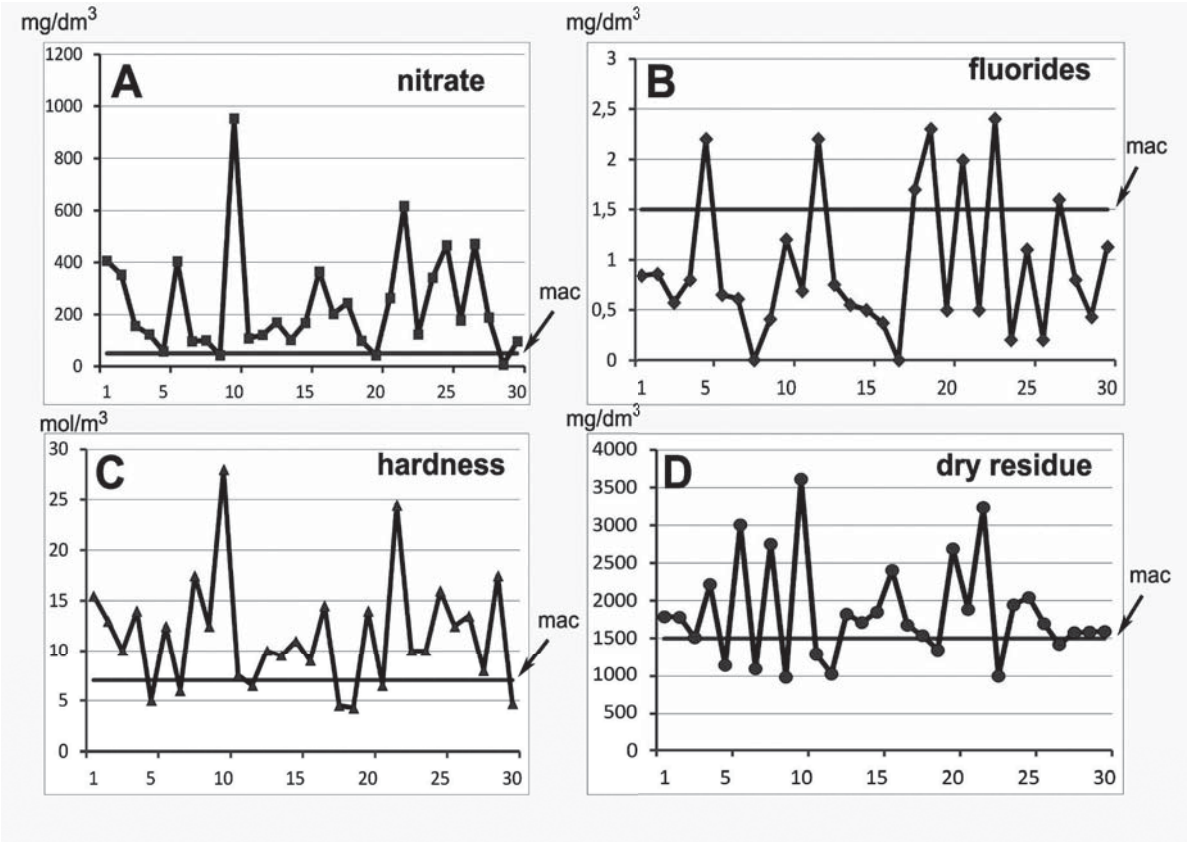

Fig.1. The results of determination of nitrates (A), fluorides (B), hardness (C) and dry residue (D) in the water of 30 wells in v. Myndreshty, Telenesti district

The abscissa shows the number of wells. Arrows indicate the maximum allowable concentration (mac) for each of the analyzed parameters. Figure 1 shows that the content of each of the analytes varies from well to well. Thus, the nitrate content varies from 6.6 to 953.8 mg/dm^3. Fluoride ranges from <0.2 to 2.40 mg/dm^3. Hardness varies from 4.25 to 28.0 mol/m^3, dry residue - from 990 to 3238 mg/dm^3. Nitrate concentrations did not exceed the maximum allowable concentration in only three of the 30 (10%) wells in v.Myndreshty.

In the rest of the wells, excess of concentration ranged from 2 to 18 times. Hardness does not exceed the maximum allowable concentration in eight (26,7%) wells, dry residue - in 8 (26,7%) wells and fluorides in 24(80%) wells.

If the dry residue is evaluated according to GOST 2874-82, only one well meets the requirements of the standard on the content of dry residue. Of the 30 wells analyzed, none of them meets the requirements of Governmental Decision № .934 for at least one of the four parameters. The content of strontium in these wells ranged from 0.57 to 4.40 mg/dm^3, that does not exceed the maximum allowable concentration. These data suggest that even within the same locality one cannot conclude over the quality of water according to the analysis of one or two wells.

Table 1 presents the results of the analysis of water from wells in various villages of Cahul district.

Only one of seven wells (14%) in various villages of Cahul district has concentration of nitrates not exceeding the maximum allowable concentration; hardness was greater than the required standards in all wells, and dry residue was in 5 (71%). Concentration of ammonium exceeded the maximum allowable concentration in 3 (60%) wells. In this case, no wells complied with the standard requirements.

Table 1

The content of some indicators of the Chemical composition of water in various villages of Cahul district

The sampling place	Parameter and units of measurements					
	Nitrate (NO_3^-), mg/dm^3	Ammonium ions (NH_4^+), mg/dm^3	Dry residue, (110^{o}C), mg/dm^3	Fluorides (F^-), mg/dm^3	Strontium (Sr), mg/dm^3	Hardness, moli/m^3
Maximum allowable concentration	50	0,5	1500	1,5	7,0	7,0
v.Vadul lui Isac	20,5	0,6	822	<0,2	0,37	10,5
v.Valeni	1476,0	n/d	6173	<0,2	1,75	58,0
v. Colibas	336,4	n/d	4011	<0,2	1,0	38,5
v.Brinza	174	0,24	785	<0,2	0,62	12,3
v.Crihana Veche	213,2	2,4	1723	0,4	1,50	24,5
v.Slobozia Mare	361,8	1,8	1543	0,6	1,25	17,4
v.Manta	171,5	0,3	1625	0,4	0,75	18,6

Remark: n/d – indices that were not determined.

Table 2 shows the results of determination of several chemical indexes in six artesian wells in and around Chisinau.

Table 2

The content of some indicators of the chemical composition of water in six artesian wells in and around Chisinau

Parameter and units of measurements	Maximum allowable concentration	Artesian well 55 Chişinău	Artesian well v.Carbuna, Ialoveni	Artesian well "Sanatate", Criuleni	Artesian well 68 Chişinău	Artesian well Danceni	Artesian well 4852 Cricova Noua
Hardness, mol/m^3	7,0	17,51	2,65	2,09	44,67	2,13	16,8
Dry residue, (110^{o}C), mg/dm^3	1500	1377	573,5	796,5	6500	610	1149
Nitrate (NO_3^-), mg/dm^3	50,0	<0,4	<0,4	<0,4	<0,4	<0,4	<0,4
Nitrite(NO_2^-), mg/dm^3	0,5	0,03	<0,01	0,06	<0,01	<0,01	<0,01
Ammonium ions (NH_4^+), mg/dm^3	0,5	4,38	1,97	2,2	1,9	4,2	0,7
Fluorides (F^-), mg/dm^3	1,5	0,21	1,0	2,65	0,5	<0,2	0,6
Iron total (Fe), mg/dm^3	0,3	0,25	0,3	0,62	-	0,3	0,29
Hydrogen sulfide (H_2S), mg/dm^3	0,1	2,22	0,42	-	-	-	0,57
Sulfates (SO_4^{-2}), mg/dm^3	250	553,5	143,8	121,5	1547,7	163	388,1
Chlorides (Cl^-),mg/dm^3	250	85,2	35,5	56,8	2566,6	46,6	66,6
Strontium (Sr),mg/dm^3	7,0	13,3	1,1	1,4	25	1,2	5,1
Selenium (Se),mg/dm^3	0,01	<0,005	<0,005	<0,005	<0,005	<0,005	<0,005
Zinc (Zn),mg/dm^3	3,0	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Copper (Cu), mg/dm^3	1,0	<0,1	<0,1	<0,1	<0,1	<0,1	<0,1
Molybdenum (Mo), mg/dm^3	0,25	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01
Manganese (Mn),mg/dm^3	0,05	<0,01	<0,01	<0,01	<0,01	<0,01	<0,01

The analysis of water in six artesian wells in the general evidence a higher quality of water from artesian wells compared to water from wells. The content of nitrate, nitrite, selenium, zinc, copper, molybdenum and manganese do not exceed the maximum allowable concentrations in all samples. However, the concentration of ammonium ions exceeds the maximum permissible concentration in all artesian wells, and the concentration of hydrogen sulfide in all studied wells.

Conclusions

The data indicate that only part of Moldova's population has access to safe drinking water. All analyzed water from wells and artesian wells requires additional treatment before reaching the consumer.

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POLLUTED IRRIGATION WATERS AS A RISK FACTOR TO PUBLIC HEALTH¶

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Abstract. Complex investigations of agro-ecosystems adjacent to a huge mining set of plants located within the city of Kapan were performed with a goal to identify risk factors to the health of local populace. A basic factor of pollution of agro-ecosystems is heavy metal enriched ore waters from adits and industrial water streams freely emptying into the irrigation network. Farm crops growing under conditions of pollution accumulate a series of heavy metals and microelements.

Keywords: heavy metals, irrigation water pollution, soil pollution, crops pollution.

Introduction

The impact of irrigation waters on public health is manifested indirectly; it practically always is mediated by the impact upon the quality of the obtained farm crops. A long-term use of irrigation waters polluted with toxic metals and microelements results in accumulation of such elements in farmland soils and their migration in system "soil-plant" [1, 2].

The issue of environmental pollution with heavy metals is of special topicality in mining sites where farmlands are exposed to the impact of mining plants [13].

One of Armenia's huge mining centers is located in the city of Kapan. The city lies in the southwest of the country, on River Voghchi valley. The region's territory was characterized by V.V. Kovalski [5] as a natural copper-enriched biogeochemical province.

Kapan's mining set comprises the objects of ore extraction and treatment as well as industrial waste centralized in a giant tailing repository of Artsvanik. The development of Kapan deposit started in 1846 and has been progressing since then. A long-term operation of the noted mining complex have resulted in pollution of practically all environmental compartments of the city and neighboring sites [3, 10, 11, 14]. A characteristic feature of pollution is superposition of man-made haloes of heavy metal distribution on natural geochemical anomalies [12].

Wholly, the noted region is characterized by intense agricultural development of river valleys. The vastest farmlands are the city neighboring lands of the Syunik community (Fig. 1). The farm crops growing there are a basic food products for the population of the cities of Kapan and Kajaran as well as of adjacent villages. The farmlands of village of Syunik are irrigated by the waters of River Voghchi tributaries.

With a goal of sanitary and hygienic assessment of farm crops grown within a mining plants impact zone, studied were the contents of heavy metals in irrigation waters and soils as well as in basic vegetable, herb and fruit species.

Materials and methods

Irrigation water samples were collected into plastic containers with a 0,5 L volume and then conserved with concentrated nitric acid 3. Soil and farm crops samples were collected into polyethylene bags [6] and then air - dried and treated consistent with ISO methods [1].

The analyses were performed in the ISO17025 - accredited Central Analytical Laboratory of the Center for Ecological-Noosphere Studies NAS RA on a PerkinElmer AAS AAnalist 800 following standard ISO methods [1]. Determined were the contents of Hg, Cd, As, Pb, Cu, Mo, Zn, Ni, and Cr.

No MAC values for some elements have been developed in Armenia, so those for a series of studied metals were taken from diverse literature sources (Tab.1).

¶This article is an extended abstract of a communication presented at the Conference Ecological Chemistry 2012

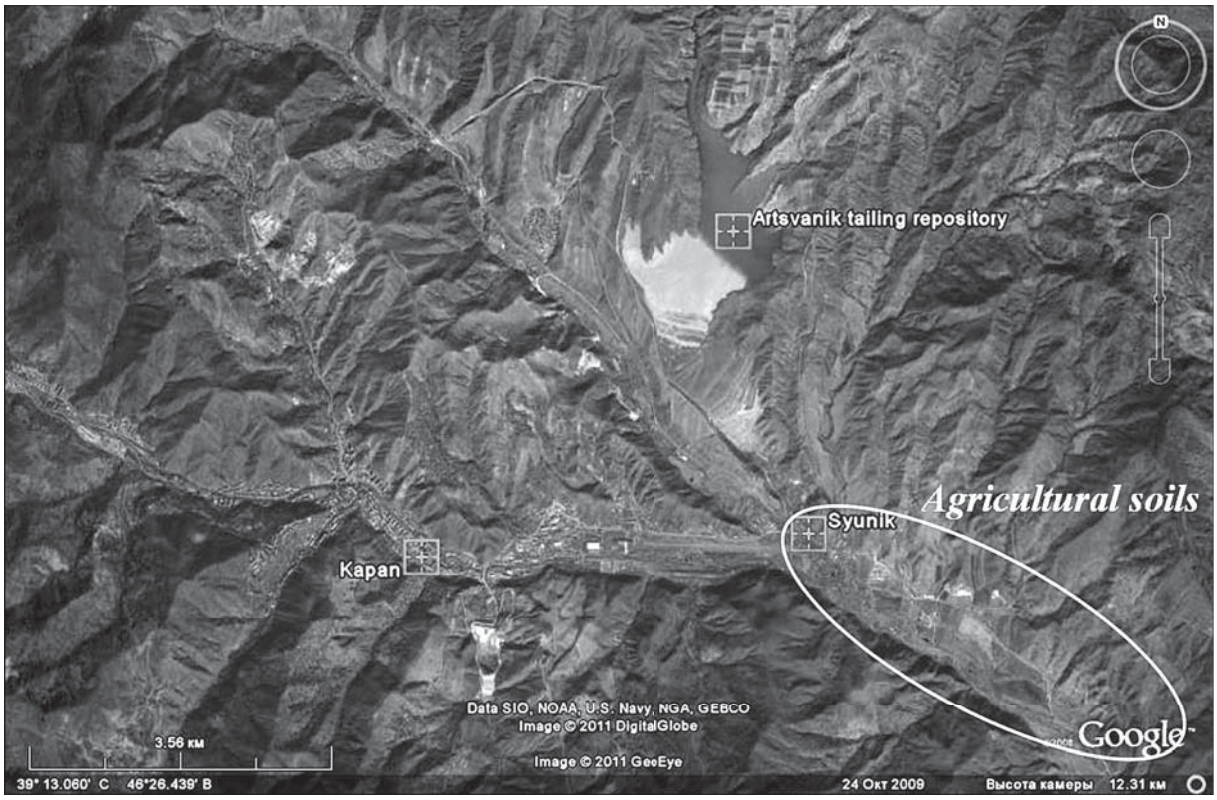


Fig. 1. The territory of the city of Kapan and adjacent farmlands

Table 1

MAC values of elements for different substrates.

Elements	Environment						
	Waters, by [4], μg/L	Soil, mg/kg		Farm crops, mg/kg			
		by [6]	by [9]	Vegetables by [7]	Fruits by [7]	Vegetables by [4]	Fruits by [4]
Hg	0,5	2,1	–	0,02	0,02	–	–
Cd	1	–	2	0,03	0,03		
As	50		2	0,2	0,2		
Pb	30		65	0,5	0,4		
Cu	1000		132	–	–	5	5
Mo	250		132			2	2
Zn	5000		220			10	10
Ni	100		80			0,5	0,5
Cr	50		90			0,1	0,1

Note: "–" – a standard either has not been developed or wasn't used in this work.

Results and discussion

The farmlands of a village of Syunik are irrigated by waters of the tributaries of the major water artery to the region – River Voghchi: the Syunik, Norashenik and Artsvanik rivulets. The noted natural water streams are mixed with untreated ore waters from adits as well as with waters from industrial aqueducts of the Kapan plant and the Artsvanik tailing repository.

Investigations of natural water streams indicated that for the studied period heavy metal contents in river water did not overstep MAC values established in Armenia for natural waters [4] (Fig. 2).

In contrast to natural water streams, the adit and industrial aqueduct waters displayed concentrations of a scope of heavy metals namely Cu, Zn, Cd, As, Hg which were notably excessive vs. MAC values (Fig. 3).

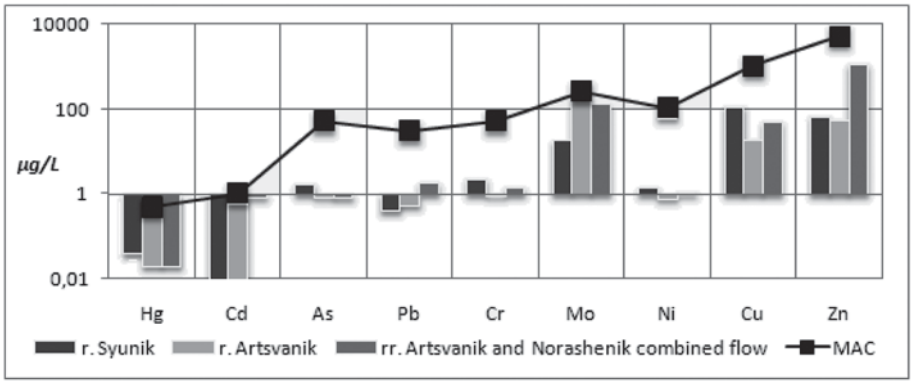


Fig. 2. Heavy metal concentrations in the waters of River Voghchi tributaries and MAC values for water [4]

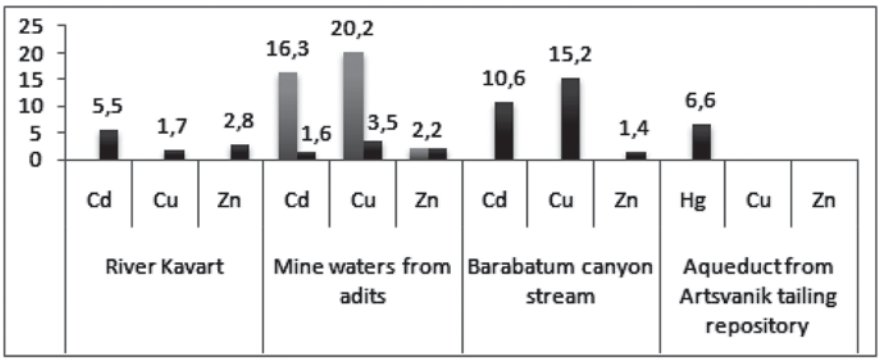
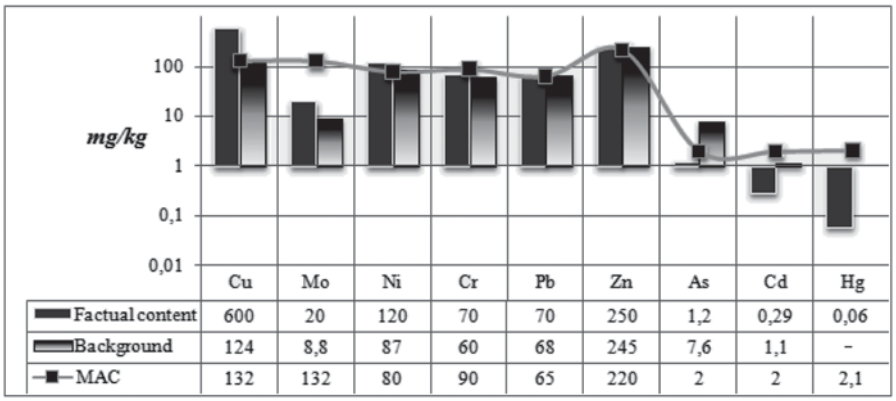


Fig. 3. Excessive concentrations of heavy metals in the waters of industrial and mixed water streams vs. MAC valus for water [4]

Factual contents of heavy metals in soils as well as their geochemical background and MAC are highlighted in Fig. 4. A geochemical series of soils ranked by excesses of factual concentrations of metals against the background is as follows: $Cu_{(4,8)} – Mo_{(2,3)} – Ni_{(1,4)} – Cr_{(1,2)}$, summary intensity of the series being equal to 9,7. Dominating pollutants of farmlands are Cu, Mo and Ni, their share in summary intensity making 66%.

As seen from the given data, the contents of most studied metals in soils exceed the geochemical background, this being a consequence of the use of irrigation waters polluted with heavy metals.

MAC – exceeding values were established for Cu – by 6, Mo – 4 and Ni – 2,4 times. It is noteworthy, that concentrations of elements of the 1st (Pb, As, Zn) and 2nd (Cr) grades of danger make 60-83 % of MAC value [6, 9]. The research indicated a presence of Cd and Hg in soils in concentration lower than MAC.



	Cu	Mo	Ni	Cr	Pb	Zn	As	Cd	Hg
Factual content	600	20	120	70	70	250	1,2	0,29	0,06
Background	124	8,8	87	60	68	245	7,6	1,1	–
MAC	132	132	80	90	65	220	2	2	2,1

Fig. 4. Concentrations of heavy metals in farmland soils vs. their geochemical background and MAC values

The performed research covered a wide scope of farm crops obtained in village of Syunik. Practically in all the studied vegetable, fruit and herb species concentrations of Cr, Ni and Pb considerably exceeded MAC values [7]. Cu, Zn and Mo concentrations were insignificant, but in some cases they, too, exceeded MAC values [4] (Fig. 5). Particularly hazardous are excessive contents of Hg vs. MAC which were indicated in farm crops grown on lands irrigated by waters from the Artsvanik tailing repository.

In the majority of farm crop samples As and Cd concentrations were either lower than MDL (Minimum Detection Limit) or did not overstep MAC values [7].

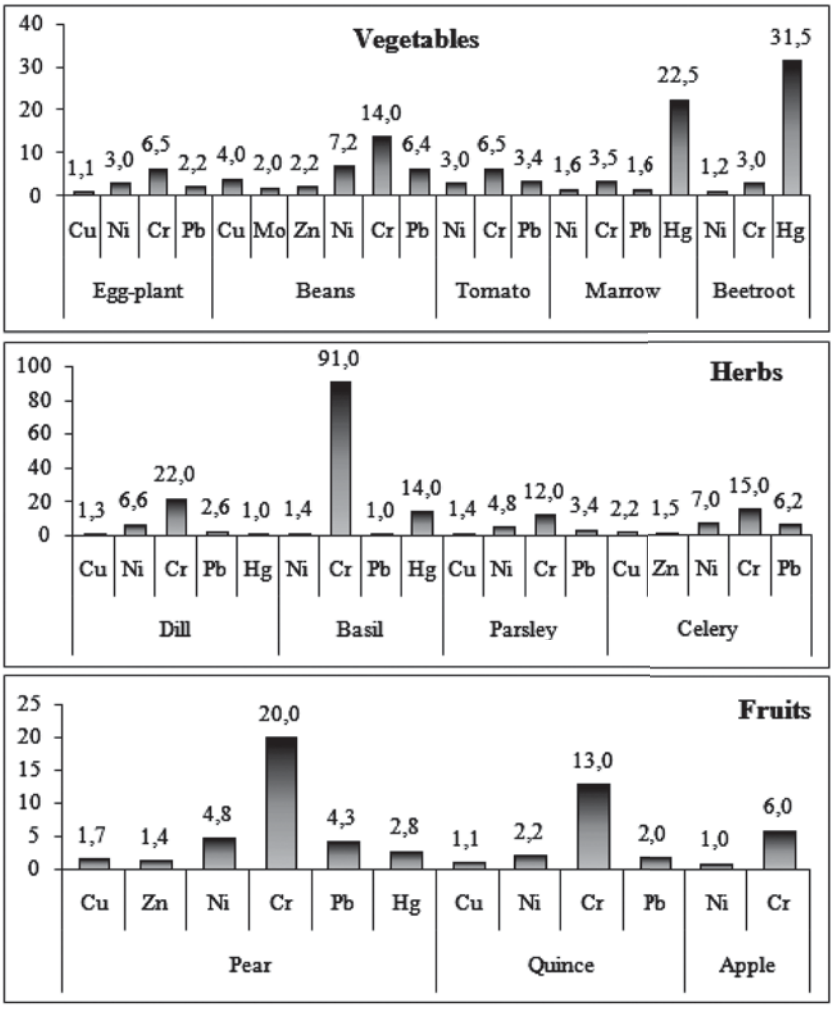


Fig. 5. MAC – exceeding concentrations of heavy metals in some farm crops [4, 7]

To characterize the intensity of heavy metal transfer from soils to plants, a biological accumulation factor (BAF) was calculated which means relation between a chemical element concentration in plant tissues and its concentration in soil: $BAF = C_{crop} / C_{soil}$ (Fig. 6).

As seen from the data, most intensively farm crops accumulate Hg. The highest BAF values were established for beet roots, vegetable marrows and green mass of basil. Hg concentrations in the tissues of the noted plants were 2-10,5 times excessive vs. those in soils. It should be noted that a mercury source is polluted irrigation water. High values of BAF were established for Mo and Cr, too, in bean peas, corn ears, and green mass of basil.

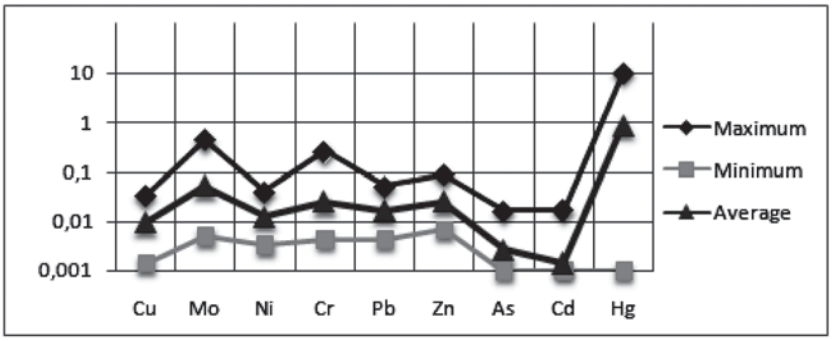


Fig. 6. A biological accumulation factor

Conclusion

The complex investigations indicated that a basic factor of pollution of huge agro-ecosystems adjacent to the city of Kapan are ore and industry-induced waters either emptying into the irrigation network or directly used for irrigation purposes. Long-term irrigation of farmlands with polluted irrigation waters results in accumulation of a set of elements on arable soil layer. The farm crops obtained in the region is hazardous to be used as it contains standard-exceeding concentrations of a set of heavy metals including such toxic elements as Pb and Hg. Intense accumulation of heavy metals in plants occurs as a result of using polluted irrigation waters, this making them a risk factor to public health.

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SELECTIVE ORGANIC SYNTHESIS FOR SUSTAINABLE DEVELOPMENT¶

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Dedicated to academician Gheorghe Duca on the occasion of his 60th birthday

Abstract. The data on the development of selective organic synthesis suitable for obtaining multifunctional organic Compounds both linear and cyclic structures using environmentally benign, inexpensive and renewable resources summarized.

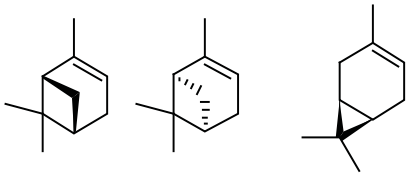
Keywords: natural chiral hydrocarbons, ionic liquids.

Content

1. Introduction

Sustainable development is a pattern of resource use, which aims to meet human needs while preserving the environment so that these needs can be met not only in the present, but also for generations [1]. Chemistry has taken on a crucial role in science and society. Since the early days the stereoselective organic synthesis has remained an important branch of chemistry. Progress of modern synthetic organic chemistry is defined by two general tendencies – profound study of biological processes and *natural product*s as the ground for creation of new effective bioregulators (*drugs, pharmaceuticals,* diagnostic materials, pesticides et al.) and the *use of natural substances* as the starting materials (raw materials) for synthesis of new optically active compounds including bioregulators [2-6]. This development reflected a growing need for an efficient synthetic methodology to produce enantio-enriched organic compounds finding application as pharmaceuticals, agrochemicals, flavours and fragrances, etc. It is knowledge that the configuration of the chiral compound often has a profound effect on its biological activity. In the pharmaceutical industry the current trend aims at developing single enantiomer drugs in the areas, where racemates are still in use, which clearly calls for the methodology for the preparation of the required active isomers in the enantiomerically pure form. One of the ways of achieving this target relies on the use of chiral pool of readily *available and renewable stock of natural products or uses them as chiral promoter* [7-52]. Based on the biological importance of chirality, pharmaceutical, agrochemical, flavour and fragrance industries invest heavily into development of asymmetric technologies where chiral pool reagents play a significant role. A family of chiral monoterpenes incorporating pinenes **1, 2** and carene **3,** due to their natural chirality and advanced skeleton, serve as a feedstock for asymmetric synthesis [53-61].

¶This article is an extended abstract of a communication presented at the Conference Ecological Chemistry 2012



1 **2** **3**

Figure 1

Importantly, nature produces these compounds in both enantiomeric series, which favorably compares with other natural sources of chirality such as amino acids and sugars, occurring predominantly in only one enantiomeric form.

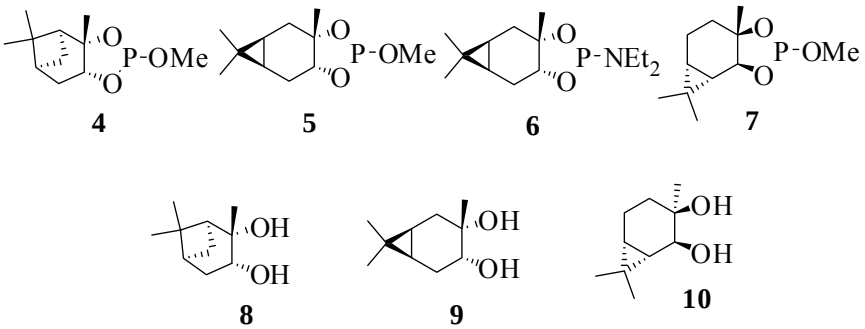
Sustainable chemistry is the design, manufacture and use of environmentally benign chemical products and processes to prevent pollution produce less hazardous waste and reduce environmental and human health risks.

2. Natural Compounds derived Chiral Phosphites for Asymmetric synthesis

Current trends in organic synthesis shown that asymmetric synthesis has become a primary focus of activity for many of the leading researchers in both academic and industrial worlds [62]. The tragedy of the thalidomide babies emphasized the importance of achieving the synthesis of optically pure drugs. Optical resolution as a method to prepare optically pure compounds is often uneconomical and impractical. Frequently the most desirable method for synthesizing optically pure materials is asymmetric synthesis.

Optically active phosphite-type compounds are very attractive and promptly developing class of phosphorus-containing ligands. As a whole, the most important advantages of chiral phosphites include their pronounced π-acidity, oxidation stability, as well as their synthetic availability and low cost. In particular, phosphites provide broad opportunities for fine tuning of their donor-acceptor and steric properties by incorporation of oxygen and nitrogen into the first coordination sphere of phosphorus and wide variation of the O- and/or N-containing building blocks. Most of phosphites can be synthesized rather simply and in high yield from a variety of optically active precursors. This makes it possible to perform the direct one-pot phosphorylation of chiral compounds, whereas the synthesis of the corresponding phosphine derivatives requires preliminary modification. In addition, these compounds exhibit higher oxidative stability because of the absence of P-C bonds. So, this makes it possible to develop protocols for the whole process that do not necessitate the use of a glovebox, including the ligand synthesis.

We designed and synthesized a library of novel *P**-chiral monodentate phosphite ligands **4-10** having five-membered phosphacycles and OMe or NEt_2 exocyclic substituents [63-65]. These can be easily prepared by direct phosphorylation of the appropriate bifunctional compounds and purified by vacuum distillation. They possess modular properties, allowing fine-tuning of their steric and electronic characteristics. The starting optically active diols **5-7**, were synthesised from pinene as well as (+)-2- and 3-carenes.



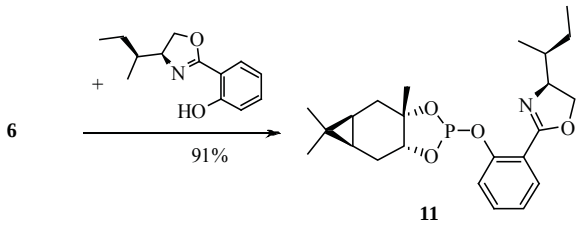
4 **5** **6** **7**

8 **9** **10**

Figure 2

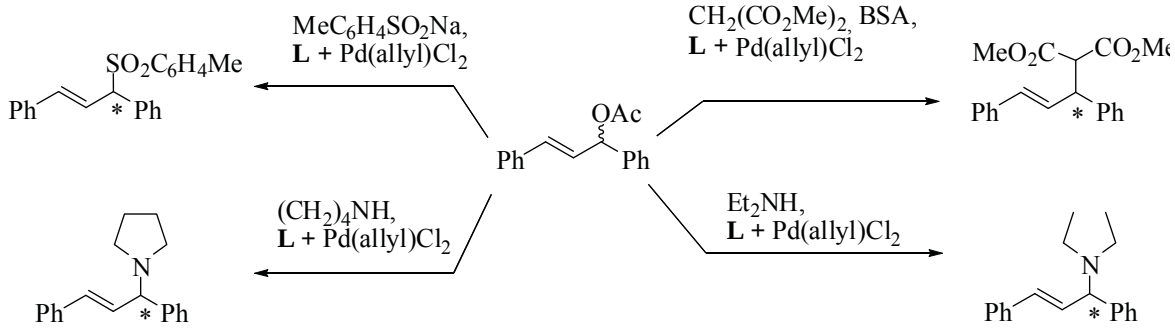
3-Carene-based compounds **5,6** are characterized by rather small contents of the minor epimer. In the last case, the major stereoisomer has the *R* - configuration at the *P** - stereocenter. Another approach to enhance the asymmetrising activity of *P**-chiral phosphite-type compounds is the synthesis of the respective *P,N*-bidentate ligands with additional *C** - stereocenters in the peripheral *N*-containing group. In particular, oxazolinophosphite **11** has been prepared using phosphoramidite **6** as a phosphorylating reagent (scheme 1).

Scheme 1



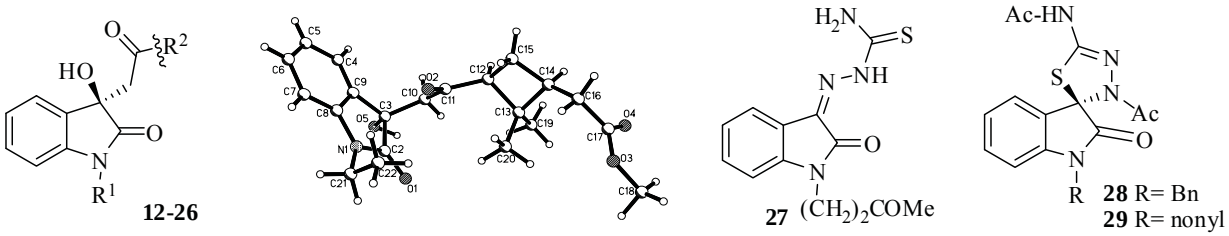
The synthesis of the novel phosphite is highly diastereoselective. The new ligands are stable enough to allow manipulation in open air and can be stored under a dry atmosphere for several months without degradation. It is noteworthy that owing to the easiness of each step, as well as the easy-to-handle nature of all the related intermediates, diamidophosphites can be prepared on multigram scales. Ligands based on terpene alcohol, induce high enantioselectivities (*ee*'s up to 99%) in Pd-catalyzed allylic substitution reactions (scheme 2).

Scheme 2



3. Natural compounds derived chiral oxindoles with anti-HIV-1 activity

The twentieth century has been characterized both by a drastic reduction in the mortality caused by infectious diseases and by a rise in the control of neoplastic pathologies [6]. The treatment of infectious diseases still remains an important and challenging problem. The therapeutic problem has achieved increasing importance in hospitalised patients, in immuno suppressed patients with AIDS or undergoing anticancer therapy and organ transplants. A decade ago we initiated a program on synthesis of chiral oxindoles. In this account we are discussing our progress in field of antiviral activity evaluation of new oxindoles **12-21**, **27-29** and an enantio-pure analogues **22-26** [2,66-69].



12 R^1= Bn, R^2=Me
13 R^1= CH_2CH_2Cl, R^2=Me
14 R^1=$CH_2N(CH_2CH_2)_2O$, R^2=Me
15 R^1=$CH_2N(CH_2CH_2)_2NPh$, R^2=Me
16 R^1= Pr, R^2 = C_6H_5
17 R^1= Me, R^2 = 2,4-Cl_2-C_6H_3
18 R^1= Et, R^2 = 2,4-Cl_2-C_6H_3
19 R^1= Bu, R^2 = 2,4-Cl_2-C_6H_3
20 R^1= hexyl, R^2 = 2,4-Cl_2-C_6H_3
21 R^1= decyl, R^2 = 2,4-Cl_2-C_6H_3
22 R^1= H, R^2= $CONH_2$
23 R^1= H, R^2= CN
24 R^1=H, R^2= CO_2Me
25 R^1= Et, R^2= CO_2Me
26 R^1= Bn, R^2= CO_2Me

Figure 3

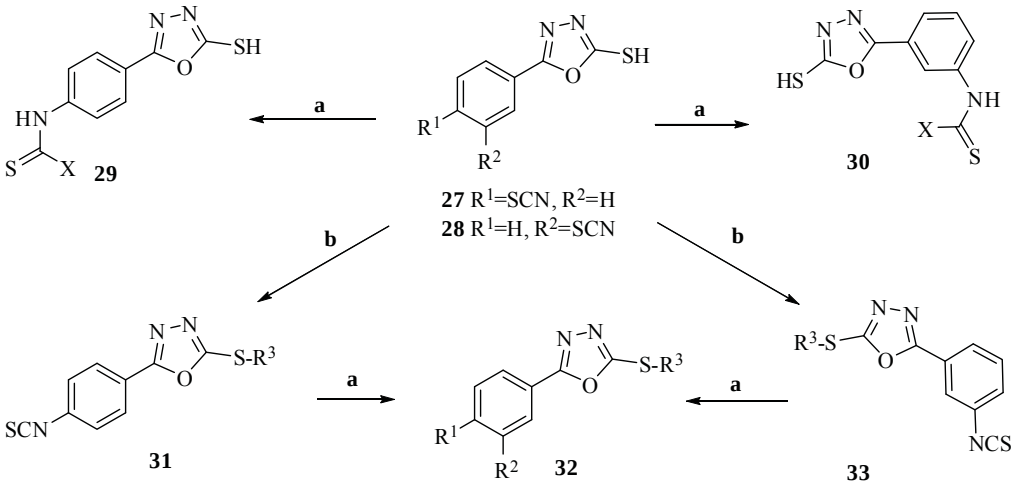
The synthesized compounds exhibited different cytotoxicity, in particular, oxindols **12**, **13**, **14**,**15**, **16**, **17**, **18**, **19**, **20**, **21** turned out to be the most cytotoxic for MT-4 cell lines. The compounds **19** and **25**, are more toxic than reference compound Efavirenz. As far as the antiviral activity is concerned, none of the title compounds turned out active against Reo-1, Sb-1, VSV, RSV, YFV and VV viruses. The results obtained against Bovine Viral Diarrhoea Virus (BVDV) showed that six compounds **14**, **20**, **21**, **24**, **27**, **28**, **29** resulted moderate active. Among all of them, the most potent compound was **27**, with EC_{50} of 6.6 μM. Studies of effect of synthesized compounds against Coxsakie Virus (CVB-2) revealed that only compound **21** exhibit moderate activity (EC_{50} >40 μM). It should be noticed that compounds, **12**, **13**, **15**, **16**, **17**, **18**, **19**, **20**, **21, 22** and **29** showed moderate activity against HIV-1 (EC_{50} >16 – m >59μM).

4. New 5-Aryl-2-thio-1,3,4-Oxadiazoles with Antituberculosis Activity

Tuberculosis remains the number one killer infectious disease affecting adults in developing countries. TB is a global emergency. The situation is more complicated when one considers countries such as India where TB disproportionately affects the young. India accounts for one-third of the global TB burden, with 1.8 million developing the disease each year and nearly 0.4 million dying due to TB annually.Until 50 years ago, there were no medicines to cure TB. Now, strains that are resistant to single drugs have been documented in every country surveyed, and, unfortunately, strains of TB resistant to all major anti-TB drugs have emerged. Drug-resistant TB is caused by many factors: inconsistent or partial treatment and lack of compliancy (sometimes patients do not take all their medicines regularly for the required period because they start to feel better), prescription of wrong treatment regimens, or unreliable drug supply. A particularly dangerous form of TB is that caused by multidrug-resistant TB (MDR-TB), defined as bacilli resistant to at least isoniazid and rifampicin, the two most powerful anti-TB drugs. While drug-resistant TB is generally treatable, it requires extensive chemotherapy (up to two years of treatment) that is often prohibitively expensive (often more than 100 times more expensive than treatment of drug-susceptible TB), in addition to being more toxic to patients. Noteworthy, the development of drug resistance also involves other poverty-related diseases, such as malaria and HIV / AIDS. 1,3,4-oxadiazoles form an important class of five-member heterocyclic compounds with a wide range of biological activities. The importance of the oxadiazoles' nucleus is well established in agricultural and pharmaceutical chemistry as far as its corresponding derivatives are used as antipyretic, analgesic, antidepressant, antimicrobial, antiviral, fungicidal, antineoplasic, anti-inflammatory agents, central nervous system stimulants, and anticonvulsive, anticancer, and antihypertensive agents. Their important structural characteristic is the presence of two aromatic rings spaced by a heteroatom.

The knowledge of quantitative relationships between chemical structure and biological activity is an essential prerequisite for the effective search for biologically active compounds. Earlier our group reported that a number of 2,5-disubstituted-1,3,4-oxadiazoles have good anti-tuberculosis activity against *M. tuberculosis H37Rv* [70]. Shortly a series of 82 5-Aryl-2-thio-1,3,4-oxadiazole derivatives were screened for their antimycobacterial activities against *Mycobacterium tuberculosis* H37Rv. The synthesis of target 1,3,4-oxadiazoles **29-33** was accomplished by the following synthetic route (scheme 3) [71].

***Scheme* 3**

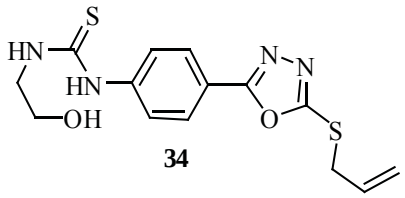


a: amines; **b**: Hal-R^3, K_2CO_3/DMF or Et_3N/acetone;
X=NH_2, NH-Me, NH-Et, NH-p-tolyl, NH-allyl, $NH(CH_2)_2OH$, NHBn, 4-$PyCH_2NH$, $ArC(O)CH_2NH$, piperidine, morpholine, 4-amino-1,2,4-triazole, hydrazones

Due to the presence of two high reactive groups in molecules **27,28**, the –SH group alkylation reaction and addition of different nucleophiles to isothiocyanate group can lead to a large number of new compounds. At the outset of this study it was observed that reactions of 2-mercapto-5-(4-isothiocyanatophenyl)-1,3,4-oxadiazole **27** with different amines in refluxing benzene gave corresponding substituted thioureas **29** with 68% up to 92 % yield. Sulfides **30** can be prepared by treatment of thiol **27** with alkyl halides. Depending on the nature of the alkylating reagent, the synthesis was carried out either in acetone in the presence of Et_3N or in DMF in the presence of K_2CO_3. Further, reactions of 2-S-substituted-5-[(4)-isothiocyanatophenyl]-1,3,4-oxadiazoles **31,33** with amines have led to thioureas **32** with high yields. The seven synthesized compounds appeared to be the most active derivatives exhibiting more than 90% inhibition of mycobacterial growth at 12.5 μg/mL. Structure-activity relationships study was performed for the given series by using the Electronic-Topological Method combined with Neural Networks (ETM-NN). A system for the anti-mycobacterial activity prediction was developed as the result of training associative neural network (ASNN) with weights calculated from projections of a compound and each pharmacophoric fragment found on the elements of the Kohonen's self-organizing maps (SOM). From the detailed analysis of all compounds under study, the necessary requirements for a compound to possess antituberculosis activity have been formulated. The analysis has shown that any requirement's violation for a molecule implies a considerable decrease or even complete loss of its activity.

Computer-assisted molecular modelling (CAMM) plays an essential role in the design of potential ligands that are both sterically and chemically compatible with the binding site of a target bio-macromolecule. Docking was performed with default settings to obtain a population of possible conformations and orientations for the inhibitors at the binding site. Knowing the binding site conformations helps to show the important interactions that stabilize the complex.

Molecular docking studies of the compounds allowed shedding light on the binding mode of these novel antimycobacterial inhibitors (figure 4).



34

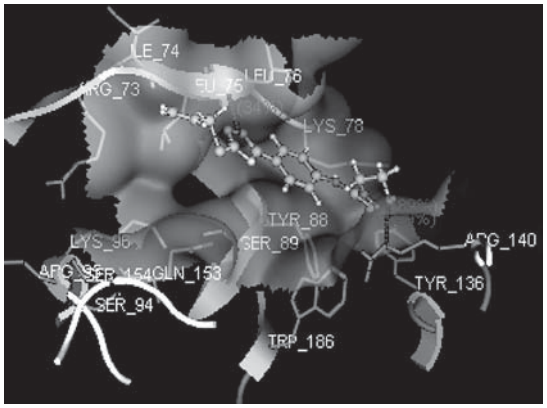


Figure 4

Docking of active compound **34** (figure 4) in Rv1155 shows that this compound docks well in the active site that consists of such amino acids as Ser144, Arg140, Lys78, Tyr88, Ile74, Leu75, and Leu76. The oxygen of the hydroxyl group forms hydrogen bonds with Ser 144 (distance 2.70Å) and Arg140 (distance 2.64Å) of the protein. The oxadiazole ring is located in the vicinity of amino acids Leu76 and Leu75. The nitrogen of oxadiazole forms a hydrogen bond with Leu76 (distance 2.65Å). Four of amino acids show a sidechain and backbone *acceptor* properties. Three of amino acids (Leu75, Leu76 and Ile74) show backbone *donor* properties. The phenyl ring settles in a hydrophobic cavity lined by Lys78, Tyr88, and Ser89.

5. New binary systems of β-cyclodextrin with a highly potential anti-mycobacterial 5-aryl-2-thio-1,3,4-oxadiazole

The studies performed by our group have lead to discovery of another group of substances with high anti-mycobacterial activity from the class of 5-aryl-2-thio-1,3,4-oxadiazole derivatives. Among the studied substances, 2-phenyl-5-{[2-phenyl-1,3-dioxolan-2-yl)methyl]sulfanyl}-1,3,4-oxadiazole (DIOX) (figure 5) has shown the highest level of predicted and *in vitro* activity [70]. The substance's molecular weight is 340,4, it has 5 freely rotatable bonds, 5 hydrogen bond acceptors and no hydrogen bond donors that makes it a drug able candidate according to the Lipinski rule [72]. Still, there is one important disadvantage that can influence bioavailability of the drug and reduce its pharmacological activity - a low solubility in water. In order to overcome this shortcoming, it has been proposed to study the possibility of DIOX inclusion in complexes with β-cyclodextrins. Besides, in the case of the direct contact of the inclusion complex with the *M. tuberculosis* cell, the presence of cyclodextrins in the formulation may increase permeability of mycobacterial wall for the active substance [73].

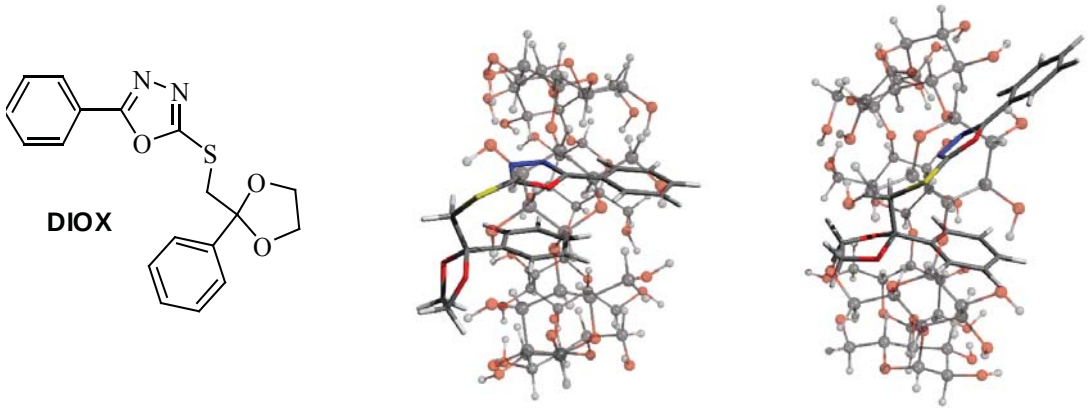


Figure 5

We have shown that 2-phenyl-5-{[2-phenyl-1,3-dioxolan-2-yl)methyl]sulfanyl}-1,3,4-oxadiazole (DIOX) interacts with βCD and the main parts of the DIOX molecule involved in the interaction process are dioxolane and benzene rings [74].

The molecular interaction between DIOX and βCD makes possible its future study in new types of antituberculosis treatment where the presence of βCD does not only provide better bioavailability and stability characteristics to the active ingredient, but also plays the role of promoter of the active substance transportation through the bacterial cell wall, increasing its permeability for the substance. The latest is possible in the case of direct interaction of the complex with the bacterial cell.

6. Green chemistry protocols

Ionic liquids are a relatively new class of compounds that have been receiving increased attention in recent years as "green" designer solvents that may potentially replace many conventional volatile organic solvents in reaction and separation processes. These unique compounds are organic salts that are liquid over a wide range of temperatures near and at room temperature. Ionic liquids have no measurable vapor pressure; hence, there has been considerable interest in using them in place of volatile organic solvents that can emit problematic vapors. The use of Ionic Liquids in organic synthesis often leads to shorter reaction times, increased yields, easier workup, all of which are important considerations for Green Chemistry protocols.

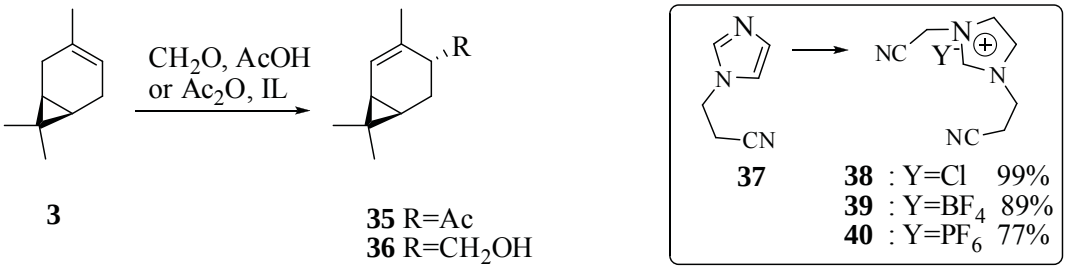
6.1. Ionic liquids as recyclable solvents/catalysts

Increased requests to ecologically pure selective chemical processes need to develop a new type of compounds, posses several useful properties (combination of solvent and catalyst for the same compound), and possibility to use of it without any additional regeneration and purification. Ionic liquids (IL) posses all above mentioned properties, but the list of it is very short. Furthermore, the use of ILs may enhance the regio- and stereo-selectivity of reactions. Well known, one of the tasks of the synthesis of a bioactive compound is preparation of required enantiomer in optically pure form. Bicyclic monoterpene (+)-3-carene **3** is widely used for resolving this type of problems. A structural future of compound **3** is the presence of the reactive C=C double bond and bicyclic bridging system. This fact opens perspectives for new synthesis with retention of the bicyclic framework of monoterpene **3**.

(+)-4a-Acetyl-2-carene **35**, and (+)-4a-hydroxymethyl-2-carene **36**, are widely used to synthesize precursors of commercially important insecticides [54-58]. Known methods for preparing 4-substiututed 2-carenes **35, 36** include heating **2a** with $ZnCl_2$ in Ac_2O solution or with paraformaldehyde in AcOH.

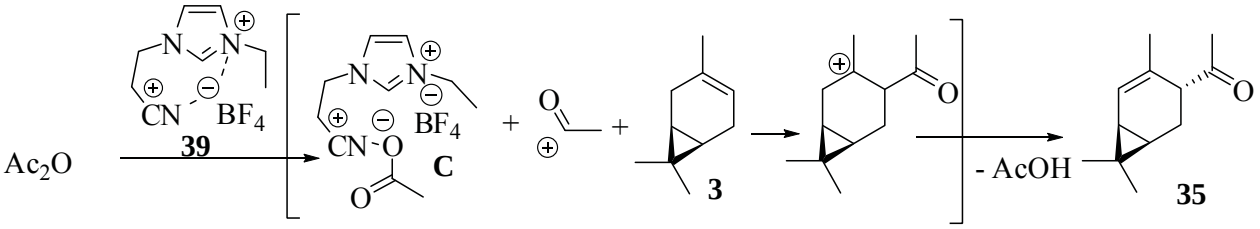
The new type of ionogenic solvents/catalysts for Kondakov's and Prins's reactions was proposed us (scheme 4) [75].

***Scheme* 4**



The salt **38** was prepared by quaternization of **37** [75-79]. The next step was the metathesis of imidazolium salt 6 with the appropriate inorganic salt (KPF_6 or KBF_4). It was observed that heating of (+)-3-carene **2a**, acetic anhydride, or with paraformaldehyde in AcOH and 6 mole % **38**, **39**, or **40** at +60°C afforded the (+)-4a-acetyl-2-carene **35** and (+)-4a-hydroxymethyl-2-carene **36** [9]. The catalytic activity of the synthesized imidazolinium salts is probably due to initial formation of an acylium ion from Ac_2O that involves the nitrile associated with the anion (see scheme 5).

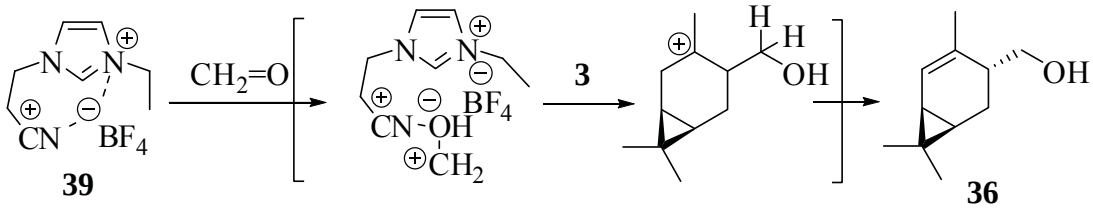
***Scheme* 5**



The carbonium ion formed by addition of the acyl-cation to the double bond of (+)-3-carene **2a** is stabilized by elimination of a proton to regenerate double bond of (+)-4a-acetyl-2-carene **38**.

On the other side the α-hydroxy carbonium ion generated *via* adding of a proton to formaldehyde reacts with (+)-3-carene **3** to give the hydroxyl-carbonium ion that is stabilized via elimination of a proton (scheme 6).

***Scheme* 6**



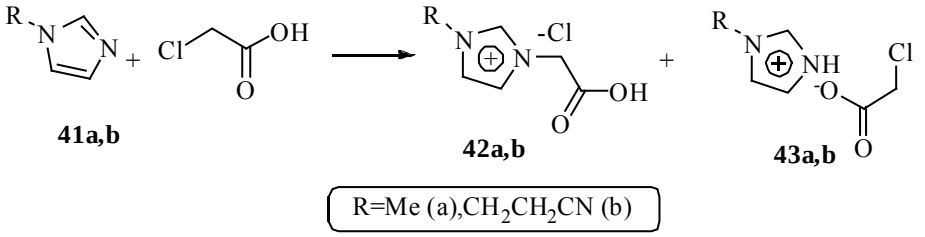
Finally the generations double bond of (+)-4a-hydroxymethyl-2-carene **36** was realizing.

6.2. Ionic liquids as recyclable catalysts

The knowledge about the three-component condensation reaction between an aldehyde, a urea or thiourea, and an easily enolizable carbonyl compound, is quite extensive. This reaction offers a straightforward approach to bioactive 3,4-dihydropyrimidin-2-(1*H*)-ones(thiones).

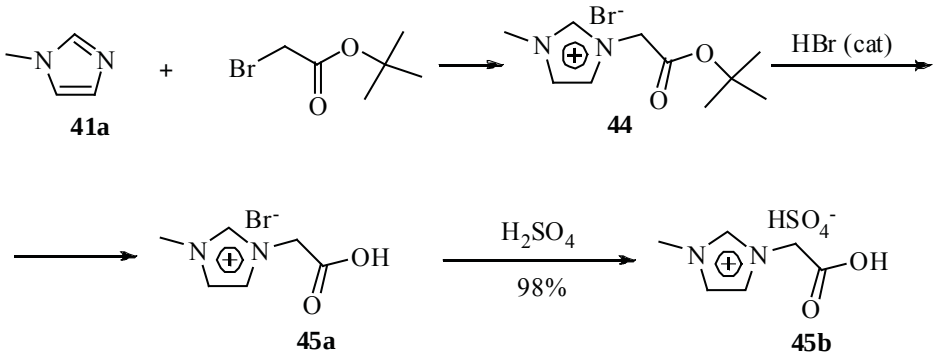
Recently we reported about the synthesis of the mixture **42a,43a** or **42b,43b** from monochloroacetic acid and N-substituted imidazoles **41a,b** [80].

***Scheme* 7**



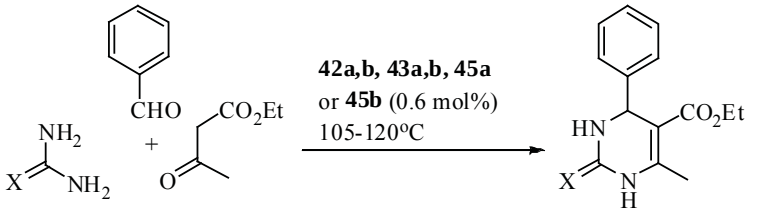
Additionally, we have looked for ionic liquids composed of imidazolium cations with "free" carboxyl group. The most logical protocol for the synthesis comprises the use the protected carboxyl group α-monohalogenated acetic acid followed by removal of protecting group [81].

***Scheme* 8**



The imidazolium bromide **44** has been prepared by reaction of the appropriate imidazole **41a** with *tert*-butyl bromoacetate, which was subsequently treated with catalytic amount of water solution of HBr to afford the 3-carboxymethyl-1-methyl-1*H*-imidazolium bromide **45a**. Oily salt **45b** was obtained by addition of one equivalent of 98% H_2SO_4 to product **45b**. We examined the Biginelli reaction of ethyl acetoacetate with benzaldehyde and urea (or thiourea) in the presence of **42a,b, 43a,b, 45a** or **45b** at elevated temperatures (scheme 9).

***Scheme* 9**



Both reactions gave rise to the corresponding 3,4-dihydropyrimidin-2-(1*H*)-ones(thiones) **46a,b** which were formed in variable yield. The reaction mechanism involves the condensation of urea with the aldehyde at high temperature to yield the corresponding iminium intermediate, which is then trapped by an aldol-type reaction with the enol derived from the ketoester. Such an effect may also be of significance in the action of "free" carboxy group of acetic acid and the enhancement of selectivity in the presence of imidazolium, as well as hydrogensulphate or bromine ions.

The yield, as well as the time of the reaction, was significantly improved by the nature of the catalyst (see Table 1).

Table 1

№ Compound	Catalyst	Time (min)	Yield (%)
46a	**42a,b**	90	56
	43a,b	60	61
	45a	30	72
	45b	10	67
46b	**42a,b**	90	55
	43a,b	80	67
	45a	60	65
	45b	10	65

It is worth noting, that our synthesized "ionic liquids" are more effective catalysts in comparison with other early published ones [82,83]. We also observed that all catalysts can be recovered during isolation of products **46a** and **46b**.

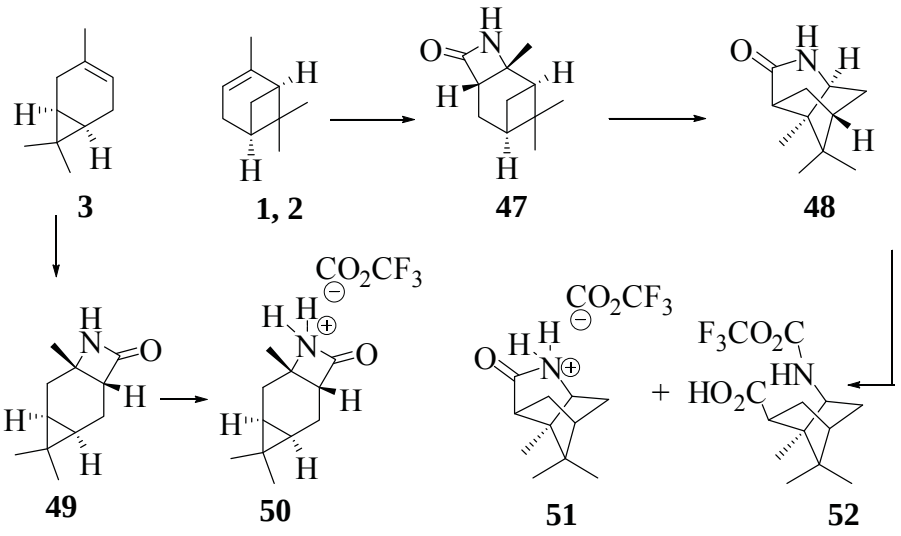
6.3. New Solvents/catalysts from renewable resources

In the current age of high dependency of chemical industries on the resources coming from oil and gas which are getting increasingly scarce, the focus is shifting towards renewable feedstock. In this prospective, natural monoterpenes produced by a wide variety of plants represent a group of inexpensive and abundant starting materials for fine chemical synthesis. It is noteworthy that used specific ionic liquids **38-40** are synthetic chemicals arising from petroleum. As this

resource continues to be consumed at a prodigious pace, and given the rather turbulent conditions present in some of the major oil-producing parts of the market, alternative materials, possible based on biorenewable monoterpenoids, are of considerable interest and great practical benefit.

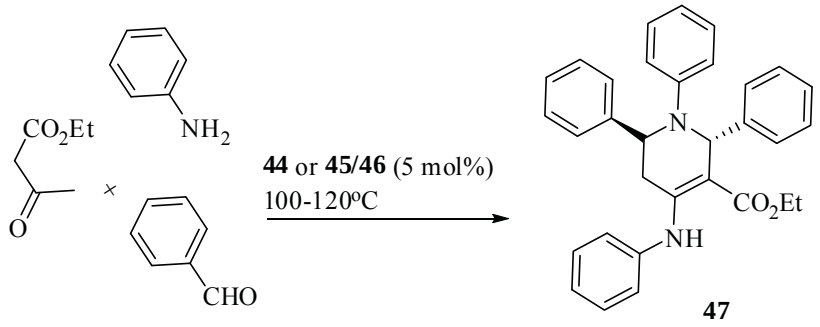
Shortly our interest in area of green solvents-catalysts from renewable resources prompted us to focus our attention on the optically active lactams **41-46** from (+)-3-carene **3** and α-pinenes **1** or **2** [84] (scheme 10).

Scheme 10



The stirring of lactams **48** or **49** in trifluoroacetic acid at room temperature gave the mixture of trifluoroacetates **51**/**52** or **50** in quantitative yields. We were proposed use of the lactam-functionalized ionic liquids **50** and **51**/**52** as catalysts to a synthesis of known [85] ethyl 1,2,6-triphenyl-4-(phenylamino)-1,2,5,6-tetrahydropyridine-3-carboxylate **53** *via* a solvent free one-pot multicomponent approach (see scheme 11).

Scheme 11



In all tested cases the product **53** was obtained with good yields. Slightly higher selectivity has been observed in the reaction between benzaldehyde, ethyl acetoacetate and aniline using mixture **51**/**52** as a catalyst in comparison with catalysts **50**. It should be to note that all catalysts can be recovered during isolation of product **53**.

7. Conclusions

In the light of the above-mentioned results, we have conclude that development of synthetic pathway suitable for obtaining multifunctional organic compounds both linear and cyclic structures using environmentally benign, inexpensive and renewable resources (ionic liquids, natural chiral hydrocarbons etc) should be continued. The overall objective of such approach is reinforcement of the sustainable chemistry in Moldova according to the contemporary needs of the European chemical and biophamaceutical sector and based on the highest European Research Area (ERA) standards.

8. Acknowledgements

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HYALURONIC ACID: OBTAINING, PROPERTIES AND APPLICATION¶

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Abstract. Properties and methods for obtaining hyaluronic acid and its derivatives from raw material of animal origin are reviewed. The importance and practical application of hyaluronic acid in various fields are discussed.

Keywords: hyaluronic acid, obtaining methods, natural sources.

1. Introduction

The production and usage of hyaluronic acid (HA) is growing worldwide. In Moldova, hyaluronic acid is less known as pharmaceutical and cosmetic component.

Currently there are many known products that contain HA, eg Juviderm, Restylane, Perlan, HYLAFORM, etc. HydraFill., and only Curiozina (Hungary) was recorded in our country.

Ever-increasing demands of hyaluronic acid for pharmaceutical, cosmetic and food industry imposes researches to study and investigate its properties; seek local sources of hyaluronic acid; develop cost-effective methods of production and domestic production of hyaluronic acid; search new chemical derivatives, medicinal and cosmetic preparations on its base.

HA is a precious natural biomaterial, biocompatible, safe and non-allergic. For these reasons HA is one of the most "agreeable" cosmetic ingredient. Emulsions based on it have a smooth soft consistency. Preparats are compatible with human skin and do not cause allergic reactions. Same properties have also sodium, potassium, calcium, zinc, copper and other salts of HA, which are successfully used in cosmetic intdustry. HA is an excellent moisturizer. A molecule of hyaluronic acid can hold up to 500 water molecules, forming a thin film on the skin, which creates an enhanced moisturizing effect. The molecular weight of the preparat increases its hydration effect.

One of the important properties of HA is that its macromolecules can be conjugated with bioactive compounds for pharmaceutical industry. So, hyaluronic acid can be a vehicle for topical medicines, which drives their absorption.

In our country we have enough cheap sources that can be used for hyaluronic acid preparation. The problem is to develop economically efficient methods of obtaining and purification of hyaluronic acid and its derivatives in quantities sufficient to be sold and used in the production of other bioactive compounds based on it.

The properties of hyaluronic acid

Hyaluronic acid (HA) was discovered about 70 years ago by scientists K. Meyer and J.W. Palmer, in vitreous of bovine eyes. It is a natural biopolymer, class proteoglycans, whose molecule is formed from the remains of β-D-glucuronic acid and N-acetyl-β-D-glucosamine linked by β-glucosidic bonds (1-3) - and β-(1-4), the long unbranched chains (Fig.1). From the primary structure of the macromolecule it can be seen that HA has a repetable disaccharide unit in which glucuronic acid is linked to glucosamine with β-glycosidic link between atoms C1 and C3, respectively [2-acetamido acid-2-deoxy-3-O-(β-D-glucopiranoziluronic)-D-glucose]. Basic structural unit is repeated in macromolecular chain is a very rigid chain segment with a length of 11.98 Å. Spatial distribution of anionic groups and their degree of ionization contribute to the conformation of the molecule due to mutual electrostatic rejection of negative duties along the polysaccharide backbone. Tertiary structure of HA in concentrated solutions, gels and solid, is influenced by the large number of hydrogen bonds inter- and intramolecular polar groups of abundant, such as: –OH, –COC–, >C=O, $–NH_2$, –COO-, etc.. Structure can be influenced also by hydrophobic interactions of protein fragments. The degree of polymerization and molecular weight varies by tissue type, the process of obtaining so.

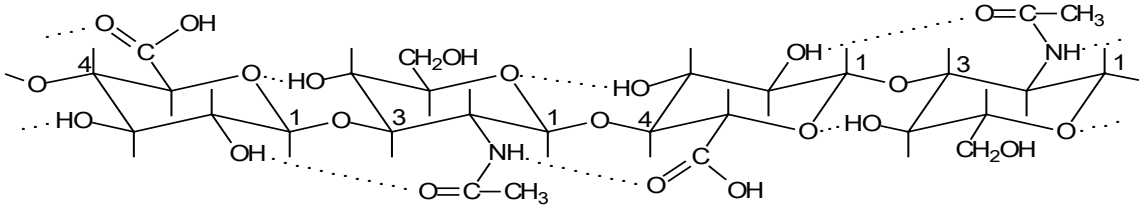

Fig. 1. Chemical structure of hyaluronic acid

The name of HA reflects its transparent nature (the Greek word for glass is hyalos) and the content of one of uronic acids (glucuronic acid).

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Hyaluronic acid is a compound present in the human body. It is one of the main constituents of the extracellular matrix of connective tissue and it is concentrated in synovial fluids, heart valves, eyes, cartilage.

In pure form HA is a white odorless powder soluble in water and insoluble in organic solvents with high viscosity. Specific rotation in aqueous solutions is –(70–80)°. HA is a polyelectrolyte which pK_{HA} = 3.21. A molecule of HA is able to retain about 200–500 molecules of H_2O. It has a high specific hydrodynamic volume. The molecule form depends on the pH and ionic strength of the solution. It was found that as the result of electrophoresis for an ionic force of 0.12 only 14% of ionized groups are effective, and for an ionic force of 0.02 already 80% of these groups are effective [1].

Chemical and spatial structure of the macromolecule, high molecular weight, high viscosity of the solution and hydrophilic qualities are important biological properties of HA. Due to these properties, HA has various biological functions and roles in animal bodies such as: participation in embryogenesis and morphogenesis processes, intercellular relationships and communication, mechanical strength of tissues, reducing friction in biomechanical systems, formation and proper function of cartilages, formation and maintenance of transparent structures of the eye, the permeability of biological membranes including vascular walls, water retention [2].

Biological researchs have shown no toxic properties, irritating, allergenic. A comparative study was conducted on biocompatibility and safety of HA obtained from three natural sources: umbilical cord, cockscomb and bovine vitreous [3].

2. The importance and usage areas of hyaluronic acid

Due to its properties, HA is one of the most attractive biomaterials for pharmaceutical and cosmetic industry [4].

Hyaluronic acid provides lubrication and hydration of connective tissues, including those of the skin. In the absence of lubrication and hydration of tissue and lose elasticity if skin leads to wrinkles and creases.

Due to its structure, HA form a film on the skin invisible, transparent and elastic, while acting in depth in the tissue, cartilages and joints. Its role is to keep the most important characteristics of young and healthy skin: suppleness, elasticity and tone. HA capacity to restore the interstitial matrix and liquid skin turgor alterations and wrinkles is successfully used for "rejuvenating" skin.

HA is a glycosaminoglycan, an essential component of extra cellular space, in which the collagen and elastin fibers are suspended. HA has an increased capacity to retain water, like a "sponge" that allows maintaining hydration, elasticity, skin firmness. Unfortunately, body's ability to produce hyaluronic acid decreases with age, and so the skin tissue becomes dehydrated, wrinkled and tonicity diminishes. HA injections are used more than 12 years in Europe and gradually replaced the injection of bovine collagen, which is sometimes complicated and required tests prior allergic reactions. Being a natural filler, it has a very low rate of allergic reactions - 0.06% vs. 3% for collagen.

HA is used to fill wrinkles and lip augmentation. Today the area of its application and usage in aesthetic medicine has expanded greatly, it is frequently used for volumization, nonsurgical facelift, correct dark circles and breast augmentation.

Also, HA is an important component of cartilage. In this role it dampens shocks in the joints, has lubrication effect and protects joints for chronic inflammation (eg arthritis). It is used to successfully heal stretching of ligaments. HA is a common ingredient in anti-osteoarthritis preparations and is frequently injected into joints, being a very effective treatment.

HA helps the immune system, acting as an antioxidant, it increases water retention in tissues, increases lubrication of heart valves, and serves as an adjunct to anti-infection treatment.

In the 1990s, hyaluronic acid began to be used in ophthalmology to treat corneal trauma.

Besides keeping joints lubricated, hyaluronic acid helps water retention in other tissues of the body, providing hydration of collagen and elastin. Interest in the use of HA as bioactive ingredient in skin care products came with the discovery that the volume of him in the skin decreases with age.

Clinically have been proven extraordinary efficiency of hyaluronic acid to fill wrinkles and smoothing. All clinical trials have shown that hyaluronic acid helps heal wounds faster and fading scars.

Currently procedures to inject cross-linked hyaluronic acid to fill wrinkles and lip augmentation, so-called fillers, are used [5]. For this purpose fits and are generally used products that are marketed as Amalian (Sweden), Perfectha (France) Remake (Italy), Aphrodite Gold (Germany) etc.

Unlike many other biologically active substances, HA shows all its valuable properties at very low concentrations (0.01 to 0.1%), which allows to create effective cosmetics, whose prices will fit producers as well as consumers. This refers to high molecular weight HA, which are now part of moisturizing creams, lipsticks and lip balms, cellulite cream, sunscreen lotions, anti-inflammatory lotion and wound healing [6, 7].

HA content in the human body is an important factor on which the physiological process of aging and immunity of the body depends upon. To strengthen the immune system and prevent various diseases including cancer various

"food additives" are prepared in which HA is used as an ingredient or as compound [8, 9]. HA is called "star" of cosmetology, "hope" of rejuvenation and "pledge" of beauty. Benefits of Hyaluronic Acid supplementation – Cosmetic effect: Skin Hydration from the inside out, correcting in this way wrinkles. – Anti-arthritic: lubricate joints, especially knees and hips ones. – Rejuvenate, anti-aging effect: for men and women between 30 and 40 who are beginning to see signs of aging mirror. The effects are felt quickly after first supplementation with hyaluronic acid.

3. Obtaining hyaluronic acid

In world literature there is more information on specialized methods of obtaining and purification of hyaluronic acid.

Hyaluronic acid is contained in animal bodies tissues, extracellular matrix and cytoskeleton. Higher concentration of contained HA can be found in: synovial fluid, umbilical cord, bird crest, the vitreous of the eye, creating, skin, heart valves, cartilage of sharks and whales [10, 11]. In all these tissues HA is accompanied by other mucopolysaccharides, which also form intermolecular compounds with collagen and other proteins. Main and most difficult problem in the process of obtaining HA is to remove as much as posible secondary components, including proteins, without affecting molecular weight of biopolymers.

For the first time HA was obtained and investigated in 1934 by K. Meyer and J. W. Palmer [12, 13], but increased interest in obtaining HA occurred after the World War II with the problem of eye vitreous substitutes search. In the monograph [14] are treated different methods of obtaining fetal umbilical cord HA based on the use of sodium acetate solution, dilute and concentrated solution of phenol, trichloroacetic acid, sodium chloride, pyridine, ferments etc. The preparation obtained was called "recovery factor", and later as a medicine it was called "Regenerator".

3.1. Isolation of hyaluronic acid from umbilical cords

One of the earliest methods of obtaining HA is described by Asatiani V.S. In his book [15] and consists of extraction of HA from umbilical cords. Umbilical cords are blood washed with water, then are dried and degrased with acetone. After grinding, HA extraction is done with water and sedimentation with acetone. Obtaining yield of this process is 6% HA and relative viscosity of 1% solution is 3.

The invention [16] Vunder P.A. and Muraşev A.N. improves the previous method. After grinding, washing, degreasing and dehydration, resulted umbilical cords are subject of extraction using boiling water for 5-15 min. For 2 g of dry weight, 200 cm^3 of water are added. After cooling, the extract is centrifuged 15 min at a speed of 6000 rpm. HA is settled in the extract obtained with acetone in 1:4 volumetric ratio. 0.28 g sediment is obtained, containing HA and protein substances. The last are determined by the Lowry method. HA mass without protein is 10.2%. Relative viscosity of the solution of 0.25% is 3.

In the patent [17] authors Lutan V. and Bezdrîgin M. from State University of Medicine and Pharmacy "Nicolae Testemitanu" of the Republic of Moldova describe the method of obtaining of biocompatible HA from umbilical cords of fetuses for the medical use. Biological material is collected and preserved in 96% ethanol. After crushing, drying and washing procedures were performed at a temperature of 0–4°C, authors extracted HA in saline (0.89% NaCl solution) to cold in three innings, then added to the obtained extract crystalline NaCl until a concentration of 25% is reached and ethanol in a proportion of 1:3 for HA sedimentation. HA obtained is recristalizat of 2 to 3 times of normal saline. Removing of the proteins is made using $CHCl_3$ in proportion of 1:2 for 3–4 rounds until the solution remains transparent. After the last procedure of removing proteins is done, HA settles with 96% ethanol (1:3). Product yield is not indicated. Protein concentration determined by Lowry method is 0.2 g/l, representing 5% of the total mass dissolved. Thus the authors consider that the content of HA is 95% (m/m). Relative viscosity of 0.2% HA solution is 5.0. Absorption of 0.5% solution of HA at 257 nm (absorption maximum of nucleotides) is equal to 0.712, and at 280 nm (absorption maximum of protein) is equal to 0.622. Optical density spectra measured HA solution in 400–700 nm shows a maximum of 520–540 nm, which is specific for hyaluronic acid. The authors have tested obtained biological product and demonstrated lack of toxicity, allergenic, hepatotoxic and cardiotoxic action, irritant action, action on the mineral metabolism.

3.2. Isolation of hyaluronic acid from bovine vitreous

Romanian patent [18] refers to a process of obtaining the HA and its sodium and potassium salts for cosmetic use from bovine vitreous humor. HA extraction is carried out with sodium para-xilensulfonat 10% weight of the material to be extracted. At a temperature of 60 °C, under vigorous stirring, until a homogeneous material is obtained (6–8 hours). After the material is cooled to 0–4 °C and with continuous stirring, it is treated with HCl solution to pH 3–3.5 or with solution of 1 mol/l NaOH, KOH properly. After 24 hours material is filtered on bed hyflosupercel. If the filtrate obtained is colored, it treated with reactive charcoal and filtered again. Perfectly clear solution is cooled and treated with a volume of isopropyl alcohol. Settled HA is kept in cold for additional 24 hours, then isolated by centrifugation. It is then washed with acetone, isopropyl alcohol and sterile, then dried over calcium chloride. Product yield from the initial volume of raw material was 0.04% (w/v). The authors state that the protein content in the product is 0.1–2%. Treatment of merial with strong acid or base during extraction period changes strongly the environment. There are clear consequences.

3.3. Isolation of hyaluronic acid in the crest of birds and other connective tissues

Balazs E.A. in 1979 reports about obtaining ultrapure high molecular weight HA from connective tissue of animal origin such as comb, umbilical cord [19]. The proposed method consists of extraction and purification of sodium hyaluronate in several consecutive steps, which takes longer and requires a large amount of work. Freshly collected raw material is washed thoroughly with water to remove blood completely, cut into small pieces and frozen at –20–40°C. While meterial os frozen it is finely grined and dehydrated in few rounds (no more than 3–4) with 95% ethanol containing traces of chloroform (bacterial agent). Each wash-dehydration procedure takes 24 hours. Mass obtained is extracted twice with a mixture of water-chloroform (20:1, v/v). An extraction takes 24 hours and is performed at a temperature of 4–25°C. On 2.5 kg of processed raw materials are added for imbibition 10 l of water and 0.5 l of $CHCl_3$, then for each extraction - at 15.75 l mixture of chloroform-water. Prepared HA is separated from tissues using nylon filters. To remove proteins, crystalline NaCl is added to obtain a 10% solution. The author states that removal of proteins from extracted HA must be done with great caution as it can easily have destructive influence on macromolecule due to various external factors (ions of iron, oxygen and other oxidants that can penetrate the solution). Conditions for separation of proteins in solution were determined empirically and HA are considered optimal by the author. To the obtained solution chloroform is added in 1:1 ratio. Shaking of the mixture is made in a glass bowl for 3–5 hours with a Teflon stirrer (at 120–300 rpm) or vibration. Acid solution is maintained at pH 4–5 by adding diluted HCl and an equal volume of $CHCl_3$.

After 3–4 hours of stirring, the organic phase ($CHCl_3$) is removed with protein sediments at the surface layer of phase separation. This process is repeated several times until the chloroform layer remains clean after shaking. If the obtained extract contains an impressive amount of protein then author proposes to use enzymes. After the second treatment with $CHCl_3$ of the extract, to the aqueous phase is added 50–100 g DNase enzymes and RNase, continuously stir a further 24 hours. After 24 hours bring the pH to 6–7 and 50–100 mg Pronase added. The solution still shake another 48 hours. In this way most of the proteins in the mixture are removed. If at the previous stage the extract was processed only with $CHCl_3$ then pH is adjusted with 0.1 N NaOH solution to pH 6–7. To the extract of HA with pH 6–7 an equal volume of chloroform is added and is shaked at 20–40 °C for five days. The author considers that full distortion of proteins and other components that can cause toxicity and inflammation occurs in five days. After removing $CHCl_3$ layer in the aqueous phase remains only sodium hyaluronate. The obtained solution is centrifuged for 4 hours, filtered through sterile teflon filter (pore diameter 0.2 μm). To the obtained filtered solution 3 volumes of ethanol are added. HA settles and separates, then redissolves in 1.5 l of doubly distilled water containing 0.1 mol NaCl and settles again. This procedure is repeated 5 times. In the second redissolvation a 1% cetylpyridinium chloride is added in solution. After last redissolvation HA settles with 3 volumes of sterilized acetone then washed several times with sterilized acetone and obtained product is dried in vacuum. HA yield is 0.08%. Protein content in the product - 0.4%. Molecular weight determined - 1586000 D. absorbance of 1% aqueous solution where HA length 257 nm is 0.243, and at 280 nm – 0.198.

His method allowed obtaining pharmaceutical preparation of HA with the trade name “Hea Lon” (Farmacia, Sweden), which up to date remains one of the best, but also the most expensive viscoprotector in ophthalmology surgery. A dose of HA required to perform a surgical operation is 0.4 cm^3 of 1% solution.

According to the method presented in reference [20] crests of cocks are extracted twice with aqueous solution of 5–25% (v/v) butyl or propyl alcohol and their isomers. Extraction takes about 32 hours. To the obtained extract crystalline NaCl was added to separate phases. HA is separated by sedimentation from aqueous phase with ethanol. Protein is less than 1%, humidity - 15%. Ultraviolet absorption in the solution of 1% at a wavelength 257 nm is less than 3.0, and at 280 nm - less than 2. pH aqueous solution of HA obtained is 5.5 to 7.5. The degree of extraction of HA is 50%, and efficiency is not indicated.

In reference [21] Laurent T.C. mentions that crests of cocks might contain up to 0.75% (m/m) HA. In the vitreous of human eyes there are approximately 0.02% (w/v) HA.

According to the invention [22] cockscombs are extracted with water fowls or solution 1–15% NaCl at a temperature of 80°C, HA yield is 2%, 9% protein content. Extraction duration is approximately 24 hours.

According to the publication [23] cockscombs are extracted with water for 1–2 min at a temperature of 90–100°C. The mixture obtained is cooled for 2–3 hours to a temperature of 4–6°C. Fat is removed, it is filtered and remnant is separated from the crests. To the obtained filtrate activated carbon is added at a rate of 1–2% of initial mass of raw material and is stirred 1–2 hours. The mixture is filtered. To the obtained filtrate penicillin is added at a rate of 1–2% of the initial mass of raw material and maintain 18 to 24 hours at 20–22°C, then dry. Yield is 3.8 to 4.0% from initial mass of raw material. Relative viscosity of 0.1% aqueous solution is 4.0, the protein content - 0.4–0.5%.

Further Stecolnicov L. I. with his team proposes a new method to extract HA [24]. Unlike his previous method [23] authors performed the HA extraction at room temperature 20–22°C, with water, in three stages, at the ratio of 1:(4–6 v) creasts : H_2O for 2–4 hours. Extracted HA is settled then with trichloroacetic acid in an amount of 1–2% to the volume of obtained extract. HA sediment is rising to the surface of the aqueous phase and protein settles to the bottom. HA is

washed 3 times with acetone and diethyl ether 2–4 l in the report to 1 kg of raw material. Sediment is dried in air and then redissolved in water (2 v for 1 kg of initial weight), filter and sublime. Yield is 5%, protein content is 0.05% and the relative viscosity of 0.1% solution at 20°C is 6.0.

The authors of the method [25] excludes alcohol for HA sedimentation from extracts. They performed extraction with 3 to 3.5 volumes of water acidulated with acetic acid to pH 3–4 and temperature of 90–100°C for 40-60 min. To the obtained extracts a 1.0 and 1.5% activated carbon and dietilaminoetilcellulose is added, corespondingly for 1–2 hours at 60–80°C. After removing of proteins the solution is filtered at a temperature of 30–40°C with filter paper, filter glass containing boron acetilcellulose sterile membranes and polyvinyl chloride. The obtained solution is dried by sublimation under aseptic conditions. HA yield obtained is 0.09 to 0.12%. The contents of protein substances does not exceed 0.1% in HA and ovalbumin does not exceed 0.001%. The content of total protein in preparations of HA was determined by Kjeldahl classical method and albumin content was determined with the solid phase immunoferment screening test with sensitivity of 0.5 ng/cm^3. Relative viscosity of HA solution 0.1% at 20°C is 12–14.

This method is powerful to obtain ultrapure HA, but it also includes some minuses like: using special glass filters from acetilceluloză and polyvinyl chloride. The method is long and difficult.

Another team of russian authors together with Samoilenco I.I. propose the following method for obtaining HA from umbilical cords and cocks comb [26]. The raw material from which the blood was removed is washed with cold water (10–15°C), finely grined at the meat grinders and freeze until –70°C. Add two parts of water to one part of frozen mass and combination is heated for 15–25 min at a temperature of 95–100°C, then is filtered through gauze layers or centrifuge. Remnant from the filter is again frozen at a temperature of –70°C, and the filtrate collected. To the frozen mass water is added again at a ratio of 1:1.5 and heat for 15–25 min at a temperature of 95–100°C. The mixture is filtered through gauze, the filtrate is collected, and the rest of the filter is frozen again and again repeated three times, each time using water at the ratio of 1:1 against the remnant mass. Filtrates are joined and HA is removed at extract acidification with acetic acid. HA yield is 5%. Protein content was determined by Lowry and it is 4%, molecular mass is approximately equal to 1 million D and it was calculated using the characteristic viscosity. Product homogeneity was confirmed using Tindal effect. For better removal of the protein, the product was redissolved in dilute NaOH solution and ultrafiltered. HA solution was lyophilised. Summary yield decreases very small (4.5%) and protein content decreases by 10 times.

In the method proposed in [27], after washing and degreasing with alcohol (1:2), peaks are crumbling and are subject to processing by ultrasound at a frequency of 16–20 kHz for 5–10 min. HA extraction is performed with water at a temperature of 45–50°C for 20–25 min. Aqueous phase separation is achieved by vacuum filtration and sedimentation HA is obtained with 95% ethanol in volume ratio of 1:3. Aqueous solution is filtered and the filtrate is evaporated over the oxide of phosphorus (V) in a vacuum. Yield is 11%. The mass of protein is 0.4%.

In [28] is presented a scheme for a process of obtaining the formaceutical form of the aqueous solution of HA with high molecular weight, for a certain concentration, sterilized.

This ultrafine purification scheme of HA is powerful, has many sophisticated stages, automated, and uses expensive technologies. In the local conditions at the present moment it is unlikely that it can be done.

In the invention [29] authors report about obtaining and purification of extra pure fractions of HA with applications in ophthalmic surgery. The proposed method is laborious and expensive. It includes the use of large amounts of solvents (ethanol, acetone), chelates use and their removal. The extraction and purification process using molecular sieves DOWEX M-15, Celita, or N-metilpirolidon or dimetilsulfoxid, methylene chloride, sodium chloride, cetilpiridină, NaBr, NaCl. Product yield is 0.6%. Proteins are removed by fermentation using for this purpose enzymes (papain, pepsin, trypsin or pronaza). Determination of protein in the final product was done by Lowry method and is - 0.2%. Were determined following parameters of the obtained product: static viscosity ranges between 14.5 to 21 D/g as measured by Viscometers Ubellodi at 25°C in solution of 0.15 mol/l NaCl, pH 7 and corresponding mass molecular medium (0.75–1.23)•10^6 D, 1% aqueous solution absorbance at a wavelength HA 257 nm and 280 nm does not exceed 1.0.

All these methods are of great scientific, but technological methods are still inconvenient because it requires large amounts of reagents, further removal of toxic reagents is difficult, yield is comparatively small.

So, the diversity of methods for obtaining hyaluronic acid in the laboratory is high, but there is no optimal method, reasonable technologies for extraction and purification of hyaluronic acid in industry.

4. Procedure for obtaining hyaluronic acid and its derivatives for practical use in the pharmaceutical industry, food and cosmetics

Properties of hyaluronic acid are determined by molecular mass, extraction mode, traces of proteins and other proteoglycans that can contaminate preparations obtained.

In research conducted hyaluronic acid was obtained from several natural sources of raw material: crest of hens (CH), cockscomb (CC), bovine vitreous body (BVB), bovine umbilical cord (BUC) [30–32]. Obtain preparations of hyaluronic acid was performed according to the scheme of Fig. 2.

In the proposed method the degreasing of raw material is carried out fully before extraction of HA, along with dehydration in Soxhlet apparatus with acetone for 2 hours. HA extraction is performed with NaCl solution only at cold (4–10 °C). From the obtained extract in cold, HA settles with 96% ethanol or acetone. The product is redissolved and proteins are remove by heating-cooling at pH 7 and pH 5 to 5.5 in $CHCl_3$ processing. From the obtained solution is settled and isolated the sodium hyaluronate, HA respectively, with corresponding efficiency.

Characteristics of obtained preparations are vary depending on source of raw materials and obtaining method. For obtained samples was determined the mass part (ω) of hyaluronic acid in the raw material which was subjected to extraction, the protein mass (Lowry method) [33], relative viscosity (Ostwald method) [34] (Tab. 1, fig. 3).

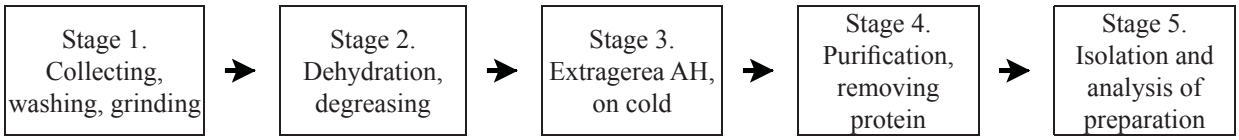

Fig. 2. Process scheme for obtaining hyaluronic acid

Table 1

Feature of HA preparations obtained from various sources of raw material

	CH	CC	BVB	BUC
ω (%)	0,5 - 0,7	1,1 - 1,5	0,1 - 0,2	1,4 - 1,8
Proteins (%)	3 - 5	1 - 3	1 - 3	2 - 4
Relative viscosity (η)	11 - 12	12 - 13	5 - 7	12 - 13

Identification of HA in the obtained preparations was made based on the infrared absorption spectra. IR spectra were recorded in the 4000–400 cm^{-1} for solid preparations, in KBr pills. (Fig. 2).

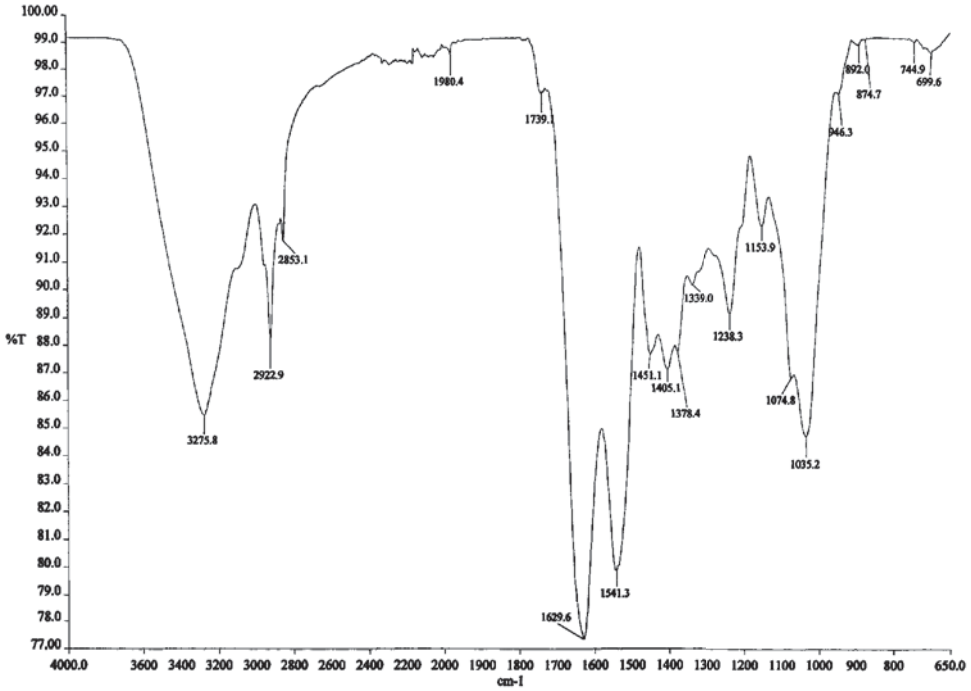

Fig. 3. IR spectrum of hyaluronic acid

In the domain 3400–3300 cm^{-1} (max 3275.8 cm^{-1}) is obvious the large absorption band of symmetrical and asymmetrical valence oscillations of OH and NH groups linked by hydrogen bonds intra-and intermolecular. Absorption bands in the domain 3000–2800 cm^{-1} (max 2922.9 and 2853.1 cm^{-1}) are characteristic for valence oscillations of CH bonds in the macromolecule chain. The intensive absorption band at 1629.6 cm^{-1} is characteristic of carbonyls of valence oscillations of carboxyl group. Absorption bands 1405.1 and 1378.4 cm^{-1} are characteristic for oscillations of valence interaction of carbonyl group and the hydroxyl deformation oscillation plane of the carboxyl group. Are well defined bands with maximum absorption at 1541.3 and 1451.1 cm^{-1} (amide I, amide II) characteristic oscillations of the carbonyl group and N-acetyl-hexozamină CN chemical bonds, CCO and NCC. The absorption at 1238.3 cm^{-1} is characteristic

of amides and is due to the valence oscillations of CN and CO bond. Summary absorption band 1100–1050 cm^{-1} with maximum 1035.2 cm^{-1} is characteristic for primary and secondary hydroxyl groups oscillations. The spectrum is well defined band 1153.9 cm^{-1} characteristic for the valence oscillations of COC bonds [35].

Taking into consideration the results of research and availability of raw material sources we have agreed to obtain hyaluronic acid from the crest of birds (hens and cocks) collected from local poultry companies [36–38].

Research result is the issuance process for obtaining HA, increasing the extraction, cost reduction, improving the quality of the final product suitable for use in medical, cosmetic and food.

Obtaining of HA was carried out in several stages as illustrated in Fig. 4.

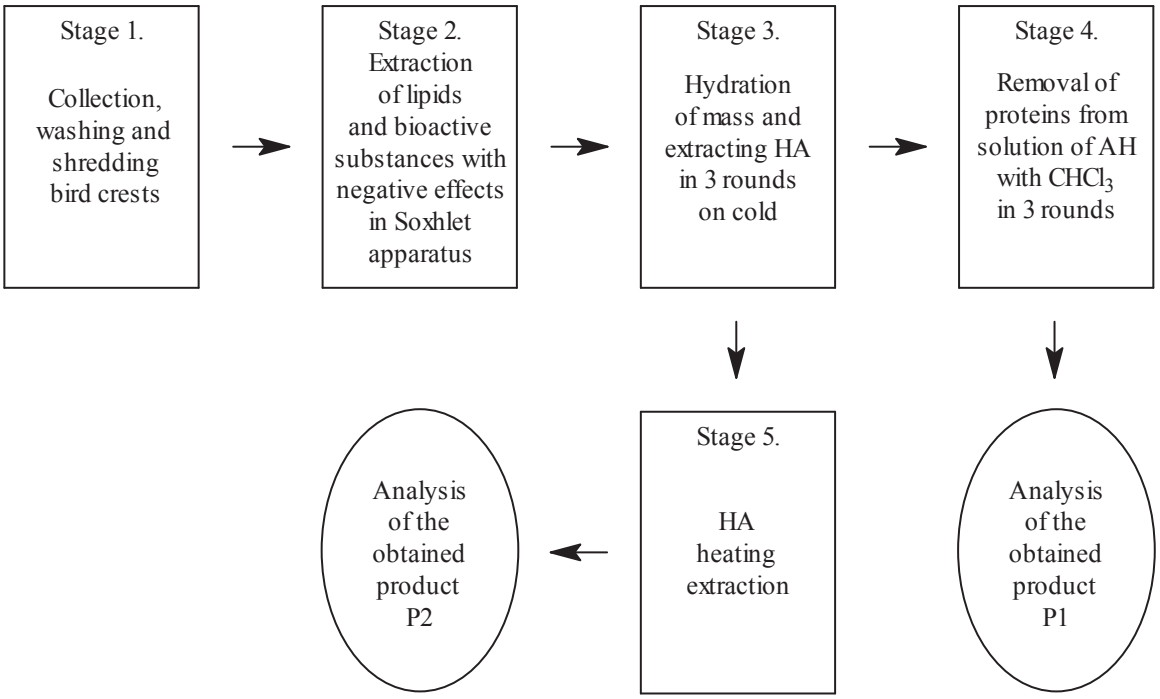

Fig. 4. Scheme process of obtaining and purification of hyaluronic acid and sodium hyaluronate

For this purpose 160 g of crests were collected from hens and cocks, immediately after healthy birds were slaughtered at the company Avicola "Roso" s. Floreni, Chisinau, were washing blood with cold water (10–15°C) in 2–3 innings for 5–6 hours and then were chopped finely, so that fragment sizes were about 1–3 mm. Grinding was performed in Moulinex Genius 2000 food processor. Shredded mass was added to acetone or 96% ethanol containing 1% $CHCl_3$ in the ratio of 1:3 and left in refrigerator for 6–24 hours at 0–4°C (stage 1). As a result, the raw material was partially dehydrated and volume decreased. The solvent was distilled and separated, and partially dehydrated mass was introduced in Soxhlet apparatus and was extracted with acetone (stage 2). As the result further dehydration took place and concomitant extraction of bioactive substances soluble in acetone (lipids, phospholipids, lipoproteins, glicolipide, glicolipoproteine, nucleotides, enzymes, etc.) which can then contaminate the final product. After removal of all harmful bioactive substances the mass was removed from the machine and to dried in ventilation niche untill smell of acetone disappeared. The mass of the dried product is 37 g.

As for HA extragent we used 1 M NaCl aqueous solution. The obtained dry mass (37 g) was added to 750 cm^3 1 M NaCl solution and 2–3 drops of $CHCl_3$ as bacteriological agent. The mixture was left in the refrigerator for 24 hours at 4–10°C. The mixture was filtered. The filtrate was collected and to the already hydrated mass of crests, a new portion of extragent and 2–3 drops of $CHCl_3$ were added. Same procedure was repeated three times (stage 3). Remnant after extraction was frozen for further processing. The three extract portions were combined together (a total of 2 liters), were filtered through nylon mesh, centrifuged at 7000 rpm for 20 min to remove insoluble impurities. The solution was cooled to 4°C, pH was set at 5 to 5.5 with 0.1 M HCl solution and 96% cold ethanol in the ratio of 1:3 was added . The formed sediment was separated and dissolved in 100 cm^3 of 1 M NaCl solution, was heated 5–10 min until the temperature of 70–80°C and then rapidly cooled to 18–20°C, pH was adjusted to 5–5.5 with 0.1 M HCl solution and 1:1 CHCl3 was added, was stirred gently with glass stirrer in one direction only, in the separating funnel. After 24 hours the upper aqueous phase was separated and was collected, the organic phase ($CHCl_3$) and proteins were sedimented at interphase and removed and the solvent was distilled. The procedure was repeated 3–4 times until the aqueous solution became transparent or slightly opalescent (stage 4). After the last procedure of removal of proteins pH was adjusted to 7.5–8.0

with dilute NaOH and hyaluronic acid sedimented from the aqueous phase with cold 96% ethanol in the ratio of 1:3 in the form of salt sodium (P1). It was obtained 0.785 g of product. Obtined product P1 yield is 0.5%. The protein mass determined by Lowry method in the final product does not exceed 1%. IR spectrum confirms the authenticity of the product obtained HA (Fig. 4).

The 1% HA aqueous solution obtained by the proposed method is a viscous liquid, eculent hard, transparent or slightly opalescent, colorless, odorless. Relative viscosity of the solution of 0.1% HA Ostwald Viscometers measured at a temperature of 18°C was equal to 12.

1% solution absorbance measured at 257 nm HA (absorption maximum nucleotide) and 280 nm (absorption maximum of protein) – is less than 0.1 a (l = 10 mm).

Obtained hyaluronic acid is kept in 96% ethanol, in closed bottles protected from light at 0–4°C temperature.

Remnant from step 3 was thawed and added to it 700 cm^3 1M NaCl solution and 2–3 drops of $CHCl_3$. The mixture obtained was heated on a water bath at a temperature of 50–60°C for 3 hours (stage 5). Then the mixture was filtered through sterilized nylon mesh. The filtrate was centrifuged at 7000 rot / min for 30 min.

It is viscous and was gelatinised in the refrigerator. To the obtained extract it was added acetone in the ratio of 1:3 (v/v). Sedimented product (P2) was separated, washed with acetone and dried. The analysis of obtained product P2 was carried using the same parameters as P1 product analysis. The results are presented in Table 2. The product P2 was carried out qualitative and quantitative analysis of protein content in amino acid analyzer. For analysis 58 mg of product were dissolved in 10 ml P2. It was found that the product P2 contains 64.7% protein with composition shown in Table 3. The 1% solution of the product P2 presents ultraviolet absorption at a wavelength 266.5 nm equal to 0.609 a. (l = 10 mm).

Hyaluronic acid was obtained from hens and cockscomb – shaped preparations:

P1 – sodium hyaluronate, P2 – hyaluronic acid-protein complex.

Table 2

Feature of obtained products

Nr/o	Obtained product	Product yield (%)	The protein mass (%)	Relative viscosity of the solution (18 °C)	Kinematic viscosity (St)
1	P1	0,5	1	12	0,13
2	P2	12	64,7	13	0,14

Table 3

Qualitative and quantitative composition of amino acids in the product P2

	μM/100мg	мg/100мg	mM/g	azot, %	azot în мg/100мg	azot în мg/0,1 мg
Aspartic acid *	24,9318	3,2935	0,2493	10,52	0,34904	0,3464
Threonine *	11,7768	1,4026	0,1178	11,76	0,16488	0,1649
Serine *	15,9810	1,6796	0,1598	13,33	0,22373	0,2238
Glutamic acid *	52,2660	7,6883	0,5227	9,52	0,73172	0,7319
Proline	13,0443	1,5014	0,1304	12,17	0,18262	0,1827
Glycine	261,0336	19,5958	2,6103	18,66	3,65447	3,6566
Alanine*	53,1720	4,7371	0,5317	15,72	0,74441	0,7447
Valine*	17,7482	2,0783	0,1775	11,96	0,24848	0,2486
Cysteine *	46,7801	5,6206	0,4678	11,66	1,30984	0,6554
Methionine	26,2385	3,9148	0,2624	9,39	0,36734	0,3676
Isoleucine	12,5406	1,6453	0,1254	10,68	0,17557	0,1757
Leucine	19,3458	2,5382	0,1935	10,68	0,27084	0,2711
Tyrosine	15,2280	2,7593	0,1523	7,73	0,21319	0,2133
Phenylalanine	11,2201	1,8536	0,1122	8,48	0,15708	0,1572
Lysine	15,0103	2,1944	0,1501	19,16	0,42029	0,4204
Histidine	7,6302	1,1839	0,0763	27,08	0,32047	0,3206
Tryptophan *	3,0765	0,6282	0,0308	13,72	0,08614	0,0862
Arginine	1,7756	0,3093	0,0178	32,16	0,09943	0,0995
Ammonia	7,5397	0,1284	0,0754	82,24	0,10556	0,1056
Σ aminoacids	608,7993	64,6242	6,0880		9,71955	9,1722

The resulting products are used in further studies.

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BIOMIMETIC STRATEGIES IN ORGANIC SYNTHESIS. TERPENES

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Abstract. The current paper represents an outline of the selected contributions to the biomimetic procedures and approaches for the synthesis of terpenes with complex structure and diverse functionalisation pattern. These include homologation strategies, cyclisations, rearrangements, as well as biomimetic remote functionalisations. Diverse groups of terpenes have been made accessible by the reported procedures: seco-eudesmanes, sacculatanes, cheilanthanes, scalaranes, rearranged labdanes and austrodoranes. Most of the presented synthetic methods represent relevant preparative value, due to the simplicity and reduced number of steps.

Keywords: terpenoids, biomimetic synthesis, cyclisation, rearrangement, oligomerisation, functionalisation.

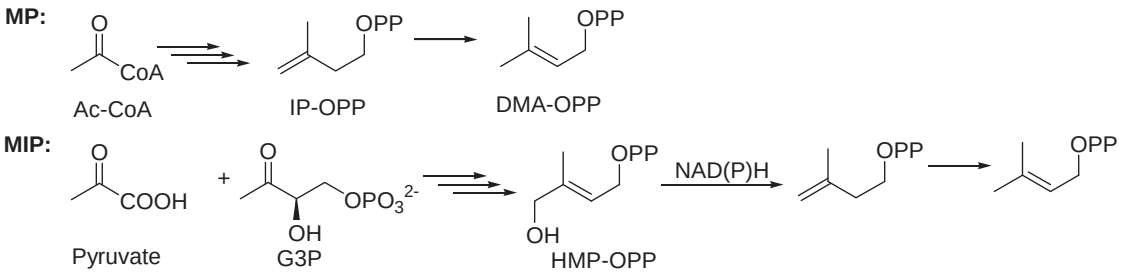
Introduction

Organic chemistry represents one of the basic areas of modern science. When we make an outline of the reasons which define the special place of this area of human knowledge, its two general aspects come into mind: cognitive and creative power. Both these aspects are quite clear. First of all we strive in our endeavours to explain all the processes which surround us. Chemists see the life through the prism of chemical reactions: reactions that occur in our own bodies, reactions that occur in the invisible micro-world, reactions that permanently affect our environment and finally reactions that take place in the outer space. This cognitive function inevitably leads to our desire to create new matter, to produce materials and substances that we might consider useful for our lives. And the pivotal role of organic synthesis in this process is generally acknowledged. It is due to the practically infinite range of compounds which can be built on the basis of organic carbon chains. But what is the strategy which leads the chemists into this creative Odisea? Again, two distinct approaches are valid: we create something that we think it might be usefull and we create what we see in the nature. The second approach in fact reflects one of the basic psychological features of humans as social beings: we build our lives by mimicking our environment. For organic chemists this means identifying natural targets and their reproduction. Following the same strategy, we try to hypothesise the *in vivo* biochemical pathways leading to the targets and then to reproduce similar synthetic sequences *in vitro*. This is called a biomimetic strategy.

A careful examination of the chemicals that have firmly entered our lives will make us conclude that most of them are mimics of natural matter: from low molecular wait bio-regulators (pheromones, drugs, agrochemicals) to polymers and supramolecular aggregates (textiles, rubber and plastics). A comprehensive study of the examples illustrating this reality would certainly do not fit the range of a journal paper. The purpose of the current report is to provide just a single example of biomimetic approach in developing synthetic strategies. This is the example of terpenes – a very large and fascinating group of natural compounds.

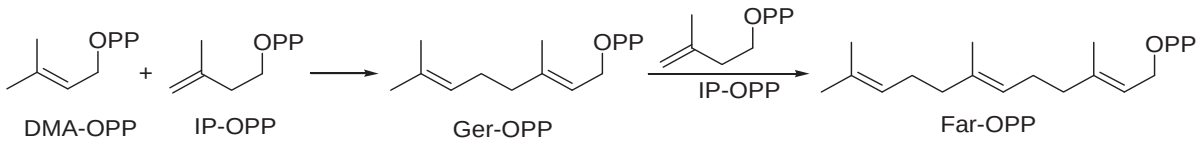
Terpenes. Structure and biosynthesis

Terpenes represent a large family of natural products with an impressive diversity of carbon skeletons and functionalisation pattern. It is believed that this diversity is due to the latter steps of terpene biosynthesis [1]. This opinion has been confirmed by numerous biosynthetic studies which proved that common precursors of all terpenic families are only several open chain oligomers of dimethylalylpyrophosphate (DMA-OPP): geranylpyrophosphate, farnesylpyrophosphate, geranylgeranylpyrophosphate and some other superior oligomers. The biosynthetic pathways to these basic building blocks include two steps. The first one is the synthesis of DMA-OPP either by mevalonate (MP) or methylerythritolphosphate (MIP) pathways (Scheme 1), which along with its precursor isopentenylpyrophosphate (IP-OPP) represent the elementary C5 terpene units.



Scheme 1. Early stage terpene biosynthesis. Mevalonate (MP) and mevalonate-independent (MIP) pathways

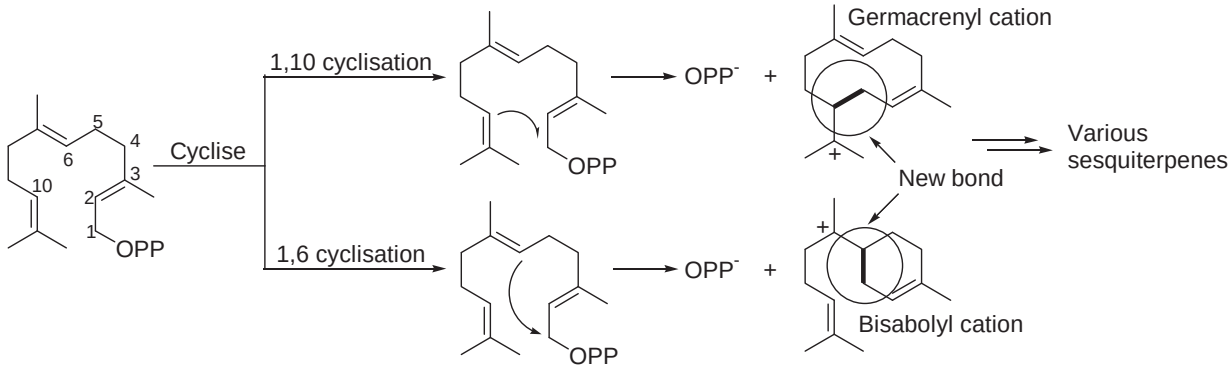
The second step of biosynthesis is the coupling of C5 fragments leading to the corresponding open chain mono-, sesqui-, di- and polyisoprenoids. DMA-OPP and IP-OPP combine in an oligomerisation process to form C10, C15, C20 and higher aliphatic chains lake geraniol (C10), farnesol (C15), geranylgeraniol (C20) and higher polyprenols.



Scheme 2. Oligomerisation of isoprene units

Both these steps are similar in all producing cells and living organisms and they bring no structural diversity. On the contrary, the next two steps of terpene biosynthesis play the crucial role in the tremendous expansion of possible terpene structures. These steps are cyclisations/isomerisations and selective functionalisations/degradations mainly by oxygenated functionalities. The enzymes responsible for these transformations are terpene cyclises and oxidases. From the mechanistic point of view, the terpene cyclases represent an interesting subject, since their action is accompanied by a huge variety of other transformations, which besides cyclisations may include hydride shifts, Wagner-Meerwein and other skeleton rearrangements. Carbonium ion intermediates are the species that ensures reaction sequences leading to carbon skeleton diversity. To date, there are two basic mechanisms of terpene cyclisations.

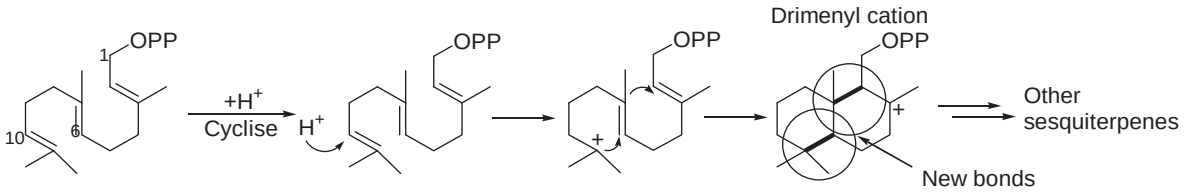
The first mechanism is given by the ability of the double bond to act as a nucleophile and to interact in a SN2 fashion with the α-terminus of the chain, leading to elimination of the –OPP group and formation of a new C-C bond.



Scheme 3. Cyclisation of terpenes by a nucleophilic substitution of the –OPP group

The Scheme 3 presents two different paths for the cyclisation of farnesyl-OPP, leading either to germacrenylor bisabolyl- cations. Both these paths occur according to this mechanism, involving either of the double bonds present in the chain.

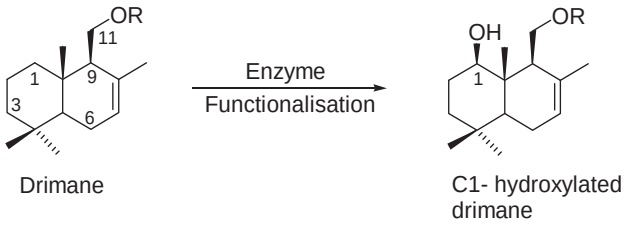
The second mechanism includes a cascade of reactions initiated by a selective protonation of the double bond, followed by a electrophilic attack of the formed carbonium ions to the other double bonds of the aliphatic chain (Scheme 4). Both mechanisms involve formation of intermediate carbonium ions, which can stabilize either by proton elimination, skeletal rearrangements or addition of other nucleophiles.



Scheme 4. Electrophilic ciclisation of terpenes by selective protonation

And the third important process which defines the structural diversity is based on the selective functionalisation of terpenes by different enzymes, which introduce additional heteroatoms, basically by an oxidative process (Scheme 5). Intercalation of cyclisations, isomerisations and functionalisations provides practically infinite opportunities for structural modification of the terpenes in living organisms.

All the enumerated biosynthetic processes have inspired organic chemists to devise synthetic methods which selectively transform the substrates exactly in the same way as enzymes do, that is to mimic the biosynthesis.



Scheme 5. Enzymatic terpene functionalisation

Surprisingly, but for some instances chemical processes have been devised long before the identification of corresponding enzymes. Baiyer-Williger oxidation of ketones and Diels-Alder cycloadditions are relevant examples. Table 1 presents a short outline of the most known processes which are successfully applied in biomimetic synthetic strategies.

Table 1

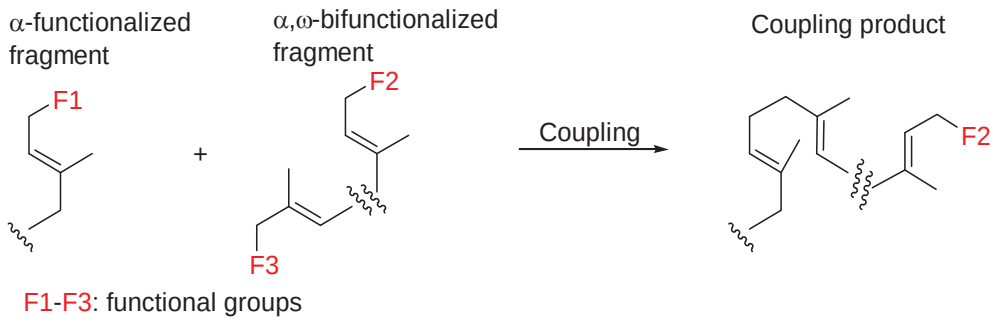
Bio- versus chemical synthesis.

Enzyme	Biomimetic process
Preniltransferaze	Oligomerization
Cyclaze	Cyclisation
Citocrom P450	Oxidation
Diels-Alderaze	Cycloaddition
Baeyer-Villigeraze	Baeyer-Villiger oxidation
Oxidoreductaze	Reduction

Examples of synthetic schemes devised according to these strategies are numerous. The following selection is provided to illustrate the most relevant ones, when used for the synthesis of complex terpenic compounds in a selective manner.

Oligomerisation

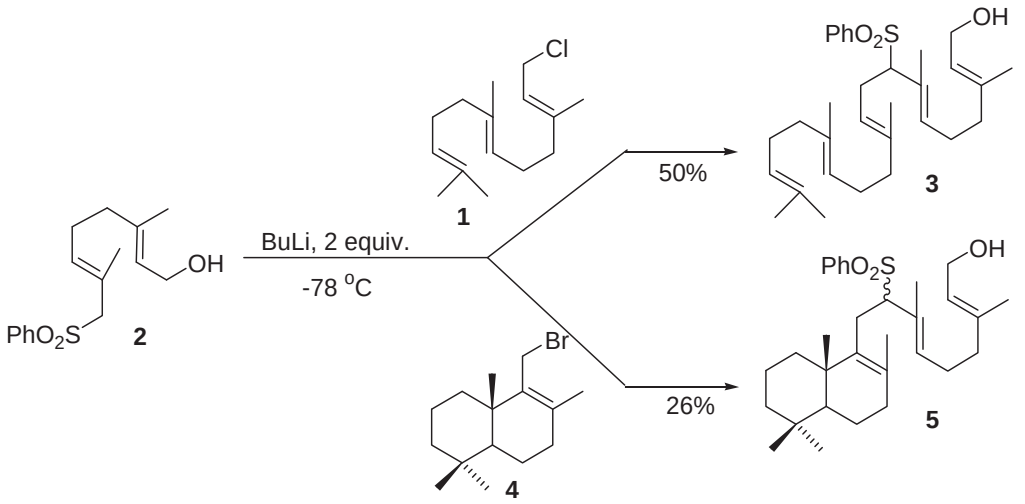
The need to do terpenes oligomerisation *in vitro* is dictated by the fact that terpene raw materials are quite limited to lower terpenes. Economically significant and renewable resources for terpene isolation are the wastes of wood processing industry first. The major part of these wastes are monoterpenes. The use of other important higher natural terpenoids like sclareol or manool is also connected to the need of oligomerisation, that's why elaboration of synthetic processes aimed to linkage of terpenic units has represented a priority of the research groups working in this field. The basic strategy relies on a biomimetic modular approach of connecting a α-functionalized building block to a α,ω-bifunctional fragment (Scheme 6). Due to practical reasons, the α-functionalized fragment bears more complexity (cyclic structures, chirality, functional groups), and the α,ω-bifunctional fragment is a simple C5 or C10 unit.



Scheme 5. Strategy of terpene oligomerisation by synthesis

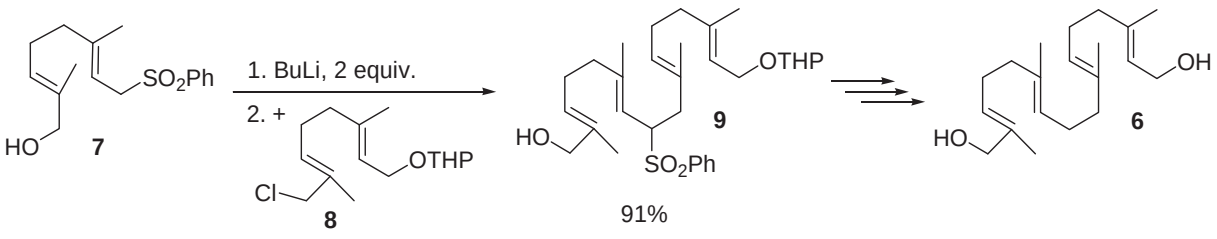
The activating functional groups F1 and F3 are selected in order to provide a couple of donor-acceptor synthons, which are able to efficiently combine and form the new C-C bond. Lithiated sulfones are used as synthetic equivalents for donor synthons and halogenides or carbonyl compounds for acceptor ones.

This strategy was successfully reported by different research groups [2] due to its several advantages. First of all the introduction of required sulfone and halogenide functional groups can be easily performed by routine techniques. The coupling yields vary from good to excellent and the products incorporate the sulfone group which can be handled in a flexible manner: either substituted for a hydrogen atom or for other functional groups [3]. In a couple of recent examples this strategy was successfully used in our laboratory [4]. The farnesylchloride **1** was coupled with the lithiated sulfone **2** derived from geraniol to provide the all-trans sesterterpene **3** (Scheme 6). Using the optically active drimenylbromide **4** as coupling partner for **2**, generated a coupling product **5**, incorporating two elements of complexity: bicyclic fragment and chirality.



Scheme 6

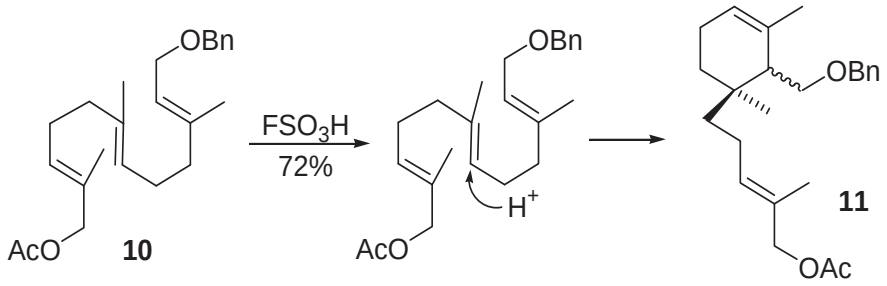
This homologation method also showed excellent regio-flexibility. The reported synthesis of natural product *trans*-16-hydroxygeranylgeraniol **6** included a similar coupling of **7** and **8**, with the sulfone group placed on the α-terminus and the chlorine on the ω-terminus [5]. The yield of **9** was excellent (Scheme 7).



Scheme 7

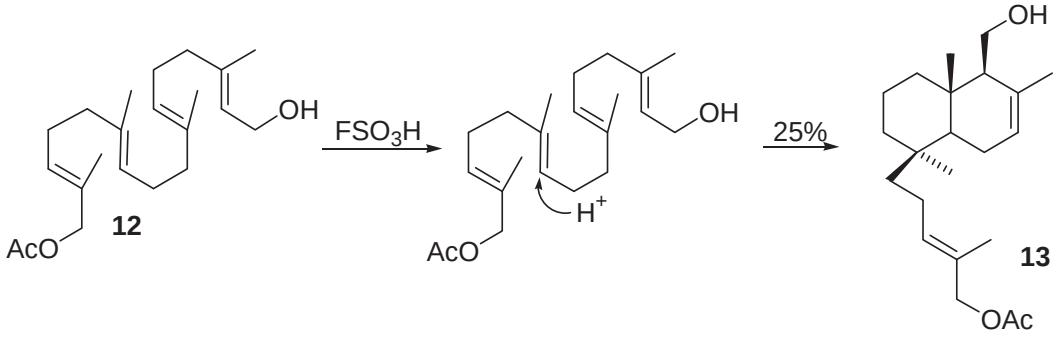
Cyclisations

Terpenoid cyclisations represent the classical example of biomimetic approach in the synthesis. The major part of the elaborated reactions is based on the mechanism including carbonium ion generation via selective protonation (second biosynthetic pathway, Scheme 4). Different cyclisation agents have been reported, most of them possesses strong acidic properties. The major challenges connected to this strategy relates to the need of controlling reaction selectivity, since the number of reaction pathways, and the range of possible products increases dramatically along with the number of isoprene units of the molecule. Therefore, cyclisation methods evolved to the use of lower reaction temperatures and stronger acid initiators. It is understandable that lower temperature influences the conformational flexibility of the molecule, in an attempt to mimic the action of the enzyme, which practically locks the substrate in one single conformation, making the cyclisation process totally specific. Solid acids like zeolites and acidic resines have been also reported to contribute to this problem solution. Their advantage consists in reaction conditions close to ambiental but unfortunately their selection is mostly empirical, they are very substrate-specific, thus limiting the process versatility. More practical from this point of view turned out to be superacids [6,7], which can initiate cyclisation sequences at temperatures as low as -78°C or even lower. Consequently, the process selectivity is beneficially influenced. A significant advancement in this field represents our finding that additional functional groups placed at certain positions of aliphatic terpenic chain, can influence the reaction course, allowing either selective initiation or termination of the cascade. The pioneering work relating in this directions deals with the superacidic cyclisation of α,ω-bifunctionalized sesquiterpenic substrates [8]. The presence in the substrate **10** of the acetoxy-group at the ω-terminus hindered the initiation of the cyclisation sequence from the extreme isoprene unit and allowed a selective protonation of the middle double bond, leaving in the resulted product **11** a pendant prenyl-group attached to the monocyclic backbone (*seco*-eudesmanic structure, Scheme 8).



Scheme 8

This reaction mechanism was further exploited for the biomimetic synthesis of sacculatane-like diterpenoids [9]. The α,ω-diterpenic open chain substrate **12** was prepared from geranylgeraniol, basing on a selective Van Tamelen epoxidation, followed by a degradation-olefination sequence. Superacidic treatment of **12** at low temperature allowed isolation of sacculatane-like bicyclic compounds as major reaction products (Scheme 9).

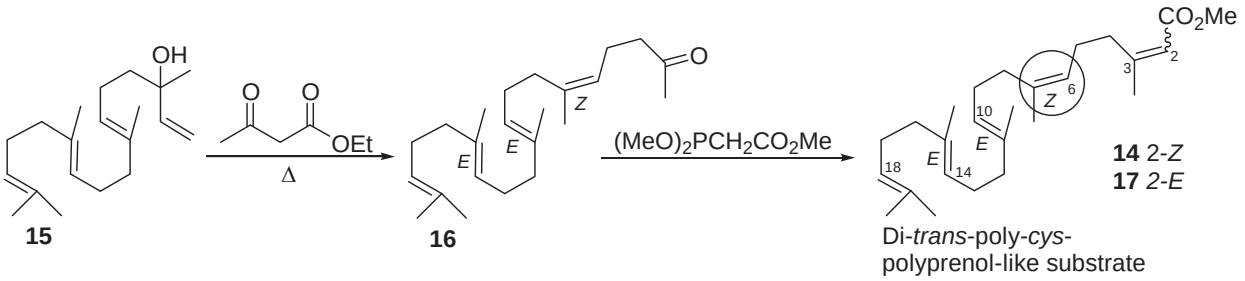


Scheme 9

Despite of the relatively low yield of the target **13**, the value of this example is given by the unique reaction mechanism, which proves the principle of functional group involvement into directing cyclisation selectivity. It is noteworthy mentioning that a substrate analogue to **12**, but without a functional group at ω-end, provides under similar conditions a totally cyclized tricyclic compound [6].

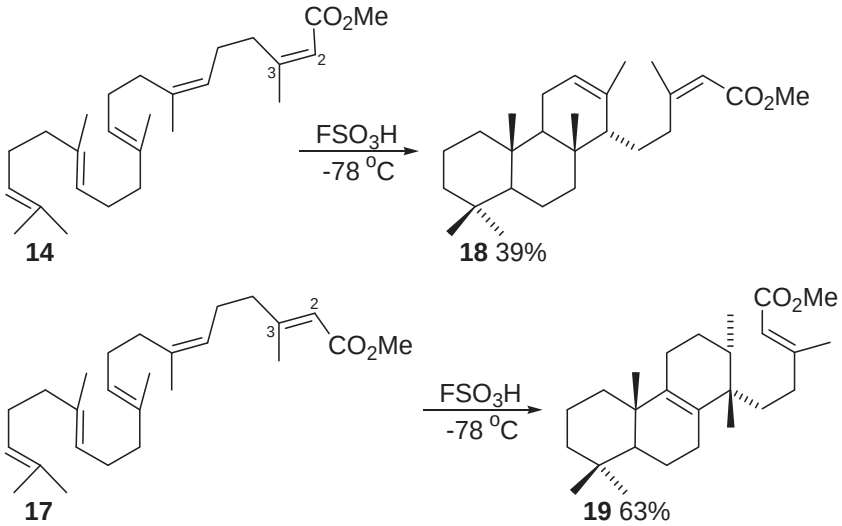
A totally opposite cyclisation pattern has been revealed on the investigation of terpenic substrates containing double bond with alternating configuration (*trans*- vs. *cis*-). The impetus for these investigations was provided by a remarkable class of natural isoprenoids – polyprenols. These compounds are higher oligomers, containing from 5 to 11 or even more isoprene units. They are usually found in plants and are potentially regarded as precursors of condensed polycyclic substances found in fossil sediments. In order to check the feasibility of this idea, we have initiated a program on superacidic isomerisation of selected polyprenols. The most representative di-trans-poly-cys- substrates have been chosen.

As it was expected, all the subsrates, independently on the chain length have shown reactivity on superacidic treatment. But the conclusions on the reaction mechanism could have been drawn only for a lowest C-25 representative **14** [10]. Selection of the esteric group as α-terminus functionality was due to its relative stability to superacidic treatment and also to the fact that long chain polyprenols showed a pronounced tendency to sediment at low temperature in the reaction solvents. The synthesis of **14** was performed from commercial geranyllinalool **15**, making use of a Carroll rearrangement followed by separation of the *cis*-ketone **16** and its standard olefination (Scheme 10). This two-step homologation procedure was preferred, since the Carroll rearrangement provided a significant ratio of the required **16**.



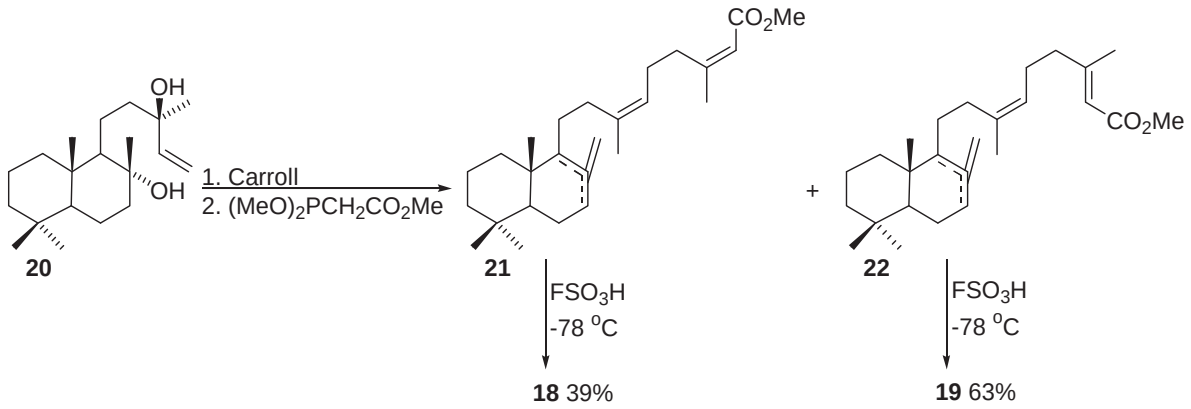
Scheme 10. Synthesis of polyprenol-like compound with the 6-*Z* double bond

The cyclisation of both substrates **14** and **17** occurred smoothly on treatment with fluorosulfonic acid (Scheme 11). Predominant reaction products in both cases were tricyclic compounds **18** and **19**, having the α-prenyl unit pendant. These compounds belong to the cheilanthane family and their biomimetical synthesis turned out to be a very efficient preparative tool. In fact, the use of substrate **14** as starting material shows clearly that cyclisation of an open chain polyprenol-like substrate can be suspended to a tricyclic compound, due to the presence of an internal *cis*-double bond. This functionality plays a crucial role in the conformational behavior of the substrate, braking-up the cyclisation cascade via a complex conformational-steric effect.



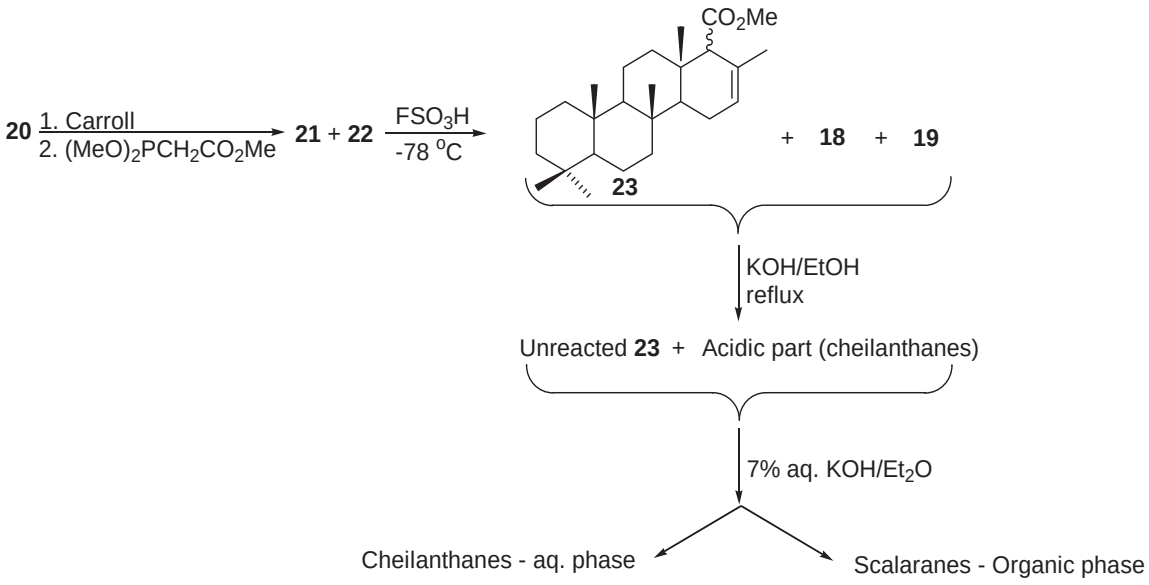
Scheme 11. Superacidic cyclisation of polyprenol-like substrates. Biomimetic synthesis of cheilanthanes

This finding led to the idea of using more available bicyclic starting materials for preparation of cheilanthanes in optically active form [11]. Labdanic diterpenoid sclareol **20** was the candidate of choice. It represents a relatively cheap compound, produced commercially as a by-product of *Salvia sclarea* essential oil processing.



Scheme 12. Biomimetic synthesis of optically active cheilanthanes

Transformation of **20** into the cyclisation substrates **21** and **22** followed the same synthetic sequence shown in Scheme 10: Carroll rearrangement, followed by Horner-Wadsworth olefination. Cyclisation of **21** and **22** proceeded smoothly, to provide cheilanthanes **18** and **19** in optically active form (Scheme 12). This approach represents a complementary addition to the alternative syntheses of cheilanthanes [12, 13] and has a great synthetic value due to its simplicity and reduced number of steps. Besides, it is also a reasonable method for the synthesis of scalaranic compounds, which are important by-products [14, 15] formed in substantial amounts along with cheilanthanes **18** and **19**. Our finding, that formation of scalaranes is not influenced by the *E-Z* configuration of the internal double bond has allowed elaboration of an efficient method for the synthesis of scalaranes and cheilanthanes in an integrated process. Its value is given by the possibility of separation the scalaranic compounds from cheilanthanic without chromatography. The flowchart of this procedure is represented in Scheme 13. After batch cyclisation of **21** and **22**, hydrolysis of the reaction products lead to scalaranic esters which do not hydrolyse under the reaction conditions, and cheilanthanic acids. Separation of the acidic and neutral part gives intact scalaranic esters in neutral fraction, while cheilanthanic acids can be recovered from the acidic fraction.

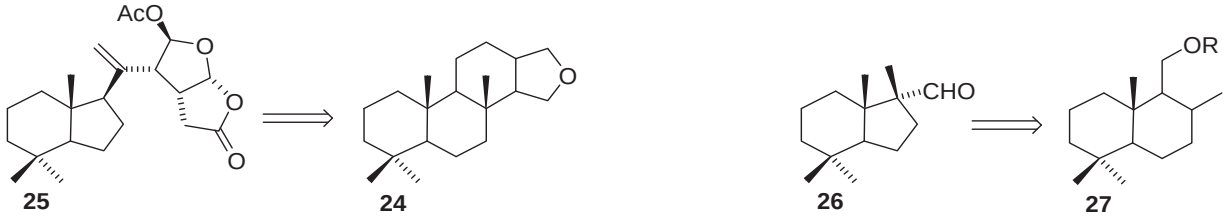


Scheme 13. A preparative procedure for the synthesis of scalaranes and cheilanthanes in optically active form

Rearrangements

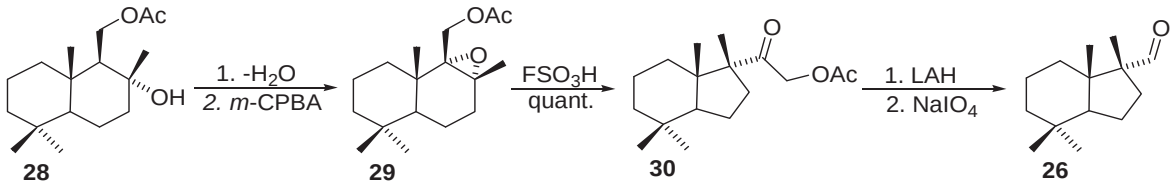
A whole range of terpenic compounds are biosynthetically formed by rearrangement reactions. These can include ring contractions, ring expansions and even functional group migrations. A good source of such rearranged terpenes are marine organisms, which provide constantly inspiration to synthetic organic chemists, due to the enormous diversity of unusual structures reported from this relevant source of natural compounds. Spongiane diterpenoids derive from sponges and besides their normal tetracyclic framework **24** , a lot of these derivatives resemble degraded skeletons.

Norrisolide **25**, a complex Golgi fragmentation agent isolated from the nudibranch *Chromodoris norrisi*, was postulated to derive biosynthetically from spongianes, by a complex degradation-rearrangement sequence [16]. The simpler nor-sesquiterpenic austrodoral **26**, isolated from the dorid nudibranch *Austrodoris kerguelenensis* was also suggested to arise biosynthetically from the drimane skeleton **27** by a ring-contraction process [17].



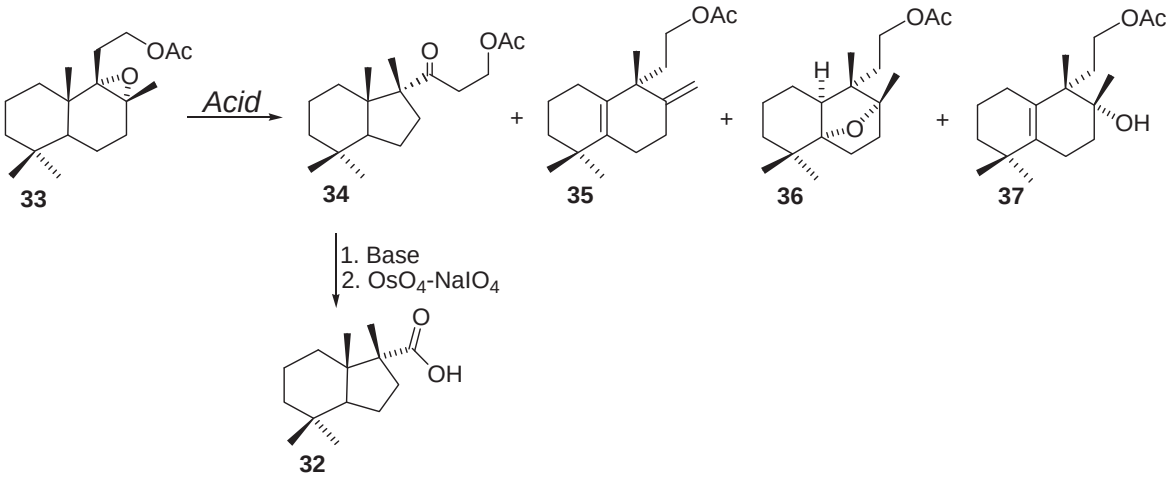
Basing on this hypothesis, a biomimetic procedure for the synthesis of **26** was devised, starting from drimanic or hoomodrimanic starting materials [18, 19].

The sequence of key transformations leading to **26** is represented in Scheme 14. The key reaction was a biomimetic ring contraction of the suitable constructed epoxide under the action of a Lewis acid to a ring contracted, perhydrindanic ketone **30**.



Scheme 14. Synthesis of austrodoral **26** from drimanes by a biomimetic ring contraction strategy

Elimination of the additional carbon atoms was performed by a standard periodate cleavage of the corresponding diols.



Scheme 15. Rearrangements of homodrimanes

Using the homodrimanic substrate **33** as starting material, provided the related austrodoric acid **32**, but unlike drimanic epoxide **29**, the homodrimane **33** interacted with different acidic inducers in a less selective manner. The perhydrindane **34** represented the basic product, but other compounds of deeper skeletal rearrangement have also been identified in the reaction mixture (Scheme 15). It included a cascade of methyl migration-hydride shifts-proton eliminations-heterocyclisation leading to halimanic bicyclic core [20, 21]. Variation of the reaction conditions, including reaction temperature and nature of acid inducer allowed a moderate selectivity control towards either ring contraction or methyl migration paths. This example incorporates two different biogenetic pathways towards compounds of both perhydrindanic and halimanic structure. The striking diference in the reactivity of drimanic **29** and homodrimanic **33** substrates is explained by the involvement of the lateral chain in the intermediate carbonium ions stabilization.

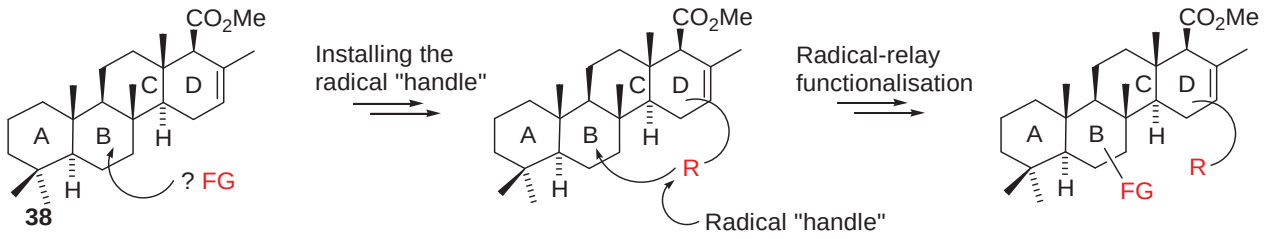
Selective functionalisation

The last chemical phenomenon under discussion is directly connected to the functional properties of terpenes, due to the heteroatoms incorporated into the carbon skeleton formed by oligomerisation-cyclisation-isomerisation sequences. Introduction of functional groups plays the crucial role in the interaction of terpenes with biological matrix leading to certain activities of the molecules. Oxigenated functionalities are the most frequently met as terpene "decoration", but nitrogen and halogens play an important role too.

Biomimetic approach can be implemented by two different ways for terpene functionalisation. The first mode includes selective functionalisation of the ready-made terpenic skeleton. The advantagies of this post-cyclisation process are connected first of all to the fact that cyclic terpenic molecules contain less reactive double bonds, and functionalisation can be more selective, at the expense of lower reactivity. Besides, cyclic molecules possess a relatively rigid molecular conformation, which can enhance steric control over functionalisation process.

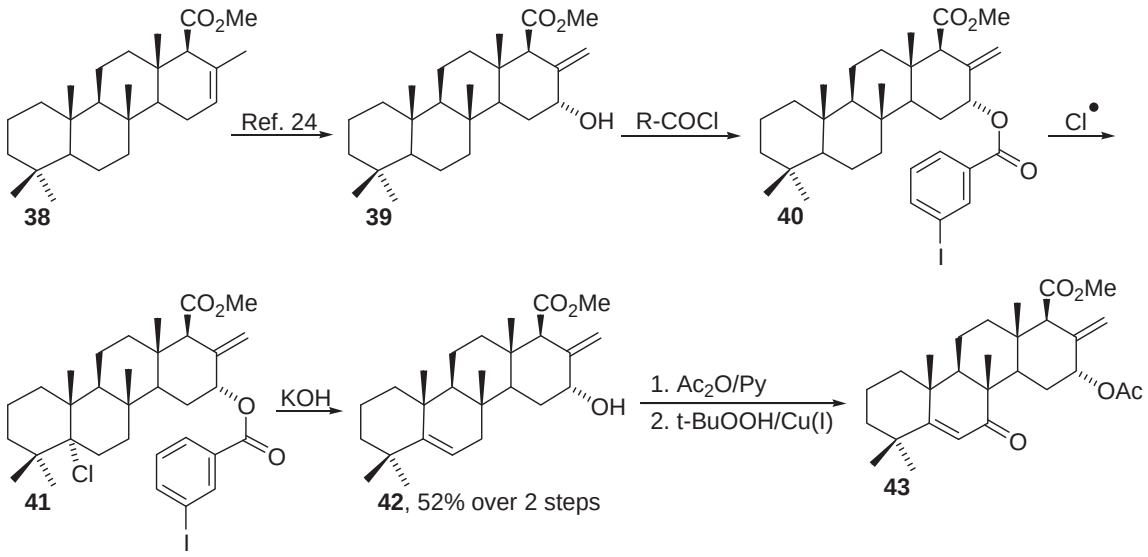
The second functionalisation strategy is based on the fact that we do not always know exactly the sequence of biosynthetical transformations and it is possible to hypothesize that functionalisation can occur before cyclisation/rearrangement in biosynthesis. In fact, additional functional groups in the substrate would inevitable control the nature and selectivity of both enzymatic and chemical transformations, including cyclisations/rearrangements. Therefore, it can be used as a tool to control the synthetic process too. On the other hand, additional functional groups in the terpenic molecule influence inevitably its steric and electronic features and shall be carefully handled. From the practical point of view, introduction of additional functionalities in the non-cyclized or partially cyclized molecule is more convenient, since the isoprene residues double bonds can be efficiently exploited for functionalisation purposes. The disadvantages of this strategy are connected to the difficulties of prediction the effect of the functional groups on the following reactivity.

Both these functionalisation pathways have been exploited in our works and the results are encouraging. First of all it was investigated the so-called remote functionalisation of a scalaranic tetracyclic compound **38**, having the carbon skeleton already established in place by a oligomerisation-cyclisation sequence [22]. Our goal was to set up an oxygenated functional group in the B-cycle of the tetracyclic system of **38**. Due to the fact, that this cycle contains only C-C and C-H bonds which can hardly be selectively transformed, the only feasible way to achieve selective functionalisation was considered the so called radical-relay halogenation (RRH) [23]. The characteristic feature of this reaction is the generation of a free radical in the molecule of the substrate, that has to contain a suitable functional group playing the role of a 'relay' for the free radical formation (radical "handle", Scheme 16), followed by the functionalization of nearby disposed C–H bond by this radical. The position of functionalization is governed both by steric and structural factors (only tertiary protons are prone to be involved into this process).



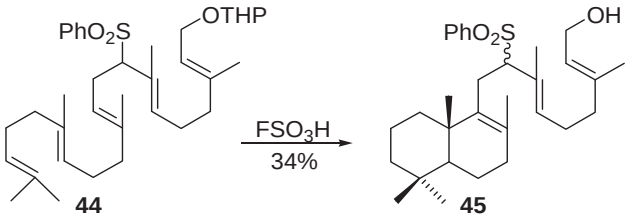
Scheme 16. Radical-relay halogenation strategy

The Scheme 17 shows implementation of the RRH for introduction of additional two functional groups in scalarane **38**. According to this scheme, the starting ester was transformed into allylic alcohol **39** [24] in order to make possible an attachment of the proper radical "handle". It was introduced further on esterification with the 3-iodo-phenylacetyl chloride. The iodine atom in the aromatic cycle was necessary in order to be able to deliver specifically the chlorine radical to the definite position in the B-cycle of **40**. The chlorine radical plays the role of the radical initiator and it efficiently substituted the tertiary proton from the B-cycle of terpenic substrate providing **41**. Following elimination of hydrochloric acid generated the double bond in the B-cycle of **42**, which can be further functionalized. In this case an allylic oxidation was performed in order to efficiently introduce the oxygen functionality in the structure **43** (Scheme 17).



Scheme 17. Functionalisation of scalaranes by radical-relay halogenation

Finally, a pre-cyclisation terpene functionalisation was also demonstrated [4]. Functionalisation step of the substrate was achieved by a sulfone-mediated homologation of farnesylchloride **1** (Scheme 6) as discussed above. The coupling product **44** incorporated two heteroatom functional groups: the THP-protected hydroxyl at the α-extremity and the phenylsulfonyl group in the middle of the molecule. Superacidic cyclisation of **44** led to a bicyclic compound with two pendant isoprenic units and two functional groups. As in the case of substrates with *cis*-configured internal double bonds described above, the phenylsulfonyl functional group contributed to suspension of cyclisation sequence, giving as the main product the sulfone **45**.



Scheme 18

Conclusions

Biomimetic strategies represent reliable tools for the elaboration of efficient synthetic pathways towards complex natural terpenes and their analogues. Most of the biosynthetical steps can be mimicked in a synthetic scheme, and different combinations of processes can lead to valuable results. Especially useful are functionalisation-cyclisation-isomerisation sequences, which can assure a flexible substrate handling in a selective manner. A careful selection and placement of functional groups has allowed achieving the following selective transformations:

- Initiation of the cyclisation cascade from an internal double bond of the open chain terpenic substrate, leading to cyclic compounds with the ω-prenyl group pendant;
- Suspension of the cyclisation cascade to partially cyclized compounds, having the α-prenyl group pendant;
- Efficient functionalization of terpenic substrates either by a post-or pre-cyclisation method;
- Rearrangement of available drimanic and homodrimanic substrates to functionalized compounds of perhydrindanic structure.

Following studies are necessary in order to estimate the influence of the functionalisation pattern on the enzymatic transformations of terpene substrates. Being combined with the enzyme engineering, this approach can contribute to new biotechnological processes for selective synthesis of complex targets.

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CYCLODEXTRINS – FIELDS OF APPLICATION. PART II¶

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Abstract. This paper represents an analysis of potential and current applications of cyclodextrins as biologically active substances in medicine. The main applications described here include use of cyclodextrins as agents that form inclusion complexes with endogenous substances (membrane lipids, cellular cholesterol), agents that form inclusion complexes with exogenous substances with their man role as guest molecules (sugammadex, FBCx), agents that block endogenous and exogenous macromolecules (ion channels, anthrax toxin, α-hemolysin), and agents which activity is based on the chemical nature of them and of their derivatives (cyclodextrin polysulphate derivatives). The first classification for medically important biological activity of cyclodextrins has been proposed.

Keywords: cyclodextrins, pharmaceuticals, medicine, inclusion complexes.

Introduction

Since the very beginning of the cyclodextrins' research and application in the pharmaceutical products, they have mainly been used as excipients that increase the dissolution rate of the active ingredients, increase their stability, mask their unpleasant taste or odor, etc. Nevertheless, an important number of studies suggested that cyclodextrins and their derivatives can be used as active drug substances in the treatment and prevention of different diseases. According to our knowledge, this is the first review that is trying to deal with all the potential and approved ways of application of the cyclodextrins due to their pharmacological properties. A similar review written by Prof. Otero-Espinar and co-authors [1] has only described the therapeutic activity of the CD's in the treatment of several host-pathogen infections.

The biological effects of the cyclodextrins important for their use in medicine can be classified as follows:

1. effects based on the ability of cyclodextrins to form inclusion complexes with endogenous substances (membrane lipids, cellular cholesterol) with their main role as guest molecules (methyl-β-cyclodextrin);
2. effects based on the ability of cyclodextrins to form inclusion complexes with exogenous substances with their man role as guest molecules (sugammadex, FBCx);
3. effects based on the ability of cyclodextrins to block endogenous and exogenous macromolecules (ion channels, anthrax toxin, α-hemolysin);
4. effects based on the chemical nature of cyclodextrins and their derivatives (cyclodextrin polysulphate derivatives).

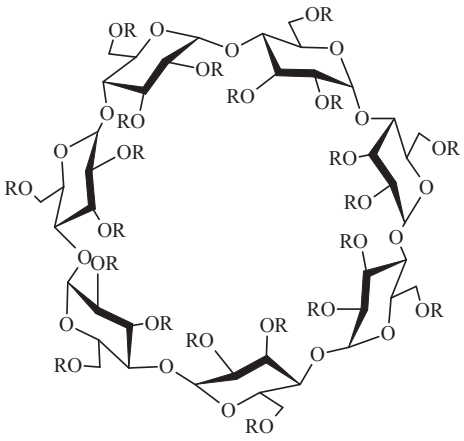
Further, all these groups will be presented with a brief description of the most used or recently developed applications of the cyclodextrins and their derivatives from each of them.

1. Biologically active cyclodextrins that form complexes with endogenous substances

A whole range of studies *in vitro* and *in vivo* have shown reduction or inhibition of entry of different infections in host cells due to the presence of cyclodextrins. This effect has been shown for the *Plasmodium* species [2], *Campylobacter jejuni* [3], poliovirus [4], human T cell leukemia virus type I (HTLV-1) and HTLV-I envelope pseudotyped lentivirus particles [5], pseudorabies herpesvirus [6], varicella-zoster [7], *Leishmania donovani* [8], hepatitis *B* virus [9], *HIV-1* [10], and many others. Many of these effects appear as a result of lipid raft disruption by cholesterol depletion in the membrane of the host cell through its extraction by cyclodextrins.

Being ideal chelators of cholesterol, cyclodextrins have been proposed for the treatment of Niemann–Pick type C (NP-C) that is a lysosomal storage disease associated with mutations in NPC1 and NPC2 genes. As a result of this disease cholesterol and glycolipids accumulate in lysosomes. This affects different internal organs and the central nervous system. The lipid-binding ability of cyclodextrins enables the sequestered lysosomal lipid to flow into the cytosolic pool in NP-C animals [11] and cells [12], relieving the cellular burden of lipids. The main cyclodextrin derivatives proposed for this purpose were 2-hydroxypropyl-β-cyclodextrin and methyl-β-cyclodextrin (fig. 1). It has been demonstrated that methyl-β-cyclodextrin is more potent than hydroxypropyl-β-cyclodextrin in reducing both cholesterol and bis(monoacylglycerol) phosphate accumulation in NPC mutant fibroblasts [13].

¶ This article is an extended abstract of a communication presented at the Conference Ecological Chemistry 2012.



methyl-β-cyclodextrin: R= -CH_3 or -H

2-hydroxypropyl-β-cyclodextrin: R= -CH_2-CH(OH)-CH_3 or -H

Fig. 1. Structures of methyl-β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin

2. Biologically active cyclodextrins that form complexes with exogenous substances

Cyclodextrin derivatives are used as a new class of selective relaxant binding agents (SRBAs). One of the first SRBAs introduced in pharmaceutical market by Organon International, a part of Schering-Plough Corporation, is sugammadex sodium (fig. 2). Sugammadex is used for a consistent and rapid termination of neuromuscular blockade induced by neuromuscular blocking agents. During the induced neuromuscular blockade, the intravenous administration of sugammadex creates a concentration gradient favoring the movement of neuromuscular blocking agent molecules from the neuromuscular junction back into the plasma, which results in a fast recovery of neuromuscular function [14].

One of the main advantages of sugammadex is a low number of side effects. It is biologically inactive, does not bind to plasma proteins, and appears to be safe and well tolerated. Additionally, it has no effect on acetylcholinesterase or any receptor system in the body [14].

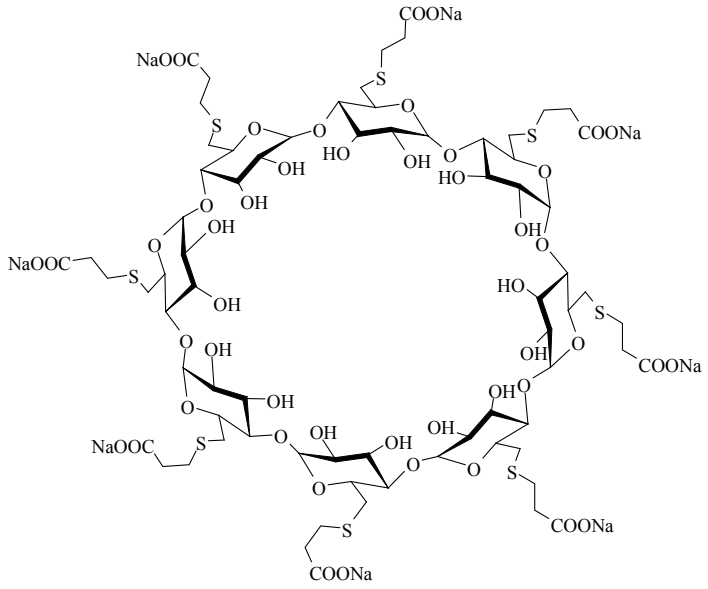


Fig. 2. Structure of sugammadex sodium molecule

The mechanism of action of the drug is based on direct encapsulation of neuromuscular blocking agents (NMBAs) as it is shown in example with rocuronium (fig. 3) [14]. The studies performed with sugammadex have shown a high level of its selectivity towards aminosteroidal NMBAs [15], and its better performance as compared to neostigmine [16].

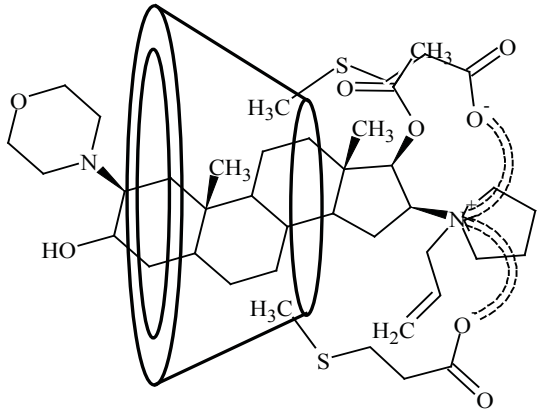


Fig. 3. Inclusion complex of sugammadex-rocuronium

α-cyclodextrin has been proposed as naturally derived fiber for the treatment of high blood lipid levels and obesity of humans. A double-blind study on obese patients with types 2 diabetes has shown that the total cholesterol had reduced by 8,2% after a 30-day treatment at the patients taking α-CD as compared to 5,2% increase in the group of patients taking placebo [17]. Another double-blind research on healthy overweight individuals has shown a decrease in total blood cholesterol by 5,3% and low-density lipoproteins by 6,7% during the two-month period. At the same time, apo-lipoprotein B levels have decreased by 5,6% and insulin levels by 9,5%, while blood glucose and leptin levels did not change [18].

3. Biologically active cyclodextrins that block endogenous and exogenous macromolecules

A group of β-cyclodextrin derivatives have been studied on their ability to inhibit anthrax lethal toxin by blocking the trans-membrane pore formed by its protective antigen (PA) subunit [19]. Most of the tested compounds have shown protective activity against anthrax lethal toxin at low or submicromolar concentrations. The activities of the derivatives in both cell protection and channel blocking have been found to depend on the length and chemical nature of the substituent groups.

A hepta-6-substituted β-cyclodextrin derivative IB201 (fig. 4) has been shown to prevent alpha-toxin mediated hemolysis of rabbit red blood cells (rRBCs), a cell type that is highly sensitive to the lytic action of the toxin. α-hemolysin (Hla) has been proved to play important role in the pathogenesis of *Staphilococcusaureus*, a bacteria that is causing severe forms of pneumonia. Under its monomeric form, Hla binds to susceptible host cell membranes and assembles into a stable transmembrane pore with a 2-nm internal diameter after a series of intra- and intermolecular interactions. Mechanism of action of IB201 is based on blocking of ion conductance through the assembled α-hemolysin pore afterlodging in it. The drug has been proved to have a high potency being active in the low micromolar concentration range. *In vivo* investigation in a murine model has detected that IB201is able to prevent mortality associated with *S. aureus pneumonia* [20].

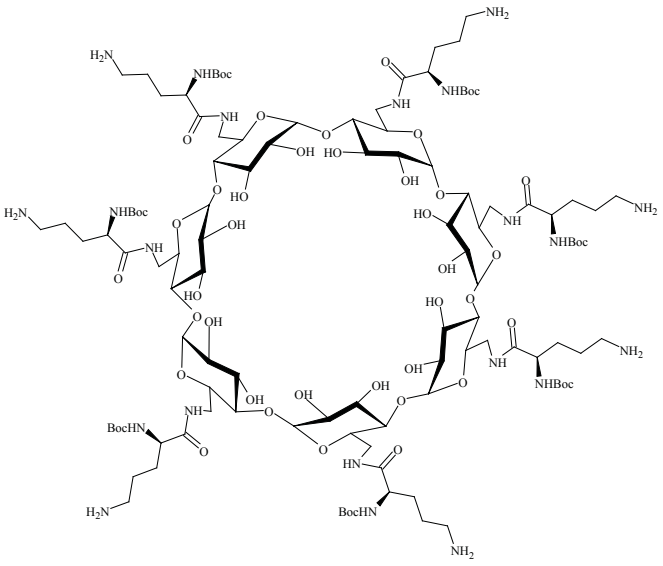


Fig. 4. Structure of IB201 molecule

Comparison of structurally related cationic cyclodextrins on ability of inhibition of *Bacillus anthracis* lethal toxin and *Staphylococcus aureus* α-hemolysin has shown that both β- and γ-cyclodextrin derivatives effectively inhibited anthrax toxin action. On the other hand, α-hemolysin was selectively blocked only by β-cyclodextrin derivatives, demonstrating that both symmetry and size of the inhibitor and the pore are important [21].

Another group of researchers [22] has designed and successfully tested *in vitro* and *in vivo* a group of heptavalent anthrax toxin inhibitors, in which β-cyclodextrin plays the role of a biocompatible core (fig. 5).

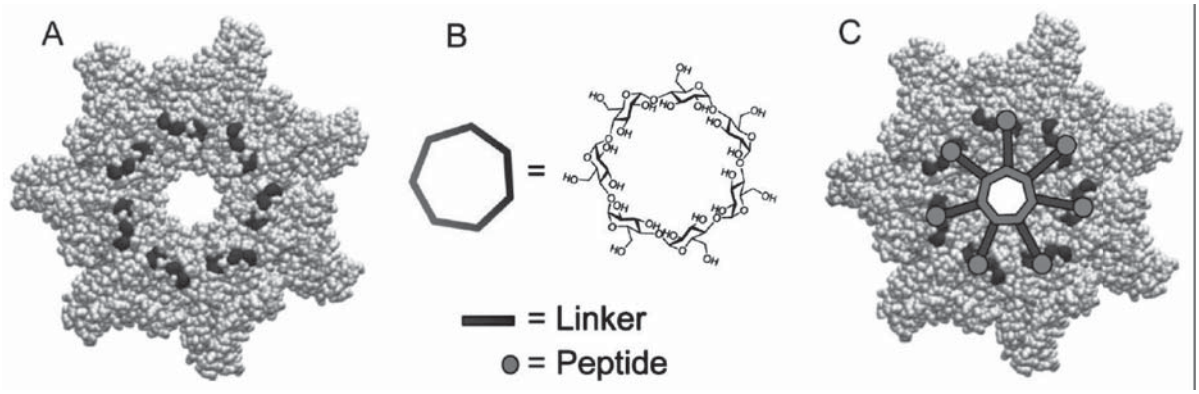


Fig. 5. Structure-based design of heptavalent anthrax toxin inhibitors. A) Structure of the L Fbinding face of [PA63]7. Residues 184, 187, 197, and 200, which form part of the peptide binding site are shown in purple. B) Structure of the core, β-cyclodextrin. C) Scheme illustrating the binding of a heptavalent inhibitor, synthesized by the attachment of seven inhibitory peptides to the β-cyclodextrin core via an appropriate polyethylene glycol linker, to [PA63]7 [22].

The process of the anthrax toxin inhibitors obtaining according to [22] is based on copper-catalyzed azidealkyne cycloaddition (click-chemistry) to facilitate the attachment of seven copies of the inhibitory peptide to a β-cyclodextrin core via a polyethylene glycol linker of an appropriate length, as it is presented in the scheme of synthesis (fig. 6).

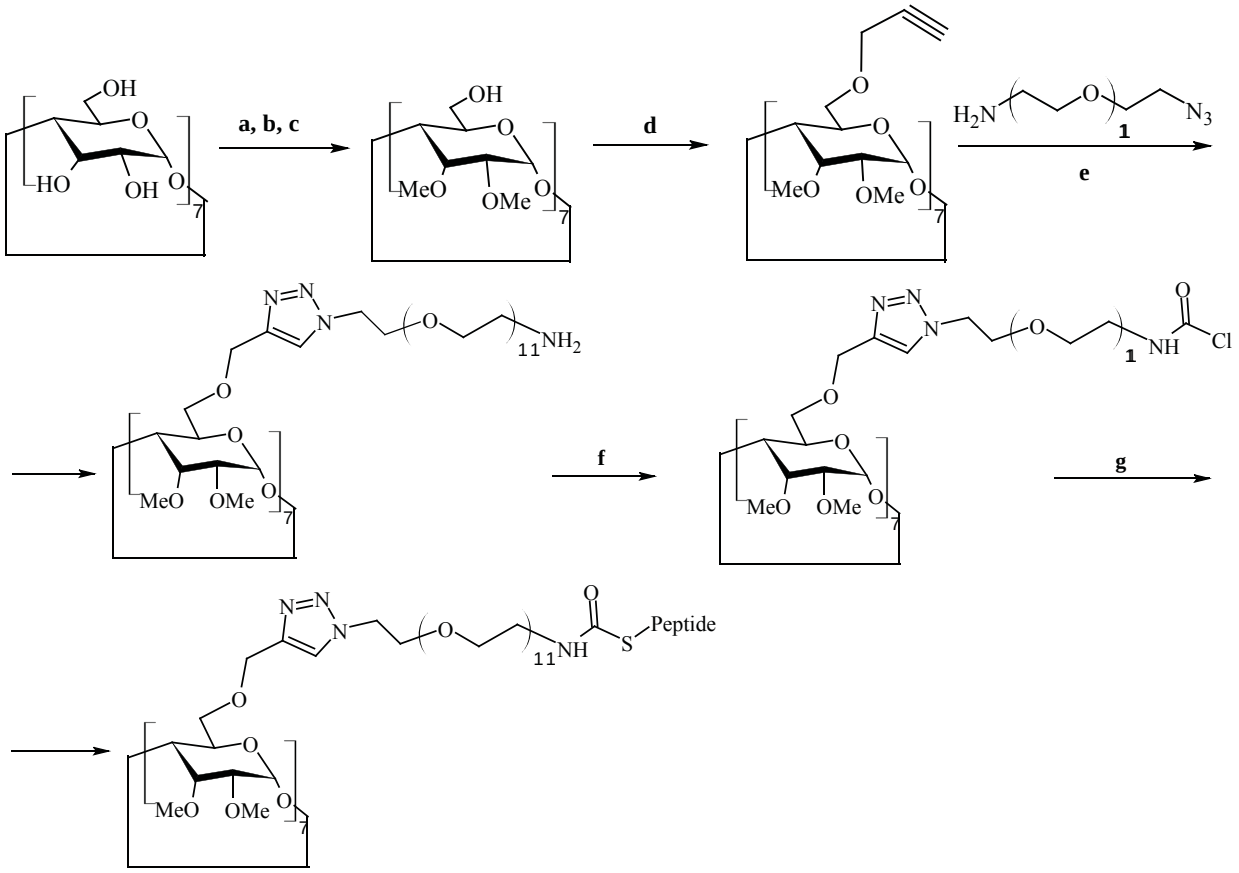


Fig. 6. Scheme of synthesis of heptavalent anthrax toxin inhibitor. (a) TBDMSCl, pyridine, 0°C - rt.; (b) NaH, MeI, THF; (c) NH_4F, MeOH, reflux; (d) NaH, propargyl bromide, DMF, 0°C - rt. (e) $CuSO_4$, sodium ascorbate, THF:H_2O:BuOH (0.5:1:1), 80°C; (f) chloroacetic anhydride, triethylamine; (g) peptide, DMF, DBU, triethylamine [22].

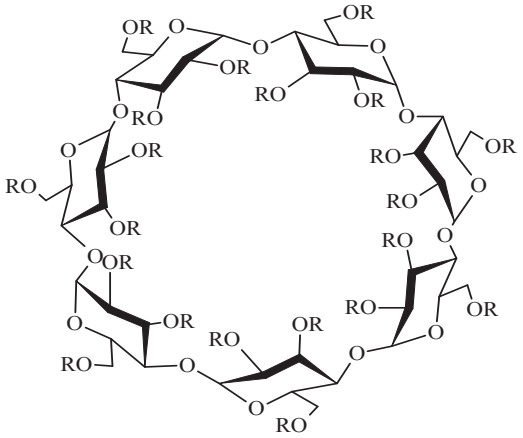
Interestingly, antibacterial activity of cyclodextrins has also been proven to have lysis activity against certain strains of Bacillus, but not against other Gram-positive or Gram-negative bacteria [23]. Another group of Japanese researchers [24] has shown inhibitory effect of dimethylacetyl-β-cyclodextrin on lipopolysaccharide-induced macrophage activation and endotoxin shock in mice.

4. Biologically active cyclodextrins with activity depending on their chemical nature

Cyclodextrin polysulphate (CDPS) has been investigated in with the aim to evaluate its chondroprotective effect in a rabbit model of experimental osteoarthritis (OA) [25]. It has been shown that subcutaneous injections of 1 mg/kg CDPS induced a marked inhibition of osteophyte formation and a significant reduction of cartilage degradation.

In order to alleviate possible heparin-related side effects of CDPS, six alkylated β-cyclodextrin polysulphates have been synthesized (fig. 7) [26]. These compounds have been tested for their capacity to restore cartilage damage *in vitro*. Besides, their effect on blood coagulation and their potency to induce thrombocytopenia through cross-reaction with heparin/PF4 antibodies have been assayed in order to determine possible heparin-like side effects.

These studies have demonstrated that namely poly- but not monosulphated cyclodextrins possess chondrocyte extracellular matrix repair potential. Also, it has been detected that from the polysulphated compounds subjected to studies (2-carboxyethyl)-β-cyclodextrin polysulphates (CE-CDPS) has the best safety profile.



ME-CD-3S: R= $-CH_3$ or $-SO_3H$

ME-CD-6S: R= $-CH_3$ or $-SO_3H$

MA-CDPS: R= $-NH_2$ or $-SO_3H$ or -H

CE-CDPS: R= $-CH_2-CH_2-COOH$ or $-SO_3H$ or -H

HP-CDPS: R= $-CH_2-CH_2-OSO_3CH_3$ or $-SO_3H$

CDPS: R= $-SO_3H$ or -H

Fig. 7. Structures of six polysulphated cyclodextrins. **ME-CD-3S -** 2,6-di-O-methyl-3-sulphate-β-cyclodextrin; **ME-CD-6S -** 2,3-di-O-methyl-6-sulphate-β-cyclodextrin; **MA-CDPS -** 6-monodeoxy-6-monoamino-β-cyclodextrin polysulphate; **CE-CDPS -** (2-carboxyethyl)-β-cyclodextrin polysulphate; **HP-CDPS -** (2-hydroxypropyl)-β-cyclodextrin polysulphate; **CDPS -** β-cyclodextrin polysulphate [26]

5. Conclusions

The main medically important biological activities of the cyclodextrins and their derivatives have been discussed. Classification of these activities has been proposed. For the best of our knowledge, this is the first classification of this type of cyclodextrins' activity proposed so far.

6. Acknowledgments: one of the authors, Veaceslav Boldescu, wishes to thank the International Innovative Nanotechnology Center of the CIS countries for a generous support in the realization of a part of this research.

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USE OF BACTERIA AND MICROALGAE IN SYNTHESIS OF NANOPARTICLES¶

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Abstract. A critical need in the field of nanotechnology is the development of a reliable and eco-friendly process for synthesis of metallic nanoparticles. A number of different organisms, including bacteria, microalgae, yeast and fungi, have shown their ability to produce metal nanoparticles. But they have some drawbacks in providing better control over size distribution, shape and crystallinity. This review article presents an overview of microorganisms (bacteria and microalga) capable of producing silver and gold nanoparticles. Also mechanisms of nanoparticles formation and parameters which influence their growth are discussed.

Keywords: nanotechnology, bacteria, microalga, nanoparticles, silver, gold.

Introduction

Nanotechnology is enabling technology with the ability to work at the atomic, molecular and submolecular levels in order to understand, create and use material structures, devices and systems with fundamentally new properties and functions resulting from their small structure [1-5].

The term *nano* is adapted from the Greek word meaning "dwarf." A nanometer (nm) is one billionth of a meter, or roughly the length of three atoms side by side. A DNA molecule is 2.5 nm wide, a protein approximately 50 nm, and a flu virus about 100 nm. A human hair is approximately 10,000 nm thick. A nanoparticle is a microscopic particle with at least one dimension less than 100 nm [6 -8].

Scientists are developing reliable, eco-friendly techniques for atom-by-atom construction of objects that have potential applications in medicine, electronics, catalysis [9], photonics, optoelectronics [10], information technology, environmental monitoring and remediation, military equipment and weapons, and so forth [11-13]. An important area of research in nanotechnology deals with the synthesis of nanoparticles of different chemical compositions, sizes and controlled monodispersity [14].

The physical and chemical methods such as chemical reduction [15, 16], electrochemical reduction [17], photochemical reduction [18], are used for nanoparticle synthesis [19-21]. They have some significant disadvantages regarding formation, monodispersity of the particles and thermodynamic stability. In some processes special pH and temperature control is required to avoid aggregation and precipitation of the particles in solution. Such nanoparticles are stabilized by additional steps involving coating with polymer layers [22, 23]. Unfortunately many organic solvents are toxic enough to pollute the environment if large scale nanoparticles are produced.

This leads to a growing awareness of the need for developing clean, nontoxic and environmentally friendly procedures [24].

Many organisms, both unicellular and multicellular, are known to produce inorganic materials either intracellularly or extracellularly [25, 26]. Microbial cells are highly organized units, regarding morphology and metabolic pathways, capable of synthesizing reproducible particles with well-defined size and structure. Advantages of biological methods include tightly controlled, highly reproducible syntheses: biocompatible particles: and the avoidance of toxic surfactants or organic solvents [27, 14]. Furthermore, biogenic nanoparticles often exhibit water-soluble and biocompatible properties, which are essential for many applications [6].

One of the principle objectives in nanotechnology is the biosynthesis of noble metal nanoparticles.

The aim of present review is overview of various reports on biological synthesis of gold and silver nanoparticles using bacteria and microalgae.

Using of microorganisms in nanoparticles synthesis

Bacteria in nanoparticle synthesis

Among the microorganisms, prokaryotic bacteria have received the most attention in the area of biosynthesis of nanoparticles [28]. Moreover, bacteria are easy to handle and can be manipulated genetically. Considering these advantages, a bacterial system could prove to be an excellent alternative of chemical methods for the synthesis of gold nanoparticles [19].

Early studies reveal that *Bacillus subtilis* 168 is able to reduce Au^{3+} ions to produce octahedral gold particles

¶ This article is an extended abstract of a communication presented at the Conference Ecological Chemistry 2012.

of nanoscale dimensions (5–25 nm) within bacterial cells by incubation of the cells with gold chloride under ambient temperature and pressure conditions [29].

Sastry and co-workers have observed that the extremophilic actinomycete, *Thermomonospora* sp. when exposed to gold ions reduced the metal ions extracellularly, yielding gold nanoparticles with a much improved polydispersity [14]. Ahmad with co-workers have showed that an alkalotolerant actinomycete, *Rhodococcus* sp., exposed to $AuCl_4^-$ ions results in the rapid reduction of the gold ions and formation of fairly monodisperse intra-cellular nanoparticles. Furthermore, the concentration of gold nanoparticles is much higher on the cytoplasmic membrane than on the cell wall. It can be seen that the average particle size is ≈ 9 nm with some particles of 10–12 nm size and a very small percentage having diameters 5, 14 and 16 nm.

Prokaryote bacteria *Rhodopseudomonas capsulata*, recognized as one of the ecologically and environmentally important microorganisms, commonly existing in the natural environment, was investigated for reducing Au^{3+} ions at room temperature with a single step process. It has been demonstrated that the bacteria *Rhodopseudomonas capsulata* are capable of producing gold nanoparticles extracellular and the gold nanoparticles are quite stable in solution [30].

Bacteria *Desulfovibrio desulphuricans*, *Escherichia coli* play an important role in fabrication of Au (0) particles of 20-25 nm size [31, 32].

Monodispersed spherical gold nanoparticles of 1.9 ± 0.8 nm size have been synthesized extracellular by reaction of aqueous $AuCl_4^-$ ions with *Bacillus megatherium* D01 [33].

Konishi et al. observed that Fe (III) reducing bacteria *Shewanella algae* can reduce Au (III) ions in anaerobic environments [34].

Pseudomonas stutzeri NCIMB 13420, *Bacillus subtilis* DSM 10, *Pseudomonas putida* DSM 291 tended to synthesize small, relatively uniform sized gold nanoparticles intracellular. The particles were observed mainly in the cytoplasm of the cells and the majority of the particles were spherical in shape [12].

The detailed study on extra-cellular biosynthesis of gold nanoparticles by the *Pseodomonas aeruginos*a was carried out in Husseiny work. *Pseodomonas. aeruginosa* is a Gram-negative bacterium that is capable of existing in multiple environmental niches and is an opportunistic pathogen, meaning that it exploits some break in the host defenses to initiate an infection [13].

Kalishwaralal et al. have reported a green chemistry approach using *Bacillus licheniformis* in the synthesis of gold nanocubes at room temperature without using any harmful reducing agents. The average particle size of nanocubes was found to be 10–100 nm [35].

The gold nanoparticles synthesized by *Geobacillus stearothermophilu* are essentially spherical and reasonably monodispersed. The mean particle size is 11nm. Some particles of 5–8 nm also could be visualized [36].

It is already established that silver is highly toxic to most microbial cells. Nonetheless, several bacterial strains are reported as silver resistant [37] and may even accumulate silver at the cell wall to as much as 25% of the dry weight biomass, thus suggesting their use for the industrial recovery of silver from ore material.

Klaus and co-workers have shown that the bacteria *Pseudomonas stutzeri* AG259 isolated from silver mine, when placed in a concentrated aqueous solution of $AgNO_3$, resulted in the reduction of the Ag^+ ions and formation of silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria [28]. The size of such nanoparticles being in the range 16 – 40 nm, with the average diameter of 27 nm.

It was shown that silver nanoparticles can be synthesized by gram-negative bacteria *Escherichia coli* [38, 39] and *Geobacter sulfurreducens* [40].

Rapid synthesis of metallic nanoparticles of Ag using the reduction of aqueous Ag^+ ions has been achieved in the culture supernatants of *Klebsiella pneumonia*, *Escherichia coli* and *Enterobacter cloacae* [41]. Recently detailed studies confirmed that synthesis of Ag can be triggered through the liquid mixing process developed in the visible light spectrum by *Klebsiella pneumonia* [42].

The silver nanoparticles synthesized by *Geobacillus stearothermophilu* generally appeared spherical. The average size of nanoparticles is 5–35 nm [37].

Biosynthesis of silver nanaparticles by Lactobacilli *acidophilus and Nannochloropsis oculata* occurred at 10^{-3} M of $AgNO_3$ while the optimum concentration for producing silver nanoparticles for *Lactobacilli casei*, and *Lactobacilli reuteri* was $2 \cdot 10^{-3}$ M. The appropriate time for incubation of all species was 24 hours [24].

Nair and Pradeep have shown that *Lactobacillus* strains present in buttermilk, when challenged with silver and gold ions, resulted in the large-scale production of metal nanoparticles within the bacterial cells [43].

Microalgae in nanoparticle synthesis

Alga is a diverse group in plant kingdom that is not only accumulates metals by chelation and chemical transformation, but is also reported to produce bio-mineral structures and metal nanoparticles [44].

A few reports are available regarding gold accumulation using algal genera including cyanobacteria as bioreagent, such as *Chlorella vulgaris*, *Spirulina platensis* [45-48]. The morphological control over the shape of Au nanoparticles has been well established using *Plectonema boryanum* UTEX485, blue-green algae, while treated with aqueous Au $(S_2O_3)_2^{3-}$ and $AuCl_4^-$solutions [47].

Kumar et al. have demonstrated the extracellular biosynthesis of silver, gold and bimetallic nanoparticles using *Spirulina platensis.* The use of blue green alga offers a means of developing 'nanofactories' for production of metal nanoparticles and it is clear that interaction of single-cell protein with inorganic materials can benefit much from effectively interfacing nanoparticles and biology [49].

Bioreduction of gold by *Rhizoclonium riparium*, *Navicula minima* and *Nitzschia obtusa* have already been reported by Nayak et al. and Pal et al. [22, 23]. In the Chakraborty's work, *Lyngbya majuscula*, *Spirulina subsalsa* and *Rhizoclonium hieroglyphicum* were exposed to radioactive and stable gold solution to study the absorption, recovery and nanogold formation [50].

Biosynthesis of silver nanoparticles by *Chlorella vulgaris* occurred at 10^{-3} M of $AgNO_3$ in 24 hours [24].

Mechanism of nanoparticle synthesis

The interactions between microorganisms and metals have been well documented and the ability of microorganisms to extract and/or accumulate metals is already employed in biotechnological processes such as bioleaching and bioremediation. But the cellular mechanism leading to the formation of nanoparticles is not well understood [51-55].

Ahmad et al. have postulated that microorganisms secrete enzymes, which may be responsible for the reduction of metal ions [56].

Nangia et al. suggested that the biosynthesis of gold nanoparticles and their stabilization via charge capping in *Stenotrophomonas maltophilia* involved NADPH-dependent reductase enzyme that converts Au^{3+} to Au^0 through electron shuttle enzymatic metal reduction process [57].

Sastry and co-workers proposed that the silver ions are reduced by enzymes present in the cell wall leading to the formation of silver nuclei, which subsequently grow by further reduction of Ag^+ ions [14].

Xie et al. demonstrated that proteins are the principal biomolecules involved in the Ag^+ reduction and silver nanoparticles anisotropic growth in *Chlorella vulgaris* cells. Hydroxyl groups of amino acids are the most active functional groups for Ag^+ ions reduction [58].

Duran et al. also proposed the involvement of enzymatic electron shuttle relationship for the formation of Ag^+ ions and the subsequent formation of silver nanoparticle [59].

Binupriya et al. found that the assumed higher organic content leached out from inactive cells can be responsible for the higher and rapid productivity of gold and silver nanoparticles [60].

However, the biochemical mechanism of nanoparticles formation and stabilization remains unexplored.

Size control over the biological synthesis of the nanoparticles

Though, microbial synthesis is regarded as safe, cost-effective, sustainable and environment friendly processes, they have some drawbacks in providing better control over size distribution, shape and crystallinity. Optimizing the conditions such as pH, temperature and incubation time, concentration of metal ions, and the amount of biological material have come up to give hope in implementation of these approaches in large scale and for commercial applications [61].

Effect of pH

Gericke et al. have shown that the particles formed at pH 3 were predominantly spherical in shape, relatively uniform in size, with the majority of the particles less than 10 nm in diameter. Nanoparticles synthesized at pH 5 included small spherical particles; in addition a large number of bigger particles with well-defined shapes, including triangles, hexagons, spheres and rods also occurred at this pH. The shapes of the particles formed at pH 7 were similar to those formed at pH 9 and included small spherical particles as well as bigger particles with irregular, undefined shapes [12].

Agnihotri and co-workers have observed for marine yeast *Yarrowia lipolytica* NCIM 3589 that acidic pH favored nucleation on the cell surfaces and the subsequent formation of gold crystals. At pH 7.0 and 9.0, no crystals were formed. The average nanoparticle size was 15 nm [62]. A report by Gurunathan et al. showed that at acidic pH the size of the silver nanoparticle synthesized by *Escherichia coli* ranged 45 nm whereas at pH 10 the size is just 15 nm [63].

Lengke et al. observed similar octahedral metallic gold using *Plectonema boryanum* UTEX 485 at pH 1.9−2.2 and 25−200 ^{0}C [47]. The nanoparticles synthesized by *Rhodopseudomonas capsulata* biomass at pH values 7 whereas a number of nanoplates were observed at pH 4 [30].

Effect of reaction temperature

Huang et al. have shown that with temperature increase reaction rate of the conversion of the metal ion to nanoparticles grow up [64].

Gurunathanet al. showed that for *Escherichia coli* at room temperature, silver nanoparticles of 50 nm are synthesized whereas at 60 ^{0}C nanoparticles of 15 nm are synthesized [63].

Gerinke et al. used 3 temperatures for gold nanoparticles synthesis: 25, 35 and 50^{0}C. At the lower temperatures, the majority of nanoparticles were spherical with an average diameter of less than 10 nm. At 50^{0}C very few small spherical particles were present. Formation of spherical particles was favored at low reduction rates, whereas high reduction rates resulted in the formation of particles exhibiting nanorod and platelet-like morphologies [12].

It is also observed that the low rate of reduction in metal ions at normal room temperature possibly facilitate the growth of anisotropic nanoparticles, and with slight modifications in the temperature and the reaction medium one can be enabled in fabricating the well-defined triangular gold nanoparticles [65].

Effect of cell-free extract and metal salt concentration

Pimprikar and co-workers analyzed effect of cell-free extract and metal salt concentration on the nanoparticle growth. 10^{11} cells/ml yeast cell exposed to different concentration of $HAuCl_4$ produce exclusive spherical nanoparticle. With 10^{10} cells/ml in addition to the spherical nanoparticles hexagonal or triangular nanoplates of gold were also observed. With a lower number of cells (10^9 ml^{-1}), the dose of the gold salt was reduced only when present in lower concentrations. This is connected with insufficient number of biomolecules which could reduce gold [66]. The same results were shown in [67, 68, 12].

In Fig.1 as example SEM micrographs of *Spirulina platensis* incubated with $HAuCl_4$ of concentration 10^{-3} and 10^{-2}M are presented.

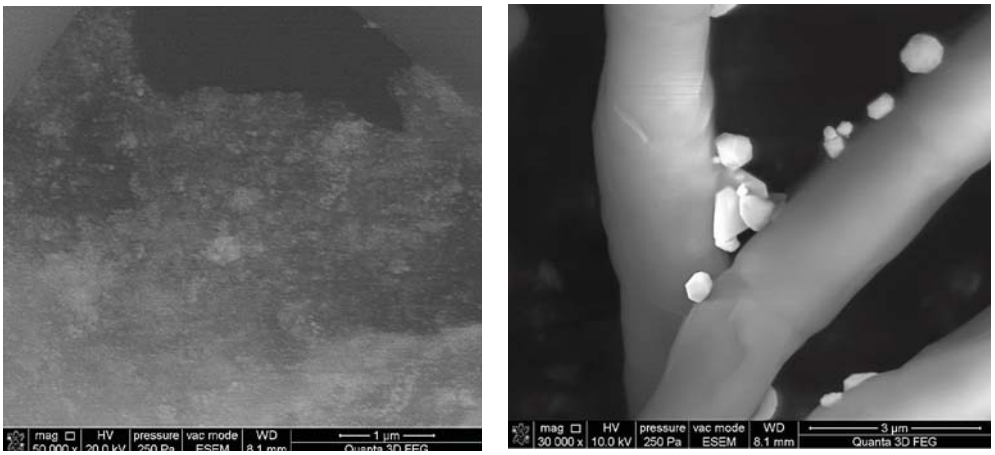

Fig. 1. SEM micrographs of *Spirulina platensis* cells exposed to $HAuCl_4$ (a) concentration 10^{-3} M; (b) concentration10^{-2}M

Effect of incubation time

Incubation time also influence nanoparticles distribution. Usually after 24 hours of the metal ion action nanoparticles are well distributed along the microorganism's surface. Large agglomerates of nanoparticles could be observed after 5−6 days of reaction. The spherical nanoparticles produced in the beginning of the reaction were stable due to the protection by sufficient biomolecules but in contrast, the crystals that were formed later on might be less stable owing to fewer protective molecules [67]. Formation of metal nanoparticles of different shapes is kinetically driven process and is a result of aggregation and rearrangement of smaller size particles, which act as nuclei for further growth into anisotropic structures [68].

As example SEM images of *Arthrobacter globiformis* 151B taken after 40 hours and 3 days of reaction with $HAuCl_4$ (10^{-3}M) are presented in Fig.2.

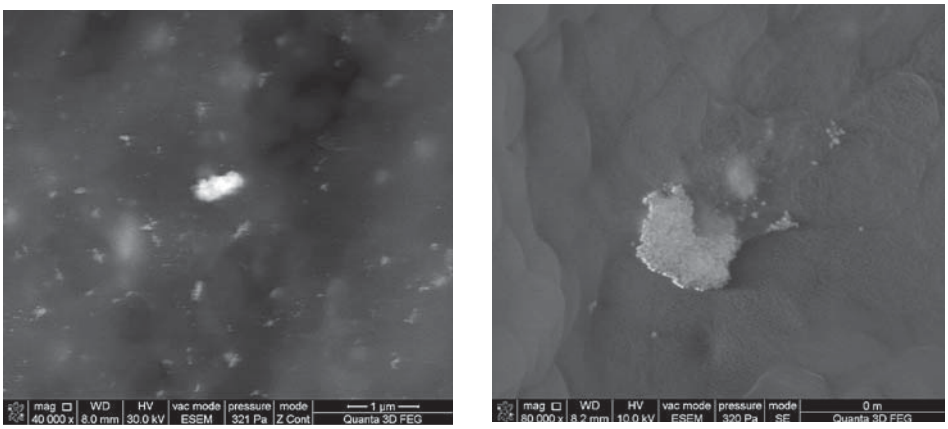

Fig. 2. SEM of A. globiformis cells (a) at 1 mM HAuCl4 for 40 hours; (b) at 1 mM HAuCl4 for 3 days

Application of nanoparticles

In the past two decades, gold nanoparticles have significant role in nanotechnology due to their potential utilisation in nanoelectronics, semiconductors, colorimetric techniques, DNA labeling [69-71], in catalysis, chemical sensing, and photonics areas, owing to their unique chemical, optical, and physical properties [2].

Gold nanoparticles can traverse through the vasculature and localize any target organ. This potentially can lead to novel therapeutic, imaging, and biomedical applications [7].

It is well-know that the polymer-gold nanoparticles composites possess the interesting electrical properties [72]. The nanocomposites of Au and biopolymer are employed as a novel biosensor [73-75]. This biosensor exhibited a fast amperometric response and wide linear range of concentrations.

Gold nanoparticles can be applied to amplify the biorecognition of the anticancer drug [76].

The silver nanoparticles have several important applications in the field of biolabelling, sensors, drug delivery system, filters [20], antimicrobial industrial processes, catalysis, mirror production, electroplating, alkaline battery production, and jewelry production [40], spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries, as optical receptors [77, 21].

Silver nanoparticles have important applications in the field of biology such as antibacterial agents and DNA sequencing.

Silver nanoparticles mainly in the range of 1 − 10 nm attach to the surface of cell membrane and drastically disturb its proper function like respiration and permeability [78].

The silver nanoparticles showed inhibition zone against *E. coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* (clinical isolate) [79].

Kim et al. reported antimicrobial activity of silver nanoparticles against *E. coli* and *S. aureus* [80].

Conclusions

The combination of biotechnology and nanotechnology created a new science nanobiotechnology. This new approach corresponds to the current scientific urge for improving the existing strategies for nanoparticle synthesis and inventing new ones. Although the ability of living cells to build up spatial structures is hardly employed in applications, there is evidence ensuring that the potential of microorganisms to produce inorganic nanoparticles will be fully exploited in technically relevant dimensions in the near future. Thus, nanotechnology promises to play an increasingly important role in many key ecological friendly technologies of the new millennium.

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A REVIEW ON BIOSORPTION OF CHROMIUM IONS BY MICROORGANISMS¶

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Abstract. Due to its widespread industrial use, chromium has become a serious pollutant in diverse environmental settings. The main source of chromium pollution including the Republic o Moldova is industry. It is a great need to develop new eco-friendly methods of chromium removal. Biosorption of heavy metals is a most promising technology involved in the removal of toxic metals from industrial waste streams and natural waters. Metal removal treatment systems using microorganisms are cheap because of the low cost of sorbent materials used and as they do not add other ions or toxic chemicals to the environment. The present review discusses hexavalent chromium biosorption properties of bacteria, microalgae and fungi, as well as adsorption isotherms (Langmiur, Freundlich, Langmiur-Freundlich) used in the evaluation of the adsorptive capacity of the biomaterial.

Keywords: chromium, biosorption, bacteria, microalgae, fungi.

Introduction

Industrial activities have led to large-scale contamination of the environment with toxic heavy metals and radionuclides [1].

Hexavalent chromium Cr(VI) is one of the most common environmental metal contaminant released primarily from industries such as leather tanning, metal plating and alloying, wood preservation [2, 3], electroplating, paint and pigment manufacturing, textile and fertilizer industries [4].

Chromium exists in 9 valence states ranging from −2 to +6. Nevertheless, only hexavalent chromium Cr (VI) and trivalent chromium Cr (III) are ecologically more important because they are the most stable oxidation forms in the natural environment [4-6].

Cr (VI) is known to be highly toxic, mutagenic and carcinogenic to living organisms including mammals. Highly water soluble Cr(VI) compounds move fairly rapidly in the subsurface and can readily enter a cell via surface anion transport systems (SO_4^{2-} channels). [2]. Cr(VI) is usually associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions [6]. Cr (III), on the other hand, is more stable and is approximately 100 times less toxic and 1000 times less mutagenic than Cr (VI) [4, 6].

Therefore there is a great need to remediate harmful Cr (VI). Though there are various physicochemical methods for the treatment of Cr (VI) such as: reduction, precipitation, ion- exchange, reverse-osmosis, electrodialysis [7], but it has been observed that these conventional methods have several disadvantages, such as high operating costs, the necessity of preliminary treatment steps, the difficulty of treating the solid waste subsequently generated, and the requirement of large quantities of chemical adsorbents [4, 8, 9].

In recent years, applying biotechnology in controlling and removing metal pollution has been paid much attention, and gradually becomes hot topic in metal pollution control practices because of its potential application. A practical and dynamic approach is the use of biological agents (microorganisms) which include bacteria, microalga, yeast, and their products [10].

Biosorption is gaining interest among researchers due to several advantages that include possibility of recovery of metal; good performance, low cost of the process [11-15]; high efficiency and selectivity for absorbing heavy metals in low concentrations, energy-saving, broad operational range of pH and temperature, easy reclamation of heavy metal and easy recycling of the biosorbent [16-19], toxicity of the metals in solution cannot affect the adsorptive function of the biomass [20], waste from the processes is readily treated and can be easily disposed by incineration. Biosorption employs inexhaustible, inexpensive and non hazardous materials [12].

Biosorption is possible with both living and non-living biomass [11]. The metal ion uptake by living and dead cells consists of two modes. The first uptake mode involves the surface binding of metal ions to cell wall and extracellular material. The second mode of metal uptake into the cell across the cell membrane is dependent on the cell metabolism and is referred to as intracellular uptake, active uptake or bioaccumulation. The first mode is common to metal adsorption

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by both living and dead cells; the second mode, which is metabolism dependent, occurs in living cells. Bioaccumulation mechanisms include precipitation, intracellular accumulation, and oxidation or reduction [10].

The main aim of this work is to summarize the results of chromium removal by microorganisms and to demonstrate the possibility of their use for solution of industrial tasks of the Republic of Moldova.

Chromium removal by microorganisms

Chromium removal by bacterial biomass

Bacteria make excellent biosorbents because of their high surface-to-volume ratios and a high content of potentially active chemosorption sites such as on teichoic acid in their cell walls. Bacteria were used as biosorbents because of their small size, their ubiquity, their ability to grow under controlled conditions, and their resilience to a wide range of environmental situations [11].

Alam et al. [10] conducted an experiment for Cr(VI) biosorption using *Exiguobacterium* sp. ZM-2, *S. maltophilia* ZA-6, *Pantoea sp*. KS-2 and *Aeromonas sp*. KS-14 and found a maximum biosorption at pH 2.5 during the first 15 min. Electron micrographs confirmed the bioaccumulation of chromium in the test bacterial isolates. Chromate sensitive isolates *Pantoea* sp. KS-2 and *Aeromonas sp.* KS-14 were not efficient chromate reducers. *Pseudomonas aeruginosa* and *Bacillus subtilis* adsorb Cr (VI) at pH 2 and temperature 32^0C [21].

Non-living biomass of *Aeromonas caviae* presents sufficient biosorption capacity for Cr(VI) anions [22]. *Chroococcus* sp. HH-11 was found to be suitable for the development of an efficient biosorbent for the removal of Cr(VI) from wastewater [23].

The greatest capacity of biosorption for Cr(VI) ions by *Pantoea* sp. TEM18. was obtained at pH 3.0. The biosorption capacity of the biomass increased first with increasing of the initial concentration of metal ions and reached a saturated value. Chromium (VI) concentration was increased from 28.9 to 245.2 mg/l approximately, the loading capacity increased from 7.81 to 53.8 mg/g of *Pantoea* sp. TEM18 [24]. *Micrococcus* sp. reported a maximum removal for Cr(VI) (90%) at pH 7.0 [25].

Cr(VI) can be efficiently removal by *Azotobacter chroococcum, Bacillus* sp. and *Pseudomonas fluorescens* [26], *S. saprophyticus* [27], *Bacillus licheniformis* [28], *E. coli* ASU 7[29], *Pseudomonas* species [30], *Acinetobacter haemolyticus* [31].

Chromium removal by microalgae

Chromium biosorption by microlgae *Phormedium bohneri*, *Oscillatoria tenuis*, *Chlamydomonas angulosa, Ulothrix tenuissima* was investigated. The maximum accumulation of Cr was shown by *Phormedium bohneri* (8550 μg/g) followed by *Oscillatoria tenuis* (7354 μg/g), *Chlamydomonas angulosa* (5325 μg/g), *Ulothrix tenuissima* (4564 μg/g), and Oscillatoria nigra (1862 μg/g); all of which demonstrated a transfer factor of >10% for Cr [32].

Adsorption of Cr(VI) by heat-dried biomass of the cyanobacterium *Phormidium laminosum* has been reported at pH 2.0 [33].

Biosorption of chromium by residual *Nannochloris oculata* after lipid extraction was investigated. Increased surface area of *N. oculata* was observed after lipid extraction. Cr(VI) removal was highest at pH 2 and it decreased with the increase in pH [34].

Travieso et al. have demonstrated better Cr removal efficiencies by *Scenedesmus acutus* than *Chlorella vulgaris* [35] *Chlorella pyrenoidosa, Spirulina maxima, Spirulina platennis, Selenastrum capriornutum* and *Scenedesmus quadricauda* [36] can be successfully used for chromium biosorption.

Chromium removal by fungi

Fungi are the organisms which are used as biosorbents for the removal of heavy metals due to the production of high yields of biomass. They grow easily under wide range of environmental situations and can also be modified genetically to produce enzymes (reductase, DNA polymerase etc.), which are helpful in higher metal removal from the wastewaters. In general, the fungal organisms are resistant to higher metal ion concentrations. A wide range of fungal species under nonliving condition have been studied by different researchers for the removal of Cr(VI) from the wastewaters [37].

Chen et al. [38] investigated the effects of pH, initial concentration, and sorption time on Cr(VI) removal by polyethylenimine (PEI)-modified *Phanerochaet chrysosporium*. The optimum pH was approximately 3.0. The maximum removal for Cr(VI) was 344.8 mg/g.

The use of the Cr-resistant fungus *Paecilomyces lilacinus* to remove Cr(VI) from two physicochemically different undiluted tannery industry effluents was studied by Sharma et al.[39]. The fungus has broad pH tolerance range and can

reduce Cr(VI) both in acidic (pH 5.5) and alkaline (pH 8.0) conditions. *Saccharomyces cerevisiae* has the ability to sorb Cr(VI) and the sorption capacity of dehydrated cells is considerably higher than that of intact cells [40]. The removal rate of Cr(VI) by biomass of marine *Aspergillus niger* was increased with a decrease in pH and an increase in Cr(VI) and biomass concentration.

A. niger exhibited the highest Cr(VI) uptake of 117.33mg/g of biomass at pH 1.0 in the presence of 400mg/l Cr (VI) at 50 ^{0}C [41].

Chromium can be successfully removed by *Aspergillus* spp [31], *Aspergillus carbonarius* NRC401121 [42], *Aspergillus* sp. [43].

Removal mechanism

The heavy metal biosorption capacity of microorganisms is good because of their large surface area and complex cell wall, which is composed of fiber-like structure and an amorphous embedding matrix of various functional groups such as carboxyl, hydroxyl, amines, phosphates sulfates, etc., which can attracts and sequester the heavy metal ions [41, 19].

In contrast to other metals, which predominantly form cationic species, Cr exists mainly in the oxyanion form (CrO_4^{2-}) and thus cannot be trapped by the anionic components of bacterial envelopes [6]. At lower pH, the negatively charged chromium species bind through electrostatic attraction to positively charged functional groups on the surface of biomass cell wall because more functional groups carrying positive charges would be exposed. As pH increased, the overall surface charge on cell walls became negative and biosorption decreased [5, 43]. Adsorption of Cr(VI) is not significant at pH values more than 6 due to dual complexation of the anions (CrO_4^{2-}, $Cr_2O_7^{2-}$ and OH^-) to be adsorbed on the surface of the adsorbents, of which OH^- predominates [7].

The Cr (VI) biosorption process included three steps as depicted in Figure 1.:

(1) the binding of anionic Cr(VI) ions with the positively charged groups present on the biomass surface;

(2) the reduction of Cr(VI) to Cr(III) by adjacent electron-donor groups;

(3) the release of the Cr(III) ions into the aqueous phase due to electronic repulsion between the positively charged groups and the Cr(III) ions, or the formation of complexes of the Cr(III) with adjacent groups capable of Cr-binding.

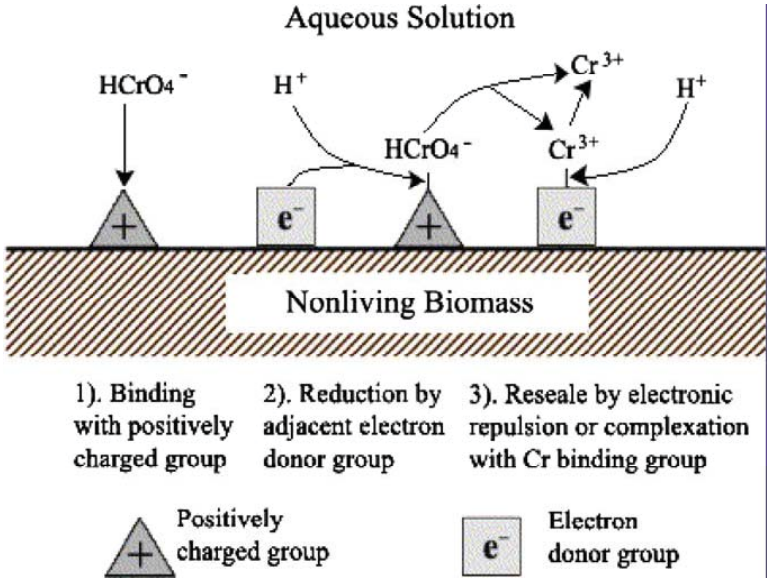

Fig. 1. Proposed mechanism of Cr(VI) biosorption by nonliving biomass [5]

Adsorption isotherms

Sorption equilibrium can be described by a number of models available in the literature [44].

Although the Langmuir and Freundlich isotherms were first introduced about 90 years ago, they still remain the two most commonly used adsorption isotherm equation. Their success undoubtedly reflect their ability to fit a wide variety of adsorption data quite well, but it may also partly reflect the appealing simplicity of the isotherm equations and the ease with which their adjustable parameters can be estimated [45, 24].

The Langmuir equation the most widely used isotherm equation for modeling adsorption equilibrium data [14, 46, 47-54]. This model is based on the assumption that the sorption energy is uniform and homogeneously distributed on the sorbent surfaces.

The Langmuir isotherm model is expressed as:

$$q = \frac{q_{max} bc}{1 + bc}$$

Here
c − is the concentration of metal ions; q_{max} − represents the maximum metal accumulation;
b − is the affinity parameter of the isotherm reflecting the high affinity of the biosorbent for the sorbate [55].

The Freundlich equation is often considered to be purely empirical in nature [50-54, 56].
The Freundlich adsorption isotherm is mathematically expressed as

$$x/m = Kc^{1/n}$$

It is also can be written as

$$\log(x/m) = \log k + (1/n)\log c$$

where
x = mass of adsorbate; m = mass of adsorbent; c = equilibrium concentration of adsorbate in solution; K and n are constants for a given adsorbate and adsorbent at a particular temperature [57].

Langmiur and Freundlich isotherms are homogeneous models. To describe adsorbtion processe in heterogeneous materials Langmuir- Freundlich (LF) model is applied [58-63].

$$q = \frac{q_{\max}(bc)^n}{1+(bc)^n}$$

Here c is the concentration of metal ions; q_{max} represents the maximum metal accumulation; b is the affinity parameter of the isotherm reflecting the high affinity of the biosorbent for the sorbate, and n is the empirical parameter that varies with the degree of heterogeneity.

Other types of adsorption isotherms are well described elsewhere [64, 18, 19, 45].

Conclusions

The present review shows that microorganisms represent an efficient and potential class of biosorbents for the removal of hexavalent chromium from industrial wastewater.

The next step of research is the assessment of the capability of microalgae *Spirulina platensis, Nostoc* paludosum and *Porphyridium cruentum* cells to remove chromium ions from wastewater of engineering company of Republic of Moldova. Elemental content of microalgae will be determined by epithermal neutron activation analysis (ENAA) at the reactor IBR-2, FLNP JINR, Dubna, Russia.

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THERMODYNAMIC STABILITY RELATIONS IN THE C-H-O SYSTEM

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In memory of Ion Vatamanu: a poet, a scholar and a man of great wisdom

Abstract. Thermodynamic stability relations in the C-H-O system are analyzed employing a recently developed approach to the stability of chemical species in multiple chemical reaction systems. The approach is based on a new quantitative definition of the overall stability of chemical species in terms of stoichiometrically unique reactions. Analytical equations for the overall stability of the species are generated and shown to be a useful and simple means to rationalize and predict the system's behavior as a function of temperature, pressure and composition.

1. Introduction

Chemical thermodynamics is an invaluable tool in determining the feasibility and optimal conditions of chemical reaction processes [1,2]. The evaluation of the equilibrium composition is now accomplished employing commercial computer software [3]. Due to a large number of independent variables that govern the equilibrium composition, however, namely, temperature, pressure and composition, it is often difficult to comprehend and rationalize the overall behavior of the system based on a large numeric output. In this respect, simple stoichiometric and graphical stability relations in complex chemical reaction systems can provide a concise picture of the expected behavior of the system as a function of the variables. Thus, the carbon deposition boundary at a given temperature and pressure may be graphically depicted employing the well-known C-H-O ternary diagram [4-6]. These diagrams, however, are still constructed based on numerical evaluation of the equilibrium compositions of the system for various values of the variables.

We have recently developed a new approach to the stability relations in multiple chemical reaction systems [7-8]. Our approach not only results in analytical expressions for stability relations of the species but, concomitantly, provides a remarkable interpretation and comprehension in terms of stoichiometric uniqueness of chemical reactions. In this contribution, we apply the overall stability approach to the analysis of the C-H-O system that is pertinent to steam and carbon dioxide reforming of methane as well as gasification of carbon.

2. Theoretical Considerations

Our overall stability approach is in fact the generalization of the fundamental thermodynamic principle according to which a single chemical reaction ρ is thermodynamically feasible (spontaneous, favorable) depending on the sign of its Gibbs free energy change, ΔG_ρ (or, affinity). That is, if ΔG_ρ is negative, the reaction should proceed from left to right, and, vice versa, if ΔG_ρ is positive, the reaction should proceed from right to left. Alternatively (and equivalently) this principle may be formulated in terms of species rather than reactions. Thus, if $\Delta G_\rho < 0$, the reactants are unstable (consumed) while the products are stable (formed). Similarly, if $\Delta G_\rho > 0$, the reactants are stable (formed) and products are unstable (consumed). It is this equivalent formulation that allows a rigorous generalization of a single reaction feasibility principle to multiple chemical reactions systems.

Below we present a succinct summary of the overall stability approach [7.8]. Let B_i (i = 1,2,…,n) be a set of chemical species involved in a multiple chemical reaction process. Let $\overline{G}_i$ (i = 1,2,…,n) be the partial Gibbs free energies of the species in an initial (before reaction) state. Let the same quantities at equilibrium (after reaction) state be $\overline{G}_i^{eq}$ (i = 1,2,…,n). For each species we define a quantitative measure of stability, $\Sigma_i = \overline{G}_i - \overline{G}_i^{eq}$ (i = 1,2,…,n), referred to as the overall stability, such that

i) species B_i is stable if $\Sigma_i < 0$
ii) species B_i is unstable if $\Sigma_i > 0$
iii) species B_i is at equilibrium if $\Sigma_i = 0$

The overall stabilities may be evaluated employing a stoichiometrically and thermodynamically constrained minimization procedure similar to that used in least square method that has been in detail presented in our previous

publications [7,8]. Here we present only the final result in terms of stoichiometrically unique response reactions (RERs) [9].

$$\Sigma_i = \frac{1}{\Delta}\sum_{\rho}\gamma_{\rho}^2 v_{\rho i}\Delta G_{\rho}\ ; i = 1,2,...,n \tag{1}$$

where

$$\Delta = \frac{1}{m}\sum_{\rho}\sum_{i=1}^{n}\gamma_{\rho}^2 v_{\rho i}^2 \tag{2}$$

and where m is the number of linearly independent reactions, $v_{\rho i}$ is the stoichiometric coefficient of species B_i in RER ρ and ΔG_{ρ} are the Gibbs free energy changes of RERs. In deriving eq 1 the convention of stoichiometrically distinct RERs has been employed, i.e., stoichiometrically equivalent RERs are considered as one RER with a lumped stoichiometric factor γ_{ρ}^2 [8].

Since Δ and γ_{ρ}^2 in eq 1 are necessarily positive quantities the sign of the overall stability Σ_i are entirely determined by the sign of the products $v_{\rho i}\Delta G_{\rho}$. This property of Σ_i allows a clear physicochemical interpretation: namely, if $\Delta G_{\rho} < 0$ then the RER ρ proceeds from the left to the right, which in turn means that the reactants ($v_{\rho i} < 0$) in this particular RER are unstable (consumed) while the products ($v_{\rho i} > 0$) are stable (formed). Similarly, if $\Delta G_{\rho} > 0$ then the RER ρ proceeds from the right to the left and, hence, the reactants ($v_{\rho i} < 0$) are stable (formed) while the products ($v_{\rho i} > 0$) are unstable (consumed). The total overall stability of a species is a sum over the RERs involving this particular species.

3. RERs in the C-H-O System

Since the overall stabilities of the species are expressed in terms of RERs we consider first the generation of a complete set of RERs along with their stoichiometric factors and Gibbs free energy changes [8]. Our starting point is the formula matrix

$$\boldsymbol{\varepsilon} = \begin{array}{c} \begin{matrix} C & H & O \end{matrix} \\ \begin{bmatrix} 1 & 4 & 0 \\ 0 & 2 & 1 \\ 1 & 0 & 1 \\ 1 & 0 & 2 \\ 0 & 2 & 0 \\ 1 & 0 & 0 \end{bmatrix} \end{array} \begin{matrix} CH_4 \\ H_2O \\ CO \\ CO_2 \\ H_2 \\ C \end{matrix}$$

Rank $\boldsymbol{\varepsilon} = 3$ and, hence, any 3 + 1 = 4 species define a RER [9]. Four species may be selected from a total of six species in 6!/4!/2! = 15 ways, i.e., the total number of RERs does not exceed 15. For instance, selecting CH_4, H_2O, CO, and, C results in the RER [8]

$$\rho\ (CH_4, H_2O, CO, C): \begin{vmatrix} 1 & 4 & 0 & CH_4 \\ 0 & 2 & 1 & H_2O \\ 1 & 0 & 1 & CO \\ 1 & 0 & 0 & C \end{vmatrix} = -2CH_4 + 4H_2O - 4CO + 6C$$

$$= 2(-CH_4 + 2H_2O - 2CO + 3C) = 0$$

with a stoichiometric factor equal to 2. Not all of the RERs, however, are stoichiometrically distinct. For instance, the following RERs are, in fact, stoichiometrically equivalent

$$\rho\ (CH_4, CO, CO_2, C): \begin{vmatrix} 1 & 4 & 0 & CH_4 \\ 1 & 0 & 1 & CO \\ 1 & 0 & 2 & CO_2 \\ 1 & 0 & 0 & C \end{vmatrix} = 4(0CH_4 - 2CO + CO_2 + C) = 0$$

$$\rho\ (H_2O, CO, CO_2, C): \begin{vmatrix} 0 & 2 & 1 & H_2O \\ 1 & 0 & 1 & CO \\ 1 & 0 & 2 & CO_2 \\ 1 & 0 & 0 & C \end{vmatrix} = -2(0H_2O - 2CO + CO_2 + C) = 0$$

$$\rho\ (H_2O, CO, CO_2, C): \begin{vmatrix} 1 & 0 & 1 & CO \\ 1 & 0 & 2 & CO_2 \\ 0 & 2 & 0 & H_2 \\ 1 & 0 & 0 & C \end{vmatrix} = -2(0H_2 - 2CO + CO_2 + C) = 0$$

As may be seen that, the stoichiometric coefficients of CH_4, H_2O and H_2 are equal to zero and, hence, all of these three RERs essentially represent the following RER

$2CO = C + CO_2$

Table 1

A Complete Set of RERs and their Stoichiometric Factors for the System C-H-O. Gibbs Free Energy Changes were Evaluated at *P* = 1 atm and Equimolar Composition of the Gas Phase Using Data from Table 2

RERs	γ_ρ^2	ΔG_ρ, kJ/mol	
		$T = 500^0$K	$T = 1200^0$K
$\rho_1 = -CH_4 - H_2O + CO + 3H_2 = 0$	1	83.15	-109.39
$\rho_2 = -CH_4 - 2H_2O + CO_2 + 4H_2 = 0$	1	63.04	-106.18
$\rho_3 = -CH_4 - CO_2 + 2CO + 2H_2 = 0$	1	103.26	-112.59
$\rho_4 = -CH_4 - 3CO_2 + 4CO + 2H_2O = 0$	1	143.49	-119.01
$\rho_5 = -H_2O - CO + CO_2 + H_2 = 0$	1	-20.11	3.20
$\rho_6 = -3C - 2H_2O + 2CO + CH_4 = 0$	1	87.61	-47.39
$\rho_7 = -2C - 2H_2O + CO_2 + CH_4 = 0$	4	10.58	8.07
$\rho_8 = -C - 2H_2 + CH_4 = 0$	6	-26.23	57.13
$\rho_9 = -C - CO_2 + 2CO = 0$	6	77.03	-55.46
$\rho_{10} = -C - H_2O + CO + H_2 = 0$	1	56.92	-52.26
$\rho_{11} = -C - 2H_2O + CO_2 + 2H_2 = 0$	1	36.81	-49.06

Upon generating all RERs and thus their stoichiometric factors, it is found that the set of stoichiometric factors may be simplified by a factor of two. For instance, the stoichiometric factors of the above three RERs are, respectively, equal to 2, 1 and 1 which gives a squared sum γ_ρ^2 equal to 6.

Table 2

Gibbs Free Energy Changes (kJ/mol) of the RERs (Table 1) in the System C-H-O for an Ideal Gas - Pure Solid Phase Model

$$\Delta G_1 = 234.36 - 0.25634\,T - \frac{4828.4}{T} + 2RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CO}x_{H_2}^3}{x_{CH_4}x_{H_2O}}$$

$$\Delta G_2 = 204.32 - 0.22699\,T - \frac{7201.2}{T} + 2RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CO_2}x_{H_2}^4}{x_{CH_4}x_{H_2O}^2}$$

$$\Delta G_3 = 264.39 - 0.28568\,T - \frac{2455.0}{T} + 2RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CO}^2x_{H_2}^2}{x_{CH_4}x_{CO_2}}$$

$$\Delta G_4 = 324.31 - 0.34431\,T + \frac{2357.7}{T} + 2RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CO}^4x_{H_2O}^2}{x_{CH_4}x_{CO_2}^3}$$

$$\Delta G_5 = -30.032 + 0.029343\,T - \frac{2373.4}{T} + RT\ln\frac{x_{CO_2}x_{H_2}}{x_{H_2O}x_{CO}}$$

$$\Delta G_6 = 178.31 - 0.17610\,T + \frac{2017.9}{T} + RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CO}^2x_{CH_4}}{x_{H_2O}^2}$$

$$\Delta G_7 = 10.724 - 0.002615\,T + \frac{581.29}{T} + RT\ln\frac{x_{CO_2}x_{CH_4}}{x_{H_2O}^2}$$

$$\Delta G_8 = -96.800 + 0.11219\,T + \frac{3891.6}{T} - RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CH_4}}{x_{H_2}^2}$$

$$\Delta G_9 = 167.59 - 0.17349\,T + \frac{1436.4}{T} + RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CO}^2}{x_{CO_2}}$$

$$\Delta G_{10} = 137.56 - 0.14415\,T - \frac{936.81}{T} + RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CO}x_{H_2}}{x_{H_2O}}$$

$$\Delta G_{11} = 107.52 - 0.11480\,T - \frac{3310.2}{T} + RT\ln\frac{P}{P_0} + RT\ln\frac{x_{CO_2}x_{H_2}^2}{x_{H_2O}^2}$$

A complete list of stoichiometrically distinct RERs and their stoichiometric factors are presented in Table 1. The Gibbs free energies of the RERs for an ideal gas and pure solid phase (graphite) model as a function of temperature, pressure and composition (mole fractions) are given in Table 2. Data were computed using the commercial software HSC Chemistry® 4.0, Outokumpu.

4. Overall Stabilities of the Species

Once the complete list of RERs, their stoichiometric factors and Gibbs free energy changes are known, the overall stabilities of the species are readily evaluated according to eqs 1 and 2. The results for the C-H-O system are summarized in Table 3.

Table 3

Overal Stabilities of the Species in the System C-H-O

$$\Sigma_{CH_4} = \frac{1}{74}\left(-\Delta G_1 - \Delta G_2 - \Delta G_3 - \Delta G_4 + \Delta G_6 + 4\Delta G_7 + 6\Delta G_8\right)$$

$$\Sigma_{H_2O} = \frac{1}{74}\left(-\Delta G_1 - 2\Delta G_2 + 2\Delta G_4 - \Delta G_5 - 2\Delta G_6 - 8\Delta G_7 - \Delta G_{10} - 2\Delta G_{11}\right)$$

$$\Sigma_{CO} = \frac{1}{74}\left(\Delta G_1 + 2\Delta G_3 + 4\Delta G_4 - \Delta G_5 + 2\Delta G_6 + 12\Delta G_9 + \Delta G_{10}\right)$$

$$\Sigma_{CO_2} = \frac{1}{74}\left(\Delta G_2 - \Delta G_3 - 3\Delta G_4 + \Delta G_5 + 4\Delta G_7 - 6\Delta G_9 + \Delta G_{11}\right)$$

$$\Sigma_{H_2} = \frac{1}{74}\left(3\Delta G_1 + 4\Delta G_2 + 2\Delta G_3 + \Delta G_5 - 12\Delta G_8 + \Delta G_{10} + 2\Delta G_{11}\right)$$

$$\Sigma_C = \frac{1}{74}\left(-3\Delta G_6 - 8\Delta G_7 - 6\Delta G_8 - 6\Delta G_9 - \Delta G_{10} - \Delta G_{11}\right)$$

Numerical evaluation of the overall stabilities of species as a function of *T*, *P* and composition allows one to determine the species that are stable (formed) or unstable (consumed). For instance, at $T = 500^0$K. $P = 1$ atm and $x_{CH_4} = x_{H_2O} = x_{CO} = x_{CO_2} = x_{H_2} = 0.2$ the overall species stabilities are: $\Sigma_{CH_4} = -4.48$, $\Sigma_{H_2O} = -5.72$, $\Sigma_{CO} = 28.91$, $\Sigma_{CO_2} = -11.60$, $\Sigma_{H_2} = 14.68$ and $\Sigma_C = -12.83$. Thus, under these conditions CH_4, H_2O, CO_2 and C are stable (formed), while H_2 and CO are unstable (consumed). At higher temperatures, e.g., $T = 1200^0$K, at the same pressure and composition, the overall species stabilities are: $\Sigma_{CH_4} = 5.48$, $\Sigma_{H_2O} = 10.87$, $\Sigma_{CO} = -27.56$, $\Sigma_{CO_2} = 8.34$, $\Sigma_{H_2} = -21.83$ and $\Sigma_C = 13.74$. Thus, under these conditions the situation is reversed, i.e., CH_4, H_2O, CO_2 and C are unstable (consumed), while H_2 and CO are stable (formed). This behavior can be easily rationalized in terms of contributions coming from RERs.

Consider, for example, the overall stability of H_2. As can be seen from Table 4, at low temperatures ($T = 500^0$K) all of the RERs involving H_2 but one are thermodynamically favored toward consumption of H_2

$CO + 3H_2 = CH_4 + H_2O$ $\quad \Delta G_1 = -83.15$ kJ/mol

$CO_2 + 4H_2 = CH_4 + 2H_2O$ $\quad \Delta G_2 = -63.04$ kJ/mol

$2CO + 2H_2 = CH_4 + CO_2$ $\quad \Delta G_3 = -103.26$ kJ/mol

$C + 2H_2 = CH_4$ $\quad \Delta G_8 = -26.23$ kJ/mol

$CO + H_2 = C + H_2O$ $\quad \Delta G_{10} = -56.92$ kJ/mol

$CO_2 + 2H_2 = C + 2H_2O$ $\quad \Delta G_{11} = -36.81$ kJ/mol

The only RER that, under these conditions, is thermodynamically favored toward the formation of H_2 is the water-gas shift reaction

$H_2O + CO = CO_2 + H_2$ $\quad \Delta G_5 = -20.11$ kJ/mol

Overall, however, the H_2 consumption reactions are dominant and, hence, under these conditions, H_2 is thermodynamically unstable (consumed). As the temperature increases ($T = 1200^0$K), the Gibbs free energies of the above reactions change sign (Table 1) thus producing H_2 via all RERs

$CH_4 + H_2O = CO + 3H_2$ $\quad \Delta G_1 = -109.39$ kJ/mol

$CH_4 + 2H_2O = CO_2 + 4H_2$ $\quad \Delta G_2 = -106.18$ kJ/mol

$CH_4 + CO_2 = 2CO + 2H_2$ $\quad \Delta G_3 = -112.59$ kJ/mol

$CH_4 = C + 2H_2$ $\quad \Delta G_8 = -57.13$ kJ/mol

$C + H_2O = CO + H_2$ $\quad \Delta G_{10} = -52.26$ kJ/mol

$C + 2H_2O = CO_2 + 2H_2$ $\quad \Delta G_{11} = -49.06$ kJ/mol

except the reverse water-gas shift reaction

$CO_2 + H_2 = H_2O + CO$ $\quad \Delta G_5 = -3.20$ kJ/mol

in which H_2 is consumed. Since H_2 producing RERs are dominant, H_2 is overall stable (formed) under these conditions.

A major problem in steam and carbon dioxide reforming is the deposition of carbon. The carbon formation region may be also easily rationalized in terms of contributions associated with RERs. Thus, employing the data presented in Table 1, it is seen that at low temperatures ($T = 500^0$K) carbon formation is thermodynamically favored in five RERs, namely

$2CO + CH_4 = 3C + 2H_2O$ $\quad \Delta G_6 = -87.61$ kJ/mol

$CO_2 + CH_4 = 2C + 2H_2O$ $\quad \Delta G_7 = -10.58$ kJ/mol

$2CO = C + CO_2$ $\quad \Delta G_9 = -77.03$ kJ/mol

$CO + H_2 = C + H_2O$ $\quad \Delta G_{10} = -56.92$ kJ/mol

$CO_2 + 2H_2 = C(gr) + 2H_2O$ $\quad \Delta G_{11} = -36.81$ kJ/mol

versus only one RER that is thermodynamically favorable toward carbon consumption

$$C(gr) + 2H_2 = CH_4 \qquad \Delta G_8 = -26.23 \text{ kJ/mol}$$

Overall, however, at low temperatures ($T = 500^0$K) the carbon formation reactions are dominant. As the temperature increases ($T = 1200^0$K), four of the RERs become thermodynamically favorable toward consumption of carbon, namely carbon gasification

$$3C + 2H_2O = 2CO + CH_4 \qquad \Delta G_6 = -47.39 \text{ kJ/mol}$$
$$C + CO_2 = 2CO \qquad \Delta G_9 = -55.46 \text{ kJ/mol}$$
$$C + H_2O = CO + H_2 \qquad \Delta G_{10} = -52.26 \text{ kJ/mol}$$
$$C + 2H_2O = CO_2 + 2H_2 \qquad \Delta G_{11} = -49.06 \text{ kJ/mol}$$

while in the two remaining RERs

$$CO_2 + CH_4 = 2C + 2H_2O \qquad \Delta G_7 = -8.07 \text{ kJ/mol}$$
$$CH_4 = C + 2H_2 \qquad \Delta G_8 = -57.13 \text{ kJ/mol}$$

carbon is formed. Overall, as can be easily verified from the stoichiometry and thermodynamics of the RERs, the carbon consuming RERs are dominant at the higher temperatures.

5. Graphical Representation of the Overall Species Stabilities

The overall stability relations may be best visualized by plotting the equilibrium lines of the overall stabilities of the species in various coordinates. As an example, consider the overall stability of carbon (graphite). Employing the relations in Tables 2 and 3, the following expression is generated for the overall stability relation for carbon (graphite)

$$\Sigma_C = -17.44 + 0.01589T - \frac{519.26}{T} - \frac{5RT}{74}\ln\frac{P}{P_0} - \frac{17RT}{74}\ln x_{CH_4} + \frac{25RT}{74}\ln x_{H_2O} - \frac{19RT}{74}\ln x_{CO} - \frac{3RT}{74}\ln x_{CO_2} + \frac{9RT}{74}\ln x_{H_2} \qquad (3)$$

The surface $\Sigma_C(T, P, x_{CH_4}, x_{H_2O}, x_{CO}, x_{CO_2}, x_{H_2}) = 0$ separates the space (T, P, $x_{CH_4}, x_{H_2O}, x_{CO}, x_{CO_2}$ and x_{H_2}) into a stable ($\Sigma_C < 0$), unstable ($\Sigma_C > 0$) and equilibrium ($\Sigma_C = 0$) field. For instance, at constant, equimolar composition of the gas phase, i.e., $x_{CH_4} = x_{H_2O} = x_{CO} = x_{CO_2} = x_{H_2} = 0.2$, the T,P carbon deposition boundary is given by

$$-17.4388 - 519.296/T + 0.016795\,T - 0.001292\,T \log P = 0$$

This line is depicted graphically in Figure 1. Further, it is evident from eq 3 that CH_4, CO and CO_2 aid in the formation of C, while H_2O and H_2 deter it, especially H_2O.

A complete graphical representation of the equilibrium surfaces (lines) for all species is, of course, not practical. It is more useful to plot regions in which the overall stability of a given species is dominant. Such type of graphical construction may be referred to as "overall predominance diagrams" and may be constructed based on the following considerations:

A) A species, say B_p, is dominant in a certain region if its overall stability Σ_p in this region is non-positive and lower than the overall stability of any other species.

b) The stability regions of two species, say B_p and B_q, are separated by a line, called predominance line, such that the overall stabilities of B_p and B_q at each point on this line are non-positive and equal, i.e., $\Sigma_p = \Sigma_q$. A predominance line separating the stability regions of two species B_p and B_q is stable if their overall stabilities on the predominance line are non-positive, equal and lower than the overall stabilities of any other species.

c) The stability regions of three species, say B_p, B_q and B_r, intersects at a point, called the triple point, such that the overall stability of the species B_p, B_q and B_r at this point are non-positive and equal, i.e., $\Sigma_{B_p} = \Sigma_{B_q} = \Sigma_{B_r}$. A triple point is stable if the overall stabilities of the species B_p, B_q and B_r at this point are non-positive, equal and lower than the overall stabilities of all other species.

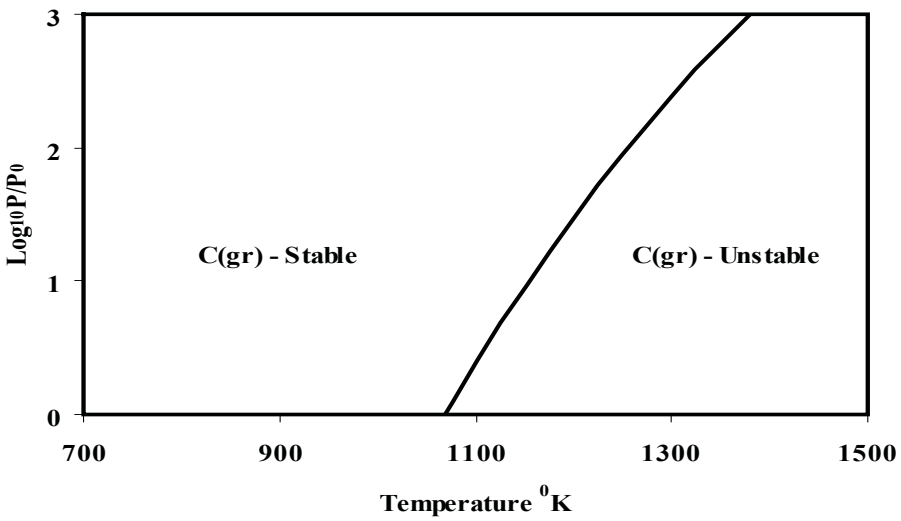

Figure 1. Overall stability boundary of C(gr) for equimolar composition of the gas phase

The easiest way to construct an overall predominance diagram is to generate first the stable triple points. Once the location of the stable triple points is known, the final topology of the stability regions of the species may be deduced by determining the stable predominance lines.

Next, we illustrate the approach by constructing an overall predominance diagram in the coordinates T - P at equimolar composition of the gas phase species. For these particular conditions, the overall species stabilities are

$$\Sigma_{CH_4} = -18.7462 + 539.057/T + 0.0239731\ T - 0.00335931\ T \log P$$
$$\Sigma_{H_2O} = -10.2556 + 338.492/T + 0.0112517\ T - 0.00180886\ T \log P$$
$$\Sigma_{CO} = 62.1143 + 298.97/T - 0.0702836\ T + 0.00749384\ T \log P$$
$$\Sigma_{CO_2} = -25.9293 - 318.731/T + 0.029516\ T - 0.00284249\ T \log P$$
$$\Sigma_{H_2} = 47.748 - 1416.61/T - 0.0591979\ T + 0.00852747\ T \log P$$
$$\Sigma_{C} = -17.4388 - 519.296/T + 0.0167945\ T - 0.00129204\ T \log P$$

For the CH_4(g) - H_2O(g) - CO(g) - CO_2(g) - H_2(g) - C(gr) system the number of possible triple points is equal to the number of ways three species may be selected from a total of six, i.e., 6!/3!/3! = 20. Consider, for instance, the position of the triple point CH_4 - H_2O - CO_2. Solving simultaneously the equations $\Sigma_{CH_4} = \Sigma_{H_2O} = \Sigma_{CO_2}$ gives T = 1097.2^{0}K and log P = 3.3. The overall stabilities of the species at this point are

Σ_{CH_4} = -4.2 kJ/mol Σ_{CO_2} = -4.2 kJ/mol
Σ_{H_2O} = -4.2 kJ/mol Σ_{H_2} = 12.6 kJ/mol
Σ_{CO} = 12.6 kJ/mol Σ_{C} = -4.2 kJ/mol

As can be seen, it so happens that the overall stabilities of four species CH_4, H_2O, CO_2 and C(gr) are equal, negative and lower than the overall stabilities of the remaining two species at this point. In fact, this means that four triple points, namely, CH_4 - H_2O - CO_2, CH_4 - H_2O - C(gr), CH_4 - CO_2 - C(gr) and H_2O - CO_2 - C(gr) coincide and all of them are stable.

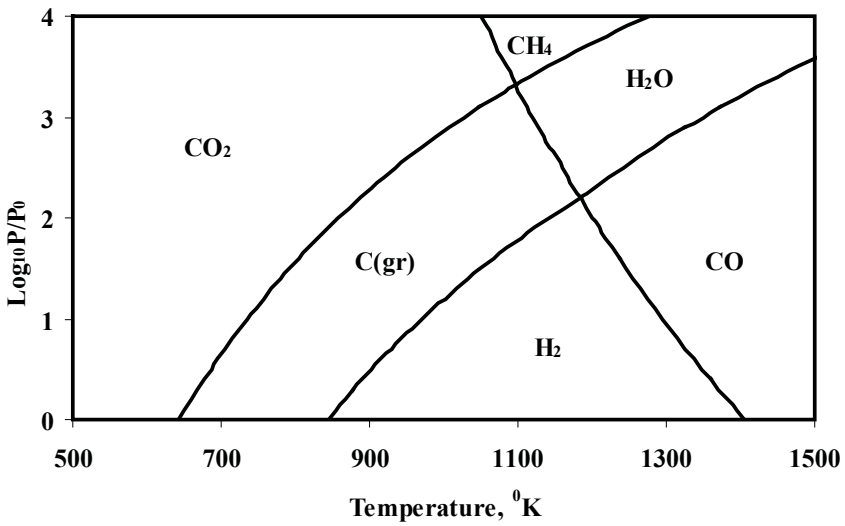

Figure 2. Overall stability predominance diagram for the system C-H-O for equimolar composition of the gas phase

Consider next the triple point CO - CO_2 - C(gr). The solution of $\Sigma_{CO} = \Sigma_{CO_2} = \Sigma_{C(gr)}$ is $T = 2157.9$ and log $P = 5.7$. At this T and P the overall stabilities of the species are

Σ_{CH_4} = -8.0 kJ/mol $\quad$ Σ_{CO_2} = 2.7 kJ/mol

Σ_{H_2O} = -8.0 kJ/mol $\quad$ Σ_{H_2} = 24.1 kJ/mol

Σ_{CO} = 2.7 kJ/mol $\quad$ Σ_{C} = 2.7 kJ/mol

Since the overall stabilities of CO, CO_2 and C(gr) are positive, this triple point is unstable and should not appear on the diagram. Continuing the above procedure over the remaining triple points shows that four more triple points, namely, H_2O - CO - H_2, H_2O - CO - C(gr), H_2O - H_2 - C(gr) and CO - H_2 - C(gr), are stable and they coincide. Hence, only 2 distinct intersections should appear in the predominance diagram. The final overall predominance diagram is presented in Figure 2.

6. Concluding Remarks

The equilibrium composition of a multiple chemical reaction system is a complex function of the thermodynamic variables. Except for the case of a single chemical reaction system, the prediction and rationalization of the system's response to a change in variables may be obtained only via numerical simulation of the equilibrium composition which is difficult to interpret. In this respect, the overall species stability approach in multiple chemical reaction systems discussed above may be viewed as the generalization of the simple thermodynamic principle valid for a single chemical reaction system. Thus, the overall stabilities of the species in a multiple chemical reaction system may be partitioned into a linear sum of contributions associated with distinct RERs, such that each individual term retains the same mathematical form as that valid for a single reaction system. In this work, we applied this new approach to the analysis of stability relations in the C-H-O system. The overall stabilities of the species generated here are shown to be useful in predicting and rationalizing the system's behavior as a function of pressure, temperature and composition.

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ION VATAMANU

Doctor of Chemistry, Head of the Analytical Chemistry Department
(75 years after his birth)

Ion Vatamanu was a chemist, writer and public figure. He was equally passionate about both his chosen fields of activity: chemistry and poetry. Chemistry, with its perfect equilibrium of logic and precision, provided inspiration for lyrical creativity, whereas poetry writing enlivened his imagination and passion for chemistry. He loved his parents. He adored his wife Elena, whom he often gifted a sea of flowers. He loved his daughters Mihaela, Mariana, and Leontina. He loved life, and he loved people...

Ion Vatamanu was born on May 1, 1937 in the village of Costiceni of the Hotin County (now in the Chernivtsi Oblast, Ukraine). Following school graduation, he worked for one year as an assistant in the laboratory of chemistry of the local school. After that he became a student at the Department of Chemistry of the Chişinău State University (now the Moldova State University). Having obtained his college degree, Ion Vatamanu started his scientific career in the Analytical Chemistry Laboratory of the Institute of Chemistry of the Academy of Sciences of the Republic of Moldova, led by Professor Yuri Lyalikov. His research concerned the oscillographic polarography with linear variation of the electrode potential. The oscillographic polarography is a type of voltammetry that uses a dropping mercury electrode with oscillographic scanning of the applied potential. This technique is usually employed for measuring the concentration of electroactive species in solutions. An increased interest toward this method is explained by its great possibilities in the study of kinetics of electrochemical processes, such as high sensitivity and high resolution capability. The use of compounds able to form complexes with metal ions as background in the oscillographic polarography allows for a capacity augmentation in method resolution, an adjustment of mutual influence of ions, and facilitates the difficult task of analyzing mixtures of substances.

Dr. Vatamanu studied the composition and stability of complexes of metal ions with a series of organic and inorganic ligands, as well as the kinetics of their discharge on the electrode. Reversible reduction of complexes results in oscillographic characteristics based on the same theoretical principles as those used in classical polarography. A more difficult task consists in studying complexes that are discharged irreversibly on the electrode. Lack of methods for mathematical interpretation of experimental data precluded the use of oscillographic polarography for that purpose.

Composition and stability of predominant complexes in solution, as well as kinetics of discharge of complex species on the electrode can be characterized on the basis of the exchange current, equilibrium potential of reaction, and transfer coefficients α and β. These parameters were also necessary for approving the choice of complexation compound for analysis of metal ions that reduced irreversibly.

Ion Vatamanu proposed a set of mathematical expressions that employed experimental data for determination of the exchange current rate and equilibrium potentials. The deduced equations were then used to study the behavior of complexes in solution and kinetics of their discharge on the electrode. This method was employed to successfully characterize a wide series of reversibly reducing complexes of Bi^{3+} with chlorides, trioxyglutaric acid, tartrates etc., as well as compounds whose discharge on the electrode was irreversible, such as Zn^{2+} and Sb^{3+} complexes with ammonia and a series of organic acids.

For exact measurement of potentials, Ion Vatamanu developed a tandem instrument ensuring an accuracy of 1 to 2 mV. To experimentally verify the derived equations for determination of equilibrium potentials and exchange current, a novel and original construction of the universal cell was proposed. It allowed for simultaneous oscillo-polarographic determination, in the same solution, of small quantities of substances with accumulation on the mercury stationary electrode, and large amounts of compounds on the hanging mercury drop electrode.

The experimentally obtained values of equilibrium potentials were compared with data measured by other methods. It was proved that equilibrium potentials calculated through the use of equations proposed by Ion Vatamanu correlated satisfactorily with the most accurate results obtained by the thermodynamic method and differed from those by only $\pm$ 6 mV.

The research performed under the mentorship of Dr. E. Chykryzova was finalized in the PhD dissertation "The oscillographic study of complexes of Bi^{3+}, Zn^{2+} and Sb^{3+} with various ligands and their use in analysis", which Vatamanu successfully defended at the Department of Chemistry of the National University of Lviv (Ukraine) in 1971. Later, several PhD students of Professor A. Komlev (T. Vrublevsky, V. Marina and others) from Lviv University came to the Institute of Chemistry of the Academy of Sciences of the Republic of Moldova to train and study the

methods for calculating equilibrium potentials and exchange current, which they applied for data collection in their own research.

When studying the process of bismuth complex formation with trioxyglutaric acid (pH 2-4), it was observed that the oscillo-polarogram had the shape of an isosceles triangle. Such a shape is characteristic for processes of high complexity due to adsorption of depolarizator on the electrode. These processes can be influenced by a variety of experimental parameters.

Analytical application of phenomena of complex reduction from the adsorptive state opens the possibility for a significant increase in sensibility of polarographic identification of various metal ions, whereas the use of tensioactive compounds allows for individual detection of metals with comparable reduction potentials.

The use of tetrabutylammonia (as an inhibitor) and iodide ion (as an accelerator) for the polarographic determination of cadmium in the presence of palladium and indium, as well as lead in the presence of tin by I. Vatamanu and his co-workers (V. Mereanu, B. Pintilie, I. Grama and others) showed that tetrabutylammonia indeed inhibited the discharge of Cd, Pb, Sn, Pd and In, and the iodide ion is suitable as an accelerator for the detection of some metals in the presence of others. The system cadmium–lead–iodide ion is characterized by anion-induced adsorption, resulting in an accelerated discharge of cadmium and lead. At the same time, this acceleration is quantitative in nature, which allowed for the development of a quick, simple and sensitive method of determination of a series of metals in the presence of large amounts of other metals. These principles were employed in developing methods for determination of Pb in clays and limestone, and of Bi in the semiconductor system Bi-Sn, as well as of Bi and Pb in copper alloys. Dr. Vatamanu and colleagues contributed significantly to the development of polarographic and other electrochemical methods of analysis and proposed principles and concepts that led to substantial advances in analytical chemistry. Polarography (especially the oscillographic and alternative current versions) as a research method holds a distinctive place among electrochemical methods. Currently, polarographists are focused on the task of developing methods of analysis for difficult technical objects and implementing the newest experimental advances at the industrial scale.

Ion Vatamanu directed the collaborators of the Laboratory of Analytical Chemistry (V. Merean, L. Kopansky, L. Chiriac, I. Grama, B. Pintilie and others) to perform a series of important research studies of electrode adsorption processes involving numerous metal ions - As, Bi, Sn, Pb, etc. with organic ligands. The contribution of adsorption within the general electrode process was also estimated. Valuable information regarding the kinetics of discharge of adsorbed complex species was thus obtained. Selection of ligands favoring metal ion complex formation also contributed to the development of analytical methods of high sensitivity and selectivity. These methods were applied for analysis and accreditation of standards for heavy metal content in alloys and samples on the basis of Ni, Zn, Cu, and were summarized in the monograph “Polarography in the analysis of samples of heavy metals and alloys”. They found practical application in many plants of the former USSR for quality control of raw material and initial production. Drs. Ion Vatamanu and Ilie Fishtik studied a variety of complexes whose electrochemical reduction is characterized by prevalent adsorption processes. Experimental conditions and measurements were optimized in order to accelerate or inhibit surface processes. This research direction was based on a novel principle in polarography and opened up new perspectives in electro-analytical chemistry.

Theoretical studies conducted by Dr. Vatamanu and Dr. Fishtik in collaboration with colleagues at the Institute of Electrochemistry of the Academy of Sciences of former USSR aimed at developing new and diverse experimental models that would predict and closely approach real systems. These studies showed great promise in explaining the nature of highly complex processes. The expression for the isotherm of adsorption and chemical potential with the possibility of calculating different configurations of the binary complexes in the compact layer, as well as the expression for the electrostatic potential of chemically adsorbed particles were derived through this particular project. Ion Vatamanu had an essential contribution in the development of theoretical methods intended for optimization of conditions for performing chemical analysis (with Dr. I. Fishtik and Dr. I. Povar).

The theoretical group of researchers proposed a different approach to the problem of complex chemical equilibria. By introducing the definition of generalized equation of the reaction, Drs. Fishtik, Povar and Vatamanu managed to solve a series of problems of chemical thermodynamics as well as a number of problems in applied analytical chemistry. They proposed the use of change in Gibbs energy in real conditions instead of traditional approaches for calculation of complex chemical equilibria in solutions in the presence of solid phase. Besides a simplified calculation procedure, this method determined thermodynamic probabilities for chemical processes in solution to occur in the expected direction. The thermodynamic formulae for other functions such as enthalpy, entropy and heat capacity were also deducted.

The results of these studies were generalized in a monograph (written with Dr. Ilie Fishtik) and were presented in a PhD thesis (by Igor Povar). During the last years of his scientific activity Ion Vatamanu applied quantomechanical calculations (in collaboration with Dr. Tudor Spataru) to estimate the composition of complexes formed in solution and discharged on the electrode.

The research accomplishments of Dr. Ion Vatamanu were published in prestigious international scientific journals. Our narrative is only a brief summary of his many important contributions to analytical chemistry. His scientific legacy and vision is kept alive through the research successfully conducted by the collaborators of the Laboratory of Analytical Chemistry, now the Laboratory of Physicochemical Methods of Analysis and Research.

Dr. hab. I. Povar, Dr. L. Chiriac and Dr. T. Cazac

Ion Vatamanu (1937-1993)

In memoriam Ion Vatamanu

(Commemorating the 75th birth anniversary)

"I have studied the valences of the world: the bound and the unbound,/ The limiting and the endless/ Captured in the circular bond of thought" [1] – these verses represent a confluence of *Ars Poetica* and the scientific method, a relentless and impassioned quest for knowledge through the exploration of metaphor. To the uninitiated, scientific research may be synonymous with painstaking drudgery in the confines of the laboratory, somewhat detrimenting creativity and intellectual fulfillment. Albert Einstein once said: "Imagination is more important than knowledge. For knowledge is limited to all we now know and understand, while imagination embraces the entire world, and all there ever will be to know and understand". Ion Vatamanu's afore quoted poem similarly invokes the universal valences of creativity. It also reveals the quintessence of his own personality: a true thinker and Renaissance man.

Ion Vatamanu's destiny would be marked by an indissoluble bond to the history of his motherland. He was born on May 1, 1937 in the village of Costiceni, Hotin County, in the Kingdom of Romania. The heavy reality of traumatically established post-World War II borders would be etched deeply in Ion Vatamanu's childhood. The beautiful and breathtaking landscapes of Bucovina and the nurturing love of his family – Ioan and Maria Vatamanu – molded the personality of the future scientist and poet. Following high school and a brief teaching job, the young Vatamanu enrolled at Moldova State University in Chişinău where he received his bachelor's degree in Chemistry in 1960. In the same year, Ion married Elena Curicheru, whose friendship and love would be a haven and muse for his creativity throughout his life. The journey from Bucovina to Moldova, from homeland to homeland, produced the literary first snow (*"Primii Fulgi"*, debut volume, 1962).

Fresh out of college, Ion Vatamanu dedicated himself to scientific research. In 1971 he successfully defended his doctoral thesis "Oscillopolarografic investigation of bismuth, zinc, and antimony complexes and their applications in analytical chemistry" at Lviv University, Ukraine. In 1973 he was appointed principal investigator and laboratory head at the Institute of Chemistry of the Academy of Sciences of the Republic of Moldova. As a chemist, Ion Vatamanu authored and co-authored over 150 scientific publications in analytical chemistry. His research yielded observations of practical value and earned him five patents on oscillopolarographic applications (1980-1989), whose underlying methodologies were thereafter implemented at industrial scale. In collaboration with his colleagues, Ion Vatamanu published several analytical chemistry books (for a summary, see [2]). In 1988 he co-authored (with Dr. Ilie Fiştic) the monograph "The thermodynamics of metal ion hydrolysis".

In scientific circles, Ion Vatamanu was thus known for his accomplishments in analytical chemistry, yet he also emerged as a distinct literary voice. Chemistry and poetry intertwine in an inseparable duality in Ion Vatamanu's life, not unlike the wave-particle nature of light. From inception, Vatamanu's poetry would irreversibly change Chişinău's literary landscape, inducing a paradigm shift. His poems transpose the reader to a universe of multifaceted emotion, with titles crafted in inspiration that encourage lyrical and philosophical reflection. In an eloquent literary analysis, the academician Mihai Cimpoi praised the genuinely innovative spirit of Ion Vatamanu's poetry (Foreword to *"Altă iubire nu este"*, Biodova, 2001). The numerous poetry books and literary essays that Ion Vatamanu authored over the years have become an integral part of the national cultural patrimony. The esthetics of his works continues to provide literary critics and theorists with venues for exploration.

This portrait into Ion Vatamanu's life would prove incomplete with no mention of his journalistic work – during 1989-1991 he led, along with writer Leonida Lari, the newspaper *"Glasul"*, the first post-World War II publication in the Republic of Moldova to use the Latin alphabet, and in 1991-1993 he was the director of the journal *"Columna"*. Additionally, he translated poems by American, Greek, Latvian, Lithuanian, Russian, and Ukrainian authors. As a member of the first Parliament of the Republic of Moldova (1990-1993), within which he also acted as head of the

Parliamentary Commission for Culture and Religion, he participated in numerous events of historical note, including the signing of the Declaration of Independence of the Republic of Moldova. His talent as an orator was on full display during the Great National Assembly in Chişinău, wherein he delivered vibrant patriotic speeches. Ion Vatamanu's unexpected death on August 9, 1993 would be an early end for his creative endeavors. Reaching into my own memories, I try to recall how I got to know Ion Vatamanu. My parents' passion for literature introduced me to his poems at a young age, and I enjoyed revisiting his unmistakable metaphors in my classes as well. I remember, as an undergraduate at Moldova State University, a particularly inspiring talk-show conversation between Ion Vatamanu and the chemistry and philology students making up the invitees, covering topics ranging from chemistry to poetry and philosophy. Later on, as I started my research career as a chemist, I had the pleasure of knowing Ion Vatamanu during his tenure at the Institute of Chemistry of the Academy of Sciences of the Republic of Moldova. I have always admired his imagination, eloquence, honesty, and passion for knowledge, while our shared intellectual interests gave rise to a great lifelong friendship.

A dreamer in his creative solitude, an objective and lucid analyst of history and contemporaneity, an energetic and decisive leader with an uncanny ability for crisis management – all these describe Ion Vatamanu. His wife Elena and daughters Mihaela, Mariana, Leontina treasure a personal universe in which the magical spark of Ion Vatamanu's love and joy of life meld the everyday in and out of poetry. Ion Vatamanu's instantaneous connection to the audiences and deeply felt words still touch the hearts of his many colleagues and friends. But perhaps the most vivid and overwhelming memory is captured in the title of the documentary produced by his daughter Leontina, *"Dor de Ion Vatamanu"* [3]. This succinct metaphor encompasses all the love and gratitude with which Ion Vatamanu will forever be remembered by all those who had the privilege to have known him.

Dr. Sergiu Petru Palii
University of Florida, USA
May 1, 2012

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[1]. Original Romanian text: *"Am studiat valenţele lumii: ceea ce se leagă/ şi ceea ce nu se leagă,/ Mărginirea şi nemărginirea/ Într-o legătură rotundă a gândirii"* (Ion Vatamanu, *"Cuvinte de cretă"*).

[2]. Tudor Lupascu, Pavel F. Vlad, Aculina Aricu, Maria Cocu. Achievements of the Institute of Chemistry of the Academy of Sciences of Moldova at 50 years anniversary, Chemistry Journal of Moldova, 2009, 4 (1), 8-16.

[3]. Documentary *"Dor de Ion Vatamanu"* directed by Leontina Vatamanu. OWH TV Studio, Moldova, 2007. The Romanian word *"dor"* evokes a combination of longing, love, and sadness. It is both a concept and a metaphor and thus eludes adequate translation.

PECULARITIES OF PHOSPHORUS FORMS DYNAMICS IN BOTTOM SEDIMENTS OF RIVER DNIESTER (MOLDOVA)¶

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Abstract. Seasonal, spatial and multi-annual dynamics of phosphorus forms in bottom sediments and their interstitial water for river Dniester was evaluated. The spatial dynamics of phosphorus forms in the bottom sediments along river had, in general, the same trend during of 2004 and 2009 years, being recorded the increasing tendency along river Dniester for inorganic phosphorus and decreasing tendency for organic phosphorus. Multi-annual dynamics for 2004-2010 years showed a tendency of increasing of all phosphorus forms in bottom sediments; the highest values being registered during 2010 year. Spatial and seasonal dynamics of phosphorus forms in bottom sediments and their interstitial water were generally in correspondence, although sometimes with some differences.

Keywords: bottom sediments, interstitial water, and phosphorus forms.

Introduction

Phosphorus concentrations in rivers result from both external inputs and internal loading from the bottom sediments [1]. Its chemistry in sediments is greatly influenced by the oxidation-reduction status (redox potential) [2]. Under oxidized conditions, ferric and manganese oxides and hydroxides are important adsorption sites for phosphorus; however, under reducing conditions these minerals are unstable. Mobilization-immobilization processes on the particle surface of sediments occur through interstitial water and its participation [3]. During the desorption process from sediments, phosphorus compounds are accumulated in interstitial water, then according to environmental factors (pH, oxidation-reduction potential Eh, ionic strength, or water mineralization etc.) can be immobilized in the water horizon overlying the bottom sediments. Reverse process, the immobilization from water in sediments, also occurs through interstitial water. Concentrations of phosphorus compounds in the water horizon overlying the bottom sediments and in interstitial water of bottom sediments can vary greatly, and the direction of mobilization-immobilization processes determines pollution - self-purification processes of water bodies.

The objectives of this paper were (i) to evaluate peculiarities of spatial and seasonal dynamics of phosphorus forms in bottom sediments from the Dniester River during 2004, 2009 and 2010, (ii) to perform comparative analysis of phosphorus forms contents in interstitial water and bottom sediments, and (iii) to establish the desorbed amount of phosphorus forms during re-suspension of bottom sediments performed in field and lab conditions.

Case study

Samples of bottom sediments were collected during the spring and summer of 2004, 2009 and 2010 years along river Dniester (sites Oxentia, Malovata, Vadul lui Voda, Fig. 1).

According to World Health Organization (WHO), phosphorus compounds occurred in natural waters are classified into 12 phosphorus forms (Fig. 2), by chemical type – (i) orthophosphates, (ii) acid hydrolysable phosphates (poly- and pyrophosphates), (iii) organic phosphorus, (iv) total content, and by physical state – (i) dissolved (filterable), (ii) particulate, (iii) total content [4]. Additionally, this scheme was tested for estimation of phosphorus content in bottom sediments being determined (i) inorganic phosphorus (orthophosphate plus condensed forms - polyphosphates and pyrophosphates), (ii) organic-phosphorus and (iii) the total amount of phosphorus [5].

In order to determine content of inorganic and organic phosphorus compounds in the bottom sediments, fresh (wet) samples were used. Scheme for determination of phosphorus forms in bottom sediments presents a summary of existing methods being determined (i) inorganic phosphorus (orthophosphate plus condensed forms), (ii) organic phosphorus and (iii) the total amount of phosphorus in sediments. The amount of total phosphorus was determined according to U.S. Geological Agency [6]. The content of inorganic phosphorus was determined according to WHO methods for inorganic particulate phosphorus [4]. The amount of organic phosphorus was obtained by subtracting inorganic phosphorus from the amount of total phosphorus.

Content of phosphorus forms in interstitial water was determined after centrifugation of fresh (wet) sediments. There were established amount of orthophosphates, condensed and organic phosphorus according to World Health Organization recommendations [4].

Re-suspension was performed in natural conditions by „aquarium" method [7]. To perform desorption from sediments in lab condition, fresh (wet) samples were stirred in a portion of distilled water for 2 hours.

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Fig. 1. Hydrographical basin of Dniester River. Sampling sites - Oxentia, Malovata, Vadul lui Voda

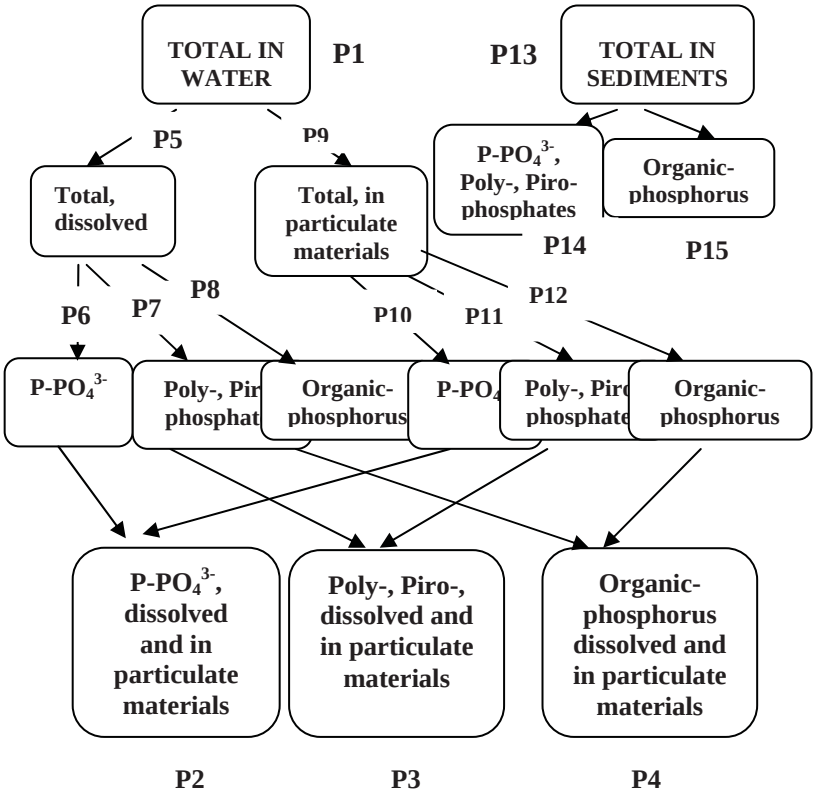

Fig. 2. Phosphorus forms in natural waters for the entirely system "water-particulate materials-bottom sediments". Scheme for analysis of the phosphorus forms in water and particulate materials according to World Health Organization classification (forms P1-P12, [4]). Supplemented scheme for analysis of the phosphorus forms in sediments (forms P13-P15, [5])

Results and discussion

The spatial dynamics of phosphorus forms in the bottom sediments during summer of 2004 and 2009 years had, in general, the same trend (Fig. 3). Thus, the content of inorganic phosphorus (Pinorg) had been increased in bottom sediments along Dniester River. The content of organic phosphorus (Porg) had been decreased along the river, the highest values of their content being recorded in bottom sediments of Dubasari Lake (sites Oxentia and Malovata).

Dynamics of the content of phosphorus forms in the bottom sediments was different during the years. Multi-annual dynamics of inorganic (Pinorg) and organic (Porg) phosphorus forms in the bottom sediments is presented in figure 4. Thus, for Vadul lui Voda location (site) the tendency of increasing of all phosphorus forms in bottom sediments was characteristic. The ratio of Pinorg : Porg forms in bottom sediments was not homogeneous during these years (Fig. 5), although more frequently the percentage of inorganic phosphorus (Pinorg) prevailed over organic (Porg) phosphorus.

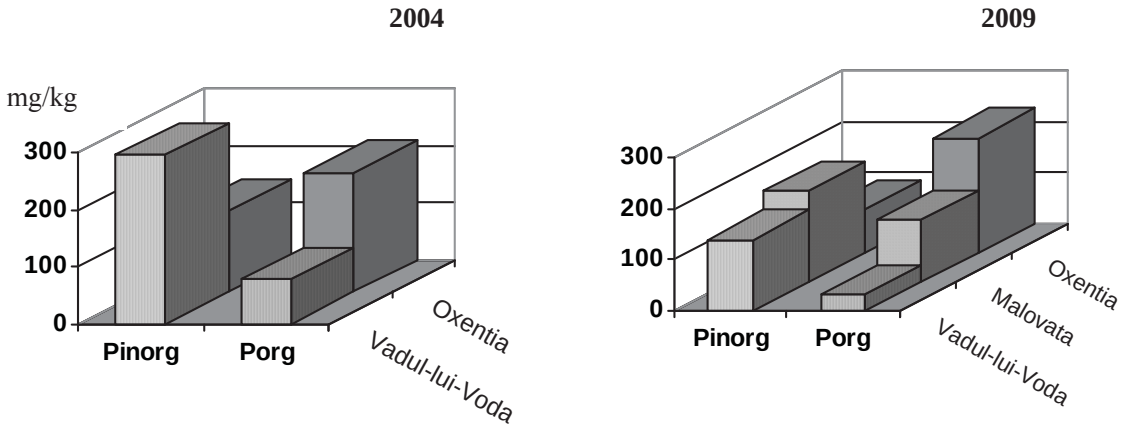

Fig. 3. Spatial dynamics of inorganic (P inorg) and organic (P org) phosphorus forms in the bottom sediments along river Dniester during of 2004 and 2009 years (summer)

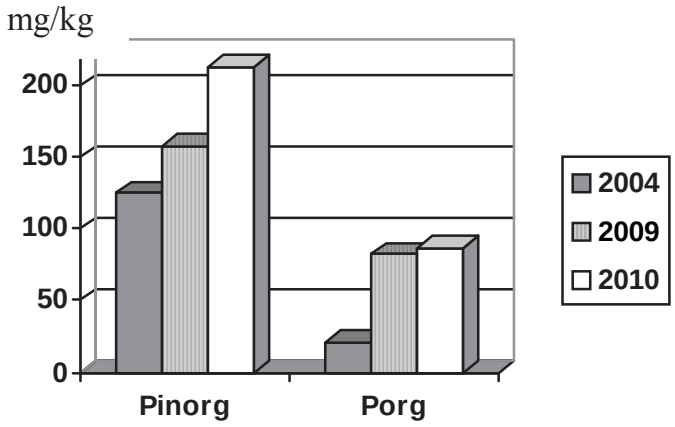

Fig. 4. Multi-annual dynamics of inorganic (P inorg) and organic (P org) phosphorus forms in the bottom sediments during of 2004, 2009 and 2010 years (site Vadul lui Voda, spring)

The seasonal dynamics was also less distinct. The content of inorganic phosphorus for sediments sampled in lake Dubasari (site Oxentia) decreased and organic phosphorus increased from spring to summer during researched years (Fig. 5). For bottom sediments from Vadul lui Voda site the dynamics of these forms was rather somewhat inverted.

Spatial dynamics of phosphorus forms in interstitial water was different as compared for bottom sediments. During summer of 2009 year the content of inorganic phosphorus decreased, while the content of organic phosphorus increased in interstitial water along Dniester (Fig. 6). For Vadul-lui-Voda site, seasonal dynamics of phosphorus forms in bottom sediments and their interstitial water was generally in correspondence (Fig. 7). As whole, the tendency of increasing of inorganic phosphorus in interstitial water was registered from the spring to summer (Fig. 8).

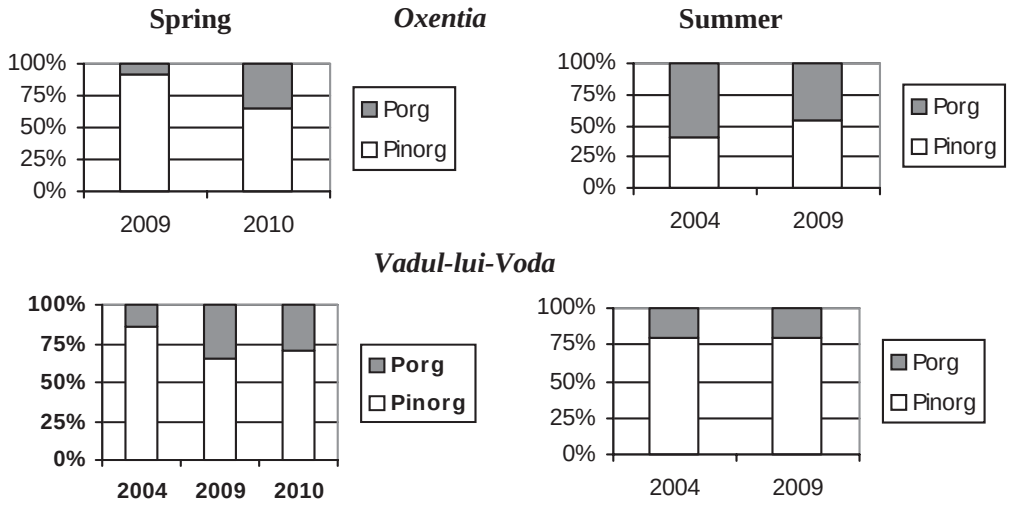

Fig. 5. Proportions of inorganic (P inorg) and organic (P org) phosphorus in bottom sediments along river Dniester during spring and summer of 2004, 2009 and 2010 years

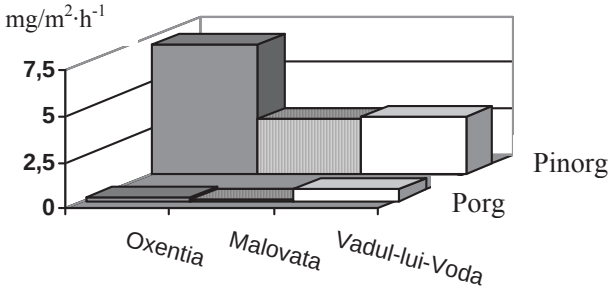

Fig. 6. Spatial dynamics of phosphorus forms (inorganic and organic) in interstitial water of sediments during of summer of 2009 year. Data computed per layer of sediments with 5 cm of thickness (h) and an area (S) of $1m^2$

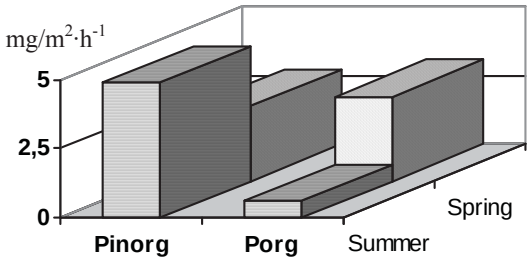

Fig. 7. Seasonal dynamics of phosphorus forms (inorganic and organic) in interstitial water of sediments during of 2009 year (Vadul-lui-Voda site). Data computed per layer of sediments with 5 cm of thickness (h) and an area (S) of $1m^2$.

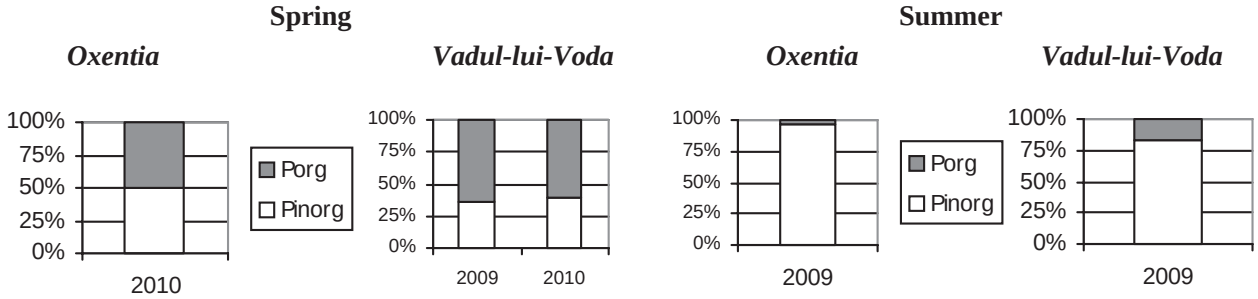

Fig. 8. Proportions of inorganic (P inorg) and organic (P org) phosphorus in interstitial water of bottom sediments along river Dniester during of spring and summer of 2009 and 2010 years

During the turbulent moments (e.g. winds), the bottom sediments are re-suspended in water horizon above sediments, i.e. the re-suspension phenomena (RES) takes place. In such conditions the content of phosphorus orthophosphate in water layer (W) above sediments can increase for about 3,1 times, compared with its content in quiet conditions (Fig. 9). The content of poly- and pyrophosphates may increase for about 1,4 times, which constitutes about 43% from its content in interstitial water (IW).

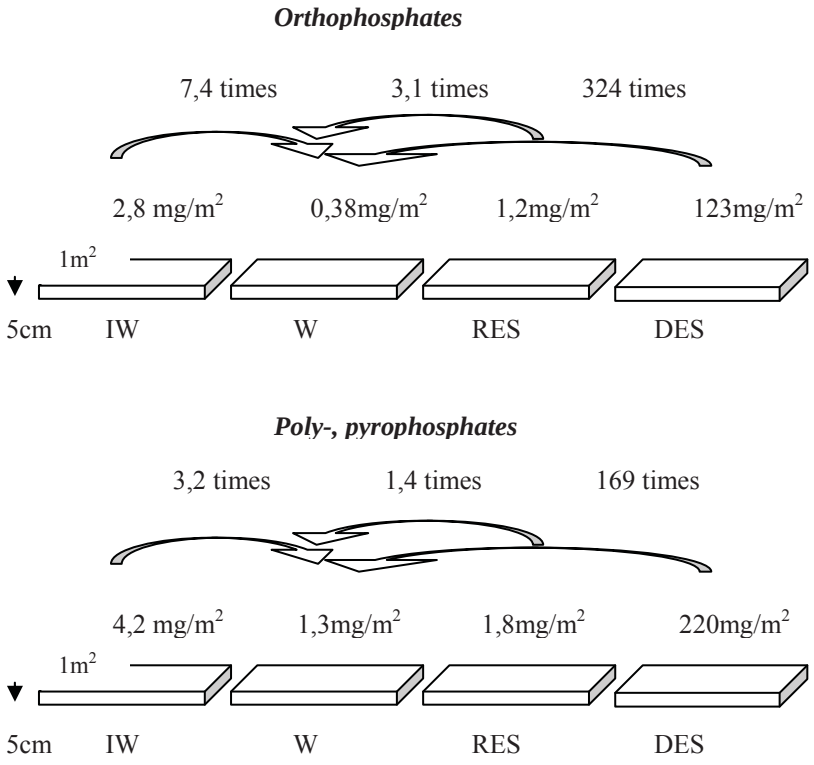

Fig. 9. Phosphorus forms dynamics in water (W) and interstitial water (IW) of sediments along Dniester River during of summer of 2009 year (site Oxentia). Phosphorus mobilization during the re-suspension of sediments in field conditions (RES) and desorbed amounts (DES) from sediments in lab conditions. Data computed per layer of sediments with 5 cm of thickness (h) and an area (S) of $1m^2$

Lab modeling of re-suspension of bottom sediments shows that considerable amount of phosphorus is desorbed from bottom sediments (Tab.). Results presented in the table demonstrate that under such conditions about 1,5-29% from total quantity of inorganic phosphorus and 0,2-27% from total quantity of organic phosphorus can be desorbed from sediments.

Table

Phosphorus amount desorbed from bottom sediments of the Dniester River (lab modeling).

Site	P inorganic desorbed,%	P organic desorbed,%
Spring		
2004		
Vadul lui Voda	29	-
2009		
Oxentia	1,5	19
Vadul lui Voda	3,4	3,8
Summer		
2004		
Oxentia	22	0,5
Vadul lui Voda	16	27
2009		
Oxentia	8	0,2
Malovata	6,6	0,3
Vadul lui Voda	3,6	0,6

Conclusions

The spatial dynamics of phosphorus forms in the bottom sediments had, in general, the same trend during summer of 2004 and 2009 years, being recorded along river the increasing tendency for inorganic phosphorus and decreasing

tendency for organic phosphorus. Multi-annual dynamics for 2004-2010 years showed a tendency of increasing of all phosphorus forms in bottom sediments, the highest values being registered during 2010 year. Spatial and seasonal dynamics of phosphorus forms in bottom sediments and their interstitial water were generally in correspondence, although sometimes with some differences. The results suggest that during of re-suspension the bottom sediments can become a relevant source of phosphorus, being mobilized significant amounts of phosphorus in water horizons above the sediments.

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