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# New Adamantane Analogues - Synthesis And Antiviral Activity

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**Abstract:** A series of adamantane derivatives with peptidomimetics containing thiazole and bistiazole ring were synthesized and investigated for their antiviral activity against influenza virus H1N1. N, N-dicyclohexylcarbodiimide (DCC) served as coupling reagent for their synthesis. The rimantadine analogues with thiazole ring showed moderate activity against influenza virus A/Hongkong. The remaining compounds were considerably less effective.

**Keywords:** Amantadine, Rimantadine, Peptidomimetics, Influenza Virus

## 1. INTRODUCTION

There are several specific antiviral agents against influenza viral infections - inhibitors of M2 ion channel and neuraminidase inhibitors [1]. Inhibitors of M2 ion channel amantadine and rimantadine (Fig. 1) are the first antiviral drugs developed in the 70s of the last century. They are effective in respect of some influenza A viruses (H1N1, H2N2, H3N2), but are not active against influenza B viruses, because do not possess M2 protein. However, many influenza virus strains have developed resistance to adamantanes and/or oseltamivir (the only orally bioavailable neuraminidase inhibitor), highlighting a major health risk [2]. For example, after four decades of effective use of amantadine, resistance by influenza viruses of the A/H3N2 subtype currently exceeds 90% in the United States, and virus mutants are as fit as the wild-type (wt) virus. The situation is even worst with the new 2009 pandemic H1N1 influenza. In both strains, the basis for resistance is a single Ser to Asn amino acid replacement (S31N) in the matrix M2 ion channel, which interferes with the drug's ability to block

M2 ion channel activity and viral replication [3]. Modification of antiviral agents by peptidomimetics, with chemical structures different from the natural peptides but maintaining the same ability to interact with specific receptors, is of great interest [4]. Based on the known structure/activity relationship we designed a new series of analogues of amantadine and rimantadine with the natural amino acid glycine containing thiazole and bithiazole ring.

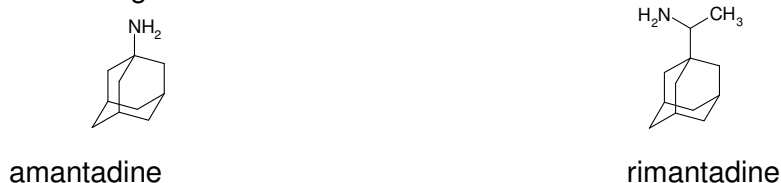


Fig. 1: Adamantane derivatives

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

The rimantadine, amantadine were purchased from Sigma, and DMAP and dicyclohexylcarbodiimide hydrochloride (DCC) from Merck. TLC analysis was performed on aluminum silica gel sheets 60 F254 plates (Merck) and spots were detected using an UV lamp at 254 nm. NMR Spectroscopy: Bruker Avance DRX-500 spectrometer; chemical shifts referenced to the solvent peaks [(1H, ([D6]-DMSO) = 2.49 and (<sup>13</sup>C, [D6]-DMSO) = 39.5.

### 2.2. Synthetic procedures

#### 2.2.1. Boc-Gly-Thz-amantadine (4a)

A mixture of amantadine hydrochloride 0,150 g (80 mmol) and N, N-dicyclohexylcarbodiimide (DCC) 0,165 g (80 mmol) in tetrahydrofurane (THF) was stirred for 1 h at 0 °C under a nitrogen atmosphere (Boger at al. 1999). A solution of Boc-Gly-Thz-OH 0,206 g (80 mmol) and 4-(N,N-dimethylamino)-pyridine (DMAP) 0,098 g (80 mmol) was added to the reaction mixture and stirring continued for 24 h. Then THF was evaporated *in vacuo* and the residue was chromatographed on silica gel, using heksane:ethyl acetate (4:5). 500MHz 1H NMR(in DMSO-d6, in ppm): 8.12 (s, 1H, ArH), 7.82 (t, J= 5.9 Hz, 1H, NH-CH2), 7.52 (d,J=10.0 Hz, NH-CH), 4.41 (d,J= 5.9 Hz, 2H, NH-CH2), 3.71 (m,1H, NH-CH-CH3), 2.0 -1.45 (15H,cycl.systema), 1.40 (s,9H,3xCH3),1.04 (d,J= 6.8Hz, NH-CH-CH3). 100MHz 13C NMR spectra ((in DMSO-d6, in ppm): 171.8(C=O), 159.7

(C=O), 155.9(Cq), 149.8(Cq), 123 (1C, C=), 78.9 (O-C(CH<sub>3</sub>)<sub>3</sub>), 52.0 (1C, NH-CH), 41.5 (1C, NH-CH<sub>2</sub>), 37.8 (3xCH<sub>2</sub>), 36.4 (3xCH<sub>2</sub>), 35.7(Cq), 27.5(3x CH<sub>3</sub>), 27.3 (3xCH), 14.0(NH-CH-CH<sub>3</sub>).

### 2.2.2. Boc-Gly-Thz-rimantadine (**4b**)

A mixture of rimantadine hydrochloride 0,150 g (69.5 mmol) and N, N-dicyclohexylcarbodiimide (DCC) 0,143 g (69.5 mmol) in tetrahydrofuran (THF) was stirred for 1 h at 0 °C under a nitrogen atmosphere (Boger at al. 1999). A solution of Boc-Gly-Thz-OH 0,228 g (69.5 mmol) and 4-(*N,N*-dimethylamino)-pyridine (DMAP) 0,084 g (69.5 mmol) was added to the reaction mixture and stirring continued for 24 h. Then THF was evaporated *in vacuo* and the residue was chromatographed on silica gel, using heksane: ethyl acetate (4:5). Yield: 0.063g, (63.5 %). <sup>1</sup>H-NMR (500MHZ, d-DMSO): δ 8.0 (s), 7.34 (NH-Phe), 7.11 (H-Phe), 3.63-3.53 (m, 2H-Phe), 1.29-2.2(m, 9H,Phe). <sup>13</sup>C-NMR (500MHZ, d-DMSO), 170.17, 170.14 (CO), 132, 71, 130.89, 130.84, (aromatic), 115.52, 115. 36, (C-5), 80.37(C-4) 56.42 (aC), 53.20, 53.14, 38.21 (bC), 28.29, 26.02, 25.32, 14.37.

### 2.2.3. Fmoc-Gly-Thz-Thz-amantadine and Fmoc-Gly-Thz-Thz-rimantadine (**5a-b**)

**5a-b** was prepared as described for compound **4a-b**. **5a** Yield: 0,055g (55 %). 500MHz <sup>13</sup>C NMR spectra ((in DMSO-d<sub>6</sub>, in ppm): 162.4(C=O), 161.8 (C=O), 148.7(Cq), 126.4 (1C, C=), 54.3 (1C, NH-CH), 39.5 (1C, NH-CH<sub>2</sub>), 38.1 (3xCH<sub>2</sub>), 36.4 (3xCH<sub>2</sub>), 35.7(Cq), 27.9 (3xCH), 13.1 (NH-CH-CH<sub>3</sub>).

**5b**. Yield: 0,055g ( 55 %). <sup>1</sup>H-NMR (500MHZ, d-DMSO<sub>3</sub> d-DMSO): δ 7.01 (s), 6.50 (s, 2H), 7.95 (NH-Phe), 7.94 (s), 7.07-7.17 (H-Phe), 5.59 (s,N), 4.12 (m), 3.53 (m), 2.72 (αCH<sub>2</sub>-Phe), 1.78 (m, H, 9H,Phe). <sup>13</sup>C-NMR (500MHZ, d-CHCl<sub>3</sub>), 172.17, 173.14, 132, 71, 130.89, 130.84, 115.52, 115. 36, 80.37, 56.42, 38.21, 28.29, 25.32, 14.36.

### 2.2.4. Removing Boc-protecting group

The resulting white solid, **4a-b** were dissolved in 20 ml of TFA and stirred at 0 °C for 1 h to remove the BOC group. Following removal of the TFA in vacuum, 20 ml of ethyl acetate was added to the oily residue. The solution was then added drop-wise to the cold diethyl ether. After filtration, compounds (TFA salt) was collected as white solid with high purity (>95%). Four milliliters of triethylamine (TEA) was added to new compounds and kept for 15 min before dissolving it in 15 ml DMF. 500MHz <sup>1</sup>H NMR(in DMSO-d<sub>6</sub>, in ppm): 8.18 (s, 1H, ArH), 4.51 (s, 2H, NH-CH<sub>2</sub>), 3.64 (m, 1H,

NH-CH-CH<sub>3</sub>), 2.0 -1.45 (15H,cycl. system), 1.40 (s,9H,3xCH<sub>3</sub>), 1.06 (d, J= 7.0 Hz, NH-CH-CH<sub>3</sub>).

### 2.2.5. Removing Fmoc-protecting group

The resulting white solid, **5a-b** were dissolved in solution of piperidine/DMF 20 ml of TFA and stirred at 0 °C for 10 min to remove the Fmoc group. The solution was then added drop-wise to the cold diethyl ether. After filtration, compounds was collected as white solid with high purity (>95%). Four milliliters of triethylamine (TEA) was added to new compounds and kept for 15 min before dissolving it in 15 ml DMF. 500MHz <sup>1</sup>H NMR(in DMSO-d<sub>6</sub>, in ppm): 8.05 (s, 1H, ArH), 4.89 (s, 2H, NH-CH<sub>2</sub>), 3.89 (m,1H, NH-CH-CH<sub>3</sub>), 2.0 -1.39 (15H,cycl.system), 1.40 (s,9H,3xCH<sub>3</sub>), 1.06 (d, J= 7.0 Hz, NH-CH-CH<sub>3</sub>).

## 3. RESULTS AND DISCUSSION

Rimantadine and amantadine are rather old, therefore it is not a great surprise that hundreds of derivatives have been synthesized and pharmacologically tested. *In fact, soon after the publication of the antiviral activity of amantadine by du Pont de Nemours' researchers [5], several amantadine derivatives were synthesized and evaluated as anti-influenza agents. Most of these analogs are alkylaminoalkyl derivatives of adamantane, although some derivatives featuring additional polar groups, such as alcohols, amines, ethers, or derivatives lacking an amino group were also synthesized and tested. However adamantane analogues (rimantadine and amantadine) containing peptidomimetics are not known at all. In order to obtain new analogues with more desirable characteristics, we synthesized adamantane analogues with peptidomimetics containing thiazole and bithiazole ring.*

### 3.1. Synthesis of adamantane analogues

A mixture of amantadine (**1a**), rimantadine (**1b**) and DCC in THF was stirred for 1h at 0°C under nitrogen atmosphere. A solution of Boc-Gly-Thz-OH (**2**) or Fmoc-Gly-Thz-Thz-OH (**3**) and 4-*N*, *N*-(dimethylamino)-pyridine (DMAP) was added to the reaction mixture and stirred for 24 h. Then THF was evaporated *in vacuo* and the residue was chromatographed on silica gel, using 4:5/hexane:ethyl acetate (Fig. 2).

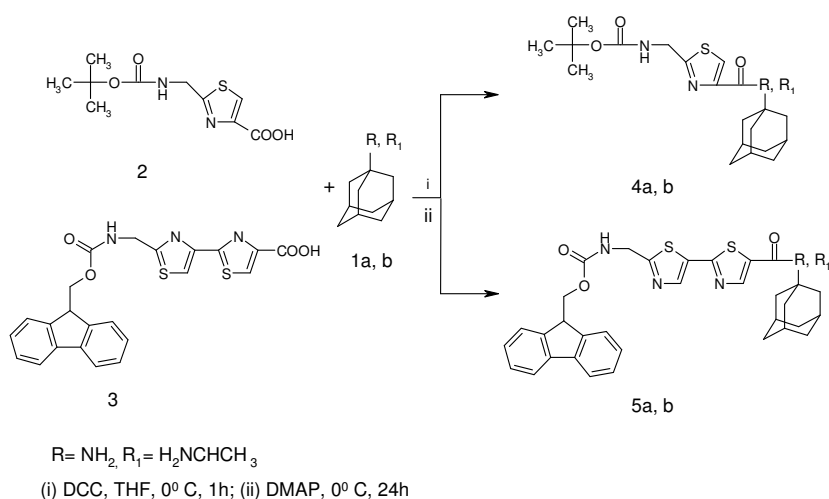


Fig. 2: Synthesis of new adamantane analogues

The resulting white solid, Boc-Gly-Thz-amantadine (**4a**), Boc-Gly-Thz-rimantadine (**4b**) were dissolved in 20 ml of TFA and stirred at  $0^\circ\text{C}$  for 1 h to remove the BOC group and Fmoc-Gly-Thz-Thz-amantadine (**5a**), Fmoc-Gly-Thz-Thz-rimantadine (**5b**) were dissolved in piperidine/DMF to remove the Fmoc group followed by neutralization with ammonia and adding HCl to obtain the desired compounds as hydrochloride (Fig. 3). The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR, mass-spectra were consistent with desired structure.

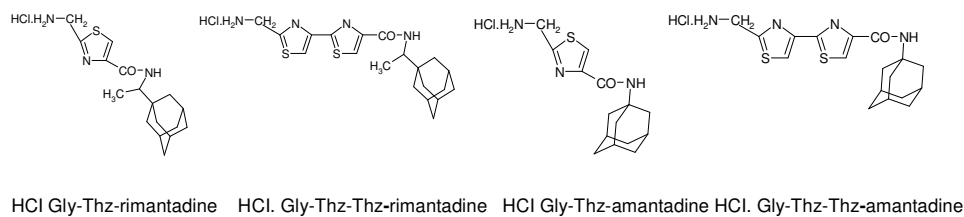


Fig. 3: Adamantane analogues with peptidomimetics

### 3.2. Cells and viruses: MDCK cells and influenza virus A/Hongkong/68

The replication of influenza viruses in MDCK cells induces the complete destruction of host cells, a distinct cytopathic effect (CPE). The virus-induced CPE can be inhibited by addition of antiviral compounds (100  $\mu\text{l}$ /well; 2 parallels/concentration, dilution factor 2). Untreated (virus control) and compound-treated confluent monolayers of test cells were infected

with a multiplicity of infection that induces a complete CPE in virus control 24 h after virus addition. Thereafter, adherent cells were fixed and stained with a crystal violet/formalin solution. After elution of the stain, inhibition of virus-induced CPE was quantified by optical density (OD) determination in a Dynatech microplate reader. The percentage of antiviral activities of test compounds was calculated. Based on the mean dose response curve of at least 2 assays, the 50 % CPE inhibitory concentration (IC<sub>50</sub>) was calculated (Tab. 1).

Tab. 1: Cytotoxicity and anti-influenza activity in MDCK cells

Compound	CC <sub>50</sub> [μg/ml]	MTD [μg/ml]	IC <sub>50</sub> [μg/ml]	IS
HCl.Gly-Thz-Thz-rimantadine	50	3.6	not active	455
HCl.Gly-Thz-rimantadine	50	9.7	0.11	455
HCl.Gly-Thz-amantadine	23.7	8.8	not active	455
HCl.Gly-Thz-Thz-amantadine	36.6	13.6	not active	455

Novel rimantadine (1, 2) and amantadine (3, 4) analogues have been synthesized with amino acids containing thiazole and thiazolyl-thiazole rings and their activity on the Influenza virus A/Hongkong/68 have been explored. The rimantadine analogues with thiazole ring showed moderate activity against influenza virus A/Hongkong. The remaining compounds were considerably less effective.

#### 4. ACKNOWLEDGMENTS

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# Quantum-chemical calculation of O-H Bond Dissociation Enthalpy in flavones

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**Abstract:** Bond dissociation enthalpy is an important descriptor of radical-scavenging activity. Here we present the calculated values of the bond dissociation enthalpy for all possible mono hydroxyl derivatives of flavones. The purpose is to see how the position of the hydroxyl group affects its ability for homolytic dissociation. DFT/B3LYP functional in combination with 6-31G\*\* orbital basis was used for the geometric optimization and frequencies calculations.

**Keywords:** bond dissociation enthalpy, quantum-chemical calculations, flavones.

## 1. INTRODUCTION

The flavonoids probably possess the greatest variety of biological activities among natural organic compounds. They are competitive inhibitors of the protein tyrosine kinases. An inappropriate or enhanced expression of this enzyme may contribute to the transformed state of cells in many human malignancies [1-6].

Some flavones show vasorelaxing, antioxidative, and chemopreventive effects [7-9]. A number of studies suggest that flavonoids manifest anti-inflammatory action via their ability to modulate free radical production by phagocytic leukocytes [10-12].

Some flavonoids are good aldose reductase inhibitors and have been linked with numerous detrimental complications [13].

Flavonoids, which contain more than one hydroxyl group, show antiperoxyl radical activities several times stronger than trolox and other  $\alpha$ -tocopherol analogues [14-16]

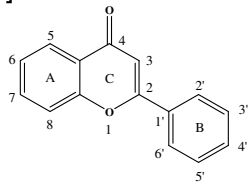


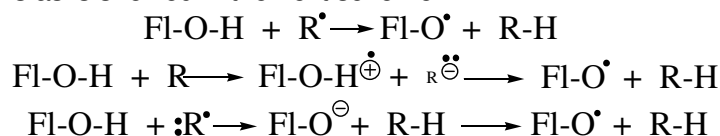
Fig. 1: Basic structure of flavones [2-(1'-phenyl)-chromen-4-one].

Flavones are type flavonoids having as a basic structure 4-chromenone (1-benzopyran-4-one) moiety connected to a phenyl ring at 2 position: 2-(1'-phenyl)-chromen-4-one or 2-phenyl-(1,4-benzopyranone) (See Fig. 1).

Many authors postulate that the most effective inhibitors of lipid peroxidation are flavonoids (see Fig. 1) with: (i) hydroxyl groups at positions 3' and 4' in ring B [17]; (ii) hydroxyl group at position 3 in ring C [17]; (iii) C2-C3 double bond and keto-function at 4 position in ring C [18,19].

There are a lot of studies in the literature which focus on the number and position of functional groups in the flavonoid in relation to their biological activity. Only flavonoids with natural origin are considered however, in these studies [20-22].

The antioxidant activity of the flavonoids is associated with their ability to bind metal ions and to trap free radicals. The radical-trapping (scavenging) activity takes place in one step transfer of hydrogen atom or in two-steps mechanisms as is showed in the next scheme:



Scheme 1. Mechanisms of radical-scavenging.

A measure (descriptor) of the chemical bond strength is the bond-dissociation enthalpy (BDE). Therefore BDE is a descriptor of radical-scavenging activity.

In this investigation will be discussed the BDE of the O-H bonds at different positions in the basic structure of the flavones. We have never encountered quantum-chemical study of this type in the literature. All possible mono-hydroxy derivatives of the flavones are included in the investigation.

## 2. COMPUTATIONAL DETAILS

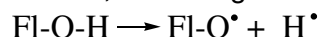
The calculations were carried out using the density functional theory as implemented in the Gaussian09 program package [23]. The optimization of the geometry was performed by Becke's 3-Parameter hybrid functional combined with the Lee–Yang–Parr correlation functional (B3LYP), with the standard 6-31G(d,p) basis set [24]. Unrestricted B3LYP was used for the geometry and vibrational frequency calculations of the phenoxyl radicals, and the H atom. Restricted B3LYP was used for the closed-shell molecules.

The optimization was achieved without any geometry constraints. Harmonic vibrational frequencies for all structures were

computed to confirm the true minima on the calculated potential surface.

In the initial geometries all possible intramolecular interactions were taken into account.

BDE was calculated as a difference in total enthalpy of the corresponding radical, (formed after H abstraction) hydrogen atom and the flavone, according to the following reaction:



### 3. RESULTS AND DISCUSSION

Tab.1. Calculated BDE of all mono hydroxyl flavones

Derivative	Flavon Enthalpy	Radical enthalpy	BDE Hartree	BDE kcal/mol
2'-OH-flavone	-803.078	-802.451	0.129264	81.11355
3'-OH-flavone	-803.08	-802.448	0.134198	84.20965
4'-OH-flavone	-803.082	-802.452	0.132277	83.00421
5'-OH-flavone	-803.081	-802.449	0.133962	84.06156
6'-OH-flavone	-803.081	-802.446	0.137354	86.19005
3-OH-flavone	-803.085	-802.454	0.132598	83.20564
5-OH-flavone	-803.096	-802.44	0.158353	99.36698
6-OH-flavone	-803.078	-802.449	0.130905	82.14328
7-OH-flavone	-803.081	-802.448	0.135448	84.99403
8-OH-flavone	-803.078	-802.451	0.129806	81.45365

\*Calculated enthalpy of hydrogen atom = -0.49791 H

The task we set ourselves within this quantum-chemical investigation is to elucidate how the position of the hydroxyl group in flavones affects their O-H BDE.

The smallest BDE has the hydroxyl group located at the position 2' in ring B. Larger, but very similar BDE have hydroxyl groups located at the 8 and 6 position in ring A (the difference between the first and the third isomer is about 1 kcal/mol).

This is a bit surprise because the antioxidant activity is associated mainly with the hydroxyl groups positioned at 3' and 4' carbon atoms in the ring B. In many articles is announced that the number and positions of OH groups in ring B are the most significant determinants of reactive oxygen species scavenging [14-16]).

The next isomer in that order of increasing BDE is that with hydroxyl group at the 4' position (at about 2 kcal/mol from 2'-OH), which is usually

assumed to be responsible for the antioxidant activity of these type of compounds.

After that, with higher BDE are hydroxyl groups at 3, 5' and 3' position (at about 4 kcal/mol from 2'-OH).

Follows the hydroxyl groups at 7 position and after that hydroxyl group at 6' position (at about 5 and 6 kcal/mol from O-H group at 2' position).

With the highest BDE is the hydroxyl group at 5 positions. BDE of the hydroxyl group at this position differs dramatically from others. It is greater by about 18 kcal/mol than BDE of the O-H group at 2' position.

The hydroxyl group at the 5 position is involved in strong hydrogen bond with the keto oxygen at the 4 position and therefore is not surprise that BDE of this group is highest. Other authors have found similar results. The distance between the hydrogen of hydroxyl group and the carbonyl oxygen is very close: 1.705 Å. Removing of a hydrogen atom from the hydroxyl group also means the rupture of the hydrogen bond which stabilizes the parent compound and this increases the BDE. The same, but to a lesser extent is true for the BDE of the hydroxyl group at 6' position. Here the hydrogen from the hydroxyl group can form hydrogen bond with the oxygen at position 1 in the ring C. The distance between two atoms is 1.857 Å, which indicates a strong hydrogen bond. The stability of the radical formed after H atom abstraction from the 6-OH derivative is more stable than the radical formed from 5-OH-flavone.

Interestingly, other hydroxyl group, which can also form hydrogen bond with the oxygen at position 1 in ring C is the hydroxyl group at 8 position. It has very low BDE. Interatomic distance OH---O here is 2.246 Å and suggests that there is not hydrogen bond.

It is interesting to compare the two isomers: 2'- and 6'-OH-flavones. Their BDEs are significantly different. The difference is 5 kcal/mol.

In the 2'-OH-flavone the oxygen from a hydroxyl group attracts the hydrogen atom at the 3 position in ring C because of the partial charges (respectively +0.139 for the hydrogen and -0.554 for the oxygen). This interaction makes the molecule planar. The interaction is available in the corresponding radical too. It makes the radical flatness and stable.

The spin density in the radical derived from the flavon-2'-ol is 0,369, while in the radical derived from the flavon-6'-ol is 0.416. The main difference between both radicals is that in the first radical there is a significant spin density at carbon atoms 2 and 3 in ring C, while in the second radical the delocalization remains within the ring B, which does not lie in one plane with ring C.

#### 4. CONCLUSION

As have already become clear the trend for homolytic O-H bond dissociation among the hydroxyl groups in the ring B is  $6' < 3' < 5' < 4' < 2'$ . In ring A the trend is as follows:  $5 < 7 < 6 < 8$ .

With the exception of the hydroxyl group at the 5 position BDE of all other hydroxyl groups do not differ significantly. The reasons for these trends are the participation of OH group in hydrogen bonds and ability to form stable radical.

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**Supplemental materials** (\*.log files) are available to everyone:

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# BIOLOGICAL ACTIVITY OF ADAMANTANE ANALOGUES

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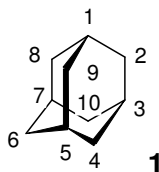
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**Abstract:** After the discovery of the first synthetic anti-influenza compound – amantadine (1- adamantanamine), the “birth” of medicinal chemistry of adamantane analogues has been started. Adamantane derivatives attracted the attention of scientists, having the potential as chemotherapeutic agents (against influenza and HIV viruses, as well as antibacterial, antifungal, antiinflammatory, etc. activities). In continuation to our research work on the chemical and pharmacological properties of such molecules, herein we report our results on the synthesis and biological activity of the newly synthesized aminoadamantane derivatives.

**Key words:** Adamantane derivatives, rimantadine, amantadine, influenza A

## 1. INTRODUCTION

The adamantane nucleus (or tricyclo [3.3.1.1<sup>3,7</sup>] decane, Fig. 1) is found to be an important motif in many antiviral drugs, displaying various mechanisms of action.



**Fig. 1**

The incorporation of an adamantyl moiety into several molecules in many cases resulted in improving the therapeutic profile of the parent compound [1].

The intensified functionalization and pharmaceutical studies of adamantanes has started with the discovery of aminoadamantane (amantadine, **Fig. 1**) and continue to do so to date. Numerous of applications of adamantane derivatives have been found in medicinal chemistry. Usually, to improve the pharmacokinetics of the drug, adamantane modifications should enhance its lipophilicity and stability. On the other hand, it is known that replacing the amino group in amantadine with functional groups like:  $-\text{OH}$ ,  $-\text{SH}$ ,  $-\text{CN}$ ,  $-\text{CO}_2\text{H}$ ,  $-\text{Cl}$ , or  $-\text{Br}$  leads to inactive compounds [2].

### 1.1. Antiviral, antibacterial and antifungal activities of Adamantanes

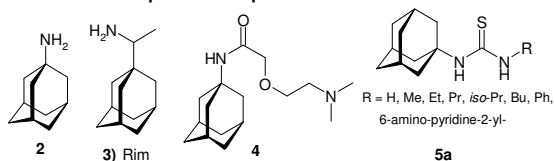
Amantadine (1-adamantanamine hydrochloride, Symmetrel®, **2**, **Fig. 2**) was the first compound, demonstrating the capacity to inhibit the replication of influenza A [3]. Despite amantadine effectiveness in both prevention and prophylaxis of influenza A virus infections, its clinical application is limited in view of the fast selection of resistant virus mutants as well as some side effects - nausea, vomiting, loss of concentration. Those side effects have been significantly reduced when amantadine methylated analogue - rimantadine ( $\alpha$ -methyl-1-adamantanemethylamine hydrochloride) is applied (**3**, **Fig. 2**), due to the fact that the latter compound does not pass through the blood-brain barrier. Later, rimantadine is further developed to a more potent and less toxic alternative to amantadine with the same mechanism of antiviral action [4].

Unlike amantadine, tromantadine (*N*-(adamant-1-yl)-*N*-[2-(dimethyl amino) ethoxy] acetamide hydrochloride) inhibits the replication of herpes simplex virus type 1.

Moderate activity against herpes Simplex viruses (HSV-1) is found also for the 1-(adamant-1-yl) thiourea derivatives (**5a**, **6**, **7**, **Fig.2**) [5].

A recent report exists for novel *in vitro* antiherpetic adamantane derivatives [6], containing also adamant-1-yl-monothioureas, which derive from diaminocyclohexane (**5b**) and bis-urea (**5c**).

Adamantyl thiosemicasbazones **6** and **7** [7] are also useful as inhibitors of herpes simplex and vaccinia viruses.



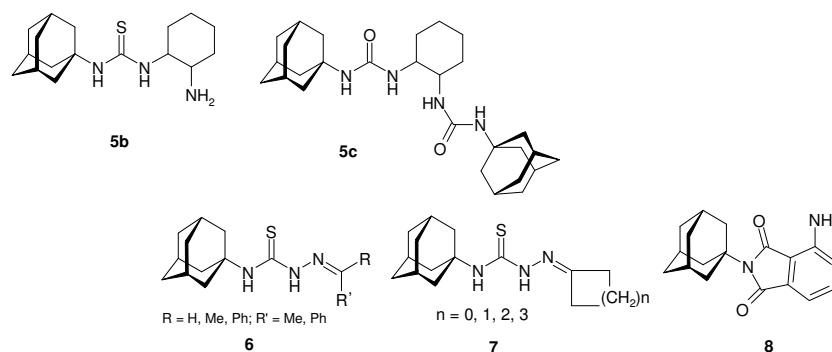


Fig. 2

Bactericidal and fungicidal effects are also amongst the biological activities of adamantane derivatives. In addition to similar effects, compounds as *N*-(dialkylaminoalkyl) adamantane-1-carboxamides (**9**) **Fig. 3** are reported to exhibit antiprotozoal, anti-inflammatory and analgesic activities as well [9].

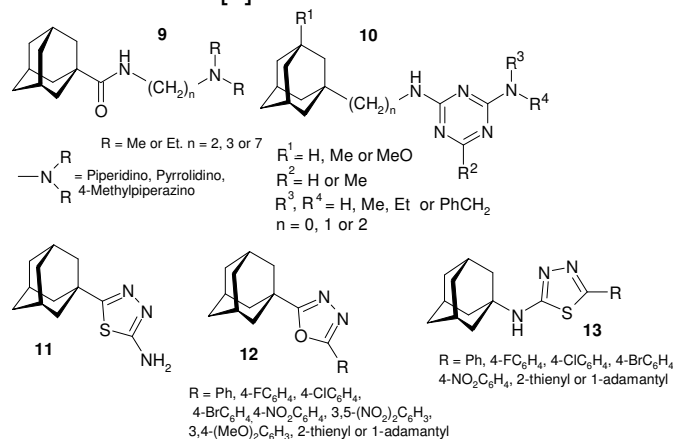


Fig. 3

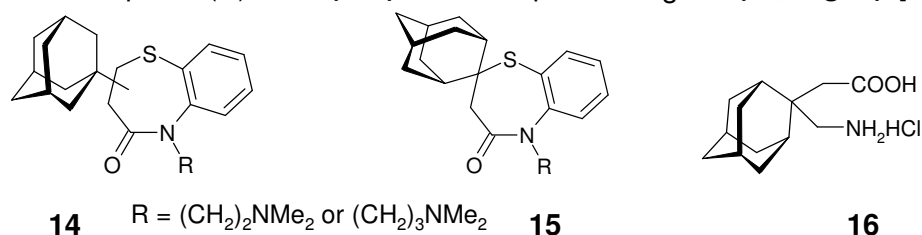
2-(Adamant-1-yl or 1-adamantylalkylamino)-4-amino-s-triazines (**10**) possess potent also antibacterial and hypoglycemic activities [10].

2-(Adamant-1-yl)-5-amino-1,3,4- thiadiazole (**11**) are found to be a typical general fungal disinfectant [11], whereas a series of compounds (**12,13**) are shown to possess a good antimicrobial activity particularly against the tested Gram-positive bacteria *Bacillus subtilis* and moderate activity against the yeast-like pathogenic fungus *Candida albicans* [12].

### 1.2. Effects on the central nervous system of Adamantane Derivatives

Being highly lipophilic, adamantane nucleus enables passing through the blood-brain barriers and thus affecting the central nervous system. That is one of the reasons amantadine and lately a number of aminoadamantanes to be suited to combat symptoms of Parkinson disease. Meanwhile 3,5-dimethylaminoadamantane (memantine) have been approved by EMA and FDA for the treatment of Alzheimer and Parkinson diseases [13].

A potent antidepressant activity is observed for adamantyl 1,5-benzothiazepine-4(H)-ones (**14**) and its spiro analogues (**15**, **Fig. 4**) [14].



**Fig. 4**

Analgesic and antipyretic activities are also reported for several adamantane derivatives.

Recently the adamantane-aminobutyric acid (AdGABA) (**16**) is reported to possess strong anticonvulsant and analgesic activities [15].

The adamantyl benzothiazolyldene derivatives are found to be potent anticonvulsant agents too [16].

### 1.3. Miscellaneous effects of Adamantane Derivatives (antitumor, antiinflammatory, enzyme inhibitory, etc.)

Several adamantanes are reported to possess an anti-inflammatory activity as their main biological activity. Among a series of tested 3-(Adamant-1-yl)-4-substituted-5-mercapto-1,2,4-triazoles for their anti-inflammatory and analgesic activities [17], the compound presented on **Fig. 5** (**17**) is the most potent ones— its activity is found to be comparable to the activity of indomethacin.

11  $\beta$ -hydroxysteroid dehydrogenase type 1 (11  $\beta$ -HSD1) is an NADPH-dependent enzyme which reduces inactive cortisone to the its active form-cortisol, stimulating glucocorticoid receptors [18]. Therefore, finding selective 11  $\beta$ -HSD1 inhibitors would be an important therapy for controlling non-insulin-dependent diabetes, hyperglycemia, obesity, insulin and etc.

Adamantly bearing triazole nucleus **18**, and its cyclic analogues **19** are identified as potent selective inhibitors of 11 $\beta$ -HSD1 *in vitro*, and *in vivo* (**Fig. 6**). The most active compounds are **18** (R' = Me, R'' = Ph) and **19** (n = 3), presented on Fig. 6 [19].

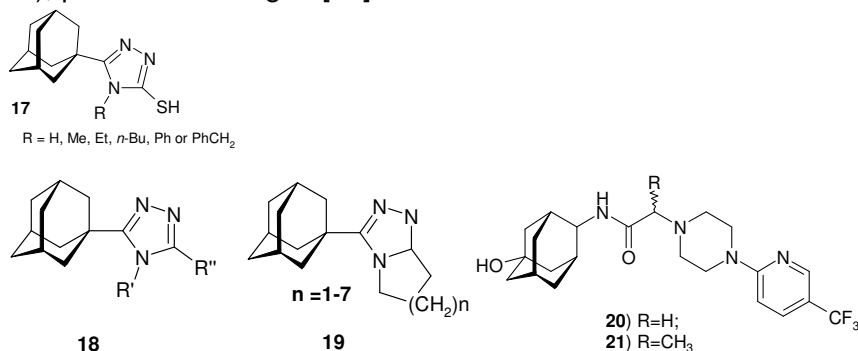


Fig. 5

Fig. 6

N-(adamant-2-yl) acetamide derivative (**20**) and its ( $\pm$ )-methylated derivative (**21**, **Fig. 6**), are discovered to be highly potent 11 $\beta$ -HSD1-selective inhibitors, too [20].

Antitumour activity is observed for some adamantane analogues (*S*)-1-(3- and 4-pyridyl) ethyl adamantane-1-carboxylate (**22**) and (**23** **Fig. 7**)[21].

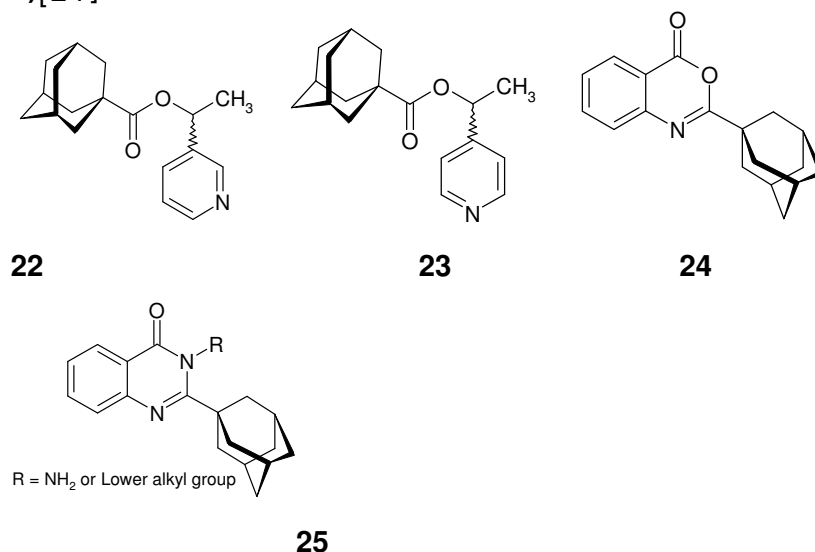


Fig. 7

Marked antitumour and anti-HIV-1 activities are demonstrated also for 2-(adamant-1-yl)-4*H*-3,1-benzoxazin-4-one (**24**) and 3,4-dihydroquinazolin-

4-one analogues (**25**, **Fig. 7** [22]).

Various other biological activities have been also estimated for some adamantane derivatives.

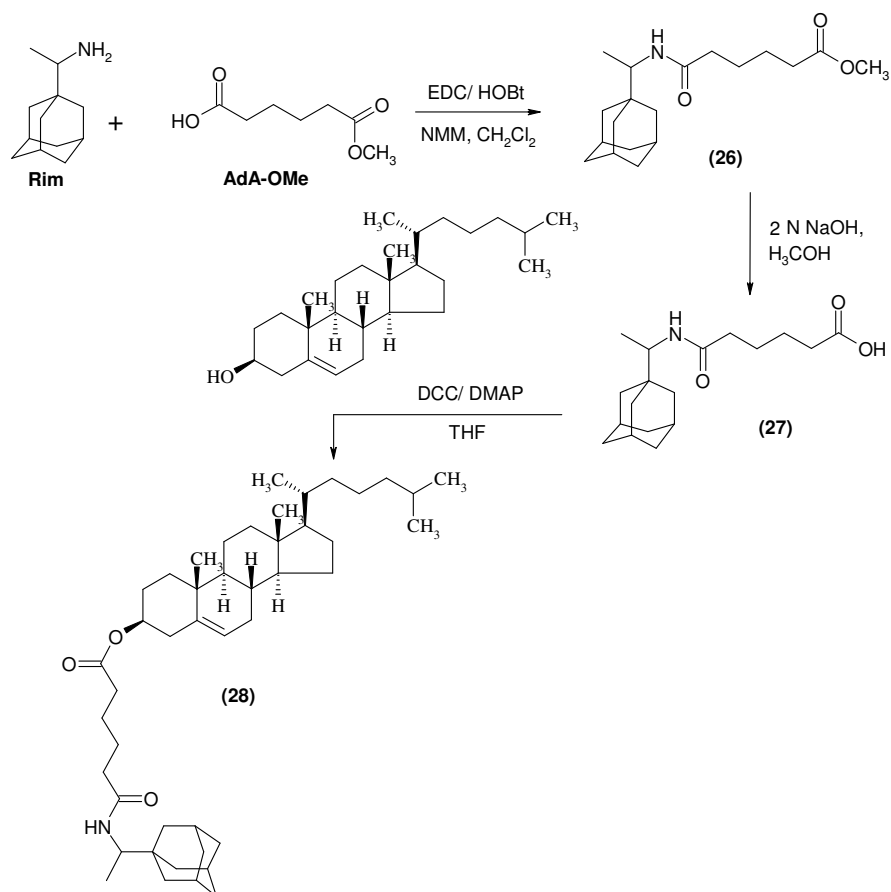
## 2. RESULTS

Over the last decade, an increasing attention has been paid to the design of drug-transport molecules. Continuing the chase for novel anti-influenza agents, herein, we modified rimantadine with cholesterol. By attaching the highly hydrophobic cholesterol (a part of the natural cell membrane) to rimantadine, a promising structure for penetrating into the lipid bilayer membrane of the virus envelope could be achieved.. Because of the absence of a suitable functional group in the cholesterol skeleton, the connection with rimantadine was conducted using a two-step procedure. Firstly, cholesteryl bromoacetate was obtained. Then the latter had to be attached to rimantadine, but to our regret this reaction failed.

As outlined in **Scheme 1**, our second attempt to modify the cholesterol nucleus with the anti-influenza drug, included amidation of rimantadine (**Rim**) with mono methyl adipate (**AdA-OMe**) using the standard method in peptide chemistry (EDC/HOBt). Then, after alkaline hydrolysis of ester, compound **26** was converted into **27**, which in turn was esterified with cholesterol to give compound (**28**).

The aim of the next modifications of rimantadine was the obtaining of the following Schiff-bases (**Fig. 8**) and amino acid amides of aspartic and glutamic acid derivatives (**Fig. 9**):

The newly synthesized compounds were tested *in vitro* for their anti-influenza activity against A/H3N2 and A/H1N1. The preliminary results of this study showed a promising antiviral activity. All the synthesized compounds have to be tested for other biological activities ahead.



Scheme 1.

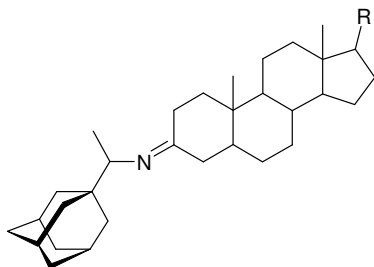


Fig. 8.

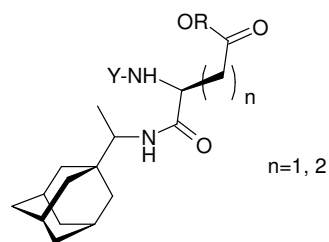


Fig. 9.

### ACKNOWLEDGEMENTS

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## Synthesis and IR-spectral characterization of dipeptide threonyl-methionine

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**Abstract:** *The dipeptide L-threonyl-L-methionine (Thr-Met) was synthesized and studied. The peptide was obtained using active ester procedure. The conventional and linear-polarized IR-spectroscopy of oriented colloids in nematic host is applied as a part of modern approaches for spectral and structural investigation of peptides. It was established the role of intermolecular hydrogen bonding on conformational behavior and spectroscopic properties of compound studied in solid-state. The obtained results for the characteristic IR-bands of amide O=C-NH fragments and other vibrations can be provides a structural information about the configuration of amide group in the peptide molecules.*

**Key words:** *threonyl-methionine, active esters method, IR-spectroscopy*

### 1. INTRODUCTION

Amino acids are the building blocks of peptides and proteins. The structure and conformers number of amino acids are the subjects of considerable attention, both in experimental investigations [1-5] and in theoretical calculations [6-9]. Although less structurally complex than larger protein molecules, peptides have significant biological activities. Moreover, they are involved in a variety of signal transduction processes. L-Threonine containing di- and tripeptides are very important in metabolism of some pathogenic microorganisms due to specific L-threonine recognition by cell membrane ensuring by this the peptide uptake [10]. In order to design mimetics excellent knowledge of three dimensional structure of the peptides is needed. The best strategy to design peptide mimetics is modification and stabilization of peptide backbone, but preserving the essential three

dimensional pharmacophores necessary, which is essential for receptor recognition and desired biological activity [11].

Many different analytical methods were developed for analysis of organic and biological active compounds. Infrared spectroscopy is often used for obtaining both structural and conformational information from biological samples, especially proteins and amino acids. However, peptides possess the complicated spectroscopic IR-patterns, requiring the application of specific not conventional techniques for analysis. The possibilities of linear-polarized IR-spectroscopy (IR-LD) or oriented solid samples as a colloidal suspension in nematic liquid crystal cover these difficulties in significant level. The method has been applied on peptide systems, their salts and complexes. This work presents the synthesis and IR-spectral analysis of the dipeptide Thr-Met in solid state for obtaining structural information.

## 2. EXPERIMENTAL PART

### 2.1. Synthesis

#### ***Boc-Thr-OH (1)***

Threonine (1.2 g, 10 mM) was dissolved in a solution of  $\text{NaHCO}_3$  (0.9 g, 10.5 mM) in water (25 ml) and was cooled to 0°C. Solution of  $\text{Boc}_2\text{O}$  (2.3 g, 10.5 mM) in *i*-PrOH (25 ml) was added dropwise. The reaction mixture was stirred for 24 hours at room temperature. The process was monitored by TLC  $\text{CT}_1$  ( $\text{CHCl}_3$  : MeOH :  $\text{H}_2\text{O}$ , 80 : 30: 5). At the end of the process *i*-PrOH was evaporated, aqueous solution was acidified with dry  $\text{NaHSO}_4$  to pH 3, and three extractions with EtOAc were made (3 x 30 ml). Combined organic layers were washed with saturated NaCl, dried over  $\text{Na}_2\text{SO}_4$ , filtered, and EtOAc was evaporated. Pure Boc-Thr-OH crystalized on air and 2.1 g (95 %) was obtained. M.p. 80 – 82°C,  $[\alpha]_D^{20} +24^\circ \sim +26^\circ$  ( $c = 1$  in EtOH).

#### ***Boc-Thr-OSu (2)***

Boc-Thr-OH (2.1 g, 9.6 mM) was dissolved in EtOAc (20 ml) and N-hydroxysuccinimide (1.1 g, 9.6 mM). EDAC (1.8 g, 9.6 mM) was added at room temperature. The reaction mixture was stirred for 24 hours at room temperature. The process was monitored by TLC  $\text{CT}_1$  ( $\text{CHCl}_3$  : MeOH :  $\text{H}_2\text{O}$ , 80 : 30: 5). At the end of the process, reaction mixture was washed quickly with 10%  $\text{NaHCO}_3$  (3 x 20 ml),  $\text{H}_2\text{O}$  (to pH 7), and EtOAc dried over  $\text{Na}_2\text{SO}_4$ , filtered, and EtOAc was evaporated. Crude product was used without further purification (2.9g, 96%). M.p. 134-135°C,  $[\alpha]_D^{20} -33^\circ$  ( $c=1$  in EtOAc).

#### ***Boc-Thr-Met-OH (3)***

To the solution of Boc-Thr-OSu (2.9g, 9 mM) in THF (10 ml) at room temperature solution of Met (1.3g, 9 mM) and  $\text{Et}_3\text{N}$  (1.25ml, 9 mM) in THF (10 ml). The reaction mixture was stirred for 24 hours at room temperature. The process was monitored by TLC  $\text{CT}_1$  ( $\text{CHCl}_3$  : MeOH :  $\text{H}_2\text{O}$ , 80 : 30:

5). At the end of the process THF was evaporated, and the residue was dissolved in EtOAc (10 ml) and was washed with 10 % NaHSO<sub>4</sub>, H<sub>2</sub>O dried over Na<sub>2</sub>SO<sub>4</sub>, filtered, and EtOAc was evaporated. Pure Boc-Thr-Met-OH crystallized after evaporation and 2 g (63 %) was obtained. Oil,  $[\alpha]_D^{20} -17^\circ$  (c=1, MeOH).

#### ***H-Thr-Met-OH (4)***

Boc-Thr-Met-OH (2 g, 5.7 mM) was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (2 ml) and TFA (1 ml) was added. The reaction mixture was stirred for one hour at room temperature. The process was monitored by TLC CH<sub>3</sub>CN : H<sub>2</sub>O, 4 : 1. At the end of the process solvent was evaporated and the crude product was purified by column chromatography (Silicagel, CH<sub>3</sub>CN : H<sub>2</sub>O, 4 : 1). It was obtained 1 g (61 %) crystal product. M.p. 151 °C (decomposition),  $[\alpha]_D^{20} +29^\circ$  (c=1 in water).

## **2.2. Methods**

The dipeptide *Thr-Met* was synthesized by the method describe above. IR-spectra have been measured on a Bomem Michelson 100 FTIR Spectrometer (4000 – 400 cm<sup>-1</sup>, 2.0 cm<sup>-1</sup> resolution, 150 scans) equipped with a Perkin Elmer wire-grid polarizer. Non-polarized solid-state IR spectra have been recorded, using the KBr disk technique. The oriented samples in the IR-LD spectroscopic study have been obtained as a colloidal suspension in a nematic liquid crystal (MLC-6815, Merck) in the presence of an isolated nitrile stretching IR-band at about 2230 cm<sup>-1</sup>, which additionally serves as an orientation indicator. The validation of this new orientation of the solid-state method [12-14], used in linear-dichroic infrared (IR-LD) spectroscopy, based on a colloidal suspension in nematic liquid crystal for accuracy and precision, and the influence of the liquid crystal medium on peak positions and integral absorbances of the guest molecule bands are presented.

## **3. RESULT AND DISCUSSION**

The investigation of *L*-threonyl-*L*-methionine (*Thr-Met*) dipeptide (Fig. 1) is justifiable and this paper deals with synthesis and preliminary structural analysis of the neutral ligand by solid-state IR-LD spectroscopy of oriented colloid suspensions.

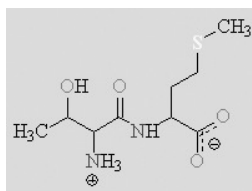


Fig. 1: Zwitterionic structure of dipeptide *L*-threonyl-*L*-methionine

### 3.1. Synthesis of dipeptide Trh-Met

The dipeptide threonyl-methionine was obtained by active esters method, according to synthetic scheme shown in Fig.2. For the protection of  $\alpha$ -amino group tert-butyloxycarbonyl group was used. Reaction was carried out at room temperature in water-alcoholic solution. Active esters procedure allows using carboxyl component without protection. This step reduced the synthetic scheme with two steps at least – protection of  $\alpha$ -carboxyl group and its deprotection.

At the final stage of the synthetic scheme, the tert.-Boc-group was removed by TFA at room temperature and the crude product was purified by

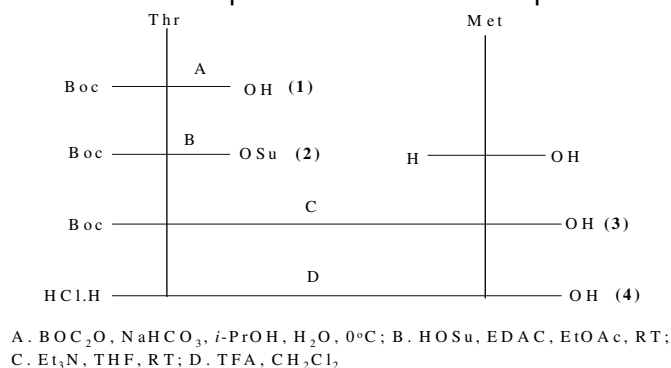


Fig. 2: Synthesis of dipeptide Trh-Met.

column chromatography (Silicagel and eluent  $\text{CH}_3\text{CN} : \text{H}_2\text{O}$ , 4 : 1). The obtained product peptide was with a high purity and yield. M.p.  $151^\circ \text{C}$  (decomposition); Yield: 61 %,  $[\alpha]_D^{20} +29^\circ$  (c=1 in water).

### 3.1.1. 3.2. IR-spectral analysis

In aqueous solution and in the solid state the dipeptides are expected to exist as the zwitterions [15,16]. In the crystal the zwitterions are stabilized by intermolecular interactions with neighbouring zwitterions, while in solution the dielectric field of the solvent, and in addition specific H-bonds with solvent molecules stabilize the zwitterions.

Zwitterions are also characterized by different molecular structures and significantly altered vibrational spectra. Accordingly, proper and valid characterizations of structures and infrared spectra of zwitterions in *ab initio* molecular orbital calculations, must take into account the local environment.

The conventional non-polarized IR- and difference IR-LD- spectra of *Thr-Met* are depicted in Fig. 3. and Fig.4 shows some typical bands characterizing zwitterionic structure of investigated dipeptide. The pairs of maxima at  $3345\text{ cm}^{-1}$  and  $3315\text{ cm}^{-1}$  which are observed, belong to the

stretching  $\nu_{\text{NH}}$  and  $\nu_{\text{OH}}$  modes of NH and OH groups in the molecule of the dipeptide.

Furthermore, the low-frequency shifting of the last band is at hand and those can be associated with the strong hydrogen bonding by hydroxyl group (-OH) of the *Thr*-side chain participation.

The broad multiple character of the IR-bands within  $3300 - 2100 \text{ cm}^{-1}$  corresponds to asymmetric and symmetric  $\nu_{\text{NH}_3^+}^{\text{as}}$  and  $\nu_{\text{NH}_3^+}^{\text{s}}$  stretching vibrations of protonated  $\text{NH}_3^+$ -group, which is formed in the zwitterionic structure.

The IR-spectroscopic region within  $1700 - 1400 \text{ cm}^{-1}$  is characterized with a series of bands belonging to a typical functional group absorbed in this region.

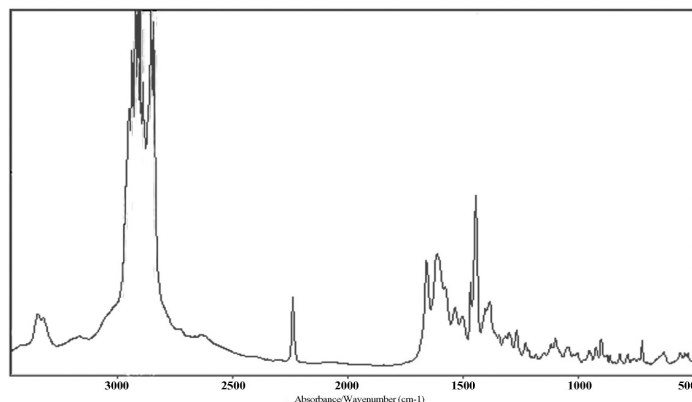


Fig.3: Non-polarized IR-spectrum of *Thr-Met*

The maxima at  $1655 \text{ cm}^{-1}$  and  $1535 \text{ cm}^{-1}$  can be assigned to  $\nu_{\text{C=O}}$ , Amide I and  $\delta_{\text{NH}}$  Amide II modes correspondingly. The bands at  $1576 \text{ cm}^{-1}$  and  $1506 \text{ cm}^{-1}$  characterized  $\text{NH}_3^+$ -asymmetric ( $\delta_{\text{NH}_3^+}^{\text{as}}$ ) and symmetric ( $\delta_{\text{NH}_3^+}^{\text{s}}$ ) bending vibration of protonated amino group ( $\text{NH}_3^+$ ). The maxima at  $1611 \text{ cm}^{-1}$  ( $\nu_{\text{COO}^-}^{\text{as}}$ ) and  $1401 \text{ cm}^{-1}$  ( $\nu_{\text{COO}^-}^{\text{s}}$ ), belong to asymmetric and symmetric stretching vibrations of carboxylic anion respectively. The obtained results correlate well with the known theoretical IR-spectral ones for pure amino acids methionine and glycine [17-20, 21].

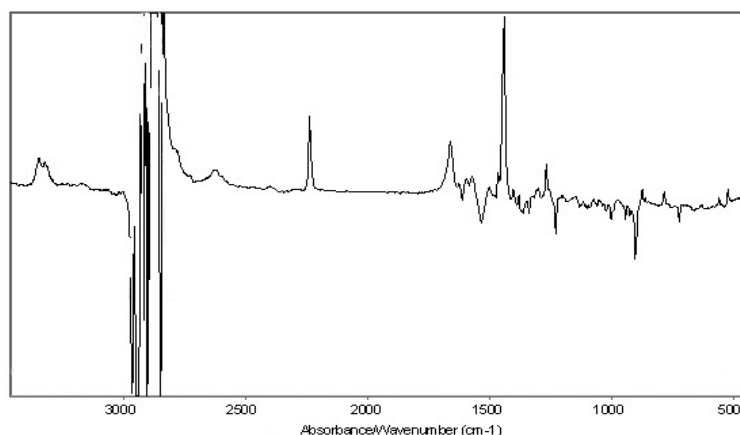


Fig. 4: Difference IR-LD spectrum of *Thr-Met*

According to the theory of linear-polarized spectroscopy, the elimination of the bands at equal dichroic ratio proposes the co-linear orientation of corresponding transition moments. Therefore, the elimination of  $\nu_{\text{NH}}$  peak at  $3345\text{ cm}^{-1}$  leads to disappearance of the Amide I band at  $1655\text{ cm}^{-1}$ , as well as the bands at  $1575\text{ cm}^{-1}$  and  $1401\text{ cm}^{-1}$ . When the bands for Amide I fragment  $\nu_{\text{NH}}$  and  $\nu_{\text{C=O}}$  are eliminated simultaneously, those supposed co-linear disposition of the  $\text{NH-C=O}$  amide fragment –probably in *trans*-configuration. Moreover, during the procedure the band at  $3315\text{ cm}^{-1}$  is strong reduced too, proposing a near to co-linear disposition of the transition moment of stretching OH vibration. It is known, that in pure methionine the stretching  $\nu_{\text{CS}}$  vibration appeared at  $725\text{ cm}^{-1}$  [21]. The elimination of the band at  $715\text{ cm}^{-1}$  in our case, leads to strong reduction of the  $\nu_{\text{COO}}^{\text{as}}$  band, which means that the band at  $715\text{ cm}^{-1}$  can be assigned to the discussed  $\nu_{\text{CS}}$  vibrations.

#### 4. CONCLUSION

The synthesis and IR-spectral elucidation of the dipeptide *L*-threonyl-*L*-methionine is carried out, using the possibilities of the IR-LD spectroscopy of oriented colloid suspensions in nematic liquid crystal.

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## Heavy Metal Complexes with the Amino Acid Phenylalanine

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**Abstract:** The complexes between the essential amino acid phenylalanine and transition metal ions ( $\text{MoO}_4^{2-}$ ,  $\text{VO}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$ ) in aqueous solutions at room temperature were obtained. The complexes by elemental analysis and IR-FT were characterized.

The geometry of the resulting compounds is optimized. Their electronic structures are calculated. The semi-empirical quantum chemical method ZINDO/1 was used for all calculations. The resulting complexes are flat-square structures with  $D_{2h}$  symmetry and are low-spin complexes. Characterized complexes were investigated as catalysts in the oxidation reaction of cyclohexene with tert-butylhydroperoxide. The reaction products were identified by GC/MS.

**Keywords:** metal complexes, phenylalanine, oxidation

### 1. INTRODUCTION

Quantum chemical calculations and analysis of the electronic structure of coordination complexes of transition metals can explain their physical and chemical properties at the electron level as well as facilitate estimation of some of their properties. Such analysis is needed due to their use in catalysis and their essential role in biological processes.

The interaction of heavy metals with amino acids, peptides and proteins is a subject of current interest. Amino acids are interesting and important compounds among chelating reagent. Some metal ions which react as catalysts in biochemical reactions are often combined with amino acid residues within proteins. Therefore, structural data of amino-carboxylate

complexes is very important in elucidating mechanisms of biochemical reactions.

The aim of the present work is to study of electronic structures of complexes of amino acid phenylalanine with heavy metals and their catalytic activity in the model oxidation reaction.

## 2. RESULTS AND DISCUSSIONS

Geometry optimization and electronic structure calculations were performed for six of the obtained coordination complexes of  $\text{MoO}_2^{2+}$ ,  $\text{VO}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  with the amino acid phenylalanine in vacuum. All calculations were performed by the semi-empirical quantum chemical method ZINDO/1 of the software package HyperChem 6.0 [4], using standard parametrization for the metal ion as well as all other atoms [3]. The structures were optimized in advance by Molecular mechanics (MM2). Quantum chemical calculations are performed by variation of all chemical bond lengths, valence and torsion angles by the optimization algorithm of Fletcher-Reeves. Calculation results for some bond lengths of the coordination complexes are presented in Table 1.

The following is known for the stereochemistry of coordination complexes of transition metals with electron configuration  $d^0$ ,  $d^1$  и  $d^2$  or multiply bonded ligands, such as the complexes of  $\text{MoO}_2^{2+}$  [1, 2]:

- multiply bonded ligands ( $\text{O}=\text{}$ ,  $\text{N}\equiv\text{}$ ) are in *cis*-position in reference to each other;
- Introduction of a multiply bonded ligand in an octahedric complex contributes to a significant elongation of the bond in *trans*-position in reference to the substituent.

Tab. 1: Calculated bond lengths (Å) metal - ligand of coordination complexes of phenylalanine with some transition  $\text{Me}^{n+}$  ions

Complex	Me = O	Me- N <sub>trans</sub>	Me - N <sub>cis</sub>	Me- O <sub>trans</sub>	Me- O <sub>cis</sub>
MoO <sub>2</sub> -(Phen) <sub>2</sub>	1.755 2.105	2.339	2.277	2.363	2.328
VO-(Phen) <sub>2</sub>	2.284	2.396	2.339	2.351	2.287
Fe-(Phen) <sub>2</sub>	-	2.155	2.143	2.039	2.012
Co-(Phen) <sub>2</sub>	-	2.039	2.053	1.929	1.890
Ni-(Phen) <sub>2</sub>	-	1.982	1.970	1.839	1.807
Cu-(Phen) <sub>2</sub>	-	2.005	1.992	1.858	1.843

Results in Table 1 show that the calculated geometry represents correctly the above specifics – the bonds in *trans*-position in reference to the ligand are longer those in *cis*. The bond lengths metal-ligand in all complexes do not differ significantly due to the lack of a conjugated bond system, which leads to insignificant contribution of the various substituents to coordination.

The small differences in bond lengths can be attributed to the influence of steric factors. In comparison to data from X-ray structure analysis of  $\text{MoO}_2\text{-(o-hydroxybenzimidazole)}_2$  bonds are longer, which correlates to the parameters of Mo atom in the applied quantum chemical method.

The obtained complexes of  $\text{Cu}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  with the amino acid phenylalanine have flat square structure with  $D_{2h}$  symmetry, where the main molecule skeleton is a single plain. They are low-spin complexes.

$E_{\text{HOMO}}$  and  $E_{\text{LUMO}}$  values are shown in the last two columns of Table 2. Also called border MO, they determine to a large extent the properties of the molecule system, such as steric behavior for ligand coordination and chemical reactivity.

Tab. 2: Calculation results of electronic structure of complexes of transition metals with phenylalanine

Complex	$q_{\text{Me}}$	$q_{\text{Ntrans}}$	$q_{\text{Ncis}}$	$q_{\text{Otrans}}$	$q_{\text{Ocis}}$	$E_{\text{HOMO}}$ (ev)	$E_{\text{LUMO}}$ (ev)	$\Delta E$ (HOMO- LUMO) (ev)
$\text{MoO}_2\text{-(Phen)}_2$	0.214	-0.187	-0.140	-0.390	-0.307	-5.287	3.542	8.829
$\text{VO-(Phen)}_2$	-0.295	-0.191	-0.181	-0.483	-0.471	-2.054	3.300	5.354
$(\text{Phen})_2\text{-Fe}$	0.218	-0.207	-0.202	-0.459	-0.455	-6.408	2.713	9.121
$(\text{Phen})_2\text{-Co}$	0.130	-0.193	-0.183	-0.448	-0.441	-6.477	3.472	9.949
$(\text{Phen})_2\text{-Ni}$	0.139	-0.183	-0.176	-0.470	-0.448	-6.410	4.219	10.629
$(\text{Phen})_2\text{-Cu}$	-0.128	-0.209	-0.178	-0.419	-0.413	-6.242	3.054	9.296

In all coordination complexes the HOMO orbital consists of atom orbital of the ligands. The LUMO orbital of all complexes, as opposed to the HOMO one, consists mostly of AO of the metal ion and partly  $p_y$ -AO of oxygen and nitrogen atom of the amino acid (Fig. 1 and Fig. 2).

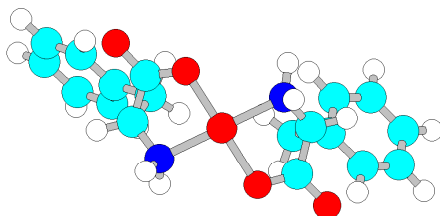


Fig.1: complex  $\text{Fe-(Phenylalanine)}_2$  – ZINDO/1  
Symbol: Fe- red centre, O-red, N-blue, C- light blue, H- white.

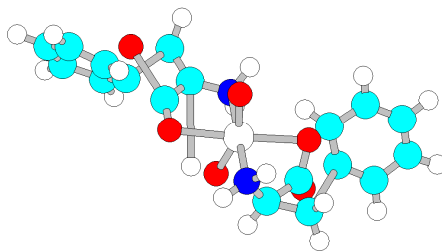


Fig.2: complex  $\text{MoO}_2\text{-(Phenylalanine)}_2$  – ZINDO/1  
 Symbol: Mo- white centre, O-red, N-blue, C- light blue, H- white.

The Table 3 shows the characteristic bands of the amino acid and its complexes. As can be seen from this, the characteristic bands at  $1614\text{ cm}^{-1}$  and  $1419\text{ cm}^{-1}$  attributed to the to the asymmetric and symmetric vibrations of the  $\text{COO}^-$  were shifted to  $1620\text{ cm}^{-1}$  and  $1407\text{ cm}^{-1}$  for vanadium and  $1619\text{ cm}^{-1}$  and  $1395\text{ cm}^{-1}$  for molybdenum, respectively. The deformation vibrations of the  $\delta\text{ NH}$  group at  $1550\text{ cm}^{-1}$  were not registered after the formation of a complex with vanadium, while for molybdenum-complex they were shifted to  $1497\text{ cm}^{-1}$ . After complexation with vanadium, bands at  $949\text{ cm}^{-1}$  and  $1008\text{ cm}^{-1}$  were observed, which could be attributed to  $\text{V=O}$  bond. For the molybdenum complex, a band at  $745\text{ cm}^{-1}$  corresponding to  $\text{Mo-O-Mo}$  and  $920\text{ cm}^{-1}$  corresponding to  $\text{Mo=O}$  bond were observed [5-9].

Tab. 3: Characteristic bands for complexes,  $\text{cm}^{-1}$

Phen	Phen+V	Phen+Mo	Functional group
3059	5159	3196	$\nu\text{ }^+\text{NH}_3$
1614	1620	1619	$\nu_{\text{as}}\text{ COO}^-$
1550	-	1497	$\delta\text{ NH}$
1419	1407	1395	$\nu_{\text{s}}\text{ COO}^-$
-	949,1008	-	$\text{V=O}$
-	-	920	$\text{Mo=O}$
		745	$\text{Mo-O-Mo}$

Cyclohexene was subjected to oxidation with *tert*-butylhydroperoxide in a batch glass reactor, equipped with a magnetic stirrer at  $80^\circ\text{C}$  for 90 min. Certain amount of the complex, corresponding to concentration of  $5 \cdot 10^{-3}\text{ mmol metal/l}$  was initially placed into the reactor, which was followed by addition of cyclohexene (0.8 g,  $9.8 \cdot 10^{-3}\text{ mol}$ ), *tert*-butylhydroperoxide (0.081 g,  $8.79 \cdot 10^{-4}\text{ mol}$ ) and 4.0 ml toluene as solvent. The reaction was conducted under stirring.

The metal complexes were studied as catalysts in the reaction of oxidation of cyclohexene with *tert*-butylhydroperoxide. The results presented in Table 4 show that the high yields of cyclohexene oxide were obtained with molybdenum and vanadium complexes, 36.5% and 19.2% respectively, while the yields with Cu, Co, Fe and Ni were about 2%. These metals have high oxidation potential and initiate the homolytic decomposition of organic hydroperoxides but do not have significant effect on the epoxidation reaction.

Tab. 4: Oxidation of cyclohexene with *tert*-butylhydroperoxide

Complex	Cyclohexene oxide (%)	2-Cyclohexene- 1-ol (%)	2-Cyclohexene- 1-one (%)
Phen+Mo	36.5	2.1	1.8
Phen+V	19.2	5.1	9.1
Phen+Cu	5.3	3.9	1.1
Phen+Co	3.2	7.3	8.8
Phen+Fe	1.5	7.7	7.8
Phen+Ni	1.8	14.3	1.4

### 3. CONCLUSIONS

The following conclusions are based on the obtained experimental results:

1. The semi-empirical method ZINDO/1 is used to optimize the geometry of six of the complexes. The probable structures of the complexes are obtained.
2. The obtained results for the structures coincide with data obtained from physical-chemical analysis of similar chemicals.
3. It was found out that the order of the catalytic activity of the complexes of phenylalanine in the reaction of epoxidation of cyclohexene was as follows:  $\text{MoO}^{2+} > \text{VO}^{2+} > \text{Cu}^{2+} > \text{Co}^{2+} > \text{Fe}^{2+} \sim \text{Ni}^{2+}$

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## Generation and Selection of Likely Active Conformers of Metal Complexes with Amino Acid (Phenylalanine)

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**Abstract:** While many drug molecules are “organic” in nature, elements in the periodic table, particularly metals, offer a much more diverse chemistry and have important therapeutic applications. The mononuclear molybdoen- zymes play a pivotal role in the regular metabolism of biological system. These enzymes catalyze specific reactions and they are indirectly involved in the global recycling of sulphur, nitrogen and carbon. The different metal complexes interact with amino acids and modulate compounds with possible activity. The aim of this work was to generate and select possible active conformers of metal complexes ( $\text{MoO}_2^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{VO}^{2+}$ ) with amino acid (phenylalanine) and using molecular parameters found to be associated with the chemical bilks and other steric distances.

**Keywords:** conformers, metal complexes, amino acid, phenylalanine

### 1. INTRODUCTION

Metal ions play an important role in the structure and function of many biomacromolecules. They can be found at active sites of enzymes [8], have an important role in photosynthesis [2], act as secondary messengers [1], promote and stabilize native conformations of nucleic acids [11], and facilitate protein-DNA binding [9]. Metal-based compounds may also be used as potent antibacterial, antifungal, and anticancer drugs [10], or as imaging agents [7].



Recently, metal ions have been also used in metallization of biomacromolecules [4]. The molecular mechanism of the metallization process has been studied by means of quantum chemical calculations of metal ion-amino acid interactions [12]. It has been found that high chemical affinities of specific sites within the macromolecules towards the metal ion usually depend on the interplay of at least two amino acid moieties. In some cases, one of the amino acids involved in the metal ion binding may have a rather small affinity towards that ion, but its combination with a second amino acid may result in a combined high metal affinity leading to a strong binding [13].

Metal aminocarboxylate complexes have been the subject of extensive research for many years. They are used to study phenomenon of the structure, stability, magnetic properties and non-covalent interactions important in chemical reactions, molecular recognition and regulation of biochemical processes, for a better understanding of enzyme-metal ion-substrate complexes, which play an important role in metalloenzyme-catalysed biochemical reactions [6].

The aim of this work was to generate and select possible active conformers of metal complexes ( $\text{MoO}_2^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{VO}^{2+}$ ) with amino acid (phenylalanine) and using molecular parameters found to be associated with the chemical bonds and other steric distances.

## 2. MATERIAL AND METHODS

Metal complexes ( $\text{MoO}_2^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{VO}^{2+}$ ) with amino acid (phenylalanine) were generated. Details of procedure used for conformer generation can be found in Bradbury et al., [3]. The number of conformers initially generated for these chemicals was reduced by not permitting rotation around the two most peripheral C-C bonds. Up to 500 of the sterically most distinct points from the conformational space for each chemical were selected. Geometric dissimilarity was assessed based on Euclidean distances between the sums of interatomic distances for the conformers. All generated conformations of each chemical were submitted to strain minimization techniques and geometric optimizations [3].

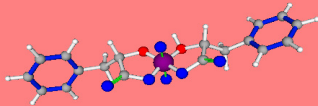
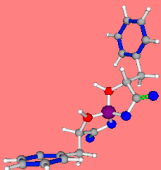
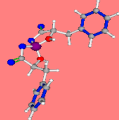
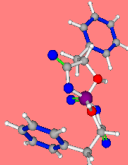
Van der Waals surface and volume calculations were chosen as calculated by Connolly et al. [5]

## 3. RESULTS AND DISCUSSIONS

Conformer generation of metal complexes ( $\text{MoO}_2^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{VO}^{2+}$ ) with amino acid (phenylalanine) were done. The number of conformers generated was 30. Of them one conformer was selected by parameters

(VAN\_D.\_WAALS\_SUR [ $\text{\AA}^2$ ] and MAX DISTANCE [ $\text{\AA}$ ]). Conformer with a maximum value of VAN\_D.\_WAALS\_SUR was selected. Results are presented in Table 1.

Tab. 1: Generated and selected conformer of metal complexes with amino acid phenylalanine by parameters

Metal complexes with amino acid (phenylalanine)	Parameters	Conformer
MoO <sub>2</sub> <sup>2+</sup>	VAN_D._WAALS_SUR = 344.05 $\text{\AA}^2$ ; (van der Waals surface) MAX DISTANCE = 16.94 $\text{\AA}$ ; (steric index; maximal distance in the molecule [ $\text{\AA}$ ])	
Fe <sup>2+</sup>	VAN_D._WAALS_SUR = 340.50 $\text{\AA}^2$  MAX DISTANCE = 14.34 $\text{\AA}$	
Ni <sup>2+</sup>	VAN_D._WAALS_SUR = 337.05 $\text{\AA}^2$ ; MAX DISTANCE = 13.92 $\text{\AA}$	
VO <sup>2+</sup>	VAN_D._WAALS_SUR = 339.06 $\text{\AA}^2$ ; MAX DISTANCE = 12.00 $\text{\AA}$	

#### 4. CONCLUSION

It is well known that some metal ions are essential for living organisms. They play important roles in the synthesis and transport of organic molecules and in catalysing acid-base and redox processes of biological systems. The results of this work have shown that metal complexes with the amino acid phenylalanine can have different conformers conditions (30 conformers are generated) but potentially actives are just some of them. Selection of active conformers occur through molecular parameters found to be associated with the chemical bilks (VAN\_D.\_WAALS\_SUR, Å<sup>2</sup>) and other steric distances (MAX\_DISTANCE, Å).

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# Synthesis and Structural Characterization of 1,1'-Dihalo-2,2'-spirobiindanes

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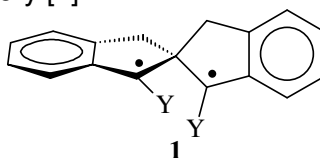
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**Abstract:** Starting from 2,2'-spirobiindan-1,1'-dione, 1,1'-dibromo-2,2'-spirobiindanes and 1,1'-dichloro-2,2'-spirobiindanes were synthesized via two-steps using standard chemical transformations. For each of these derivatives six stereoisomers are possible (three pairs of enantiomers). In the case of 1,1'-dichloro-2,2'-spirobiindanes two of the diastereomers were separated and purified, and using  $^1\text{H}$  NMR and single crystal X-ray crystallography their relative configuration was determined. The stereochemical assignment of the 1,1'-dibromo-2,2'-spirobiindanes was accomplished by analogy to the dichloro derivative.

**Keywords:** 1,1'-Dihalo-2,2'-spirobiindanes, relative configuration, synthesis

## 1. INTRODUCTION

As a part of our study of 2,2'-spirobiindane derivatives as potential diradical precursors [1,2], we have focused our attention on synthesis of photolabile derivatives such as dihalides. The starting material of choice was, 2,2'-spirobiindan-1,1'-dione, because its synthesis was well documented in the literature [3] and we had experience with its large-scale preparation in our laboratory [4].



Our primary goal was to focus on the preparation of the 1,1'-Dihalo-2,2'-spirobiindane (**X(HH)D**) systems ( $\text{X}=\text{Cl}, \text{Br}$ ), in order to gain understanding of the stereochemical and reactivity issues and apply it to the more challenging precursors (spirocyclopropane derivatives). The choice of photoremovable **X** substituents was chlorine or bromine, because these are known in the literature to give diradicals either by direct photolysis [5,6] or by photolysis in the presence of amines [7,8]. Once the 1,3-dihalides are prepared they can serve as convenient diradical precursors and synthetic precursors for the spirocyclopropanes.

## 2. MATERIALS AND METHODS

The title compounds were purified and characterized using standard procedures. All NMR spectra were recorded on Bruker DRX-400 spectrometer (400 MHz for proton) in deuterated chloroform ( $\text{CDCl}_3$ ) using TMS as internal standard. The reported X-ray structures were obtained using X-ray intensity data measured at 98 K (cooled by Rigaku MSC X-Stream 2000) on a Bruker SMART APEX CCD area detector system equipped with a graphite monochromator and a MoK $\alpha$  fine-focus sealed tube ( $\lambda = 0.71073 \text{ \AA}$ ) operated at 1600 W (50 kV, 32 mA).

## 3. RESULTS AND DISCUSSION

### 3.1 Practical assignment of relative stereochemistry

In the course of this study both enantiomers are always present in equal amounts (all materials are racemates). Since the tetrahedral stereogenic centers on the carbons 1 and 1' are equivalent in terms of their substituents, only six stereoisomers (three pairs of enantiomers) are expected. The three diastereomeric racemates of the synthetic precursors should in principle have different enough physico-chemical properties, both chromatographically and spectroscopically, to be separable and identifiable. From the point of view of the NMR techniques, inspection of models indicates that there are two symmetrical and one non-symmetrical diastereomers (symmetrical in terms of chemical shift equivalency of the "halves"). The non-symmetrical isomers would yield two sets of signals (the two spiro sub-systems will be non-equivalent). The non-symmetrical isomer ( $C_1$  space group), will always correspond to mixed label, **X(mHH)D**. On the other hand, the two symmetrical isomers will have only one set of signals (for both subunits). These isomers are  $C_2$  symmetric, and will always have the designation anti, **X(aHH)D** or syn **X(sHH)D**. The only method to distinguish is single crystal X-ray crystallography. Additional potential complications arise from the fact that in the non-idealized structures five-membered rings are not flat but adopt a so-called "envelope" conformation. This deviation from planarity becomes evident from structural calculations and literature X-ray structure of 2,2'-spirobiindan-1,1'-dione [9] as well as those obtained in our laboratory [4]. In solution, however, there is conformational flexibility.

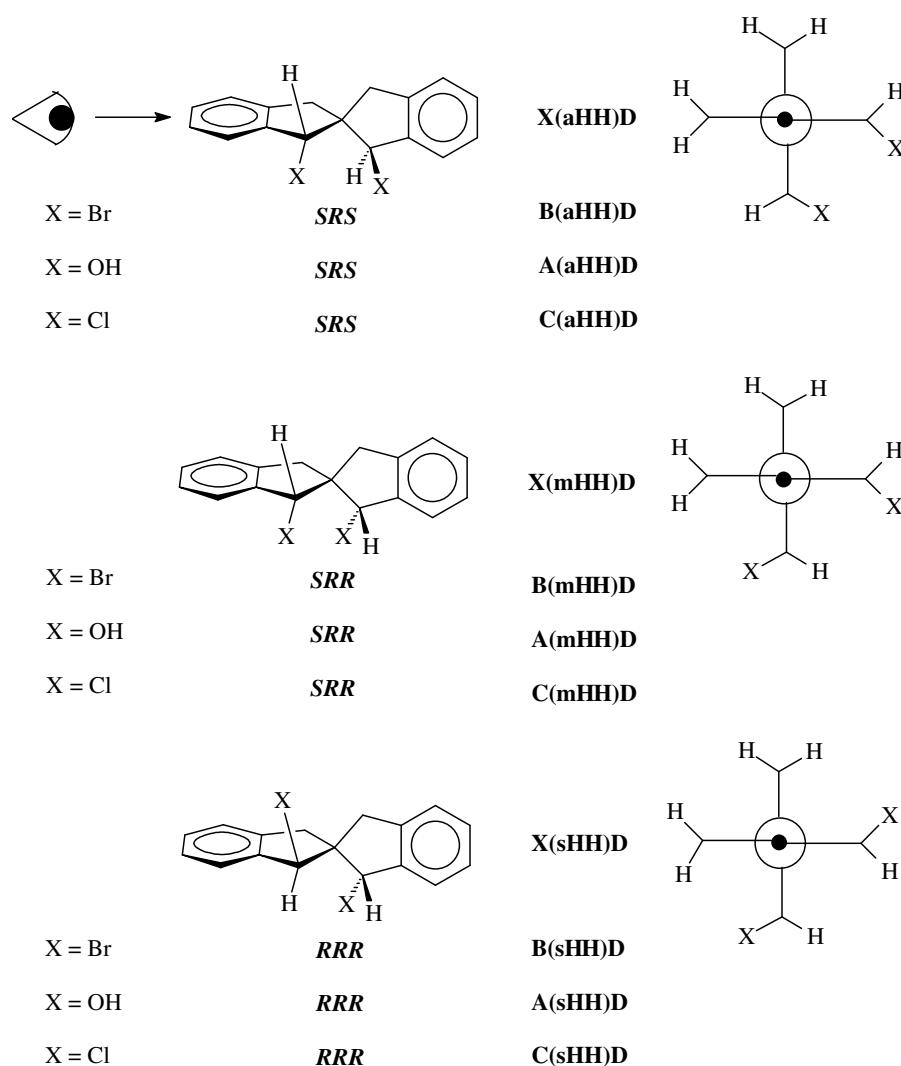


Fig. 1: Three possible diastereomers of 1,1'-(diX)-2,2'-spirobiindane, **X(HH)D**, system. Only one enantiomer of each pair is shown.

The energy differences between extreme conformers are low [10,11], so the interconversion in solution at 25 °C is rapid [12,13]. As indeed was observed, the rings rapidly (faster than the NMR time-scale) swing between the extremes, giving “time averaged” signals simplifying the analysis based on the idealized “flat” structures. This kind of behavior has been observed for the 2,2'-spirobiindan-1,1'-dione derivatives.

### 3.2. Synthesis of the 1,1'-Dihalo-2,2'-spirobiindanes

This simplest system in the series offered rather easy access to the desired dichlorides **C(HH)D** and dibromides **B(HH)D**. The synthetic strategy followed conventional logic: reduction of 2,2'-spirobiindan-1,1'-dione, **2**, followed by treatment with  $\text{SOCl}_2$  or  $\text{PBr}_3$  to obtain the desired **C(HH)D** and **B(HH)D**, respectively. The synthesis of 2,2'-spirobiindan-1,1'-diols (**A(aHH)D**, **A(mHH)D**, **A(sHH)D**) and their absolute and relative configuration were reported in the literature [14-19]. However, the syntheses employed lithium aluminum hydride, DIBAL-H, or lithium *t*-butyldiisobutylaluminum hydride, not particularly convenient reagents, especially for large-scale reactions. We developed a simple and convenient procedure utilizing sodium borohydride giving a mixture of diastereomeric alcohols (**A(aHH)D** : **A(mHH)D** : **A(sHH)D** 1.0:3.75:1.5) in 90% isolated yield.

There was a grain of concern at this stage, since these specific diols were found by Nieman and co-workers [14] to be acid-sensitive (rearranged to 11*H*-benzo[*b*]fluorene). Addition of the above-mentioned diol mixture to an excess of neat thionyl chloride and subsequent work-up resulted in a mixture of three diastereomeric dichlorides (**C(aHH)D** : **C(mHH)D** : **C(sHH)D** 1.2:3.6:1.0) in 71% isolated yield after column chromatography. Two of the isomers (**C(aHH)D** and **C(mHH)D**) were further purified by recrystallization and the relative stereochemistry assignments for this group were determined via X-ray crystallography.

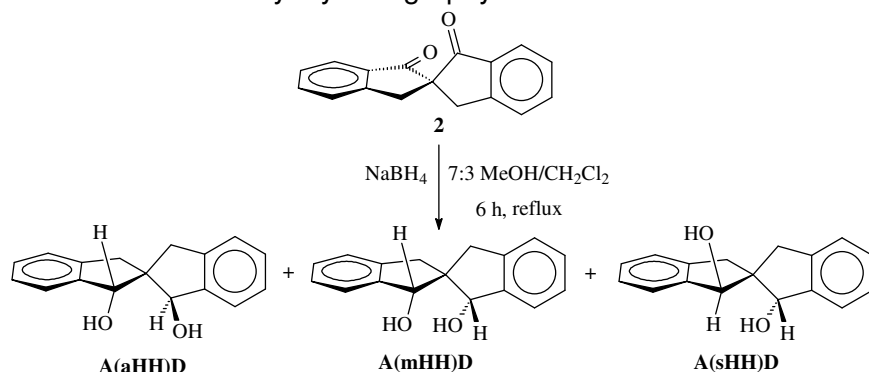


Fig. 2: Reduction of **2** with sodium borohydride. The three diols were obtained in 90% yield and their ratio was **A(aHH)D**:**A(mHH)D**:**A(sHH)D** = 1.0: 3.75 : 1.5.

In similar manner, the mixture of diols was added to an excess of neat phosphorous tribromide and the following aqueous work-up resulted in a mixture of three diastereomeric bromides (**B(aHH)D** : **B(mHH)D** : **B(sHH)D** 1.0:3.4:1.5). All recrystallization attempts failed. The bromides were susceptible to solvolysis, and were found to be labile to silica gel



chromatography. The stereochemical assignment was accomplished by analogy to the chloride system.(Fig. 3).

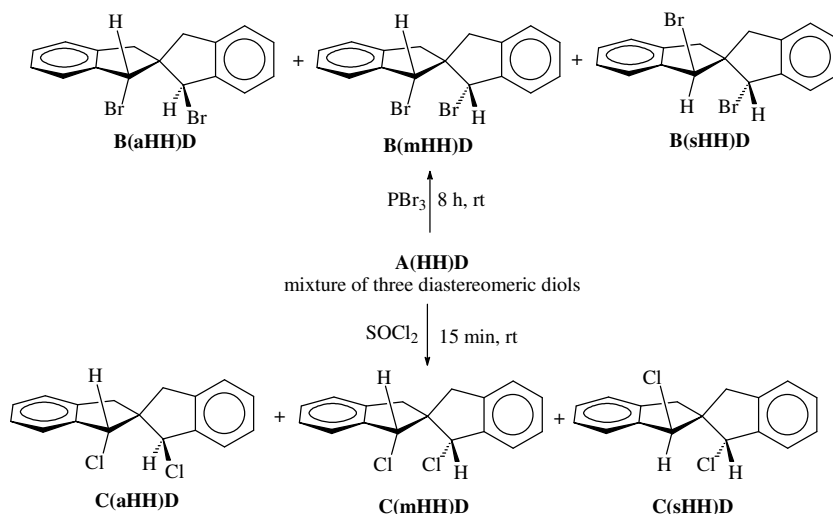


Fig 3.: Syntheses of 1,1'-dihalo-2,2'-spirobiindanes.

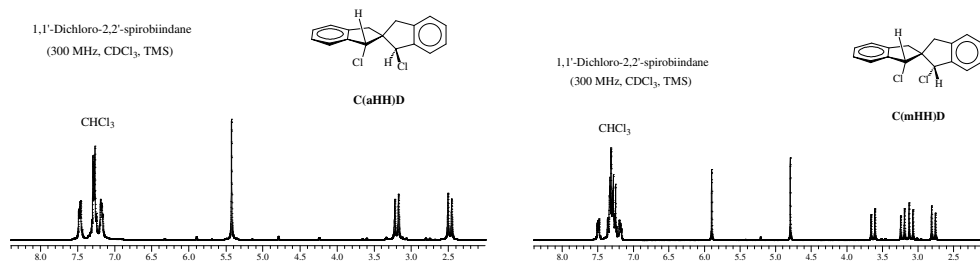


Fig. 4:  $^1\text{H}$  NMR spectra of two diastereomers of 1,1'-dichloro-2,2'-spirobiindane, **C(aHH)D** (left) and **C(mHH)D** (right) in  $\text{CDCl}_3$  (400 MHz).

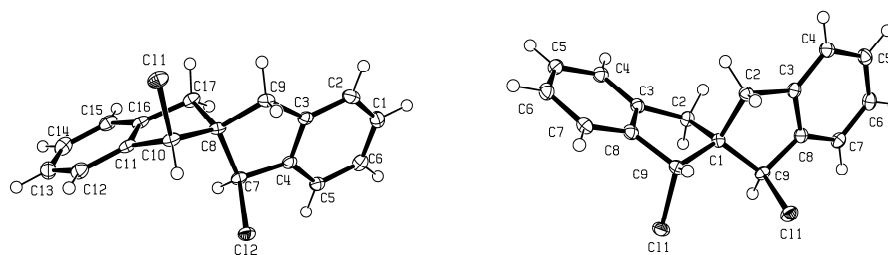


Fig. 5: ORTEP drawings of **C(aHH)D** (left) and **C(mHH)D** (right). Displacement ellipsoids are drawn at 50% probability level.

#### 4. ACKNOWLEDGEMENT

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# Synthesis and Deoxygenation of 1,1'-Diaryl-2,2'-spirobiindan-1,1'-diols

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**Abstract:** *In the course of functionalization of the 2,2'-spirobiindane skeleton, 1,1'-diaryl-2,2'-spirobiindane-1,1'-diols were synthesized as a versatile synthetic intermediates (Ar = Ph, 4-FPh). It was found that most of the conventional deoxygenation methods cause the 1,1'-diarylspirodiol to undergo a facile Lewis-acid catalyzed dehydration. Employing the method developed by Lau and co-workers, using zinc iodide and sodium cyanoborohydride in 1,2-dichloroethane, the desired deoxygenated product(s) were obtained along with deoxygenated ring-opened products. Based on the structure of the intermediate, the absence of monoalcohols, and the presence of the ring-opened by-products tentative mechanism for this transformation is proposed. The structure and the relative stereochemistry of the desired deoxygenated products, 1,1'-diphenyl-2,2'-spirobiindane and 1,1'-bis(4-fluorophenyl)-2,2'-spirobiindane were determined using  $^1\text{H}$  NMR and single crystal X-ray crystallography.*

**Keywords:** *1,1'-diaryl-2,2'-spirobiindane-1,1'-diols, synthesis, deoxygenation, 1,1'-diaryl-2,2'-spirobiindanes*

## 1. INTRODUCTION

As a part of our systematic study of spirobiindane derivatives as potential precursors for reactive intermediates [1] (such as diradicals), we have focused our attention on introduction of groups that would increase the persistency of the diradicals [2]. We have chosen to build the spirocenter early in our synthetic sequence and then to introduce aryl groups in the 2,2'-spirobiindane framework. Previous work [3] clearly indicated the sensitivity of 2,2'-spirobiindan-1,1'-dione and related systems to nucleophiles and reaction conditions were optimized to avoid the destruction of the spirosystem. Additionally, it was found that the 1,1'-diaryl-2,2'-spirobiindane-1,1'-diols were susceptible to ring-opening via acid-catalyzed dehydration (Figure 1). This meant that the diarylspirodiols could not be converted to the desired dichlorides or dibromides due to their acid

sensitivity. We have resorted to deoxygenation of the diaryl spirodiols. Herein, we would like to report the syntheses of 1,1'-diaryl-2,2'-spirobiindane-1,1'-diols (Ar = Ph, 4-FPh) and their deoxygenation to the corresponding 1,1'-diaryl-2,2'-spirobiindanes.

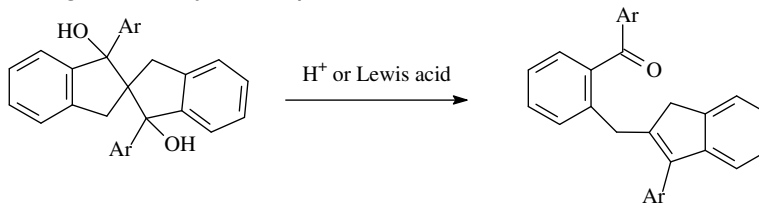


Fig. 1: Lewis-acid catalyzed rearrangement of 1,1'-diaryl-2,2'-spirobiindane-1,1'-diols.

## 2. MATERIALS AND METHODS

The title compounds were purified and characterized using standard procedures. NMR spectra were recorded on Bruker DRX-400 spectrometer (400 MHz for proton) in deuterated chloroform ( $\text{CDCl}_3$ ) and were reported in ppm with respect to tetramethylsilane (2 drops per 100 g  $\text{CDCl}_3$ ). Infrared spectra were recorded on Perkin Elmer Model 1600. as thin films between sodium chloride plates (reported as film). The reported X-ray structures were obtained using X-ray intensity data measured at 98 K (cooled by Rigaku MSC X-Stream 2000) on a Bruker SMART APEX CCD area detector system equipped with a graphite monochromator and a  $\text{MoK}\alpha$  fine-focus sealed tube ( $\lambda = 0.71073 \text{ \AA}$ ) operated at 1600 W (50 kV, 32 mA). 1,1'-Dihydroxy-1,1'-diphenyl-2,2'-spirobiindanes were prepared by literature procedure [10].

**Preparation of 1,1'-bis(4-fluorophenyl)-2,2'-spirobiindan-1,1'-diol.** 2,2'-Spirobiindan-1,1'-dione was dissolved in dry tetrahydrofuran in a Schlenk flask and cooled to  $-78^\circ\text{C}$ . In another Schlenk flask 2.2 equivalents of 4-fluorophenylmagnesium bromide (ether solution) solution was cooled also cooled to  $-78^\circ\text{C}$ . The dione was slowly added to the Grignard reagent over a period of 10 minutes. The resulting solution was stirred at  $-78^\circ\text{C}$  for 1 hour and allowed to warm-up to ambient temperature and then stirred for additional 2 hours. The excess of Grignard reagent was quenched by dropwise addition of water, followed by ammonium chloride and dichloromethane. The resulting two-phase mixture was partitioned between dichloromethane and saturated ammonium chloride aqueous solution. After the separation, drying and evaporation of organic solvent, white solid was obtained in 79% yield and  $^1\text{H}$  NMR analysis revealed the presence of two

diastereomeric alcohols (one symmetrical and one non-symmetrical) in 1.0:1.1 ratio. **3a**:  $^1\text{H NMR}$  (360 MHz,  $\text{C}_6\text{D}_6$ ): 7.66 - 6.8 (m, 16 H), 4.46 (s, 2 H, OH), 2.92 (d,  $J = 16$  Hz, 2 H), 2.44 (d,  $J = 16$  Hz, 2 H); **3b**:  $^1\text{H NMR}$ : 7.7 - 6.72 (m, 16 H), 5.00 (s, 1 H, OH), 4.63 (s, 1 H, OH), 4.38 (d,  $J = 16.2$  Hz, 1 H), 3.32 (d,  $J = 16.2$  Hz, 1 H), 2.59 (m, 2 overlapping doublets, 2 H). The mixture of diols (**3a** and **3b**) was used in the next step without further purification.

**General deoxygenation procedure for 1,1'-diaryl-2,2'-spirobiindane-1,1'-diols.** Zinc iodide (0.0868 mol) and sodium cyanoborohydride (0.428 mol) were placed in a 1 L Schlenk flask and dried for 4 hours at 70 °C. The Schlenk flask was removed from the oil bath and was cooled to room temperature. A mixture of 1,1'-dihydroxy-1,1'-diaryl-2,2'-spirobiindanes (0.0248 mol) were added to the flask, followed by 1,2-dichloroethane (550 mL) and the resulting suspension was brought to reflux. The suspension was refluxed for 60 hours, cooled to room temperature and filtered through a sintered funnel. The solid residue on the funnel was rinsed with dichloromethane (4×100 mL). The filtrate was washed with saturated ammonium chloride containing 8% concentrated hydrochloric acid (300 mL), saturated ammonium chloride (300 mL), water (3×300 mL) and dried over sodium sulfate. Removal of the solvent via rotary evaporation gave yellow oil, which was triturated with hexane. The hexane was removed and the residue was dried in vacuo to give on average 21 g of yellow foam. The product distributions were determined by  $^1\text{H NMR}$  analysis. In the case of deoxygenation of 1,1'-dihydroxy-1,1'-diphenyl-2,2'-spirobiindane, one of the desired 1,1'-diphenyl-2,2'-spirobiindane products, **H(sPhPh)D**, was obtained by recrystallization from methanol/benzene (1:1) from the reaction mixture and X-ray structure was obtained. **(SSS) + (RRR)-1,1'-diphenyl-2,2'-spirobiindane, H(sPhPh)D**. mp 241 - 242 °C;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , p. 285): 7.24 (d,  $J = 7.4$  Hz, 2 H), 7.15 (t,  $J = 7.3$  Hz, 2 H), 7.08 (t,  $J = 7.3$  Hz, 2 H), 6.99 (d,  $J = 7.4$  Hz, 2H), 6.95 (d,  $J = 7.3$  Hz, 2 H), 6.88 (m, 4 H), 6.50 (d,  $J = 7.3$  Hz, 4 H), 4.06 (s, 2 H), 3.48 (d,  $J = 15.4$  Hz, 2 H), 2.97 (d,  $J = 15.4$  Hz, 2 H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ): 148.90 (C), 143.80 (C), 141.24 (C), 127.82 (CH), 126.92 (CH), 126.66 (CH), 125.34 (CH), 125.19 (CH), 60.64 (C, spiro), 59.24 (CH), 49.03 ( $\text{CH}_2$ ); **EI-MS** ( $m/z$ , relative intensity): 373 ( $\text{M}^+ + 1$ , 16%), 372 ( $\text{M}^+$ , 50%), 215(6%), 203(6%), 202(8%), 192 (20%), 191 (46%), 181 (29%), 180 (100%), 179 (81%), 178 (38%), 166 (11%), 165 (35%); In the case of deoxygenation of **3 (a and b)**, one of the desired 1,1'-bis(4-fluorophenyl)-2,2'-spirobiindane products was obtained by recrystallization from methylene chloride and X-ray structure was also obtained [10]. **H(s4-FPh4-FPh)D**. mp 171 - 173 °C;  $^1\text{H NMR}$  (400 MHz,  $\text{CDCl}_3$ , p. 285): 7.35-6.40 (m, 16 H), 4.04 (s, 2 H), 3.43 (d,  $J = 15.5$  Hz, 2 H), 2.97 (d,  $J = 15.5$  Hz, 2H);  $^{13}\text{C NMR}$  (100 MHz,  $\text{CDCl}_3$ ): 162.49 (C), 160.06

(C), 148.69 (C), 141.16 (C), 139.98 (C), 139.95 (C), 129.31 (CH), 129.24 (CH), 127.30 (CH), 127.13 (CH), 125.69 (CH), 125.35 (CH), 114.95 (CH), 114.74 (CH), 60.68 (C, spiro), 58.69 (CH), 49.12 (CH<sub>2</sub>); **EI-MS** (m/z, relative intensity): 409 (M<sup>+</sup> + 1, 3%), 408 (M<sup>+</sup>, 9%), 199 (23%), 197 (68%), 196 (20%).

### 3. RESULTS AND DISCUSSION

In order to avoid ring opening we have optimized reaction conditions for the synthesis of the 1,1'-diaryl-2,2'-spirobiindane-1,1'-diols (Ar = Ph, 4-FPh) by "reverse addition" i.e. slowly add a solution of the 2,2'-spirobiindandione (**1**) to a slight excess (2.1-2.3 equivalents) of a pre-cooled phenyllithium solution or p-fluorophenylmagnesium bromide followed by mild protonation with aqueous ammonium chloride. The above mentioned reverse addition gave the desired products, **2** and **3** in 69% and 72% yields respectively (mixture of two diastereomers, **2a** : **2b** = 2.5 : 1.0 and **3a** : **3b** = 1.1 : 1.0). Several attempts of obtaining X-ray quality crystals of **2a** have failed. The stereochemistry of **2a** and **2b** was established based on <sup>1</sup>H NMR data and via structural correlation with the 1,1'-dimethoxy-1,1'-diphenyl-2,2'-spirobiindane, for which we have obtained single crystal X-ray crystallographic data.

We have come to a conclusion, based on the spectral data, that the spirodiol, **2a**, is sensitive to the acidic impurities in the deuterated chloroform. The <sup>1</sup>H NMR spectroscopy indicated quantitative conversion of **2a** (or **2b**) to **6**.

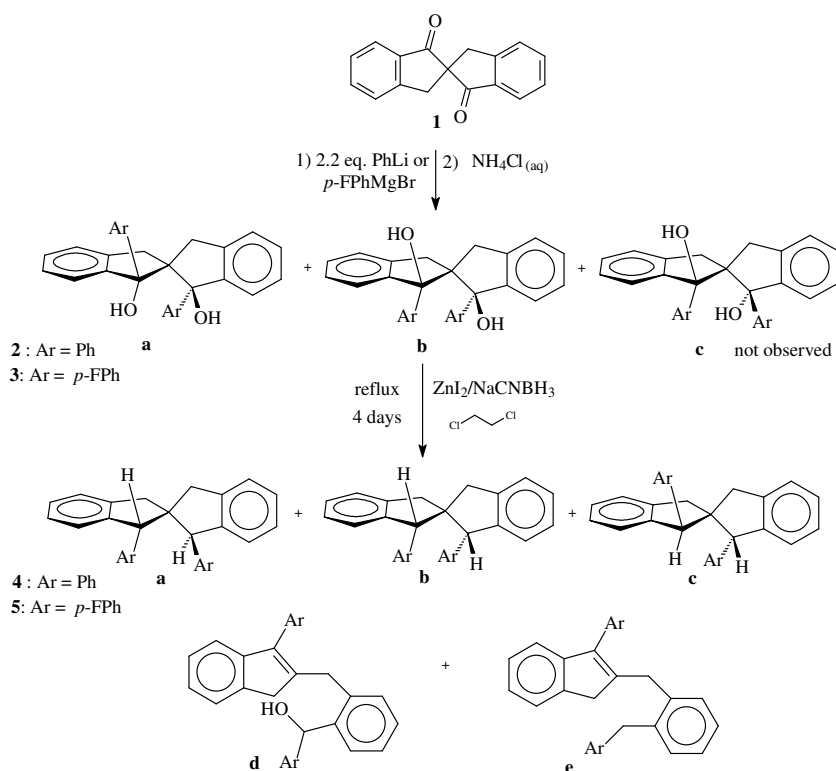


Fig. 2: Synthesis of 1,1'-diphenyl-2,2'-spirobiindane-1,1'-diols (**2** and **3**) and the products (**4a-e** and **5a-e**) of their deoxygenation with NaCNBH<sub>3</sub> in presence of ZnI<sub>2</sub>.

We decided to proceed to find alternative protective groups for the tertiary alcohols, possibly such that could serve as radical precursor (Barton-type deoxygenation chemistry[4,5]). We have attempted preparation of the cyclopropane derivative using the methodology developed by Walborsky [6] involving reductive coupling of 1,3-diols using low valent titanium (McMurry reagent,[7,8] TiCl<sub>3</sub>/LiAlH<sub>4</sub>) to afford the cyclopropane. Subjecting **2a** to these conditions resulted in rearrangement product **6**. The presence of traces of Lewis acid, (we suspect unreacted TiCl<sub>3</sub>) was sufficient to trigger the rearrangement. We have decided to remove the OH groups and proceed with functionalization without them. There are many methods for deoxygenation, of which several were tried without success (Pd/C catalytic hydrogenation under acidic conditions, EtOH/Na, PDCl<sub>2</sub>/CH<sub>3</sub>OH). Convenient deoxygenation was achieved by a method developed by Lau and co-workers [9], utilizing NaCNBH<sub>3</sub> in presence of ZnI<sub>2</sub> in 1,2-dichloroethane. Analysis of the crude reaction mixture revealed presence of three deoxygenated isomers **4a**, **4b** and **4c** and two major side products **4d** and **4e** (Fig. 2).

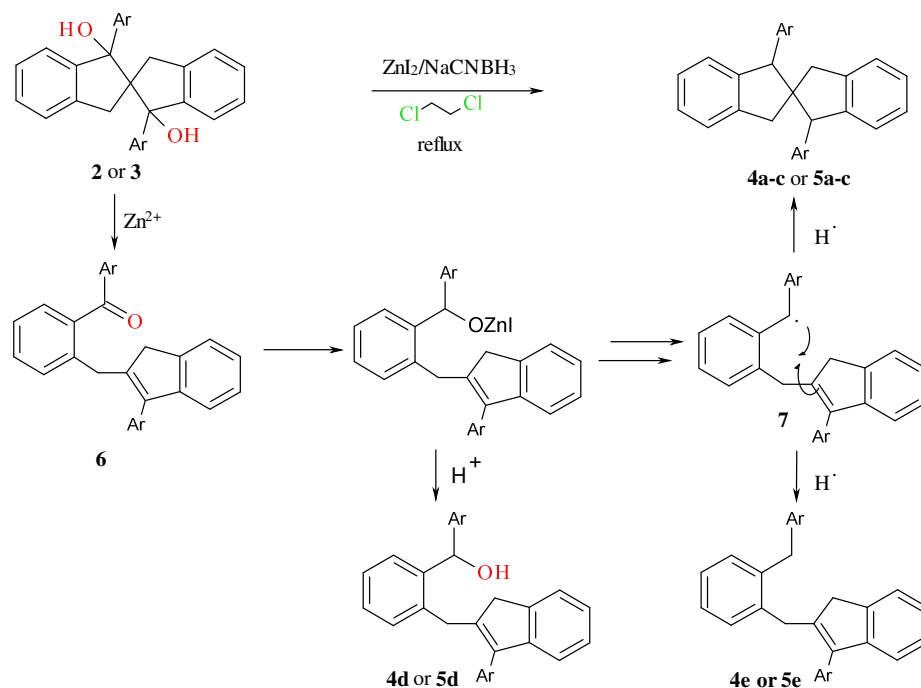


Fig. 3: Proposed mechanism for the deoxygenation of 1,1'-diphenyl-2,2'-spirobiindane-1,1'-diols (**2** and **3**) with  $\text{NaCNBH}_3$  in presence of  $\text{ZnI}_2$

Frequent and careful monitoring of the progress of the reaction revealed that the conversion to the desired hydrocarbons did not proceed via “direct” stepwise deoxygenation, but through previously described Lewis-acid-catalyzed rearrangement of the diol (Fig. 1). Reduction of the ketone **6** (the product of such rearrangement) followed by generation of a radical, **7**, and its cyclization results in the desired hydrocarbons. Hydrogen abstraction by the intermediate radical, **6**, results in the ring opened hydrocarbon **4e**. In support of our proposed mechanism is the isolation of the ring opened alcohol, **4d**, and the absence of spiro monoalcohol. The convincing evidence for such a mechanism was obtained when **4d** was subjected to the reaction conditions, giving the identical hydrocarbon products in a similar ratio.

Direct recrystallization of the crude reaction mixture resulted in **4a** as a benzene solvate. X-ray analysis firmly established the stereochemistry of this group of compounds. It is a remarkable feature that from four isomeric hydrocarbons (three spiro and one ring-opened) only one forms benzene solvate. The other two hydrocarbons **4b** and **4c** were obtained as a mixture after column chromatography.



This methodology was extended to another diaryl system (Ar = p-FPh). Deoxygenation ( $\text{ZnI}_2/\text{NaCNBH}_3$ ) of the mixture of diols (**3a** and **3 b**) resulted in a mixture of three hydrocarbons along with side products analogous to those found in the diphenyl system (Fig. 2). One of the desired hydrocarbons **5a** was recrystallized, and X-ray analysis revealed that the stereochemistry is the same as that of the isomer that crystallized in the diphenyl system (**4a**). It is interesting to note that the product distribution was also similar.

#### 4. ACKNOWLEDGEMENT

The author expresses deep gratitude to Dr. Pshemak Maslak (Pennsylvania State University) for his help and valuable advices and to Dr. Alan Benesi and Dr. Robert Minard from Pennsylvania State University for their help with the acquisition of NMR and mass spectra.

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## Preliminary study on the kinetics of the reaction between antioxidants and test radical – DPPH

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**Abstract:** *There are evidences that the disturbed balance between antioxidants and active free radicals in the human organisms plays a major role in the pathogenesis of many diseases. Methods for fast evaluation the effectiveness of various natural and synthetic antioxidants are usually based on their reactions with test radicals (e.g. DPPH). The present work is an initial part of a larger project for investigation of kinetics and mechanism of such processes. Better understanding of the influence of various factors e.g. nature of solvents, acidity etc. could increase the reliability and compatibility of the antioxidant efficiency estimations.*

**Keywords:** *antioxidants, kinetic of reactions, molecular design, quantum chemistry, UV-VIS Spectrometry.*

### 1. INTRODUCTION

In healthy human organisms there is a balance between anti- and pro-oxidants (active free radicals). When this equilibrium is disturbed in favor of the oxidants, the condition is known as oxidative stress [1]. There are abundant evidences that the oxidative stress triggers various undesired processes at cellular, tissue and organism levels and plays a major role in the pathogenesis of many human diseases like ischemia/reperfusion syndrome, atherosclerosis, chronic renal failure, etc. [2]. Therefore, antioxidants are commonly administered as prevention against undesired chemical damage caused by the oxidative stress [3].

The protective role of antioxidants against these diseases and the design of new, healthy, synthetic antioxidants can be evaluated only after thoroughly studying the chemical mechanisms of interaction between antioxidants and active free radicals (radical scavenging).

One widely used method for rapid assessment of the propensity of a compound to interact with radicals was proposed more that 50 years ago by

Marsden Blois [4]. To this end, he suggests using a stable radical - 1,1-diphenyl-2-picrylhydrazyl (DPPH). The popularity of the method is due to the ease of monitoring of the reaction with it and the availability of DPPH on the market as stable crystals.

Many things for the reactions of DPPH are not sufficiently clarified. It is not entirely clear what is the main mechanism of the reaction with DPPH [5-7], how the absorption of DPPH solution is changing with the change of acidity/alkalinity of the medium, the solvent type or frequency of exposure to UV light.

In this article we will try to clarify these issues. This will help us in future structural and kinetic studies on the reactions between antioxidants and active radicals.

## **2. EXPERIMENTAL**

All of the spectrums were observed using Agilent 8453 UV-Visible spectrophotometer with photodiode array detector and 10 mm cuvette. DPPH was purchased from Sigma-Aldrich.

## **3. RESULTS AND DISCUSSIONS**

Correct kinetic investigation implies preliminary study checking the behavior of the studied system under different conditions. It is very important to avoid mixing of kinetic and external effects.

### **3.1. DPPH Spectrum**

In the spectrum of the DPPH radical there are two spectral bands: at  $\lambda_{\max}=325$  nm in the UV region and at  $\lambda_{\max}=516$  nm in the Visible region. Since almost all antioxidants have absorption bands in the UV region overlapping the DPPH one, for practical purposes is more important the visible peak. Observed spectrum is in a good agreement with the literature data.

### **3.2. Linearity of the calibration function**

The calibration function is linear ( $R^2=0.9994$ ) at least in the concentration region from 0.01 to 0.1 mmol/l. This allows simplifying the future kinetic experiments replacing in the equations concentrations with absorbance.

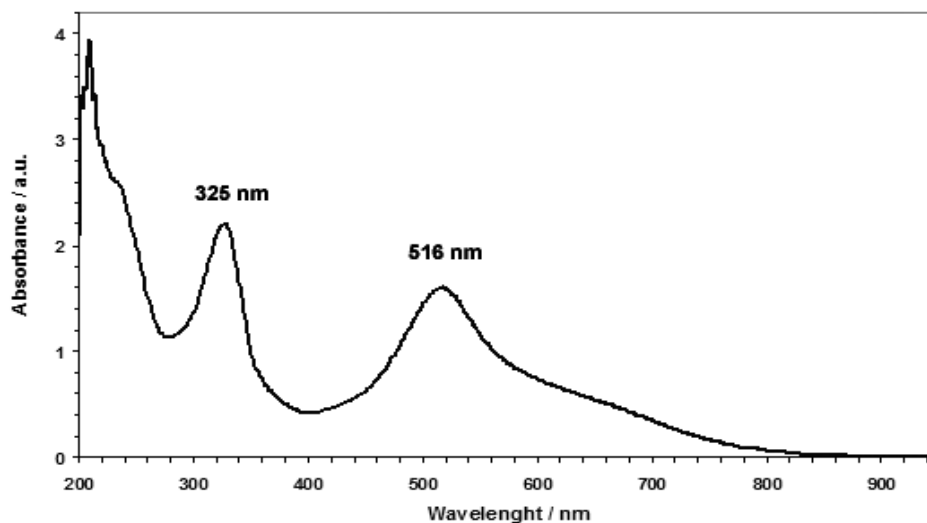


Fig. 1: DPPH spectrum in UV and visible region between 200 and 900 nm.

### 3.3. Influence of the solvent

One of the factors which could be changed strongly during the kinetic experiments is the polarity of the solution. In order to check this possible influence the DPPH spectra were recorded in pure ethanol and in ethanol – water mixture. The effect of increased polarity causes on one hand – shift of the visible absorption band to higher wavelengths and decreasing of sensitivity on the other. The peak height absorbance in the mixture is approximately 56% of those in pure ethanol (Fig. 2). Such strong effect indicates that in any further experiments change or mixing of solvents should be avoided.

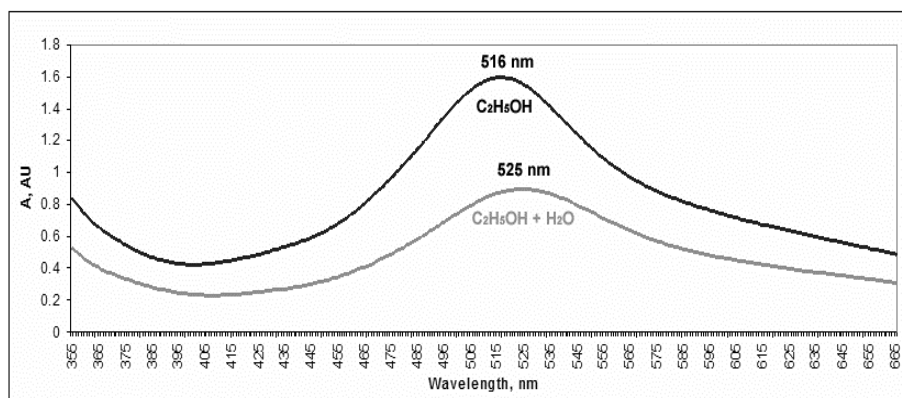


Fig. 2: Spectra containing the same concentration DPPH (approximately 0.085 mmol/l) dissolved in pure ethanol and in mixture C<sub>2</sub>H<sub>5</sub>OH:H<sub>2</sub>O=2: 1.5.

### 3.4. Influence of the acidity and alkalinity

Many of the compounds having antioxidant activity are weak or moderately strong organic acids or bases. Thus it is important to know if the changes in proton activity in the solutions may affect the DPPH spectra. For this purpose were prepared two sets of solutions containing 2.0 ml of the DPPH stock solution and 1.5 ml water solutions of HCl and NaOH with different concentrations covering the interval from  $10^{-3}$  mol/l to 1 mol/l. Spectra of these solutions are presented in Fig. 3 and Fig. 4. In concentrations below  $10^{-2}$  mol/l (for HCl) and  $10^{-3}$  mol/l (for NaOH) no effects were observed. In presence of higher concentrations of acid is clearly observed depression effect on sensitivity approximately proportional to  $\lg C$  (Fig. 3). The visible spectral band was registered at  $\lambda_{\max}=525$  nm and no further shift were observed. In case of basic solutions a process of irreversible destruction of DPPH takes place (Fig. 4). Even after subsequent acidifying of the solution the system does not restore to the initial status.

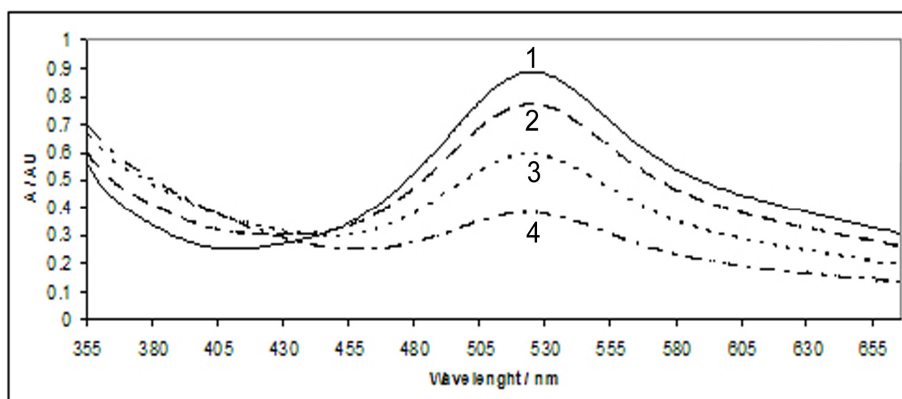


Fig. 3: Influence of the acidity. 2 ml DPPH of the stock solution are added to 1.5 ml water solutions of HCl with concentrations: 0.01 M (1); 0.1 M (2); 0.5 M (3); 1 M (4).

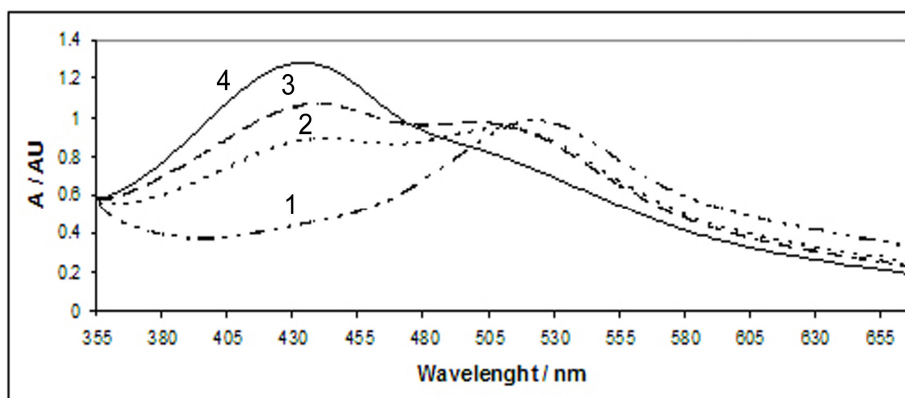


Fig. 4: Influence of the alkalinity. 2 ml DPPH of the stock solution were added to 1.5 ml water solutions of NaOH with concentrations: 0.001 M (1); 0.005 M (2); 0.01 M (3); 0.1 M (4).

### 3.5. Effects of the UV-Vis irradiation

Significant activity of the test radical makes possible photochemical transformations caused by the spectrometer's radiation sources. An ethanol solution of DPPH (c.a. 0.035 mmol/l) was used to study this possible effect. Multiple measurements of absorbance at 516 nm were done without replacing the sample with different interval between replicates. The observed trends are clearly indicating participation of DPPH in reversible photochemical process. Increasing of time interval between irradiations leads to decreasing of observed effect. The decreasing of the signal in all studied cases is within 5%. This effect should be taken into account since it may distort the results of the kinetic study.

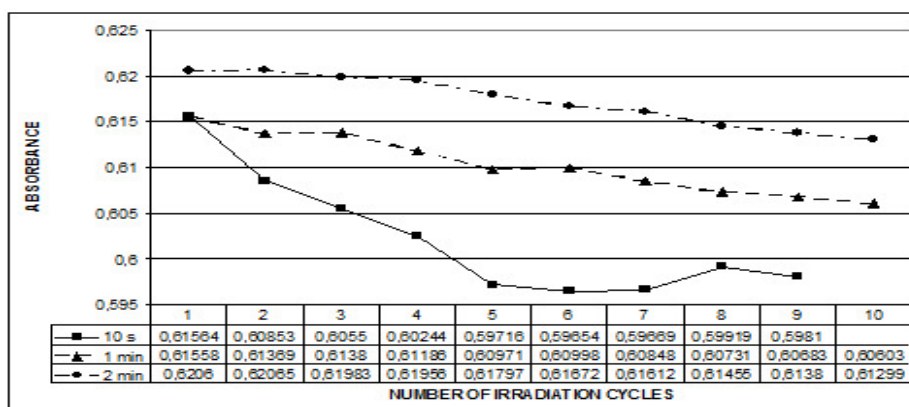


Fig. 5: The effect of irradiation on the DPPH signal. Ten replicate measurements of the same sample with time between irradiations 10 s, 1 min and 2 min.

#### 4. CONCLUSIONS

Many factors may affect the analytical signal for the DPPH test radical. Neglecting of these effects may lead to completely wrong conclusions in study of the kinetics of the reaction between DPPH and antioxidants as well as in evaluation of the antioxidant activity of the natural and synthetic antioxidants. All these effects should be evaluated in order to obtain reliable, traceable and comparable results for the antioxidant activity of the compounds.

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# The effect under exploration properties at use of low octane component for obtaining of contemporary gasoline

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**Abstract:** *Fuels, including gasoline, diesel, and kerosene (jet fuel), are the most valuable products from petroleum. To enhance the quantity of these fuels produced from a single barrel of crude, heavier streams are cracked, or broken down into smaller molecules. Fluid catalytic cracking (FCC) typically utilizes a solid acid zeolite catalyst, often promoted with rare earth metals in a fluidized bed. Large molecules are broken down to create additional material in the naphtha range in order to produce more gasoline, a valuable product. The "cracked naphtha" stream often contains larger amounts of sulfur than virgin naphtha, since much of the sulfur in crude is in the form of heavy polynuclear aromatic molecules present in the FCC feed stream. The aim of this work was to evaluate and investigate a new component for contemporary gasoline such as low octane component and its effect under exploration properties of the mixture.*

**Keywords:** *gasoline, contemporary fuels, component*

## 1. INTRODUCTION

A modern refinery is a highly integrated industrial plant, the main task of which is to efficiently produce high yields of valuable products from a crude oil feed of variable composition. Employing different physical and chemical processes such as distillation, extraction, reforming, hydrogenation, cracking and blending the refinery converts crude oil to higher value products. The main products are liquid petroleum gas, gasoline, jet and diesel fuels, wax, lubricants, bitumen and petrochemicals. Energy and hydrogen for internal and external use are also produced in a refinery. Currently, refineries meet changing societal needs concerning product specifications and quality by upgrading existing technologies and continuously developing advanced technologies [1].

Environmental restrictions regarding the quality of transportation fuels produced and the emissions from the refinery itself are currently the most important issues, as well as the most costly to meet. The primary goal of the recently proposed regulations (by the Directive of the European Parliament [2] and the Environmental Protection Agency (EPA) Clean Air Act (Tier 2) [3]) is to reduce the sulphur content of transportation fuels. The CO<sub>2</sub> emitted by the refinery into the atmosphere is limited by the Kyoto protocol [4]. According to various estimation models, \$10–15 billions in the European refinery industry and up to \$16 billion in US and Canadian refineries will be invested in direct response to the new environmental clean-fuel legislation [5,6]. Gasoline, diesel and non-transportation fuels account for 75–80% of the total refinery products. Most of the desulphurisation processes are therefore dealing with the streams forming these end products. Sulphur present in the fuels leads to SO<sub>x</sub> air pollution generated by vehicle engines. In order to minimize the negative health and environmental effects of automotive exhaust emissions the sulphur level in motor fuels is minimized. New sulphur limits of 10 ppm for gasoline and diesel marketed in the European community and the USA will be introduced starting from January 1, 2007 [10]. Bulgaria has even passed legislation limiting the sulphur in diesel and gasoline to 10 ppm as of January 2010 [10]. In fact, zero-emission and, as a consequence, zero levels of S are called for worldwide in coming 5–10 years. Such ultra low-sulphur fuels requirements have consequences for the refinery. Efficiency of the desulphurisation technologies becomes a key point. Conventional hydrodesulphurisation (HDS) processes cannot currently produce such zero sulphur level fuels, while maintaining other fuel requirements such as oxygen content, vapour pressure, benzene content, overall aromatics content, boiling range and olefin content for gasoline, and cetane number, density, polynuclear aromatics content, and distillation 95% point for diesel fuel [11].

Utilities are not the only source for atmospheric sulfur. Automobiles are also adversely affected by sulfur compounds. Sulfur levels in automotive fuels have a profound effect on the efficacy of catalytic converters. Sulfur affects these emission control devices by strongly adsorbing to the precious metal catalysts, preventing the adsorption and reaction of hydrocarbons, nitrogen oxides, and carbon monoxide. The EPA estimates that reducing sulfur levels from 400 ppm to 50 ppm reduces emissions of hydrocarbons by 45.9%, NO<sub>x</sub> by 7.01%, and CO by 31.12% (based on Tier 1 running specification) by reducing the poisoning effect of sulfur. Obviously, emissions of SO<sub>x</sub> are also reduced by an amount equivalent to the sulfur reduction. The US national average sulfur level in automotive fuel in 1997 was 339 ppm. Producing energy in a clean and responsible manner can be accomplished in a number of ways. The use of non-fossil fuel energy sources such as solar, wind, and nuclear power will eventually replace fossil

fuels. However, many of these technologies will require many years before they are able to provide the amounts of energy needed. In the immediate future, fossil fuel-based energy production will continue, and new technologies need to be developed in order to produce clean fuels to power our societies. Fuels, including gasoline, diesel, and kerosene (jet fuel), are the most valuable products from petroleum. To enhance the quantity of these fuels produced from a single barrel of crude, heavier streams are cracked, or broken down into smaller molecules.

Fluid catalytic cracking (FCC) typically utilizes a solid acid zeolite catalyst, often promoted with rare earth metals in a fluidized bed. Large molecules are broken down to create additional material in the naphtha range in order to produce more gasoline, a valuable product. The “cracked naphtha” stream often contains larger amounts of sulfur than virgin naphtha, since much of the sulfur in crude is in the form of heavy polynuclear aromatic molecules present in the FCC feed stream. Two additional processes are used to improve the quality of the resulting fuels, particularly gasoline. Reforming uses Pt based catalysts to isomerize linear paraffins, such as *n*-hexane, to higher octane number branched paraffins like 2,3-dimethylbutane. Pt supported on chlorided alumina, sulfated zirconia, and zeolites are all used.<sup>6</sup> The support alters the activity of the catalyst, with alumina being most active and zeolites being least active. However, high activity catalysts are more susceptible to poisoning by sulfur and water. Removal of sulfur compounds before reforming gasoline streams is therefore required.

The second process used to improve the quality of gasoline is alkylation. Alkylation reacts *n*-butene with isobutane to create 2,2,4-trimethylpentane, also called iso-octane, and other branched paraffins. Alkylation also uses an acid catalyst, but due to excessive coking, only liquid acid catalysts are currently used. Alkylation reactors blend either sulfuric or hydrofluoric acid with the butane/isobutene stream to create alkylate, a high quality gasoline that is blended into other gasoline streams.

## 2. MATERIAL AND METHODS

For the purpose of our work we used pure gasoline fractions from different technological processes, produced by “Lukoil Neftohim Burgas” – Burgas. The physical-chemical properties of these raw-materials are given in Table 1.

Tab. 1: Physical-chemical properties of pure gasoline fractions

Parameters	Reformate	FCC gasoline	Alkylate
1.Density, g /cm <sup>3</sup>			
at 20 °C	0.8003	0.7369	0.7000
at 15 °C	0.8041	0.7412	0.7045
2.Sulphur, ppm	0.3	23	14
3.Hydrocarbon content (FIA), % v/v.			
Alkanes + naftenes	32.4	42.92	100.00
alkens	0.5	36.25	-
aromatics	67.1	20.83	-
4.Benzene, %	1.8	0.6	-
5.RON	99.5	92	97.1
MON	88.1	80.5	91.4
6.Distillation curves, °C			
i.p.	47	39	38
e.p.	205	200	190
-70°C distilled, % v/v.	7	28	7
- 100°C distilled, % v/v.	20	52	23
-150°C distilled, % v/v.	71	82	92

It was used bioethanol as oxynageted component, too. Its physical-chemical properties are given in Table 2.

We used a pure gasoline fraction, produced from “Bulgarian Petroleum Reffinery” – Sofia as a low octane component. It was given physical - chemical characteristics in Table 3.

Tab. 2: Physical-chemical properties of bioethanol

Parameters	Value
Density at 20 °C, kg /m <sup>3</sup>	791.3
Total contaminants, mg/kg	neal
Water content, % mass	0.16
Sulphur content, mg/kg	neal
Chlorides, mg/kg	neal
Organic acids, mg/dm <sup>3</sup>	12.5
Methanol content, % v/v.	neal
Ethanol content, % v/v.	99.96
High molecular alcohols, mg/dm <sup>3</sup>	8
Appearance	Clear liquid

Tab. 3: Physical-chemical properties of low octane gasoline fraction

Parameters	Low octane gasoline fraction
1.Density, g /cm <sup>3</sup> at 15 °C	0.749
2.Sulphur, ppm	0.5
3.Hydrocarbon content (FIA), % v/v.	36.8
Alkanes + naftenes	2.5
alkenes	60.7
aromatics	
4.Benzene, %	0.9
5.RON	77.5
MON	66.7
6.Distillation curves, °C	
i.p.	41
e.p.	187
-70°C distilled, % v/v.	23
- 100°C distilled, % v/v.	46
-150°C distilled, % v/v.	83

### 3. RESULTS AND DISCUSSION

It was made gasoline-alcoholic mixtures with low octane gasoline so called BAS /mixture 1, mixture 2 and mixture 3/ according to technical standards and methods for mixing and handling of gasoline fractions. The new mixtures are consisted by receipts from [7]. We investigated the effect of low octane component BAS under exploration properties of gasoline – alcoholic mixtures. The obtained data is given in Table 4.

Tab. 4: Physical-chemical properties of new gasoline – alcoholic mixtures

Parameters	Mixture 1	Mixture 2	Mixture 3
1.Density, g /cm <sup>3</sup> at 20 °C	0.7556	0.7545	0.7561
2.Sulphur, ppm	7.30	6.11	6.55
3.Hydrocarbon content (FIA), % v/v.	34.6	42.92	100.00
Alkanes + naftenes	0.3	36.25	-
alkens	63.2	20.83	-
aromatics			
4.Benzene, %	0.9	0.7	-
5.RON	98.1	92	95.0
MON	87.2	81.2	85.2
6.Distillation curves, °C			
i.p.	40	42	41
e.p.	190	192	188
-70°C distilled, % v/v.	23	22	23
- 100°C distilled, % v/v.	53	52	51
-150°C distilled, % v/v.	83	82	82

#### 4. CONCLUSION

The obtained data showed that it's very possible to add low octane gasoline components like BAS without change of exploration properties of gasoline-alcoholic mixtures and to obtain a contemporary gasoline, according to BSS EN 228:2010.

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# The Potential Risk of Petroleum Propyl Mercaptan in the Environment

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**Abstract:** Sulfur compounds in petroleum streams can have detrimental effects on the performance and longevity of the catalysts used in hydrocarbon processing. Furthermore, the toxicity and odor associated with sulfur compounds is of significant environmental importance. To protect both processing equipment and the environment, the ability to quantify sulfur compounds to ppb levels is imperative. The aim of this work was to predict the potential risk of petroleum propyl mercaptan in the environment, using QSAR tools.

**Keywords:** petroleum propyl mercaptan, environment, acute toxicity

## 1. INTRODUCTION

Natural gas and petroleum industries deal with raw materials containing variable concentrations of acid gases (CO<sub>2</sub>, H<sub>2</sub>S) and traces  $x=(1 \text{ to } 4) \cdot 10^{-4}$  of organic sulphur species like mercaptans and dimethylsulfide. Treatment processes have to remove not only H<sub>2</sub>S and CO<sub>2</sub> but also sulphur species because worldwide regulations for environmental protection are forcing the petroleum industry to decrease the sulphur content in petroleum fluids [2]. Mercaptan belong thiol-group of compound which contain an –SH group bound to a radical R. Mercaptan properties are governed to a large extent by the length of this radical [2, 9].

Production rates and processing of oils and gas condensates which contain a great amount of mercaptans and hydrogen sulfid has been grown in all over the world during the recent years. Hydrogen sulphide and low molecular weight mercaptans (C<sub>1</sub>-C<sub>3</sub>) are toxic and volatile, they have irritant odour and high corrosivity. For an ecological and technological safe storage, transportation and processing, these crude oils and gas



condensates should be treated for removing hydrogen sulphide and low molecular weight mercaptans [4].

The aim of this work was to predict the potential risk of petroleum propyl mercaptan in the environment, using QSAR tools.

## 2. MATERIAL AND METHODS

*Compounds.* Petroleum propyl mercaptan was collected for its acute toxicity (terrestrial species).

*Acute Terrestrial Toxicity Data.* The experimental data for rat (oral LD<sub>50</sub> value) was collected from the literature [8].

*Log P.* Data for the logarithm of the 1-octanol-water partition coefficient (log P) were obtained from the KOWWIN software [7]. Where possible measured log P values were verified and used in preference to calculated values.

*Baseline models.* In this study a model was used for non-polar compounds to terrestrial species to determine the acute toxicity of propyl mercaptan (Table 1).

Baseline model (saturated alcohols and ketones) of Rat (oral) [5]:

$$\log(1/LD_{50}) = 0.805 \cdot \log P - 0.971 \cdot \log(0.0807 \cdot 10^{\log P} + 1) + 0.984 \quad (1)$$

$n = 54 \quad R^2 = 0.824 \quad s = 0.208 \quad F = 35.3$

*Excess toxicity.* The property - excess toxicity - was used to define the toxicity of chemicals (reactive or nonreactive) [5]. The extent of excess toxicity was determined as the toxic ratio (TR), which was calculated by the following equation 2 [5, 6]:

$$TR = (\text{predicted baseline toxicity}) / (\text{observed toxicity}) \quad (2)$$

*Criteria used by the PBT Profiler.* The PBT Profiler is a screening-level tool that provides estimates of the persistence, bioaccumulation, and chronic fish toxicity potential of chemical compounds. It is designed to be used when no data are available. In order to help interested parties make informed decision on a chemical's PBT characteristics, the PBT profiler automatically identifies chemicals that may persist in the environment and bioaccumulate in the food chain. These chemicals are identified using thresholds published by the EPA [3].

## 3. RESULTS AND DISCUSSION

A number of reliable baseline equations are available for different organisms (aquatic (*Tetrahymena pyriformis*) and terrestrial (Rat)) and endpoints

(IGC<sub>50</sub>, LD<sub>50</sub>). A Baseline model (eq 1) for terrestrial species was applied to propyl mercaptan (Table 1).

On the basis of calculated and experimental values for acute toxicity, the toxicity ratio (TR) as the ratio of the calculated baseline toxicity over the experimentally determined value was calculated. A TR-value less than one could indicate rapid hydrolysis and/or biotransformation of the parent compound by the organism to non-toxic metabolites [1].

Tab. 1: Experimental and predicted values of propyl mercaptan

Name of compound	logP	Exp. Oral Rat LD <sub>50</sub> mmol/kg	Pred. Oral Rat LD <sub>50</sub> mmol/kg / TR
Propyl mercaptan	1.81	23.50	46.87/ 1.99

Chemicals that are persistent, bioaccumulative, and toxic have the potential to concentrate to levels that may cause significant adverse impact on human health and the environment. The results of estimation of propyl mercaptan for persistence, bioaccumulation and toxicity are presented in Table 2.

Tab. 2: PBT profiler estimate of propyl mercaptan

Name of compound	Persistence		Bioaccumulation	Toxicity
	Media (water, soil, sediment, air) Half-life (days)	Percent in Each Medium	BCF	Fish ChV (mg/l)
Propyl mercaptan	15; 30; 140; 0.33	67%; 25%; 0%; 8%	7.3	0.49

Analysis of data in Table 2 reveals that propyl mercaptan is toxic (Fish ChV). The PBT Profiler estimates that propyl mercaptan is not expected to persist and bioaccumulate in the food chain because it does not exceed the BCF criteria. The compound is with moderate toxicity (0.1-10 mg/l).

#### 4. CONCLUSION

The effect of a chemical is dependent on the species, route of exposure, and dose. Acute toxicity is one of endpoints used in environmental risk assessment to determine the safe use and disposal of organic chemicals. The [PBT Profiler](#) is an online risk-screening tool that predicts a chemical's potential to persist in the environment, bioconcentrate in animals, and be toxic, properties which cause concern for human health and the environment. The PBT Profiler estimates that propyl mercaptan is not expected to persistent and bioaccumulate but the compound is with moderate toxicity.

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# Investigation of the Molecular Mechanisms of Hepatotoxicity of Some Drugs in the Therapy of Autism

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**Abstract:** *There is currently no medical treatment for the core features of autism, although attempts have been made to use medications to treat symptoms and co-morbid disorders of autism such as anxiety and ADHD, as well as to increase the likelihood that children will benefit from concurrent interventions. The medications have been demonstrated to be some what effective for individuals with autism, although careful monitoring is required to measure effects and side effects: Neuroleptics/Antipsychotics, Risperidone, Selective Serotonin Reuptake Inhibitors (SSRIs), Antidepressants, Stimulants, Anticonvulsants. The aim of this work is to present a summary of the molecular mechanisms of hepatotoxicity associated with some drugs in the therapy of autism.*

**Keywords:** *drugs, hepatotoxicity, molecular mechanism, autism*

## 1. INTRODUCTION

Autism spectrum disorders (ASDs) are pervasive neurodevelopmental disorders affecting 0.5-1% of children and characterized by varying degrees of deficiencies in social interactions, concentration, language, and learning [6, 11, 12]. Such symptoms may become apparent as early as 6 months old and are often established by age 3 years. This is also the age at which many children who develop an ASD do so by regression, often after a specific event such as vaccination, even though they were developing normally [16].

Behavioral interventions alone are insufficient to address the disruptive nature of ASD symptoms and their persistence throughout life, which instead necessitate the development of effective biomedical treatments.

Drug therapy is typically used to treat irritability, hyperactivity, inattention, obsessive-compulsive symptoms, aggression, and self-injury because all of these symptoms can negatively interfere with the success of educational interventions and quality of family life [3, 7]. Many children with ASDs also develop seizures with no apparent underlying pathology [10, 18, 19] but traditional anti-seizure medications appear to worsen some ASD symptoms [8]. Overall, 70% of children with ASDs take at least 10 different drugs, dietary supplements, vitamins, or intravenous treatments [1, 2, 7, 17] including intravenous immunoglobulin (IVIG) [5] with little attention to unwanted drug-drug or drug-supplement interactions. Moreover, in many cases, as in the use of IVIG, there is a lack of well-designed double-blind studies [9] so there should be continued vigilance for the possible development of inflammatory complications [4].

Taking multiple drugs increases the risk of adverse reactions. In a recent study of spontaneous reporting of adverse drug-drug interactions in Italy, the incidence was 9.8% for two drugs but increased to 88.3% for 8 drugs or more [13]. There is less information on drug-supplement interactions. However, another study reported that 33.4% of patients using antipsychotics took traditional Chinese medicine concurrently; patients using both treatments had worse outcomes (7.2%) than those using antipsychotics alone (4.4%) [20].

This short review attempts to provide information regarding the molecular mechanisms of hepatotoxicity of some drugs in the therapy of autism. Clinicians and parents ought to carefully weigh risks of pharmacologic and non-pharmacologic interactions when deciding on a treatment plan. They should also include healthy lifestyle instructions and regular side effect monitoring in their routine clinical care. Awareness of efficacy, safety, and unwanted interactions could increase the benefits of treatment and prevent adverse effects.

## 2. MATERIALS AND METHODS

*Compounds.* Some drugs in the therapy of autism were investigated which are presented in Table 1.

*OECD (Q)SAR Application Toolbox.* (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on the hazards of chemicals, while reducing time, monetary costs and animal testing currently needed. To facilitate practical application of (Q)SAR approaches in regulatory contexts by governments and industry and to improve their regulatory acceptance, the OECD (Q)SAR project has developed various outcomes such as the principles for the validation of (Q)SAR models, guidance documents as well as the QSAR Toolbox [15].

Metabolic pathways documented for 200 organic chemicals in different mammals are stored in a database format that allows easy computer-aided access to the metabolism information. The collection includes chemicals of different classes, with variety of functionalities such aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, nitro-derivatives, and multifunctional compounds. *In vivo* and *in vitro* (predominantly, with liver microsomes as experimental systems) studies were used to analyze the metabolic fate of chemicals. Different sources, including monographs, scientific articles and public websites were used to compile the database [14, 15].

### 3. RESULTS AND DISCUSSIONS

The results of protein and DNA binding of parent structure and the probable metabolic activation in liver (observed and predicted) of some drugs in the therapy of autism are presented in Table 1.

Tab. 1: Protein and DNA binding of parent structure and probable metabolic activation of some drugs in the therapy of autism by (Q)SAR Application Toolbox

No	Drugs in the therapy of autism	Protein and DNA binding of parent structure; Observed liver metabolism by Toolbox	Liver Metabolism Simulator by Toolbox
<b>Typical antipsychotics</b>			
1	Haloperidol	Parent structure-Protein binding- Nucleophilic addition to ketones; DNA binding-No binding; 5 metabolites;	10 metabolites;
2	Pimozide	Parent structure-Protein binding- No binding; DNA binding- Ureides and other urea derivatives; 0 metabolites;	36 metabolites;
<b>Atypical antipsychotics</b>			
3	Olanzapine	Parent structure-Protein binding- Nucleophilic addition to azomethynes; DNA binding- No binding; 0 metabolites;	35 metabolites;
4	Clozapine	Parent structure-Protein binding- Nucleophilic addition to	11 metabolites;

		azomethynes; DNA binding- No binding; 0 metabolites;	
<b>Antidepressants</b>			
5	Clomipramine	Parent structure-Protein binding- No binding; DNA binding- No binding; 0 metabolites;	53 metabolites;
6	Mirtazapine	Parent structure-Protein binding- No binding; DNA binding- No binding; 0 metabolites;	33 metabolites;
7	Imipramine	Parent structure-Protein binding- No binding; DNA binding- No binding; 6 metabolites;	39 metabolites;
8	Venlafaxine	Parent structure-Protein binding- No binding; DNA binding- No binding; 0 metabolites;	16 metabolites;
<b>Indirect 5-HT receptor agonist</b>			
9	Fenfluramine	Parent structure-Protein binding- No binding; DNA binding- No binding; 0 metabolites;	16 metabolites;
<b>Anticonvulsants</b>			
10	Lamtrigine	Parent structure-Protein binding- No binding; DNA binding- Aromatic amines; 0 metabolites;	16 metabolites;

#### 4. CONCLUSION

The age at which medication for autism symptoms can be started varies. Finding the right medication or combination of medications can be a process of trial and error. It should be borne in mind that many of these medications have side effects. Awareness of efficacy, safety, and unwanted interactions could increase the benefits of treatment and prevent adverse effects with theoretical predictions.

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## Probable Hepatotoxic Actions of the Metabolites of Some Drugs in the Therapy of Autism

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**Abstract:** *Most of the pharmacological studies to date have been open trial (rather than controlled) studies with very small sample sizes. Nevertheless, a number of drugs have been shown to be promising in ameliorating certain symptoms of Autism. The aim of this work is to predict the possible metabolites of some drugs in the therapy of autism by a specialized software which can cause hepatotoxic action. This could help medical professionals to easily predict liver injury in patients with autism.*

**Keywords:** *hepatotoxicity, drugs, metabolites, autism*

### 1. INTRODUCTION

Findings from preliminary studies of major neurotransmitters and other neurochemical agents strongly suggest that neurochemical factors play a major role in the aetiology of autism. However, compared to the breadth of research into the aetiology and neurobiology of autism, there have been relatively few controlled studies that address the pharmacological treatment of ASD. Whilst there are no aetiologically based drug treatments available that specifically seek to cure autism [9], there is an extensive body of literature describing pharmacotherapy management of the symptoms and associated conditions of autism in children and adolescents, and to a lesser extent adults [8].

A variety of drugs are used to treat or manage symptoms and associated conditions of autism, including core and associated features, which are regarded as being problematic to those with autism and their carers, and which may affect quality of life and/or the ability to live independently. Several controlled studies have investigated the efficacy of a range of medications in the treatment of the associated symptoms of autism [7], including aggression, agitation, hyperactivity, inattention, irritability,

repetitive behaviours and self-injury. Treatment of these disabling symptoms may allow other more non-pharmacological interventions (e.g., educational and behavioural) to proceed more smoothly and effectively. Medication may also be useful in assisting individuals with autism to live outside institutional settings [8].

A major factor in guiding clinicians' approach to choice of pharmacological treatment is an awareness of specific conditions comorbid with autism in an individual patient [3, 4], including epilepsy, and obsessive compulsive, mood or anxiety disorders. Overall, 70% of children with ASDs take at least 10 different drugs. Taking multiple drugs increases the risk of adverse reactions.

At a regulatory level, hepatotoxicity is the main reason for postmarketing regulatory decisions including drug withdrawal [1]. Doctors involved in administering new medications must weigh potential risks vs. benefits and be aware of appropriate monitoring guidelines for hepatotoxicity. Factors which limit our understanding of drug hepatotoxicity include the relatively rare incidence of toxicity for most drugs, lack of animal models, underreporting and practical issues of drug–drug interactions which can confound the establishment of causality in cases of suspected toxicity [2]. The aim of this work is to predict the possible metabolites of some drugs in the therapy of autism by a specialized software which can cause hepatotoxicity.

## 2. MATERIALS AND METHODS

*Compounds.* Some drugs in the therapy of autism were investigated which are presented in Table 1.

*OECD (Q)SAR Application Toolbox.* (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on the hazards of chemicals, while reducing time, monetary costs and animal testing currently needed. To facilitate practical application of (Q)SAR approaches in regulatory contexts by governments and industry and to improve their regulatory acceptance, the OECD (Q)SAR project has developed various outcomes such as the principles for the validation of (Q)SAR models, guidance documents as well as the QSAR Toolbox [6].

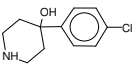
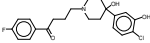
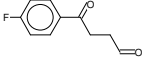
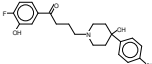
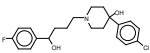
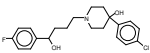
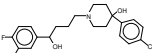
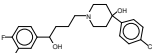
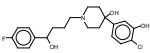
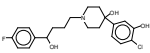
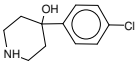
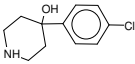
Metabolic pathways documented for 200 organic chemicals in different mammals are stored in a database format that allows easy computer-aided access to the metabolism information. The collection includes chemicals of different classes, with variety of functionalities such as aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, nitro-derivatives, and multifunctional compounds. *In vivo* and *in vitro* (predominantly, with liver

microsomes as experimental systems) studies were used to analyze the metabolic fate of chemicals. Different sources, including monographs, scientific articles and public websites were used to compile the database [5, 6].

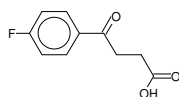
### 3. RESULTS AND DISCUSSIONS

The results of the probable metabolic activation in liver (observed and predicted) and their protein and DNA binding of some drugs in the therapy of autism are presented in Table 1.

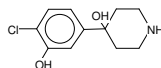
Tab. 1: The probable metabolic activation in liver and their protein and DNA binding of some drugs in the therapy of autism by (Q)SAR Application Toolbox

No	Drugs in the therapy of autism	Observed liver metabolism by Toolbox;	Liver Metabolism Simulator by Toolbox
<b>Typical antipsychotics</b>			
1	Haloperidol	5 metabolites; DNA binding – No binding; Protein binding – Nucleophilic addition to ketones (2,3,5); Schiff base formation (2); No binding (1,4)	10 metabolites; DNA binding – No binding; Protein binding – Nucleophilic addition to ketones (1,2,9,10); Schiff base formation (9); No binding (3-8)
	1)		1) 
	2)		2) 
	3)		3) 
	4)		4) 
	5)		5) 
	6)		6) 

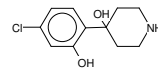
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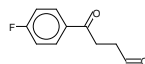
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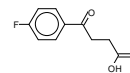
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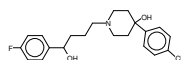
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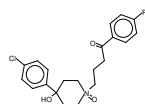
10)



9)



5)



### Atypical antipsychotics

2 Clozapine

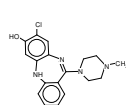
0 metabolites;

11 metabolites;

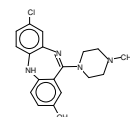
DNA binding is No binding for all metabolites;

Protein binding – Nucleophilic addition to azomethynes (1-7, 10-11);  
 Michael-type nucleophilic addition (3);  
 Schiff-base formation (6-8); No binding (9)

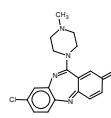
1)



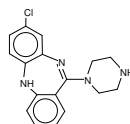
2)

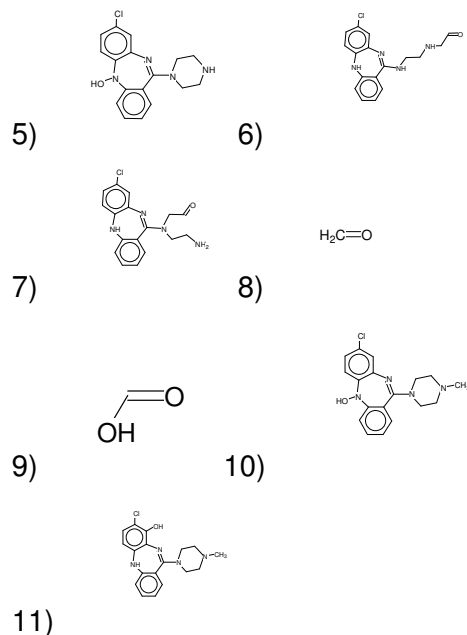


3)



4)





#### 4. CONCLUSION

Drugs can be useful in controlling some of the more troublesome symptoms seen in individuals with autism but there has been insufficient research on the doses required for people with ASDs and, on the whole, results have been disappointing. The medical practitioners utilize synthetic drugs that have a potential for side-effects in proportion to their very high pharmacological activity. Theoretical prediction about the probable metabolic activation in liver and their protein and DNA binding of some drugs in the therapy of autism can be very useful in prevent adverse effects.

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[http://www.oecd.org/document/23/0,3343,en\\_2649\\_34379\\_33957015\\_1\\_1\\_1\\_1,00.html](http://www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html)
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## NiW and NiMo Electrodeposits as Cathode Materials for Microbial Electrolysis Cell

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**Abstract:** The development of cost-effective cathodes for near-neutral pH and ambient temperature conditions is the most critical challenge for the practical application of Microbial Electrolysis Cell (MEC) technology. In this study, precious-metal-free cathodes were produced by electrodepositing NiW and NiMo on a carbon felt. The morphology and the elemental content of the developed materials was analyzed by Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS). Their electrochemical performance in neutral phosphate buffer solution (PBS) was investigated by means of Linear Sweep Voltammetry (LSV). Besides some differences in morphology, both electrodeposits exhibit similar electrocatalytic activity towards hydrogen evolution reaction (HER), much higher than that of bare carbon felt. This gives reason for further evaluation of the materials as cathodes in MECs.

**Keywords:** Microbial Electrolysis Cells, Hydrogen evolution reaction, NiW and NiMo electrodeposits, Scanning Electron Microscopy, Linear Sweep Voltammetry.

### 1. INTRODUCTION

Hydrogen production is becoming increasingly important in view of using hydrogen in fuel cells. The experts expect that the hydrogen will replace the fossil fuels [1]. Hydrogen is the cleanest fuel, there are no emissions of CO<sub>2</sub>, the only product of its burning is H<sub>2</sub>O and there is not any corrosive effect on the metals. But there are some limiting factors for hydrogen wide



application. Its storage and its transportation are risky, the producing of hydrogen is still expensive and that limits its wide application [2]. Conventional electrolysis is one of the two most common methods currently used to produce hydrogen. Hydrogen production by electrolysis is not connected with CO<sub>2</sub> emission. The insufficiency of this method is that it needs large quantity of electricity. Microbial electrolysis cells (MECs) could be a partial solution of the problem. MEC uses substrates, from renewable sources and have high conversion efficiency. It is ecologically clean, renewable and innovative technology for hydrogen production, which is related to Microbial fuel cells (MFC). A MEC is an electrochemical device that uses microorganisms to oxidize organic substrates on the anode and generate hydrogen on the cathode. [3]. Using microorganisms in electrolysis systems decreases the overpotential, makes easier the electron transfer and reduces the necessary quantity of electricity for the electrolysis. Theoretically, the hydrogen evolution on the cathode needs a potential of  $E_{\text{CAT}} = -0.41 \text{ V (vs.SHE)}$ . The anode potential of most MFCs reaches around  $E_{\text{AN}} = -0.30 \text{ V (vs.SHE)}$ . Therefore, the minimum overall cell voltage needed is  $E = -0.11 \text{ V}$  [4].

The development of cost effective cathodes for near-neutral pH and ambient temperature conditions is the most critical challenge for the practical application of MEC technology. Platinum is well known as the best cathode catalyst material used in MEC, but it is too expensive [5, 6]. The high cost of platinum is driving research into bio-cathodes as an alternative [7]. Extensive studies have been also carried out on precious metal-free catalysts for MEC. Low-cost materials as stainless steel and carbon based NiMo-, NiW-nanocomposites showed good performance as cathode in MEC [8, 9].

In this study we produced NiW and NiMo by electrodeposition on carbon felt. We characterized their morphology by scanning electron microscopy. The corrosion stability and kinetics of hydrogen evolution reaction (HER) on the newly produced nanomaterials in neutral medium was investigated by means of linear voltammetry (LV). The results obtained with different electrodes were compared and discussed.

## 2. MATERIALS AND METHODS

### 2.1. *Production and characterization of nickel-based electrodeposits*

Electrodes were prepared by electrodepositing NiMo and NiW on carbon felt (SPC-7011, 30 g/m<sup>2</sup>, Weibgerber GmbH & Co. KG) using single-compartment two-electrode cell. A piece of the carbon felt (1 cm<sup>2</sup>) was

immersed in 70% ethanol for 10 min to degrease, rinsed with water, and then used as working electrode. A platinum-titanium mesh ( $10 \text{ cm}^2$ ) was used as counter electrode. Electrolyte baths used for the electrodeposition of NiW and NiMo are listed in Table 1. In both cases the pH was adjusted to 10 by adding  $\text{Na}_2\text{CO}_3$  and  $\text{NH}_4\text{OH}$  for the electrolyte used for NiW and NiMo electrodeposition, respectively. Voltage was applied to the cell with the negative pole to the working electrode and the positive pole connected to the counter electrode using DC power supply HY 3003. Electrolysis was carried out for 90 min with a current density of  $125 \text{ A/m}^2$  for NiW and  $250 \text{ A/m}^2$  for NiMo deposition. After deposition, the electrodes were carefully rinsed with deionized water to remove residues of bath chemicals and unattached catalyst particles, and then dried at  $104^\circ\text{C}$  for 12 h [8]. The weight difference before and after deposition was used to calculate the catalyst loading based on the projected electrode surface area. The morphology and the elemental content of the developed materials were analyzed by scanning electron microscopy (SEM) using JEOL 6300 and X-ray Microanalysis (ISIS 2000).

Table 1: Electrolyte bath content for electrodeposition of NiW and NiMo

NiW bath		NiMo bath	
Chemicals	g/l	Chemicals	g/l
$\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$	20	$\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$	40
$\text{Na}_2\text{WO}_4 \cdot 2\text{H}_2\text{O}$	20	$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	25
Potassium citrate	25	Sodium citrate	45
$\text{Na}_2\text{CO}_3$	to pH 10	$\text{NH}_4\text{OH}$	to pH 10

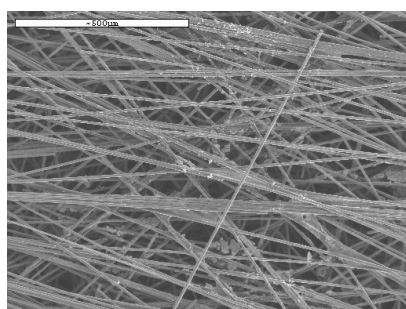
## 2.2. Experimental setup and procedure

The corrosion behavior of the newly produced materials in phosphate buffer (PBS, pH 7.0) solution was studied by means of Linear sweep voltammetry (LSV) with scan rate  $2 \text{ mV/s}$  from  $-0.4$  to  $0.6 \text{ V}$  (vs.  $\text{Ag}/\text{AgCl}$ ). Linear sweep voltammetry (LSV) from  $0$  to  $-1.2 \text{ V}$  (vs.  $\text{Ag}/\text{AgCl}$ ) was performed to evaluate the electrocatalytic activity of the studied materials towards HER in neutral PBS. The electrochemical experiments were carried out in a three-electrode cell. The electrode samples were connected as a working electrode and a platinum-titanium mesh ( $10 \text{ cm}^2$ ) was used as a counter electrode. LSV scan was repeated three times for each sample. The third scan was used for analysis of performance. The electrochemical studies were performed by using PalmSens handheld potentiostat/galvanostat.

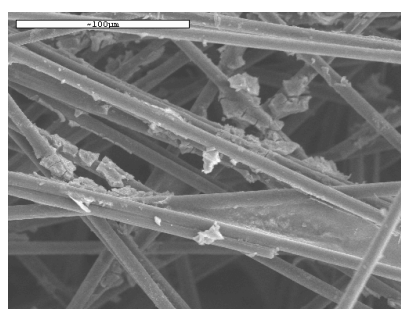
### 3. RESULTS AND DISCUSSION

#### ***3.1. Morphology and elemental content of the produced materials***

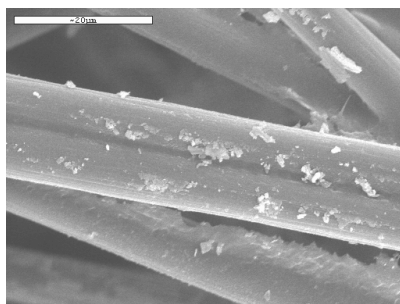
The carbon felt itself has a fiber-like structure, which provides significant active surface. After performed electrolysis in baths, described in Table 1, the carbon fibers are covered with electrodeposits. As seen from Fig.1 and Fig.2, in both cases the loading of the particles is not uniform. However, much homogeneous and dense coverage with globular nanoparticles was achieved with NiMo electrodeposits (Fig.2), while only aggregates of NiW were obtained (Fig.1).



A)



B)



C)

Fig.1 SEM images of NiW/carbon felt

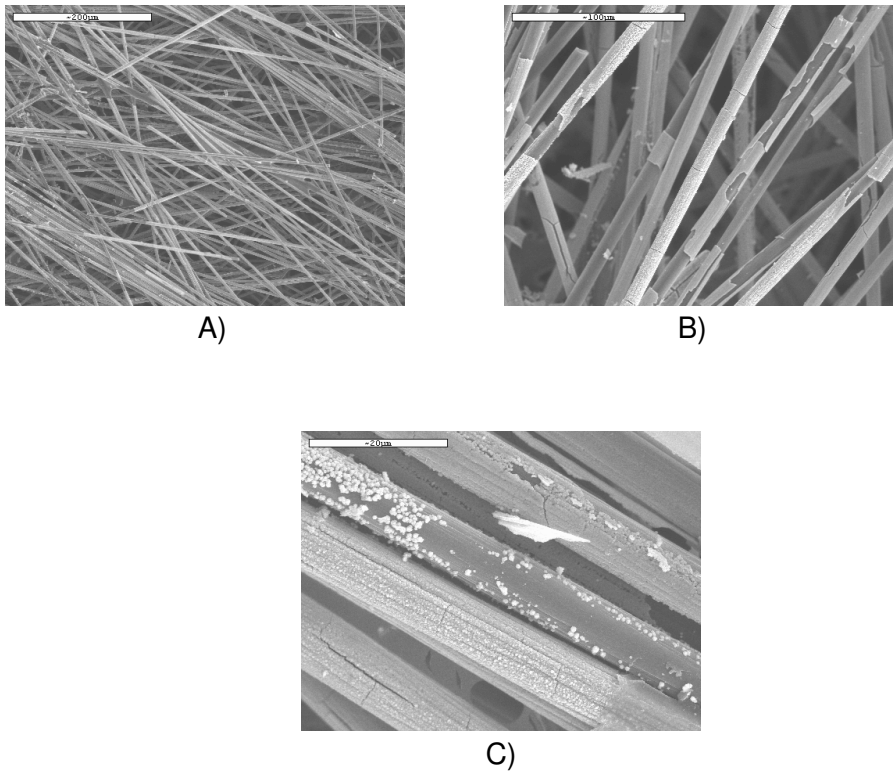


Fig.2 SEM images of NiMo/carbon felt

Local elemental analysis with X-ray EDS of the obtained composite materials confirms the presence of Ni and W or Mo in the corresponding deposits - Fig. 3. Because of the specific rough morphology of investigated materials, only qualitative information could be obtained from this analysis.

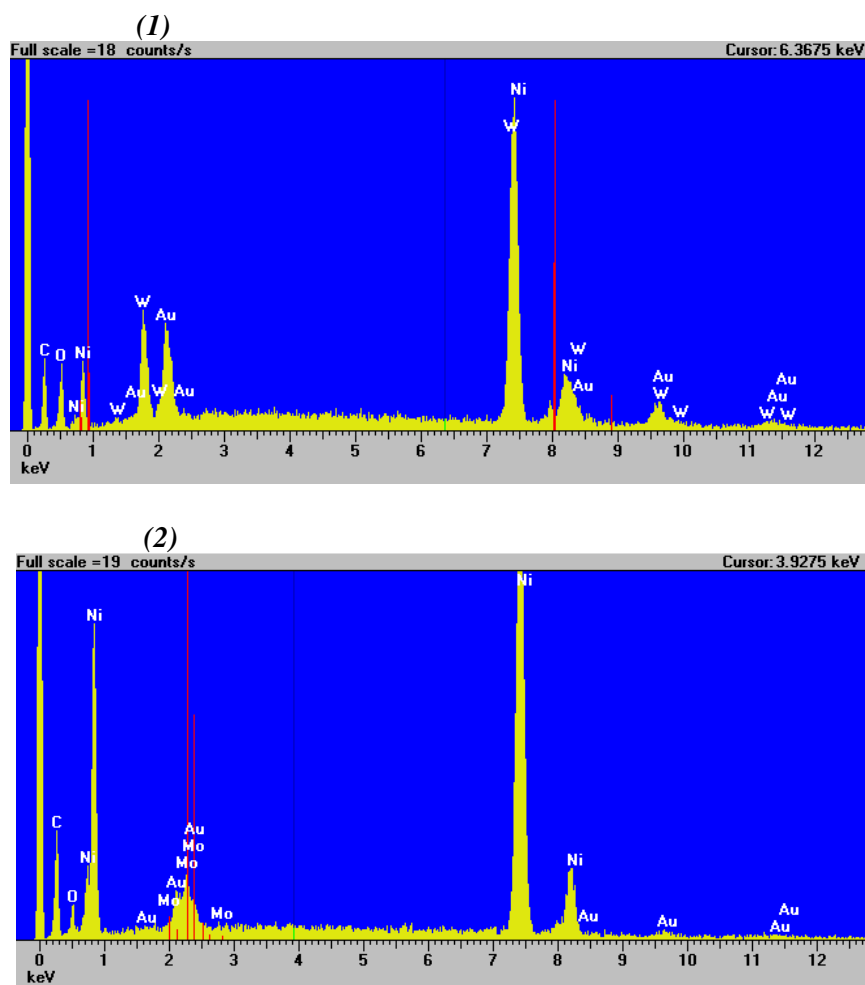


Fig.3 Qualitative analysis of NiW (1) and NiMo (2) electrodeposits

### ***3.2. Electrochemical performance of the investigated materials in neutral electrolyte***

As upper described, there is gap in the knowledge about the corrosion behavior as well as electrocatalytic activity of materials in neutral electrolytes, which is of a big importance in respect to their potential application as electrodes in MECs. Regularly, neutral phosphate buffer (PBS, pH 7) is used in bioelectrochemical systems, which determines the performed electrochemical tests in such medium.

The corrosion behavior of the produced electrodeposited materials in neutral PBS, investigated by potentiodynamic measurements, is presented as Tafel plots in Fig. 4:

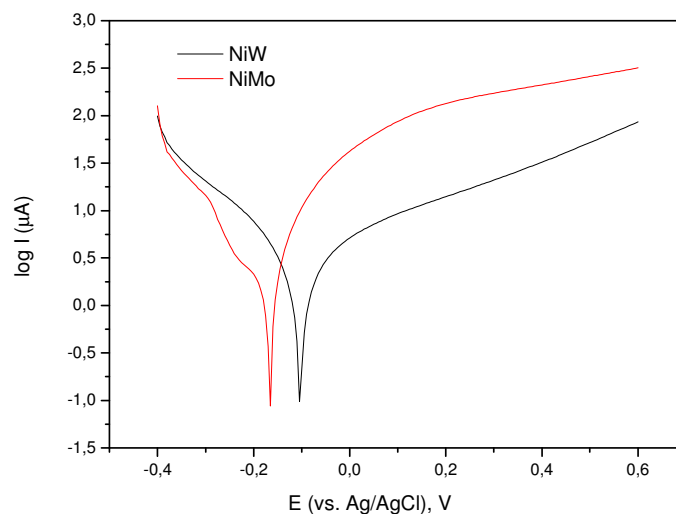


Fig 4. Tafel plots of NiMo and NiW electrodeposits on carbon felt in neutral PBS; scan rate 2 mV/s.

The estimated corrosion potentials of both materials are -0.10 V (vs. Ag/AgCl) for the NiW and -0.18 V (vs. Ag/AgCl) for the NiMo. Besides more negative corrosion potential, indicating less thermodynamic stability, higher rates of anodic dissolution is also observed for the NiMo electrodeposits. The latter is most probably connected with the much higher loading of NiMo than NiW electrodeposits, which significantly increase the number of active sites participating in the anodic dissolution. The corrosion rates, however, are relatively low, which gave reason to investigate the electrocatalytic properties of the materials towards HER in the same electrolyte.

Linear voltammograms, presenting the electrochemical performance of studied electrodes in the cathodic region of potentials, are plotted in Fig. 5:

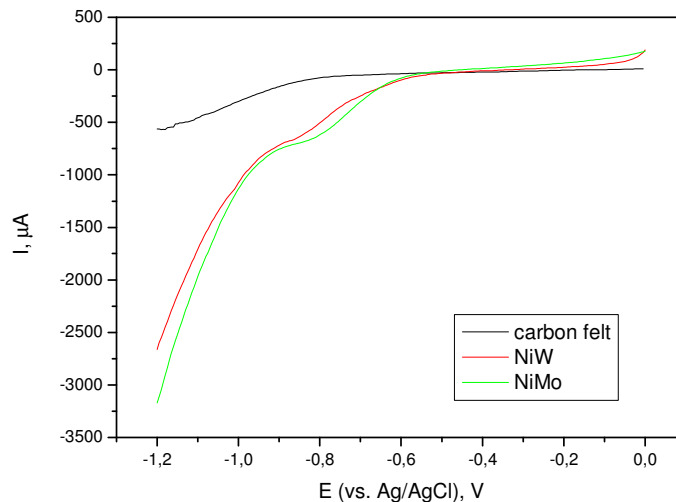


Fig. 5. Linear voltammograms obtained with NiW and NiMo electrodeposits on carbon felt in neutral PBS; scan rate 2 mV/s.

Two linear regions are observed on the LVs of both type of electrodeposited electrode. The first region is most probably connected with a reduction of surface oxide layers, formed on the electrodeposits during production or anodic oxidation. The second linear region is firmly associated with HER, which has been confirmed by visual observation of hydrogen bubbles at this potential region. The estimated values of the slope of this linear cathodic region, which is a measure of the HER rate are summarized in Table 2:

Table 2: The slope of the linear cathodic region related to HER

No	Material	Slope, mA/V
1	Carbon felt	1.46
2	NiW	6.47
3	NiMo	8.14

As seen from the data, higher rate of HER, i.e. better electrocatalytic activity, is achieved with NiMo than with NiW electrodeposits. Both materials exhibit several times higher electrocatalytic activity than that of bare carbon felt.

#### 4. CONCLUSIONS

Based on the results obtained in this study it can be concluded that the modification of carbon felt with NiMo and NiW electrodeposits results in significant increase of the electrocatalytic activity towards HER in neutral phosphate buffer electrolyte. Further studies aiming at practical application of this type of electrodes as cathodes in MECs are going to be performed.

#### 5. ACKNOWLEDGEMENTS

This work was supported by the Project "Hydrogen Economy Cooperation Network for Research - Public Awareness - Business Opportunities across Greek-Bulgarian borders – HYDECON" under the "European Territorial Cooperation" Operational Programme "Greece - Bulgaria 2007-2013".

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# ***Ab initio* study of Pd-Au Electrodeposits as Anodic Catalyst for Direct Borohydride Elelctrooxidation**

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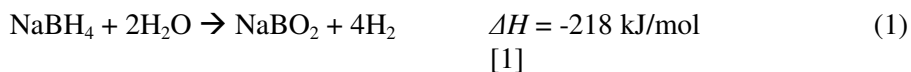
<sup>3</sup>*Department of Biochemistry and Microbiology, Plovdiv University, Plovdiv Bulgaria*

**Abstract:** Direct Borohydride Fuel Cell (DBFC) is a device that converts chemical energy of borohydrides into electricity. Fuel cells using borohydride as a fuel have received much attention because of the high energy density and theoretical working potential. Many materials are object of investigation with the importance of operating parameters on the DBFC performance, but the best catalysts are the high-cost Pt and Pd. In this study, we prepared Pd-Au electrodeposit on Ni-foam and investigated it by SEM and MS in respect to potential electrocatalysts for direct borohydride electrooxidation. They incorporated small amounts of Pd and Au, but Ni-foam provided a big surface that significantly increases the activity of the catalyst.

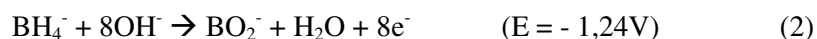
**Keywords:** DBFC, Pd-Au Electrodeposit, Anodes, Hydrolysis of borohydride.

## **1. INTRODUCTION**

Borohydrides are class of compounds containing high amounts of hydrogen. Their hydrolysis process produces hydrogen. The process takes place in ambient conditions, so it does not need any energy. The produced hydrogen can be used in the fuel cells.



Furthermore, borohydrides can be used directly as a fuel to produce electricity in so-called Direct Borohydride Fuel Cell (DBFC). Thus, borohydrides provide attractive options for the electrochemical power generation acting either as hydrogen source or anodic fuel for DBFCs. The direct borohydride fuel cell is based on a complete eight-electron reaction process and provides a high theoretical specific capacity (5.67 Ah/g, based on NaBH<sub>4</sub> [2].



Noble metals are the only selection for the electrode catalysts in the PEMFC (proton exchange membrane fuel cell), the PAFC (phosphoric acid fuel cell) and the DMFC (direct methanol fuel cell) because only they possess high corrosion resistance in acidic electrolytes and provide high electrocatalytic activity at relatively low temperatures [3, 4]. Unlike these fuel cells, the DBFC operates with alkaline electrolyte so that besides the noble metal catalysts [2], Ni-based catalysts can be also used [5].

Recently, there has been a growing interest in the preparation, characterization and the potential application of electrodes, modified by thin electrocatalytic surfaces. The electrode processes may be described by kinetic investigations, using the reaction rate and the activation energy of the processes that take place on the electrode. In general, the catalyst is introduced with the reagents in a liquid medium (the electrolyte). In many cases the catalyst is a part of the electrode material. Both homogeneous and heterogeneous reactions can be electrochemically catalyzed. It is suggested that the catalyst becomes active near the electrode surface, where the real electron transfer takes place. Therefore the deposition of thin films of active catalyst materials on the electrode surface is a promising opportunity to develop electrochemical systems with good performance [6].

In this work, Pd-Au electrodeposits on Ni-foam was prepared and characterized by scanning electron microscopy (SEM). Also the gaseous products, that were released from the very complex system: polarized investigated material immersed in an alkaline solution of sodium borohydride, were detected by mass spectroscopy (MS).

Such materials would not be so expensive, because they incorporated small amounts of Pd and Au, but Ni-foam provided a big surface that significantly increases the activity of the catalyst.

## 2. EXPERIMENTAL

### 2.1. Sample preparation

The materials were prepared by electrodeposition of Pd and Au on Ni-foam (RCM-Ni-4753.016, *Recemat Inc*). The electrodeposition was carried out at potentiostatic conditions ( $E = -0,15V$  vs. Ag/AgCl) in a three electrode cell for 10 s. A piece of Ni-foam (geometric surface of  $1\text{ cm}^2$ ) was connected as a working electrode, the potential was measured versus Ag/AgCl electrode and a Pt-mesh was used as a counter electrode. The electrolyte was a mixture of 2%  $\text{PdCl}_2$  in 0,1M HCl and 2%  $\text{HAuCl}_4$  in 0.1M HCl in equal volumes (1:1) [7]. After the electrodeposition, the prepared materials were carefully rinsed with deionized water and were ready for use.

### 2.2. SEM-investigation

The morphology and the elemental content of the developed materials were analyzed by scanning electron microscopy (SEM) JEOL 6300 with X-ray Microanalysis ISIS 2000.

### 2.3. MS-detection of the released products

A mass spectroscopy was used for determination of the gaseous products that were released from the complex system: polarized investigated material immersed in an alkaline solution of sodium borohydride. The experimental setup consisted of a three electrode hermetic cell with an outlet, connected to a quadrupole mass spectrometer (*Baltzers – Omnistar*) where the outlet stream was qualitatively and quantitatively analysed. The mass fractions corresponding to: hydrogen (m/e: 2), nitrogen (m/e: 28, 14), oxygen (m/e: 32, 16), carbon monoxide (m/e: 28, 12), carbon dioxide (m/e: 44, 28, 12), nitrogen oxides (m/e: 30) and water (m/e: 18, 17, 16) were simultaneously monitored during the experiment. The quantity of produced hydrogen was calculated, based on the areas of the corresponding mass spectrometer (MS) response. The mass-spectrometer response of hydrogen was calibrated by using pulse injections of pure hydrogen.

## 3. RESULTS AND DISCUSSION

### 3.1. SEM investigation

As seen from Fig. 1, the surface of the produced electrodeposits is not smooth, because of the porous structure of the supported material (Ni-foam).

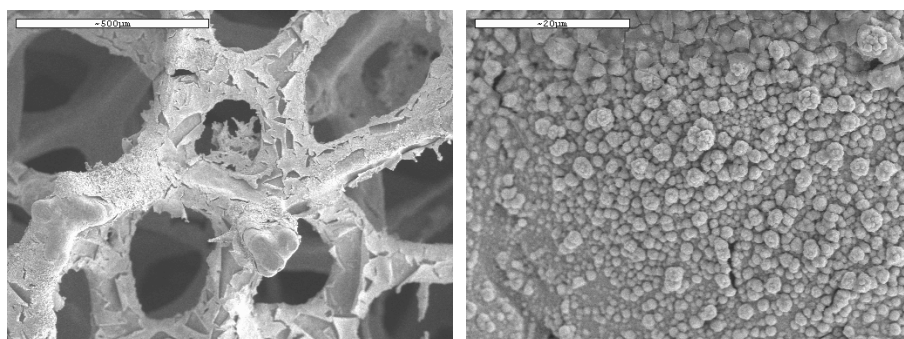


Fig. 1: SEM images of sample Pd:Au=50:50 in different enlargement.

More detailed view showed that the electrodeposits had dendrite structure and covered almost the whole surface of the supported material.

The X-ray microanalysis proved the elemental content of the electrodeposits (Fig. 2). Pd and Au lines of the electrodeposits as well as the Ni lines originating from the supported material are well detectable.

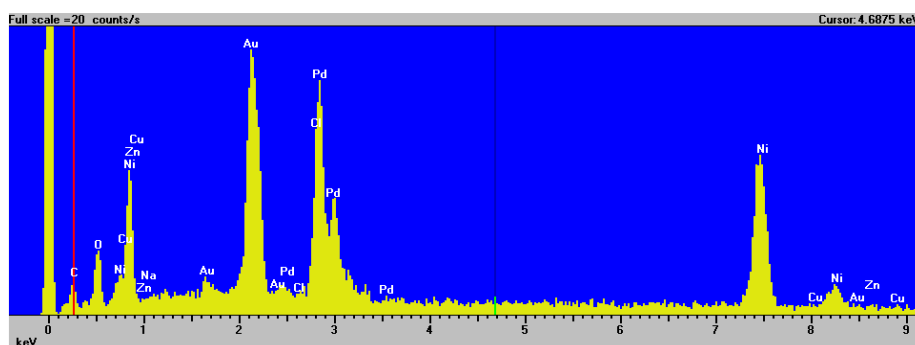


Fig. 2: X-ray microanalysis of Pd:Au=50:50 surface.

### 3.2. MS-detection of the released products

The examined material was immersed in 5%  $\text{NABH}_4$ /6M KOH solution in a three electrode cell and connected as a working electrode. The system was closed and connected with the MS detector. The temperature was kept constant at 25 °C. As seen from Fig. 3, the only gaseous product released from the reaction system was hydrogen ( $m/e = 2$ ). All the other signals did not change and were following the zero line. A small drift of the signal for hydrogen was observed and after about 30 minutes the signal rose up. For the first 30 minutes the hydrogen generation rate of 13  $\mu\text{l}/\text{min}$  was estimated and after that it increased about double to 23  $\mu\text{l}/\text{min}$ . This result could be

explained with the initial absorption and possible hydride formation of a part of the produced hydrogen by Pd. After saturation of Pd, the released amounts of hydrogen increased.

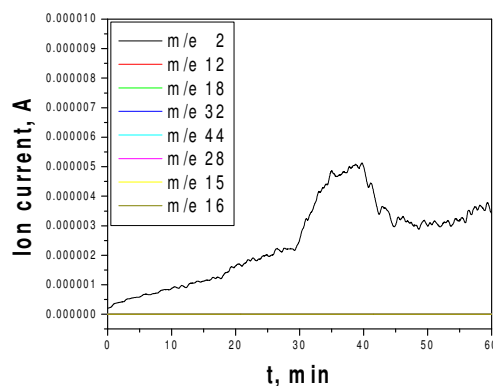


Fig. 3: MS investigation of Pd:Au=50:50 in 5%  $\text{NaBH}_4$ /6M KOH.

40 minutes after the beginning of the experiment, the investigated material, connected as a working electrode, was polarized anodically by applying a constant current of +20 mA. After polarization, the amount of the released hydrogen decreased dramatically. This indicates that at anodic polarization conditions both processes - electrooxidation and hydrolysis of borohydride are competitive and takes place simultaneously on the investigated electrodeposited catalyst.

#### 4. CONCLUSIONS

Thin layers of Pd-Au with dendrite structure were electrodeposited on Ni-foam and their performance in stabilized sodium borohydride solution was investigated. The hydrogen generation rate of  $23 \mu\text{l}/\text{min}$  from  $1 \text{ cm}^2$  geometric surface of the material indicates low catalytic activity towards borohydride hydrolysis process. Under anodic polarization, the hydrogen evolution additionally decreases, showing that sodium borohydride mainly undergoes electrooxidation at these conditions. In combination with the simple preparation procedure and possibility for production of variety of electrodeposits with different ratio of Pd and Au, the results from this *ab initio* study reveal a big potential for application of these materials as anodic catalysts in DBFCs.

## 5. ACKNOWLEDGEMENTS

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# Possibility for simultaneous electricity generation and bioremediation by using *Candida melibiosica* yeast in biofuel cell

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**Abstract:** Recently, we have proved that *Candida melibiosica* 2491 yeast strain possesses electrogenic properties and could be used as a biocatalyst in yeast-based biofuel cells. In this paper we demonstrate that when the yeast is cultivated under polarization conditions in a biofuel cell its phytase activity exceeds that obtained during cultivation in a conventional bioreactor. Furthermore, there is a correlation between the yeast phytase activity and the electrical characteristic of the biofuel cell during the different yeast growth phases. The obtained results reveal a possibility for application of *C.melibiosica* for simultaneous electricity generation and bioremediation of hardly degradable polyphosphates, especially in the regions with intensive stock-farming.

**Keywords:** Biofuel cells, yeast, *Candida melibiosica*, electricity generation, bioremediation

## 1. INTRODUCTION

Microbial fuel cells (MFCs) are considered as a promising innovative technology for simultaneous electricity generation and purification of biodegradable wastes [1]. The technology is based on the use of microorganisms capable to accomplish extracellular electron transfer to the MFC anode, which serves as a final acceptor of the electrons generated via catabolic degradation of different substrates. Most of the research in the field has been performed with bacteria, which are known to use in the nature solid (metal oxides and hydroxides, e.g. Fe(III) and Mn(IV)) or soluble (sulfates, nitrates, etc.) electron acceptors to perform their

respiration processes [2, 3]. Less attention has been paid on the possibility for utilization of eukaryotic yeasts as biocatalysts in MFCs [4].

Recently, we demonstrated that biofuel cells using *Candida melibiosica* 2491 yeast strain as a biocatalyst could generate electricity, which characteristics – current and power, depend on the nutrition medium content as well as on the growth phase of the culture [5]. Thus, for the first time we approved that *Candida melibiosica* 2491 yeast belongs to the so-called exoelectrogens. In other studies it was reported that this strain possesses high phytase activity and has a great potential for application in bioremediation of hardly degradable polyphosphates [6].

The aim of the present study was to evaluate the influence of the polarization during cultivation of *Candida melibiosica* 2491 in biofuel cells on the phytase activity of the yeast strain. For this purpose, yeast-based biofuel cells using *C. melibiosica* as a biocatalyst were operated under permanent as well as periodic polarization and the phytase activity was analyzed and compared with that of yeast cultivated as a control at normal conditions.

## 2. EXPERIMENTAL

Unified quantity of 0.3 g/l *Candida melibiosica* cells was inoculated into YP<sub>fru</sub> medium containing yeast extract, peptone and fructose as carbohydrate source. 50 ml of the obtained suspension was applied as anolyte in double-compartment MFCs with salt bridge. The same volume of 0.1M K<sub>3</sub>[Fe(CN)<sub>6</sub>] was used as a catholyte. Carbon felt (4.5 cm<sup>2</sup>; SPC-7011, 30 g/m<sup>2</sup>, Weibgerber GmbH & Co. KG) was used as both cathodes and anodes.

Two MFCs were started-up and analyzed in parallel. The first one was operated at open circuit conditions for the most of the experimental time. The second MFC was permanently polarized with 1k $\Omega$  load resistance. Every three hours polarization measurements variable resistances were carried out with both MFCs as described in [5].

The yeast cultivation at normal conditions (without polarization) was carried out as a control using the same inoculum and the same device as for the MFCs. The yeast (in MFCs and control) was grown in a thermostat at 28 °C with shaking speed 100 rpm. Each 3 hour 1.5 ml aliquots from the yeast suspensions (MFC anolyte and control) were taken for analyses of optical density and phytase activity. The yeast growth was analyzed by means of spectrophotometric determination of optical density at  $\lambda$  600nm [5] and the phytase activity - by the method of Engelen [6]. The specific phytase activity is presented as enzyme activity per gram absolutely dry biomass.

All experiments were performed in duplicate.



### 3. RESULTS AND DISSCUSION

The yeast development under different experimental conditions is presented in Fig.1:

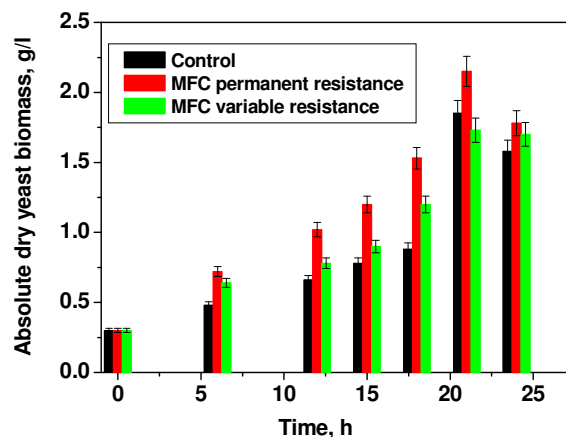


Fig.1: *C.melibiosica* yeast growth under different experimental conditions

A well-defined exponential phase of growth up to the 21<sup>st</sup> hour of the yeast cultivation is observed in all cases. The obtained results firmly prove that the applied polarization stimulates the yeast development – the highest values of biomass are achieved under permanent polarization conditions, while the lowest biomass amounts were obtained with the controlled grown yeast.

Typical polarization and power curves obtained with both studied MFCs are presented in Fig.2:

As seen from the figure, the characteristic electric parameters – open circuit voltage (OCV), maximum power ( $P_{max}$ ) and short circuit current ( $I_{sc}$ ) obtained with the MFC operated at open circuit are higher than those achieved with the MFC operated at permanent polarization. This result is quite reasonable, taking into consideration that polarization measurements at variable resistances were carried out only few minutes after the load resistance of the latter MFC was disconnected from the electric circuit, thus the system had not enough time to recover.

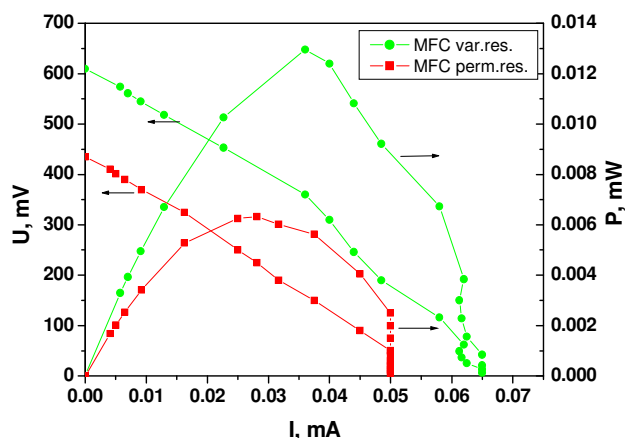


Fig.2: Polarization and power curves obtained with the studied MFCs

The variation of the maximum power, extracted from the power curves, with time is shown in Fig.3:

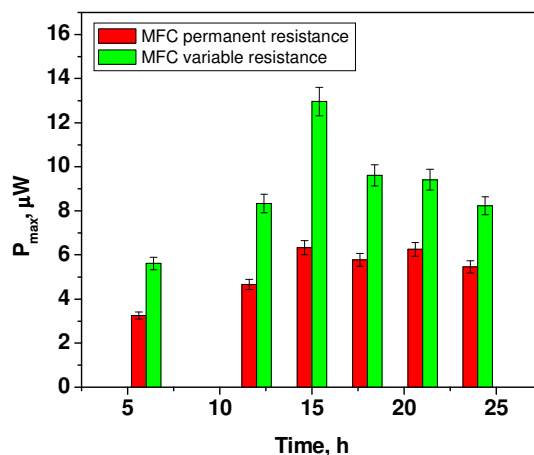


Fig.3: Variation of maximum power generated with the studied MFCs with time

The highest values of maximum power for both MFCs were achieved at the 15<sup>th</sup> hour of operation, which corresponds to the middle of the exponential phase of yeast development, when the metabolic processes are most intensive.

As reported in a previous study [6], the phytase production begins at the end of the exponential phase and beginning of the stationary phase when *C. melibiosica* is cultivated at normal conditions. The results from this study

show that under polarization conditions in MFC the phytase production starts quite earlier – at the beginning of the exponential phase, and reaches maxima at the middle to the end of this phase of yeast development – Fig.4. Furthermore, the obtained phytase activity at cultivation in MFC under variable resistances exceeds that achieved at normal cultivation.

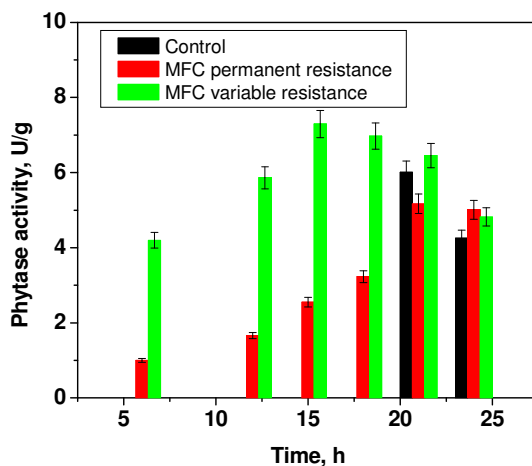


Fig.4: Variation of phytase activity obtained at different cultivation conditions with time

We suppose that the expressed higher phytase activity, even at earlier phases of the culture development, is a response to the lower biological energy yield due to the conversion of part of the total energy generated via catabolic processes into electricity. From biochemical point of view, we consider that the yeast needs additional amounts of inorganic phosphates for the phosphorylation processes, which is supplied by the intensive polyphosphate (phytate) hydrolysis.

#### 4. CONCLUSIONS

The influence of polarization on the phytase activity of the electrogenic *Candida melibiosica* 2491 yeast strain was investigated by cultivation of the culture in yeast-based biofuel cells. The obtained results show a correlation between the achieved MFC electric outputs and expressed phytase activity. In addition, under polarization conditions the yeast begins to produce phytase at earlier stage of its development and the achieved maximum of phytase activity is higher than that obtained under normal cultivation.

Besides further investigations are required, the obtained results in the present study reveal a possibility for application of *C.melibiosica* for simultaneous electricity generation and bioremediation of hardly degradable polyphosphates, especially in the regions with intensive stock-farming.

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# Long-term operation of Sediment Fuel Cells using river sediments and soil

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**Abstract:** Sediment Fuel Cells (SFCs) are considered as one of the most perspective representatives of the innovative Microbial Fuel Cell technology for power supplying electronics in remote areas or for monitoring of different aquatoria. Most of the research in the field has been performed by using marine sediments, while the fresh water sediments and soils have been paid less attention until now. In this study, results obtained eighteen months after the start-up of two column-type SFCs using river sediments and soil collected near Blagoevgrad are presented and discussed. After such long-term operation both SFCs generated one of the highest current and power values in comparison to those achieved in previous periods of the experiment.

**Keywords:** Sediment Fuel Cells, fresh water sediments, electrogenic bacteria, electricity generation, power supply

## 1. INTRODUCTION

The rising energy demands force the investigations towards development of novel, high-effective technologies for utilization of alternative energy sources. Such a technology, attracting increasing attention in the recent years, is that of microbial fuel cells (MFCs). Established with an idea for electricity generation from waste organic matter, MFCs have been recently proposed for various applications such as wastewater treatment, biosensing, bioremediation, etc. [1-3]. While the majority of MFC research is focused on the performance of laboratory reactors, the so-called sediment

fuel cells (SFCs), operating on the potential gradient at a sediment-water interface, offer a unique opportunity to investigate the efficiency of harvesting electricity from natural systems and the potentials for their real application in power generation or bioremediation in natural environments. Up to now, most of the studies have been performed with marine sediments [4-6] and a very few reports reveal the potential application of freshwater sediments [7, 8] or soil [9, 10] for electricity generation.

For this reason, we have started long-term experiments with SFCs using river sediments and soil collected near the town of Blagoevgrad, Bulgaria. In this study, results obtained eighteen months after the start-up of two column-type SFCs are presented and discussed.

## 2. MATERIALS AND METHODS

River sediments and water were collected from the basin of river Struma (GPS coordinates: 41°990354, 23.067501; Fig.1, left picture). Soil samples were taken near Blagoevgrad (GPS coordinates: +42.051209, 23.076744; Fig.1, right picture).



Fig.1: Places of samples' collection: left) for river sediments; right) for soil.

Cylindrical plastic vessels (25 cm in height, 7 cm in diameter) were used for construction of single-chamber fuel cells. Fifteen centimeters from the vessel height were fulfilled with sediments/soil. Graphite disk (diameter 6 cm, thickness 1 cm) used as anode was buried into the sediment 3 cm above the vessel bottom. Water from the place of the corresponding sample collection was poured above the sediment layer. The thickness of the water column was 5 cm. Graphite cathode with the same dimensions as the anode was placed few millimeters beneath the water surface.

Both SFCs were operated for over 18 months. Periodically, polarization measurements through constant or variable load resistances were carried

out. The cell voltage was measured with a digital multimeter and the current was estimated by using Ohm's law.

### 3. RESULTS AND DISCUSSION

A few hours after the start up the open circuit voltage of both types of SFCs began to raise slowly reaching steady-state values of 250 mV after 20 days for the soil FC and 320 mV after 15 days for the sediment FC. After this initial period, polarization measurements through variable load resistances were carried out daily and volt-ampere as well as power curves were plotted. Two months after the start up the SFCs were polarized with constant (510  $\Omega$ ) load resistance and the generated current was monitored uninterruptedly for 20 days. Such measurements were performed periodically for over a year and the results were presented and discussed elsewhere [11].

Hereafter, we present results obtained eighteen months after the start-up of the both SFCs.

Volt-ampere and power curves obtained with the studied fuel cells are plotted on Fig.2:

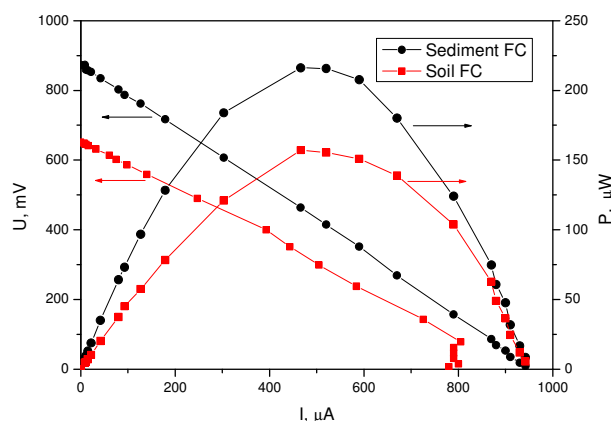


Fig.2: Volt-ampere and power curves obtained with investigated fuel cells after 18 months operation.

As in the previous periods of the long-term operation, the fuel cell using river sediments exhibit higher electrical outputs than the Soil FC. The main characteristic such as open circuit voltage ( $OCV$ ), short circuit current ( $I_{sc}$ ) and maximum power ( $P_{max}$ ) extracted from the corresponding volt-ampere and power curves are summarized in Table 1:

Table 1 Values of open circuit voltage (OCV), short circuit current ( $I_{sc}$ ) and maximum power ( $P_{max}$ ) obtained with studied fuel cells.

Parameters	OCV, mV	$I_{sc}$ , $\mu A$	$P_{max}$ , $\mu W$
Soil FC	652	1045	157
Sediment FC	873	1100	216

The efficiency of the investigated SFCs was evaluated by equation

$$\eta = 100.P_{max}/(I_{sc} .OCV), \%$$

where  $P_{max}$  was the maximum power and  $I_{sc}$  and OCV were short circuit current and open circuit voltage, respectively [12]. Using the data from Table 1, the efficiency of 23.1% and 22.5% were estimated for the Soil FC and Sediment FC, respectively. The relatively low values of estimated efficiency could be associated with the specific construction of the investigated bioelectrochemical systems resulting in high ohmic and transportation losses.

When a constant load resistance was connected in the electrical circuit, a typical voltage drop corresponding to polarization of several hundred millivolts was observed initially – Fig.3. After the initial drop, higher steady-state values of the terminal voltage were achieved with Sediment FC. The estimated by using Ohm's law values of generated current are  $196 \pm 20 \mu A$  for Sediment FC and  $157 \pm 18 \mu A$  for Soil FC.

The open circuit voltage increased steeply after disconnection of the load resistance from the electrical circuit, restoring to values close to those before polarization for about 20 hours – Fig.4. It is worth noticing that in contrary to the conventional chemical batteries and fuel cells, the studied sediment fuel cells are not damaged and are able to restore their open circuit voltage even after operating at short circuit conditions.



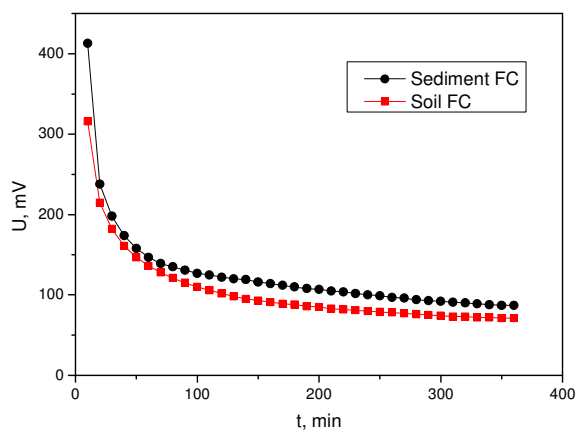


Fig.3 Variation of terminal voltage with time under polarization of 510  $\Omega$  load resistance

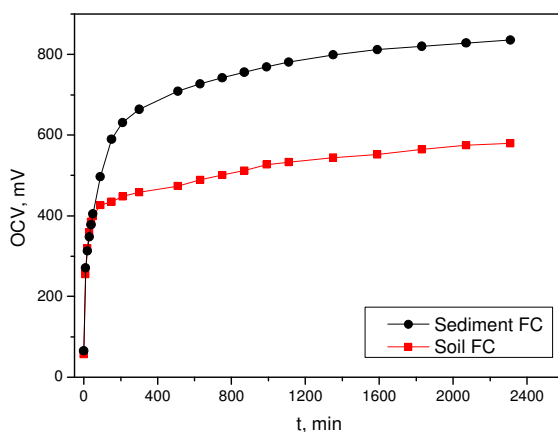


Fig.4 Recovery of the bioelectrochemical systems after disconnection of the load resistance

#### 4. CONCLUSIONS

Two sediment-type fuel cells using river sediments and soil continue to generate stable electrical outputs after 18 months operation without any maintenance except periodic addition of water for compensation of evapora-

tion losses. Since sediments or soil themselves act as a nutrient-rich anodic media, inoculum and membrane, this type of SFC is very cost-effective as the expensive proton-exchange membrane used in great number of other MFCs is not necessary.

A stack of the investigated SFCs connected in series generates current and voltage enough to supply low power consumption devices. This allows to develop SFC-based power supplies, which can operate autonomously on the field.

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## Extraction methods for speciation and quantification of Cr (III) and Cr (VI) from aqueous solution

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### Abstract

*Being as excellent metal chelates, a series of Schiff base derived from 4-Aminoantipyrine and different substituted benzaldehydes have been synthesized. The obtained ligands have been further used for modification of silica gel by physical adsorption. The analytical method based on solid phase extraction using the schiff base modified sorbents was developed, and selective sorption of Cr (VI) in presence of Cr (III) has been obtained. The eluated Cr (VI) was determined by inductively coupled plasma-optical emission spectrometry (ICP-OES). The content of Cr (III) is determined as a difference from total Cr. The validity of the proposed analytical method was checked by added/found method.*

**Keywords:** Schiff bases, preconcentration, metal speciation, trace element analysis, ICP-OES

### 1. INTRODUCTION

Chromium is naturally occurring element presents in the Earth's crust, and is found in animals and plants. It can also be released to the environment from anthropogenic sources because it is widely used in manufacturing processes. Chromium exists in the environment mainly in two stable oxidation states, Cr (III) and Cr (VI). Trivalent chromium is relatively immobile and less toxic, and is also known to be an essential trace element in mammals [1,2]. Hexavalent chromium is highly soluble and mobile in aqua media. It is considered to be a carcinogenic in humans and toxic for biological systems [1,2,3]. For this reasons, speciation and monitoring of chromium species is necessary. A various instrumental techniques commonly

used for determination of chromium such as graphite furnace and flame atomic absorption spectrometry (GFAAS and FAAS), inductively coupled plasma optical emission spectrometry (ICP-OES) and inductively coupled plasma mass spectrometry (ICP-MS) can only yield total amount of chromium. Therefore many separation/preconcentration techniques such as liquid-liquid [4,5], solid-phase [4,6] and cloud-point extractions have been widely used for the speciation of Cr (III) and Cr (VI). Recently, new types of SPE materials have been suggested for chromium speciation and preconcentration such as modified silica gel or activated carbon, chelating resins [6-8].

The aim of this work is to synthesize of new sorbents and optimize the chemical parameters for selective extraction of Cr (VI) in the presence of Cr (III). The application of analytical procedure developed for Cr (VI) determination in surface waters, will be also studied.

## **2. EXPERIMENTAL**

### **2.1. 2.1 Instruments**

The experiments were performed with a high resolution radial viewing ICP-OES system HORIBA JY ULTIMA 2 (Jobin Yvon, Longjumeau, France) equipment.

### **2.2. 2.2 Reagents and solutions**

All chemical were analytical reagent grade and are used without further purification. Stock solution ( $1000 \text{ mg L}^{-1}$ ) of chromium (III) and Cr(VI) were Merck titrizols. Working standard solutions were obtained by appropriate dilution of the stock standard solutions. Silica gel for column chromatography ( $\leq 0.063 \text{ mm}$  ( $\geq 230 \text{ mesh ASTM}$ )), 4-aminoantipyrine, substituted benzaldehydes were purchased from Sigma-Aldrich Ltd. The pH adjustment was done by addition of HCl and  $\text{NH}_3$  solutions.

### **2.3. Preparation of Schiff bases**

Synthesis of the Schiff base derivatives was carried out by using  $\text{A}_\text{N}$  reaction for the condensation of commercially available 4-aminoantipyrine and different substituted benzaldehydes in abs. ethanol. The yields of desired products varied from moderate to excellent (60–92%). The general structure of the desired Schiff base analogues is presented in Fig. 1.

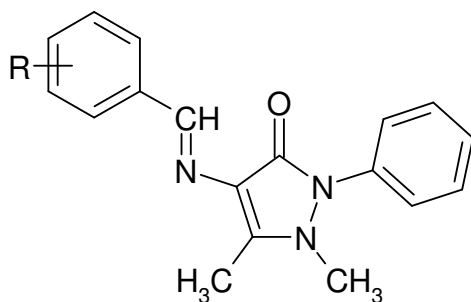


Fig. 1.

#### 2.4. Preparation of solid phase

The commercially available silica gel was activated by refluxing with concentrated hydrochloric acid for 4 h to remove any adsorbed metal ions. Thereafter it was filtered, washed with deionized water until the filtrate was neutral and dried in an oven at 150°C for 12 h to remove surface adsorbed water. The activated silica gel was refluxed with Schiff bases (9:1 w/w) in acetone for 8 h. After that mixture was vigorously stirred at room temperature to complete the solvent evaporation and then dried at 40 °C for 1 h.

### 3. RESULTS AND DISCUSSION

#### 3.1. Effect of pH

The influence of pH was studied for its effect on the determination of Cr (III) and Cr (VI). For this purpose the pH values of the solutions were varied within the range 3-10 by using HCl and ammonium solutions. The extraction yield depends on the pH at which complex formation occurs.

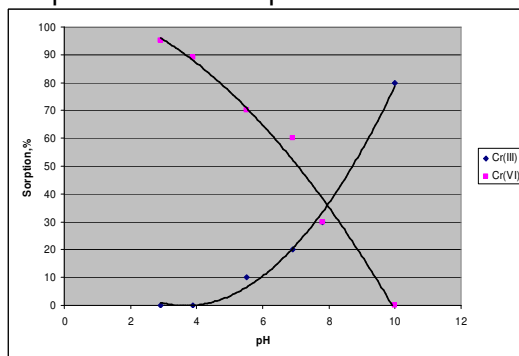


Fig 2. Effect of pH on the recoveries of chromium species on silica gel coated with SBTD

As can be seen on the graph Cr (III) does not sorb in an acid media (pH 3-4). The adsorption of Cr (III) increases with pH and obtain maximum value (80 %) at pH 10. The sorption of Cr (VI) shows the opposite behaviour – the minimum sorption was obtained for pH 10 – 0%. The quantitative recoveries (95%) of chromium (VI) were obtained at pH 3. Probably the changes in adsorption of the chromium species for different pHs can be connected with changes in the protonation/deprotonation equilibrium of the Schiff bases.

The difference in the sorption behaviour of the two species of chromium (Cr (III) and Cr (IV)) at given pH value could make it possible to separate Cr (III) from Cr (IV) and to determine Cr (IV) by adjusting the pH below 4. As the maximal sorption of Cr (III) at pH 10 is not quantitative its content could be determined as a difference from total Cr. For the subsequent experiments, pH 3 was selected as the working pH.

### **3.2. Effect of type and volume of eluent solution**

Different mineral and organic acids are used as eluents for recovery of the two species of chromium from the solid phase. Desorption of chromium (III) and chromium (VI) from the surface of the adsorbent may be taking place as a complex with the Schiff bases and/or releasing of chromium ions.

It was found 10 ml ( $1 \text{ mol L}^{-1}$ ) of nitric acid to be satisfactory for the desorption of Cr (III). On the other hand this eluent is not suitable for the desorption of Cr(VI). In this case a quantitative recovery of Cr (VI) was obtained using 10 ml ( $1 \text{ mol L}^{-1}$ ) acetic acid. In all further works, 10 ml ( $1 \text{ mol L}^{-1}$ ) acetic acid solution was used as eluent of Cr (VI).

### **3.3. Amount of adsorbent parameter that affects the recovery**

The amount of adsorbent is another important parameter that affects the recovery. A quantitative retention is not obtained when the amount of coated silica gel is less than optimum. On the other hand, an excess amount of adsorbent prevents the quantitative elution of the retained metal chelates by a small volume of eluent. In order to examine the smallest amounts of solid phase, 150-700 mg of modified silica gel were stirred in batch mode with a synthetic solution containing analytes. Quantitative recoveries values for Cr (VI) were obtained in a range 300-500 mg of coated silica gel.

### **3.4. Application of the method**

The proposed preconcentration method was applied for determination of Cr (VI) in tap water samples spiked with known amount of chromium (VI),

Table 1. A good agreement was obtained between the added and measured amounts of analyte. These results confirm the validity of the proposed method.

Tab. 1. Determination of Cr(VI) in water samples spiked with Cr(VI)

Parameter	Tap water
	Cr(VI)
Added, $\mu\text{g}$	0.10
Found, $\mu\text{g}$	0.095
Recovery, %	95
RSD, %	6-8
Detection limits, $\mu\text{g ml}^{-1}$	0.006

#### 4. CONCLUSIONS

Herein, a new application of silica gel modified with Schiff bases as the sorbent for the speciation and determination of Cr (III) and Cr (VI) was described. Conditions for quantitative and repeatable preconcentration, elution and ICP-OES determination were studied. The proposed method is simple, sensitive, accurate and repeatable. The method is successfully applied for the determination of chromium species in tap water samples. The precision and accuracy of the method are satisfactory.

It is worth to mention that the Schiff bases modified silica gel can be used as a sorbent to preconcentration and determination other metals (e.g. Pt (II), Pd (II), Au (III)), by simple varying the experimental conditions.

#### 5. ACKNOWLEDGMENTS

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## STEREOELECTROCHEMISTRY OF CALIX[4]ARENES

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Calixarene frame<sup>1</sup> is able to bear various substituents – e.g. redox centers. Tetra-nitro derivatives of calix[4]arenes are an example of molecules with more redox centers. The presence four nitro groups (on upper rim) in one molecule provokes many principal questions: What is the intramolecular electronic interaction between them? Are they reduced simultaneously or stepwisely? What is the influence of the lower rim substitution on the reduction of the nitro groups? What is the influence of the reduction on the molecular geometry? What is the influence of the molecular geometry on the reduction pattern? **Can we able to see in solution any effects observed in solid state?**

The stereochemistry of calix[4]arenes, based on X-ray data,<sup>2</sup> is well known. The four possible conformers of calix[4]arenes<sup>1</sup> are depicted in Fig.1.

In order to describe only fine deformation effects of calix[4]arenes scaffolds, any stereochemical approach should be evaluated. It is therefore necessary to find simple parameters to describe the geometry of the calix[4]arene scaffold.

The conformation of the calix[4]arene base frame (i.e. hydrocarbon skeleton without upper rim and lower rim substituents) is fully described by the values of torsion angles at the C<sub>arom</sub>-C<sub>bridge</sub> bonds. For calix[4]arenes, the relevant torsion angles visible in Fig. 2 are the angles C28-C1-C2-C3, C24-C1-C2-C3, C1-C2-C3-C4 and C1-C2-C3-C25. There are obviously two torsion angles at each bond C<sub>arom</sub>-C<sub>bridge</sub>, so there are altogether sixteen relevant torsion angles to describe the conformation of the calix[4]arene backbone. It is necessary to reduce the number of relevant parameters.

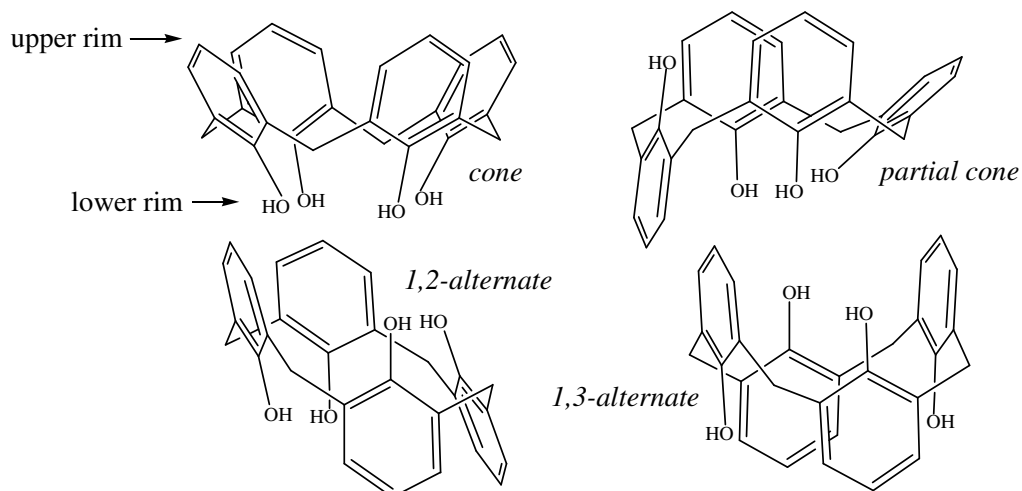


Fig.1: Conformations of calix[4]arenes

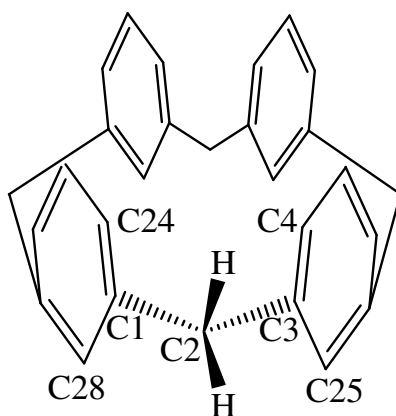
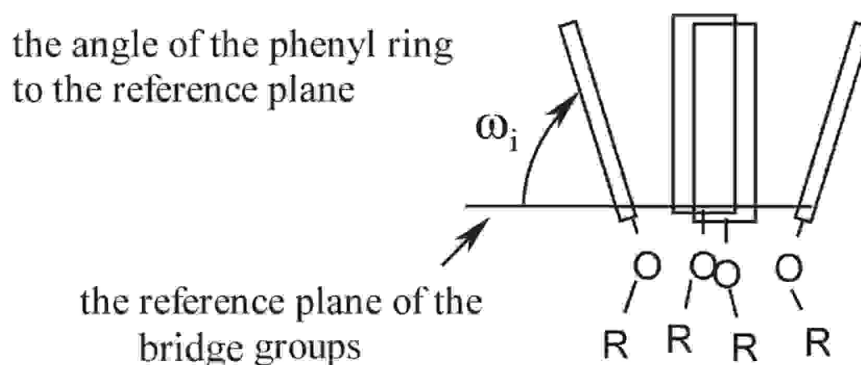


Fig. 2: Torsion angles at the calix[4]arene scaffold

The carbon atoms from  $-\text{CH}_2-$  groups have been utilized by the definition of a reference plane<sup>3</sup> to which the angles  $\varphi_1, \varphi_2, \varphi_3, \varphi_4$  of the four calix[4]arene phenyl rings are related. The angles of the phenyl rings ( $\omega_i, i = 1-4$ ) can be calculated in the scale  $0-360^\circ$ , see Fig. 3

Fig. 3: The definition of the phenyl ring angles  $\omega_i$ 

The previous sixteen torsion angle parameters have been and thus successfully reduced by our approach to only four parameters  $\omega_1$ ,  $\omega_2$ ,  $\omega_3$  and  $\omega_4$ .<sup>4</sup> However, the assignment of these four parameters is not unambiguous and depends on the numbering of the calix[4]arene base frame, e.g. there is a question which calix[4]arene phenyl ring should be assigned as  $\omega_1$ . Moreover, four parameters form a four-dimensional space that is very difficult to imagine and work with. It would be therefore more convenient to reduce the number of the parameters from four to three, since 3D space is much easier to describe. To achieve these two objectives, new parameters  $\alpha$ ,  $\beta$ ,  $\delta$  (in  $^\circ$ ) have been introduced:<sup>4</sup>

$$\alpha = 0.25 \cdot (\omega_1 + \omega_2 + \omega_3 + \omega_4) \quad (\text{average value of the phenyl ring angles } \omega_1 - \omega_4)$$

$$\beta = |(\omega_1 + \omega_3) - (\omega_2 + \omega_4)| \quad (\text{distortion of the calix[4]arene towards } C_{2v} \text{ symmetry})$$

$$\delta = |\omega_1 - \omega_3| + |\omega_2 - \omega_4| \quad (\text{distortion of the calix[4]arene towards } C_s \text{ symmetry})$$

In the following parts, the influence of substitution and inter- and/or intramolecular interactions on the stereochemistry of the *cone* calix[4]arene moiety (described by parameters  $\alpha$ ,  $\beta$ ,  $\delta$ ) is discussed on a set of structures from CCDC.<sup>2</sup>

The final geometry of the calix[4]arene scaffold in symmetrically tetrasubstituted free *cone* calix[4]arenes results in solid state from combination of the following three effects:

1. Effects of lower rim substituents

Lower rim OH-substituted calix[4]arenes are significantly more rigid than calix[4]arenes alkylated or acylated at the lower rim due to narrow ranges of parameters  $\alpha$ ,  $\beta$ . Deprotonating in lower rim hydroxyl-containing calix[4]arenes disrupts the cyclic array of hydrogen bonds present at the lower rim; therefore, the values of parameters  $\alpha$ ,  $\beta$  tend to be slightly increased in such calix[4]arenes.

### 2. Effect of a filled/empty cavity

Lower rim alkylated/acylated calix[4]arenes, the values of parameter  $\alpha$  in filled-cavity calix[4]arenes tend to be lower than the values of parameter  $\alpha$  in empty-cavity calix[4]arenes. The type, size and flexibility of the molecule inside the cavity affect the final geometry of the calix[4]arene scaffold.

### 3. Effect of upper rim substituents

The values of parameter  $\alpha$  in calix[4]arenes with unsubstituted upper rim and calix[4]arenes substituted by polar groups at the upper rim tend to be higher than the values of parameter  $\alpha$  in calix[4]arenes with bulky nonpolar upper rim substituents. The influence of nitrogoups are typical.<sup>4</sup>

The reduction of simple aromatic nitrocompounds is going on generally in two waves, as it is depicted on Fig. 4 for “one quarter” of nitrocalix[4]arene molecule.

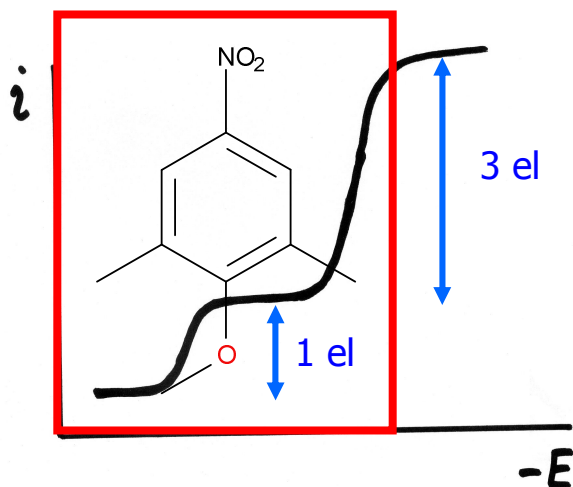


Fig. 4: The “one quarter” of nitrocalix[4]arene molecule (model compound) and its reduction pattern

**We can expect same reduction pattern (only with 4 el. + 12 el. waves) for whole tetranitrocalix[4]arene molecule, if “one multiplied by four” will be really “four”.**

Electrochemical reduction of the starts with two 2-electron reversible waves corresponding to the presence of two different couples of equivalent nitro groups.<sup>5</sup> This result reflects well the finding of the x-ray structural analysis, that the "calix" is in fact not circular, but pinched with a strong "π-stacking" of the opposite benzene rings. It corresponds with relatively high value of the parameter  $\beta$ . The electrochemical properties of the systems was discussed and interpreted in terms of molecular geometry and intramolecular electronic communication between the redox centers through the space; in the terms of the „**stereoelectrochemistry**“. The first two small waves are followed by a single 12-electron wave (large), indicating that the generated tetrakis-radical anion intermediate involves four equivalent non-communicating nitroradical anions. This result provokes questions about an electron-transfer induced change in geometry of the calix arene due to the change in the reduction state.

The experimental results mentioned above are correlated with results of quantum chemical calculations.<sup>5</sup> These calculations were performed on the tetranitro derivative (*cone*) and showed that the first two electrons reduce two nitro groups attached to two more distant benzene rings to form a biradical dianion. Consequently, next two electrons reduce the other two nitro groups to form a tetraradical tetraanion. Because its further 12-electron reduction to the final product (hydroxylamine derivative) occurs in one single step, it provokes a hypothesis that the tetraradical tetraanion has all four redox centres equivalent, thus it has symmetry of  $C_4$ .

To prove this statement by another experimental mean the **spectroelectrochemical measurements** of the series (including model nitrocompound, mono-, both di-, tri- and tetranitroderivative) were done. During the voltammetric scan both ESR and UV-Vis-NIR spectra were recorded. The ESR spectrum of a radical formed after reduction of the model compound (4-methoxy-3,5-dimethyl-1-nitrobenzene) included according to the expectations a triplet of nitrogen twicely splitted by two hydrogen atoms in the *o*-positions and by six hydrogen atoms in the both methyl groups.

**ESR spectra of radicals formed** from all five investigated nitrocalix[4]arenes showed similar pattern: splitting of the nitrogen atom and two other splittings – by two hydrogen atoms in the *o*-positions and by another two hydrogen atoms in the bridging methylene groups (from each –  $\text{CH}_2$ – unit only one hydrogen atom due to not free rotation). In all cases the formation of polyradical polyanions was observed, their ESR and difference-UV-Vis-NIR spectra were similar, so it can be concluded that each

nitrobenzene unit behaves like an electrochemically independent part of the molecule. It means, **that in the case of the intermediate, “one multiplied by four” is really “four” due to symmetry  $C_4$  of the tetraradical tetraanion.**

This is in agreement with the above mentioned hypothesis about the electrochemical (and perhaps stereochemical, too) equivalence of the nitro group radical anions in the tetraradical tetraanion.

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# DISTRIBUTION OF LEAD IN SELECTED ANIMAL ORGANS AND TISSUES IN PROBISTIP AND ITS SURROUNDINGS

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**Abstract:** *Lead belongs to the group of heavy metals and is one highly toxic. Lead polluted environments constitute a serious problem for human beings. This paper is aimed at throwing light on the concentrations of lead in the muscles, liver, kidneys, spleen and hearts of different animals (swine, sheep, and goat) from three different localities around Probistip. A total of 98 samples were collected for this purpose and lead concentrations were analyzed using atomic absorption spectrophotometer. Results showed that lead concentrations are dependent on the sampling locality, the organ and animal species. The highest concentrations of lead were detected in kidney of swine taken at the mine waste landfill near the central town area of Probistip (1.025 and 2.454mg/kg respectively). Chicken kidney taken in the Strmosh area contained the highest concentration of lead (1.125 mg/kg). The concentrations of lead in liver and kidney taken from the industrial area were higher compared to other localities from which samples of tissue were taken for analysis.*

**Key words:** *Lead, organs, animals, ecosystems, pollution, metals, contamination.*

## 1. INTRODUCTION

Heavy metals are chemical elements with specific gravity that is at least five times the specific gravity of water [31], [18]. Examples of heavy metals commonly found in the environment include lead, cadmium, mercury, zinc, arsenic, bismuth etc. These metals are particularly dangerous because they tend to bio-accumulate in the body tissues and organs [26], [5].

Lead is a ubiquitous and versatile metal which has been used by mankind for many years. It ranks as one of the most serious environmental poisons amongst the toxic heavy metals all over the world. Mankind has used it for many years because of its wide variety of applications. Human exposure to lead is from numerous sources and a myriad of pathways



including air, food, dust, soil and water [14], [19]. In the recent past, lead toxicity has emerged as an important global problem with public health consequences, particularly in children, due to its serious impact on brain functions. A higher incidence of acute intoxication among children than adults has been reported and children are exposed to higher levels of lead than are adults because of behavioral patterns (for example, characteristic mouthing of objects, pica). Also, exposures to lead from sources such as air, food and water are higher as per kilogram of body weight basis for children than for adults [4], [22].

Lead is one of toxic metals; it is dangerous to most human body organs if exposure exceeds tolerable levels [4]. Lead can affect individuals of any age, but it has a disproportionate effect on children because their behavioral patterns place them at higher risk for exposure to lead, their bodies absorb a larger percentage of the lead that they ingest and they exhibit lead toxicity at lower levels for exposure than adults [1]. Accumulation of lead produces damaging effects in the hematopoietical, hematic, renal and gastrointestinal systems [8]. Lead has been associated with various forms of cancer, nephrotoxicity, central nervous system effects and cardiovascular diseases in human. Toxicity of lead is closely related to age, sex, route of exposure, level of intake, solubility, metal oxidation state, retention percentage, and duration of exposure, frequency of intake, absorption rate and mechanisms and efficiency of excretion [27]. The inhalation of lead can permanently lower intelligence quotient (IQ), damage emotional stability, cause hyperactivity, poor school performance and hearing loss [16].

Lead is absorbed by ingestion and inhalation. Absorption varies from individuals to individuals and depends on the chemical form of lead and type of exposure. The alimentary and respiratory tracts are the main portals of entry for lead into the body. Approximately 90% of absorbed lead is reported to be stored in the bone with a half life of 600 - 3000 days. The remaining 10% is stored in soft tissues like kidney, liver and brain. The half life of lead in these tissues ranges from 40 - 50 days [13]. Lead passes through the placenta easily and fetal blood has almost the same lead concentration as maternal blood [24]. 90% of the ingested lead is excreted in the stool and urine whereas the inhaled lead is excreted through the renal pathway [30].

Lead is also eliminated through sweat and mother's milk [21]. Lead has very high affinity for red blood cells, it has been shown that lead inhibits the enzymes-aminolevulinic acid dehydratase (ALAD) and ferrochelatase of the hem synthetic pathway thus preventing conversion of ALA to protoporphyrinogen and inhibits incorporation of iron into the protoporphyrin ring respectively. This results in reduced hem synthesis and elevated levels of the precursor-aminolevulinic acid (ALA), which is a weak gamma-amino

butyric acid (GABA) agonist that decreases GABA release by presynaptic inhibition [37], [32],[28].

The presence of lead in the environment is partially due to natural processes and anthropogenic sources [2], [9], but is mostly the result of industrial wastes [11], [23]. Although atmospheric lead originates from a number of industrial sources, leaded gasoline appears to be a principal source of general environmental lead pollution. So, the heavy traffic flow of vehicles that burn gasoline with high lead content is the main cause of the high levels of lead in street dusts and in air born particles [32].

Foods may be contaminated by lead from different sources such as air, water and soil. Accurate determination of lead in food is important since intake of even low concentrations of lead can cause serious toxic effects.

The aim of the present study was to evaluate the concentrations of lead in animals (buffalo, cattle, sheep, goats and elk) meat and consumable organs (liver, kidney, spleen and heart), which are liable to be contaminated by lead. Also, the investigation provided information about the concentrations of lead in three main areas represents different localities around Probistip, i.e., industrial Zone in the town of Probistip, village Strmosh and control point

## 2. MATERIALS AND METHODS

Lead concentration was extracted from the samples (muscle, liver, kidney, spleen and heart) according to the method of [33]. Samples were homogenized separately and 5-10 g of the fresh homogenate were weighed into quartz dishes and evaporated to dryness in an oven at 100° C (~16 h). Dried samples were ached in a muffle furnace at 450-500°C for 8-12 h. Ashed samples were cooled to room temperature and 1.0 ml of concentrated nitric acid was added and the volume was adjusted to 25 ml with deionized water. The metal was measured by atomic absorption spectrophotometer (Perkin Elmer 5000). Lead was measured at wavelength 217.0 nm with Hollow Cathode Lamp of lead. The limit of detection was 0.06 mg/kg for lead. The recovery of lead was studied by adding known amounts of standard solution to different samples under investigation. The added amounts of lead were selected so that they would be close to the amounts normally found in the different samples. Recoveries in muscle, liver, kidney, spleen and heart ranged from 94-98%. All the results obtained were corrected according to the percentage of recovery.

The material for analysis (fresh tissue from muscle, liver, kidney, spleen and heart of three domestic animals – swine, sheep and goat) were taken from three localities in the vicinity of the town Probistip.

1. The first measuring point was the industrial zone in the town of Probistip – flotation of the lead-zinc ore.

2. The second measuring point was the locality in the vicinity of the waste landfill near the village of Strmos.
3. The third measuring point was the control measuring point, located at 10 km from the town of Probistip where there are no sources of pollution with heavy metals.

### 3. STATISTICAL ANALYSIS

Statistical differences between the different areas (heavy traffic, urban and industrial) were determined by one-way analysis of variance (ANOVA) according to [38]. A general linear model of Baht, et al., was performed for the analysis of variance [4].

### 4. RESULTS AND DISCUSSION

Results presented in Tables (1-3) show the mean concentrations of lead in analyzed samples collected from heavy traffic, urban and industrial areas from the different investigated animal species. The highest concentrations of lead were detected in kidney followed by liver samples. The levels of lead varied according to the species of animal and the locality (Fig. 1 and 2).

**Tab. 1:** Lead concentrations (mg/kg wet average weight) in chicken organs collected from three areas represent different ecosystems in Probistip,

Organs	Industrial Zone in the town of Probistip	Village Strmosh	Control point
Muscle	0.089	0.215	0.035
Liver	0.354	0.984	0.056
Kidney	0.568	1.125	0.120
Spleen	0.154	0.985	0.007
Heart	0.253	0.546	0.010

If we compare the results obtained for lead content in different organs and domestic animals in the three measuring points around Probistip it will be noticed that different values are obtained in terms of the tested sites, as well as of animal organs.

The highest values are measured in kidney and liver and the lowest values in spleen.

In terms of measurement sites, the lowest values are measured at the control measuring point. These results show the relation between

concentration of lead on the spot where the tested animals are kept and in tissue samples contaminated from water and soil.

Table 1 shows the results of the examination of the contents of lead in muscle, liver, kidney, spleen and muscle of chicken, where people keep them for food. In terms of the tested organs, the highest values are measured in kidney (0.120-1.125 mg/kg fresh weight), and the lowest in heart tissue (0.010 - 0.546 mg/kg fresh weight). As for the measurement sites the lowest values were obtained in the control measurement point in whose vicinity there are no sources of contamination with heavy metals, and the values were highest near the village Strmosh in whose immediate vicinity are located the old and new waste landfills where waste water from the flotation of lead-zinc ore from the lead and zinc mines "Zletovo" is accumulated. Strmosh village is located only 6 km from the town of Probistip. The concentrations of lead in liver and kidney in the industrial zone were highest in relation to other sites, so that consumption and using of organs from these sites for food should be avoided.

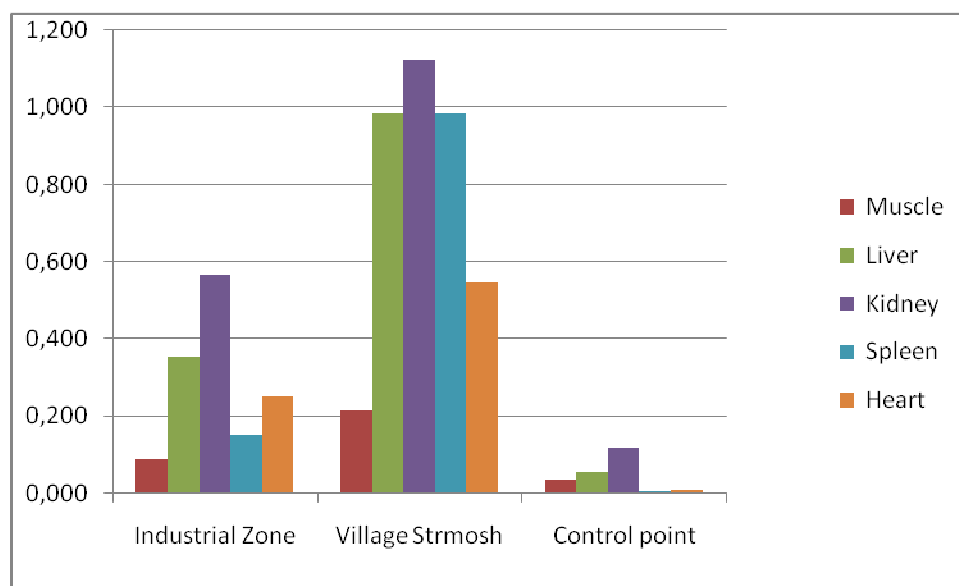


Fig. 1: Lead concentrations (mg/kg wet average weight) in chicken organs

Where should we look for the reason for keeping domestic animals at such sites that are heavily contaminated with heavy metals?

- In the fact that the population is uninformed about the consequences of eating meat with high content of lead which is inserted into the human organism and indirectly causes serious diseases.

- In the low-standard of the population that seeks way to survive during the state of crisis.

Heavy metals represent a serious problem of global pollution of the planet Earth, as well as the consequences arising from that pollution. Because of this they are the subject of much research in many countries worldwide.

Lead impairs learning, memory and audio-visual functions in children (Cohn et al., 1993). Toxic effects of lead also include Nephrotoxicity [29], Hepatotoxicity [12], cardiovascular damage. The carcinogenic effect of lead has been receiving increasing attention [20]. Research has shown that lead causes oxidative stress in the body by inducing the generation of free radicals thereby reducing the antioxidant defense system of the cells [17].

Effect of lead on reproductive systems is also well documented. Lead causes sterility in males by damaging the germinal epithelium and also spermatocytes [15]. In females, menstrual irregularities, preterm deliveries and still births have been reported (WHO, 1986).

The Netherlands [36], Brazil [3] and Finland [35]. Also, the mean concentrations of lead in sheep liver and kidneys in the present study were lower than those detected in Greece [10]. With respect to the results of urban area and by comparing them with the values of [7] the levels of lead were below this proposed limit (0.5 mg/kg). Moreover the concentrations of lead in kidneys of buffalo (0.456), cattle (0.490) and goat (0.462 mg/kg) were near the maximum value of proposed limit (0.5 mg/kg).

**Tab. 2:** Lead concentration (mg/kg wet weight) in swine organs collected from three areas represents different ecosystems in Probistip.

Organs	Industrial Zone in the town of Probistip	Village Strmosh	Control point
Muscle	0.126	0.356	0.035
Liver	0.954	1.280	0.092
Kidney	1.025	2.457	0.123
Spleen	0.098	0.185	0.012
Heart	0.355	0.894	0.015

The results obtained from our research are in close correlation with the content of lead in the areas where these animals are kept and with lead content in the examined organs.

Table 2 shows the results for lead content in the examined tissues in swine. The results obtained show the distribution of lead in tissues and at measuring points, whereupon here also the highest values were measured in kidneys and they vary from 0.123 at the control measurement point to 2.457 at the locality Strmosh.

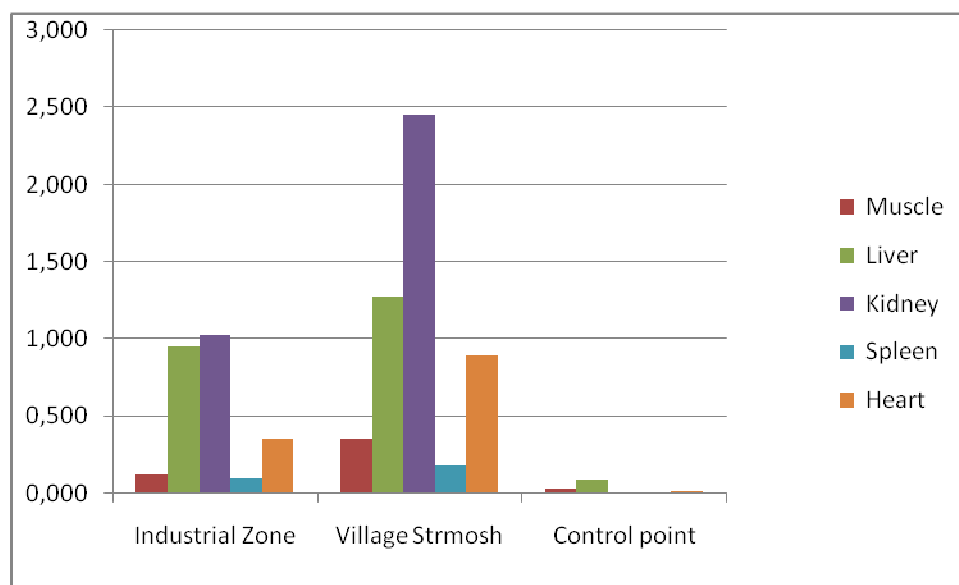


Fig. 2: Lead concentration (mg/kg wet weight) in swine organs

Table 3 gives the results for lead content in the examined tissues in goat. In respect of the measuring sites, all tissues - muscle, liver, kidney, spleen and heart were measured in the area around the village Strmosh and they vary from 0.122 in heart to 1.359 in kidney, and the lowest values were obtained at the control measuring point near which there are no sources of heavy metals pollution. They vary from 0.012 in muscle to 0.984 mg/kg fresh weight in kidney. Kidneys accumulate the highest amounts of lead, and they are not less in the analyzed spleen too.

Table 3: Lead concentration (mg/kg wet in goat organs collected from three areas represent different ecosystems in Probistip

Organs	Industrial Zone in the town of Probistip	Village Strmosh	Control point
Muscle	0.089	1.874	0.012
Liver	0.641	1.127	0.058
Kidney	0.851	1.359	0.984
Spleen	0.221	0.356	0.042
Heart	0.032	0.122	0.091

The concentration of lead at the locality Strmosh is significantly higher than those obtained at the site on which there is no nearby source of pollution with heavy metals

All this is a consequence of the position of the waste landfill in the vicinity of the village Strmosh where waste waters from the flotation of lead-zinc ore from the mine “Zletovo” are accumulated. Another source of atmospheric pollution is the re-suspension of lead dust from the old waste landfill which is also located in the vicinity of this site and which is dispersed by the wind. From the results obtained at the Strmosh site, the content of lead is much higher compared to the lead content in tissue samples collected at other measurement points.

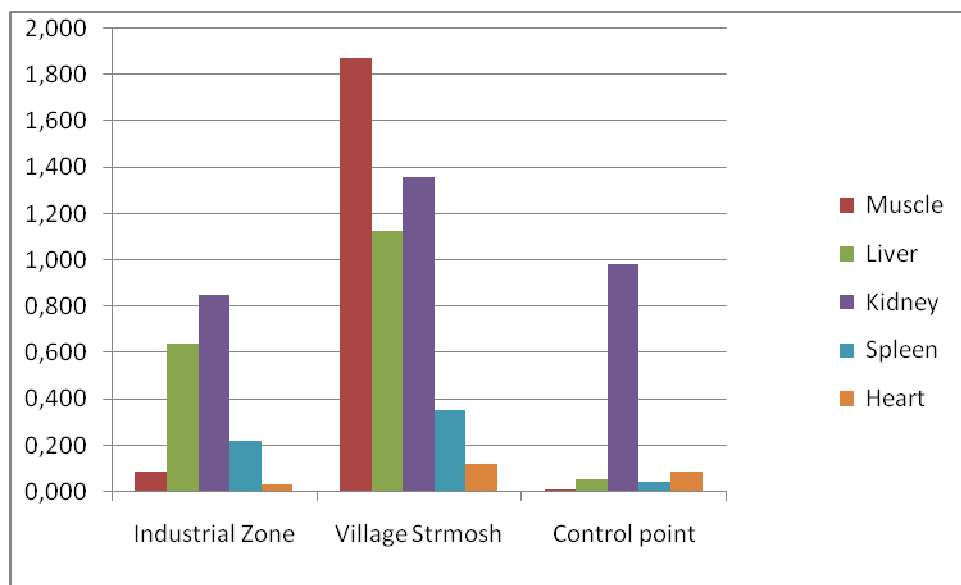


Fig. 3: Lead concentration (mg/kg wet weight) in goat organs

The difference probably result from different animals diets, whereas the animals are exposed to the influence of air pollution for longer periods where accumulate lead. Lead in the industrial area is emitted from different sources (smelters, batteries recycling, combustion of fuel for different industries).

These results show the relation between lead concentration in soil and in meat samples contaminated from water and soil. Levels of lead in the liver and kidney samples in this study were compared to the values of.

## 5. CONCLUSION

Results showed that lead concentrations are dependent on the sampling locality, the organ and animal species. The highest concentrations of lead were detected in kidney of swine taken at the mine waste landfill near the

central town area of Probstip (1.025 and 2.454mg/kg respectively). Chicken kidney taken in the Strmosh area contained the highest concentration of lead (1.125 mg/kg). The concentrations of lead in liver and kidney taken from the industrial area were higher compared to other localities from which samples of tissue were taken for analysis.

At the control point of measuring the lead content in all examined tissues from the organs of chickens is significantly low compared to the industrial zone of Probstip and the vicinity of the village Strmosh.

All the above mentioned facts require greater commitment to remediate the harmful effects of heavy metals on the environment and man.

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# Chemistry Experiments in School and Interactive Whiteboard

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**Abstract:** *The advent of interactive boards in the modern Bulgarian school requires a focused and effective enforcement to achieve the expected results. Because of limited resources to carry out chemical experiments in class, students show relatively low levels of practical skills. That's way some opportunities have been tried to find a way of using the interactive board in relation to the training of students to make learning chemistry experiment. Specific examples of tasks glassware, equipment, substances and processes have been discussed. A gallery of images is created that could be updated and enriched in accordance with specific learning objectives and technologies.*

**Keywords:** *chemistry education, interactive whiteboard, chemical experiment, problem solving.*

## 1. INTRODUCTION

One of the problems in contemporary education in chemistry and environmental protection is the lack of conditions for demonstrating chemical experiments in class. For that reason studies show a very low level of practical skills of students to work with substances and devices [1-4]. A good opportunity to compensate these shortcomings is the use of educational software - ready, prepared or fully prepared by the teacher, which can be used alone or in combination with an actual experimental work. The advent of interactive chalkboards in Bulgarian schools gives opportunities to various options for use in the learning process [5-7]. The interactive whiteboard is a convenient support for specific visualization of chemical processes and related equipment and technologies for the teacher of Chemistry and Environmental protection.

The student's successful future career depends mainly on its preparation at the university. In this respect the discipline "Method and technique of the school experiment" offers good opportunities for optimal combination of skills for a real chemical experiment and the presentation by means of the interactive board. For this purpose appropriate types of tasks are selected to be used in students' training.

## 2. RESULTS AND DISCUSSIONS

### • *Work with glassware:*

The purpose of the first type of task is to check whether students know labwares, their purpose and proper use. "Binding Arrow" is one of the functions of the board, selected from the 'toolbar', then selecting tool "forms." This tool allows painting of sections, one and two arrows, circles, rectangles. Another option is the ability of choosing the shape, color and line thickness. The advantage of the interactive boards that connect the arrow is fast, looks good, easy to check and correct.

It is possible to select different examples of referencing images to appropriate vessels names. It is better to use combinations of objects with similar names or designations, which hamper students and are often mistaken.

Another undeniable advantage of the interactive board is the possibility of using a gallery of ready images (photos, drawings, models, diagrams, etc.), which can be enriched, edited and updated. Selecting images become easy through 'drag' on the desktop as the interactive board allows rotation and image editing.

Example 1. Choose containers from the attached gallery that can't be used to measure the precise amount of liquid. Please explain your answer!

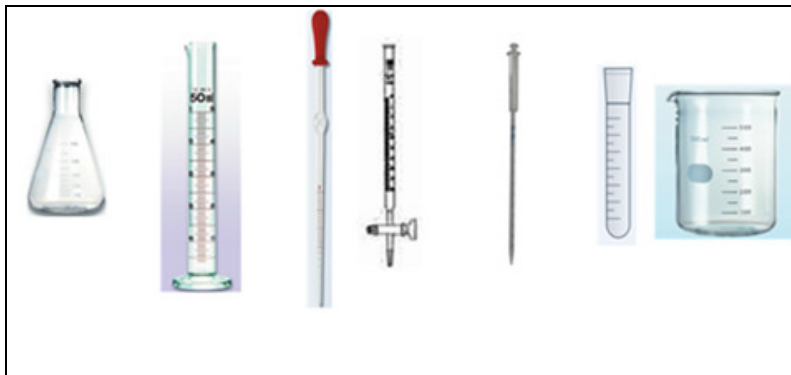


Fig.1. Containers for liquids

### • *Tasks related with equipments for gases:*

It is appropriate to perform a set of devices and the gases respective that could be obtained in them. It is possible to use the marker to mark the corresponding number of gases or equipment. Another option is moving each equipment against the corresponding gas. The advantage of the interactive board for this type of tasks is that the device sets can be varied according to options for receiving different amounts of the same gas.

Example 2. Which of the following devices you should use if you need to get oxygen, with which:

- only to prove that supports combustion;
- to fill at least two Erlenmeyer flasks.

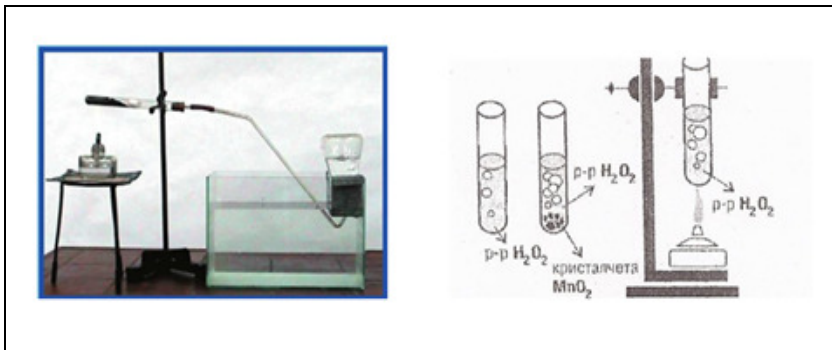


Fig.2. Apparatus for producing oxygen

In this example it can be included the function "Illumination", by which the correct answers are marked by the illumination. The object illumination can be with various shapes and sizes. In this way the attention of the audience is directed to the specific image highlighting the features and the benefits.

Complicated version of this example is the requirement to assemble separate fragments of the equipment using the interactive features on white board.

Example 3. Using the available fragments, assemble equipment appropriate for receiving:

- oxygen from potassium permanganate
- hydrogen from zinc and hydrochloric acid.

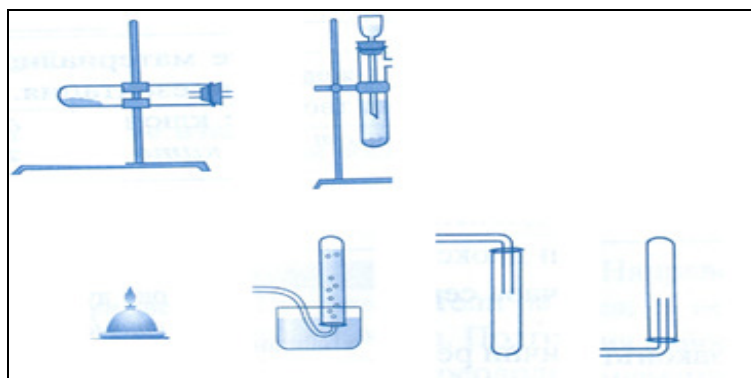


Fig. 3. Fragments of equipments

In this case other features of the interactive board are demonstrated: “moving” of fragments and “rotation”.

Another possibility for these types of tasks is to detect the errors and discrepancy in a given equipment and corrections to be entered on the image using the marker or other optional interactive board.

This allows students to test their skills through the presented models, to give proof of their choice and only then to test the equipment in a real environment.

- **Problems related to substance:**

In this type of tasks the knowledge of physical and chemical properties of substances and the conditions for their safe handling are checked.

Example 4. For each of the agents select the appropriate symbol for safe handling.



Fig.4. Signs for safety work

This task is appropriate to be presented through the interactive board because in a very easy way it can be saved as a file that can be used by other students later. Another advantage is that the file can be corrected by changing the set of agents.

- **Problems related with chemical processes:**

The application of interactive boards in the study of chemical processes is various and intermodular. It is appropriate to use models of reactions and technologies, sequences of different stages and production principles of efficiency and safety. A process can be represented through animations, films or real experiments and based on this information to build a model diagram or graph [6].

Example 5. Which of these models is:

- A) chemical degradation;
- B) chemical bonding;
- C) chemical substitution.

Write chemical equations shown by models processes.

The advantage of the interactive board is that you can specify different models of a pre-established gallery and it does not need be painted and this way - saving time and effort. In this case it is appropriate again to use the marker, writing the number of the corresponding model.

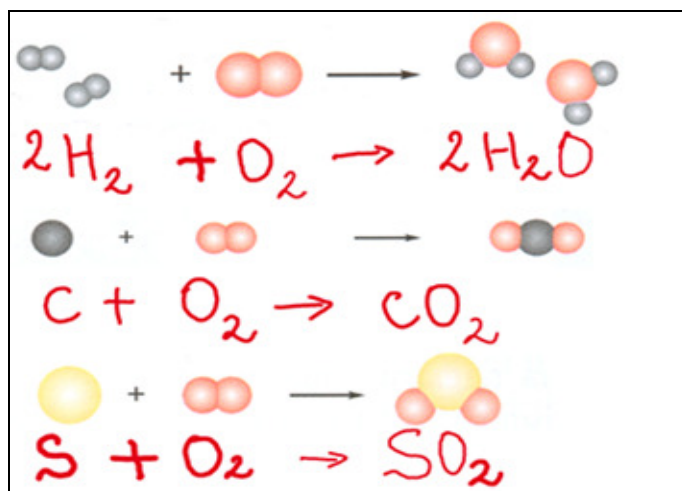


Fig.5. Models of chemical processes

A more sophisticated version of this example relates to the requirement to record the specific chemical equations for processes represented by models. The correct answers can be pre-recorded and covered by the option "wrapper" on the interactive board.

### 3. CONCLUSION

In this article are highlighted different options for optimization of the preparation of future chemistry teachers in school in the area of the educational chemistry experiment through the use of interactive boards for the discipline "Methodology and techniques of school chemistry experiment".

Four types of tasks offering concrete examples are featured and they are suitable for applying in the students' training.

Galleries of images are created: pictures, models, diagrams of equipments and its fragments, graphics, logos, etc.

The proposed options are subject to optimization implementation and enrichment of galleries, diversifying how to implement in learning, as well as adaptation in the school course “Man and Nature” and “Chemistry and the environment”.

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# Practical Application Aspect of Professional Competences of Future Teachers

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**Abstract:** *This article focuses on the formation of professional competences of students from pedagogical specialties. Interpreted in the context of the single European educational model, the professional competences of future teachers are a generalized, integrated expression of traditions and future public expectations. One of the aspects of competence forming – the practical application aspect is analyzed through the prism of the discipline „Ongoing pedagogical practice in biology”. The effect of the pragmatic training as a necessary element in the portfolio of the future teachers is commented on.*

**Keywords:** *ongoing pedagogical practice in biology, standards for training biology teachers, professional competences*

## 1. INTRODUCTION

Being an open and dynamic system the education should "meet" current and future needs as regards to the tasks set by the society. On socio-economical level, the participants in the labour market set the framework of the main "necessities" ensuring the development and prosperity of the nation. Education is an open term and it expresses the participation of society in defining the educational model [3]. The successful association of the Republic of Bulgaria as a member-state of the European Union (EU) is bound to activities in different social fields, incl. the field of education. Forming professional competences of future teachers is in the context of optimizing the educational requirements in accordance with the new realities.

## 2. STATUTORY PROVISION OF PROFESSIONAL COMPETENCES

Forming of professional competences falls within the scope of a number of documents which represent the normative instrumentarium for

management of our educational system. On Feb 02<sup>nd</sup> 2012, the Government of the Republic of Bulgaria adopted the National Qualification Framework (NQF) pursuant to the recommendations of the European Parliament and the Council on the creation of a European Qualification Framework (EQF) for life-long learning. The elaboration of national qualification frameworks in the different EU member-states in an understandable manner correlates their qualification levels to the respective levels of EQF. According to NQF, the acquisition of an education and qualification degree of "Bachelor" implies a certain level of achievement (formation) of knowledge, skills and competencies, understood as parts of one whole and an integrated entirety:

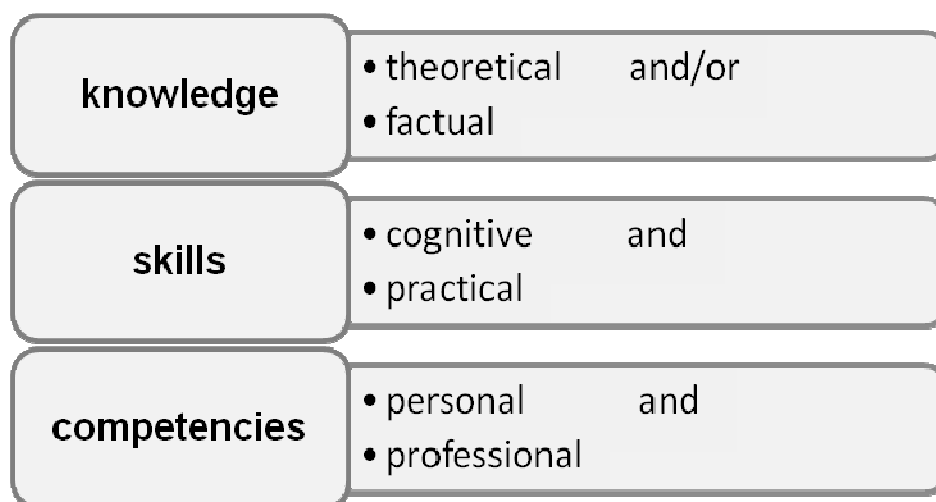


Fig. 1. Basic components defining the qualification degrees under NQF [5].

The professional competences for acquiring level 6 – b „Bachelor” are presented with a view to the level of responsibility assuming and independence of the student:

- collects, classifies, assesses and interprets data from the field for the purpose of problem solving;
- implements the acquired knowledge in new or unfamiliar conditions;
- manifests ability to analyze in broader of interdisciplinary context;
- uses new strategic approaches; forms and expresses own opinion on issues of social and ethical nature that may arise in the course of work [5].

The distinction between knowledge, skills and competences – professional and personal: is conditional to a certain extent. They enter into many direct and mediated relationships which have their qualitative new manifestation. The professional competences by themselves are natural result from training organized and institutionalized in a different aspect. „Training is the interpedagogical (didactical) correlate of education. Its content also includes those organizational and technical characteristics and components that can be contemplated and carried out only through a specialized and professional – pedagogical vision” [3].

One of the main instruments of managing the training of future teachers and a basic element of the model of this training is the standards. The standards include the system of requirements presented above (knowledge, skills and competences), posed to the level of expected training results at the end of the respective educational level. The biology teacher's professional profile is interpreted in the field of professional specialty „Pedagogy of teaching ...”, in the subject of the science „Methodology of teaching ...”. The decomposition of Standards at level training objectives for the discipline „Ongoing pedagogical practice” is presented as follows:

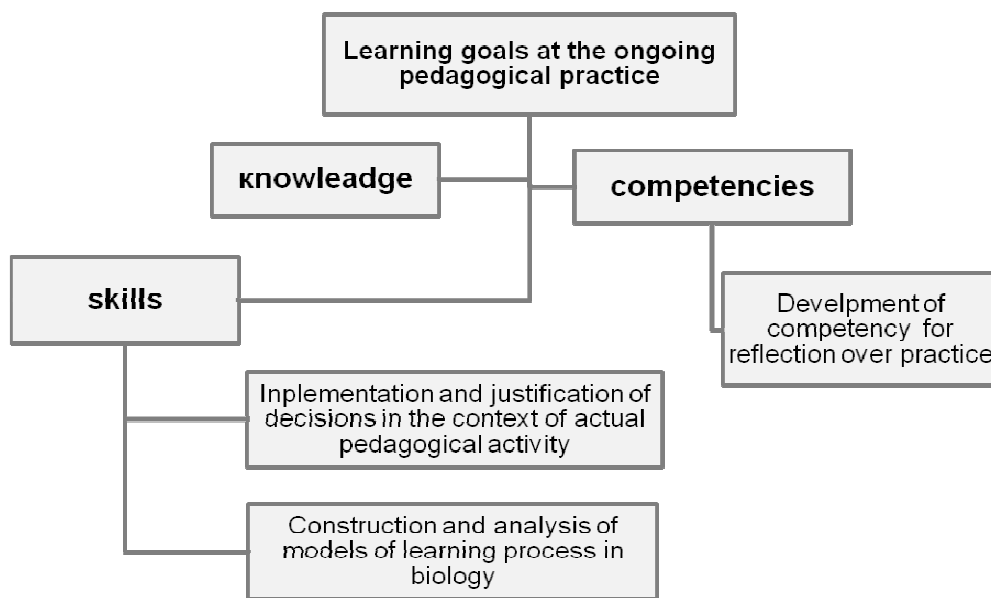


Fig. 2. Decomposition of standards at level “training objectives” in Ongoing Pedagogical Practice [4]

### **3. CURRENT PEDAGOGICAL PRACTICE- SCOPE FOR PROFESSIONAL COMPETENCES FORMATION**

The practical and applied training of students from the pedagogical specialties is reasonably possible after completion of training in the basic special scientific and pedagogical disciplines. The realization of the objectives of training in the discipline Ongoing Pedagogical Practice is done by training students in real educational environment under the guidance of a professor from the higher education establishment. Forming professional competences is understood as a conglomerate, as a system of knowledge and skills, organized and functioning not as a simple sum, but for the purpose of their successful behavior in typical specific situations [3].

Basic skill within the discipline Ongoing pedagogical practice is the creation of models of the learning process. Formation of this skill (with university instructor) presupposes analysis and research of all elements (stages, steps) with view to the successful solutions, incl. effect postponed in time in respect of building personal constructs. Planning is considered to be one of the most complicated human activities, mainly due to the need to operate with abstract information and events that have not occurred yet [1]. The realization and subsequent substantiation of the training models as a result of the pedagogical activity of students are an integral expression of re-thinking, assessment, reflexion. They are self-realized independently in real school environment.

Therefore, Ongoing pedagogical practice has mainly teaching functions in respect of the student, but it is also present through the assessment and self-assessment functions immanently involved. This, in its turn, means that the student – future biology teacher does not simply pragmatize knowledge, skills and competencies – he learns to take responsibility for the decisions taken, to choose i.e. to make self-assessment and to assess others following predefined criteria within a specific topic. A sample model for illustration:

- Self-assessment criteria – interpreted in the sense of:

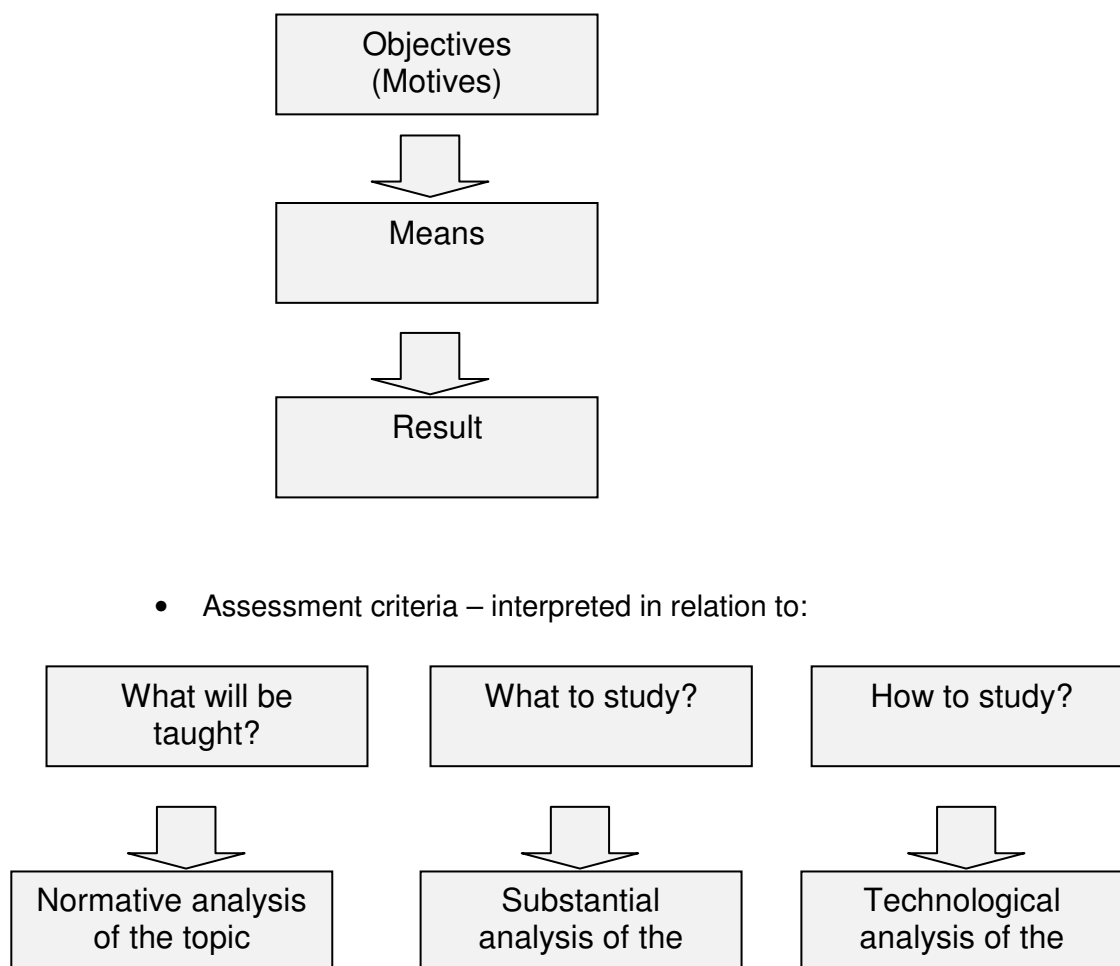


Fig. 3. Criteria for analyzing the activity of students  
in *Current Pedagogical Practice*

The above is a reference to one of the aspects of professional competences of students in their practical and applied training – creating portfolio. Despite its indisputable contribution to the current educational work, the portfolio is designed primarily as an assessment and self-assessment tool. Interpreted as something more than collection of training evidence (curricula, lesson plans, tasks etc), it includes analyses and contemplations, arguments, case studies, summaries and notes on teaching and learning. This gives grounds the authors to accept it as an overall concept for the activity of the future teacher [2].

Elaboration and maintenance of a portfolio by students within the discipline Ongoing Pedagogical Practice presupposes provision of a range of situations that directly and indirectly affect reasoning on their own teaching activity, re-arranging of priorities, re-thinking of teaching strategies, future planning. Within the discipline, the students are placed in conditions of retrospection and analysis in terms of the decisions taken. This is a kind of marker for them and a corrective of their own efforts towards achievement of expected results. Therefore, the need in pragmatizing the preparation of students places a special emphasis in developing and maintaining the portfolio of the future teacher – the formation of professional competences.

#### 4. CONCLUSION

Formation of professional competences should be an expression of the dynamics of contemporary socio-economic development. Personal competences (as an integral part of professional competences) are transformed during the active professional life, with age, with the transitions between the states of unemployment and professional career development [3]. Nowadays, there is a global demand for professionals with analytical thinking and preparedness for continuous development and self-teaching. Professional competences of future teachers must help their successful adaptation to the conditions of a competitive European environment – a goal that is addressed to all entities directly or indirectly committed to the idea of the quality of Bulgarian education at school.

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# HARMFUL FOOD ADDITIVES – A HANDBOOK FOR THE USER

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**Abstract:** *Nowadays, food additives are an essential part of the majority of foods. They improve their appearance, improve and preserve the taste and the nutritional qualities. Researches of their biological activity showed that some of them are harmful to human health. The goal of this paper is to present the available data concerning the content, the structure and the side effects of these additives. This information can be used both in extracurricular lessons in chemistry and environment and biology and health education, as well as in the user's handbook.*

**Keywords:** *food additive, bad for the health, structure, chemistry education,*

## 1. INTRODUCTION

The development of food industry and competition between manufacturers has led to more and more use of different food additives. Initially, this process occurs spontaneously and without sufficient control. As we study the influence of various substances on human health, it turned out that their use is not always safe for human health. A reverse process of limiting additives and return to natural products and raw materials has started. We are witnessing the restriction and even prohibition of some food additives due to the harmful effects on human health. The attention that is paid in biology classes and health education and chemistry and environmental on these issues is not enough, with the result that the majority of users do not pay enough attention. It would be possible to introduce a course that introduces harmful food additives, where they are represented by a purely chemical nature and their parallel education process to orient the younger generation in this important health area.

## 2. RESULTS AND DISCUSSIONS

### 2.1. *Characterization and classification of food additives*

The widest practical definition of a food additive is any substance that becomes part of a food or product. The main objectives of these substances in food are:

1. To ensure the adoption and implementation of necessary substances to the body, such as proteins, carbohydrates, fats, vitamins and minerals. Some food additives are substances possessing such value.
2. To maintain the quality and freshness of products as their nutritional value rapidly decreases and deteriorates during storage. Processes of composition change begin caused by microorganisms, bacteria and yeast, and oxidation by air.
3. To facilitate the handling of the products and to maintain the formation of permanent emulsions and homogeneous mixtures. Preserving texture by mixing several types of food is not possible without such additives.

Each additive is a chemical that in food industry is marked by a kind of code or introduction of a standard short numbering of these substances. In the Euro zone at the beginning of this code is put the so-called "E" number , prefix of Europe (tab.1)

Tab.1 Numbered food additives in categories.

E100-E199	Colours
E200-E299	Preservatives
E300-E399	Antioxidant and acidity regulators
E400-E499	Thickeners, stabilisers, emulsifiers
E500-E599	pH Regulators and anti-caking agents
E600-E699	Flavour enhancers
E900-E999	Miscellaneous
E1100-E1599	Additional chemicals



## 2.2 Method of work

Substances that have received attention are modeled by 3D visualization program HyperChem (Figure 1), which achieved the optimal performance compared to competing programs.

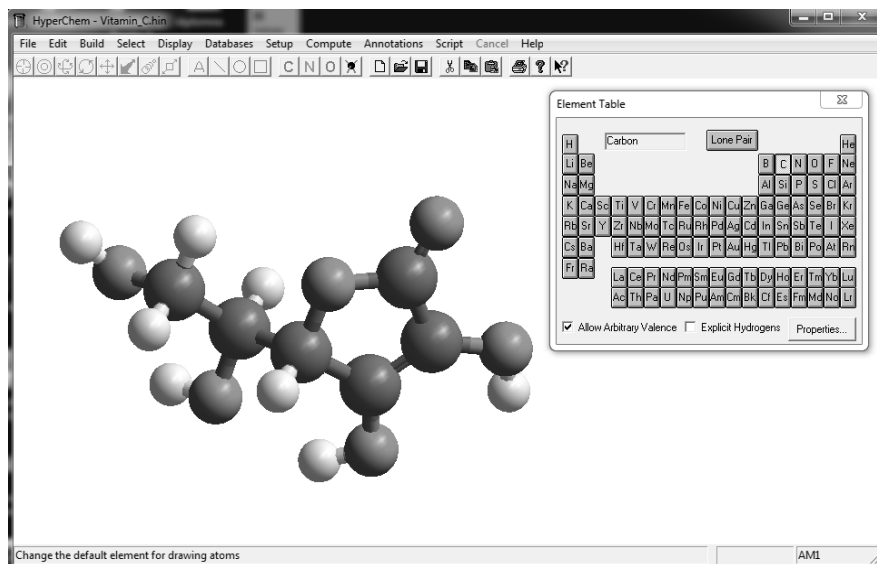


Fig. 1. Molecular model of Vitamin C, E300

The program is improved in the computation by quantum-chemical methods (Figure 2), molecular mechanics and dynamics, which are parameterized so that they make up closer to the actual geometry of the compound.

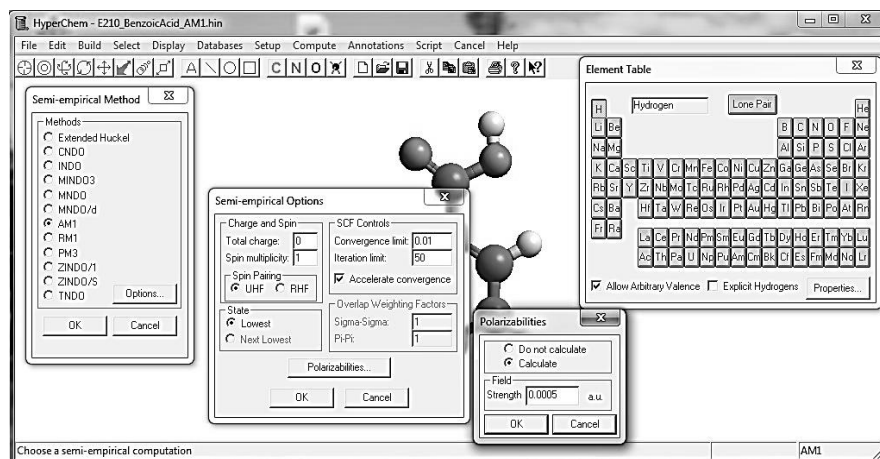


Fig. 2. Calculation chemically AM1 method of Benzoic Acid, E210.

### 2.3 • Place in Chemistry Education and Environmental

There are several applications in the experimental part, which would be essential in the training:

1. Students are given the opportunity to learn about some harmful substances used in food.
2. There is an intersecting point with the expected results at the level of curriculum that provide additional revision and pursuit of knowledge.
3. Looking at food additives through their true nature - as chemicals - it is possible to create or increase interest in chemistry as a science.

All 43 chemicals are presented by experimental pages (Figure 3), which consists of the common name of the substance (1) summary (2), table including name according IUPAC, code number, chemical formula, molecular mass, temperature melting and water solubility (3), shortened structural formula (4) full spatial structural formula (5), possible biological activity (6).

**Брилянт Черно БН**

Синтетично вещество, черно на цвят, диазо боя, получено е от катран. Представлява тетранатриева сол, добре разтворима във вода. В обикновени условия е в твърдо състояние на прах.

Наименование по IUPAC	
Tetrasodium (6Z)-4-acetamido-5-oxo-6-[[[7-sulfonato-4-(4-sulfonatophenyl)azo-1-naphthyl]hydrazono]naphthalene-1,7-disulfonate	
Кодов номер	E151
Тривиално наименование	Brilliant Black BN
Формула	$C_{24}H_{12}N_4Na_4O_{14}S_4$
Молярна маса	887.68 g/mol
Температура на топене t°	-
Разтворимост във вода при о.у.	-

Възможно е да причини алергични реакции или непоносимост, особено при хора с нетърпимост към аспирин. Провокира деца с хиперактивност. Забранен в САЩ, Белгия, Дания, Франция, Германия, Швеция, Австрия, Швейцария, Япония, Финландия и Норвегия.

Fig. 3. Experimental page E151, Brilliant Black BN.

## 2.4 • Handbook for the user

Unfortunately, very few countries have taken measures to regulate harmful food additives. People alone, through their own studies should inform themselves about the safety or potential risks that some of additives are hiding.

Tab.2 The substances with their code numbers in categories.

COLOURS	E102 E104 E107 E110 E122 E123 E124 E127 E128 E129 E131 E132 E133 E142 E151 E155 E160b
PRESERVATIVES	E210 E211 E212 E213 E214 E220 E221 E222 E223 E240 E249 E250 E251 E252
ANTIOXIDANTS AND ACIDITY REGULATORS	E320 E321
THICKENERS, STABILISERS, EMULSIFIERS	E407 E450 E495
pH REGULATORS & ANTI- CAKING AGENTS	E513
FLAVOUR ENHANCERS	E621 E635
MISCELLANEOUS	E950 E951 E952 E954

By the harmful food additives that are examined (Tab.2), a user's handbook is being developed, which will be offered to be printed or posted on the website of SWU "Neofit Rilski". It will be used in the work of students in Pedagogy of chemistry and physics.

### 3. CONCLUSION

The developed material allows students to learn or reinforce some of the expected results of mandatory training in Chemistry and Environmental, in a different, more pleasant way in a variety of sample programs for elective subjects of education.

The relationship between food additives and chemistry as a science, will facilitate increased interest in subjects related to science, but also will help to build healthy habits and critical attitude to the additives that are offered.

The topic of food additives was discussed during the development of new curricula for specialized training and was unanimously approved. It will take part in recommended elective courses.

### 4. ACKNOWLEDGMENTS

The molecular models were created using program package **HyperChem 8.0 (Student edition)** in the undergraduate course in "Molecular modeling" featuring by Dr. Zhivko Velkov.

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# INTERACTIVE LEARNING IN PROGRAMMED TEACHING OF THE SUBJECT “BASED OF NATURE SCIENCE” AT FACULTY OF EDUCATIONAL SCIENCE– R MACEDONIA

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**Abstract:** *The basic aim of interactive learning in programmed teaching is the transmission of activities from the teacher to the students, management of students’ learning, enabling students to learn together and evaluate the learning processes.*

*The paper emphasizes the need for teachers’ active participation in the creation of programmed materials for the respective subject matter of “Elements of nature science”, i.e. using pedagogical workshops in the organization of the teaching process with the application of interactive methods. Beside this theoretical approach to programmed teaching and interactive learning, we have also done a methodical shaping of the teaching unit “Photosynthesis” according to the model of interactive learning in programmed teaching and use ICT tools.*

**Key words:** ICT tools, programmed teaching, interactive learning, educative workshop, based of nature science, photosynthesis.

## 1. THEORETICAL CONTEXT OF THE PROBLEM

Teaching is an interactive process through which learning and teaching are accomplished.<sup>1</sup> This means that during the teaching process situations are created in which the student as an individual comes into direct contact and relationships, perceives classmates’ and teacher’s activities and responds to them. If we want to discuss about the quality of interaction in the classroom, we should always bear in mind that it implies some kind of exchange and it is multifaceted in terms of the participating entities, but also of the material that is taught (teacher-student, student-student, student-

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<sup>1</sup> Although teaching is essentially an interactive process, hereinafter the expression - interactive teaching will be used to highlight teaching dominated by learning and teaching through collaboration.

group of students, students-content, objects, and processes). Long ago Flanders (1963) promoted the teaching approach which he called "jug and cups" where the teacher is the jug containing all necessary knowledge to be poured into "glasses" (students), following a specific curriculum. Flander's interactive style means that the teacher speaks, i.e. teaches during two-thirds of the lesson. And everything would be all right if knowledge was enough to live a full and happy life. The time we live in requires training of a person who will be able to live in the present, provide and shape the future, and thus develop oneself and others in the spirit of humanism and democracy. So, today, teaching quality towards which science and practice aspire means interactive teaching which involves providing conditions for the transfer and development of knowledge, skills, abilities and attitudes towards the achievement of the previously indicated goal. In this context it must not be understood that teaching interaction should be reduced to routine application of interactive methodical procedures through which students will be shaped according to some pre-set templates or just to guidance without any preset goal.

Many countries, in their quest for the establishment of standards in education, attest to the importance of the aid to be given to children when they acquire not only knowledge but also skills, understanding and attitudes needed to apply knowledge in different situations. These efforts are usually directed at the request of a balance between several dominant styles of teaching interaction (teacher-student, student-student) in which the teacher is in the role of a "breeder", "sculptor" or "guide".

Interactive teaching is not only closely linked with only one role of a teacher or one approach to teaching, but it implies a balance between roles, styles and approaches, appropriate learning goals of students, their prior knowledge, abilities and skills, motivation and interests. In the most general sense, interactive teaching should provide the student's active role in the learning process through the establishment of mutual relations, which gives a social mark to teaching.<sup>2</sup> "Interactivity involves polyvalent guiding of the teaching process that is influenced by the perceived situations (reactions) of students - from rapid progression to apprehended impasse, from additional to supplemental activities, from deepening of the problem and original solutions to conducted problem solving (direct instruction)" [1].

Our long-standing teaching practice experience in working with students and mentoring students during their pedagogical practice has inspired us to find out whether and how can interactive teaching be successfully designed and implemented through programmed learning

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<sup>2</sup>In the broadest sense, social interaction is defined as a relationship of two or more persons whose perceptions and behaviors are bidirectionally or multidirectionally conditioned. (Klaic S. 1989: 282).

material, which is the essence of programmed instruction, recognizing the principles of cooperative learning and the principles of programmed instruction.

## **2. COOPERATIVE LEARNING WITH PROGRAMMED MATERIAL**

Two theoretical perspectives give significant support to cooperative learning: the theory of motivation<sup>3</sup> and the cognitive theory<sup>4</sup>.

From the motivational perspective setting and achieving group goals is a situation in which the achievement of personal goals is closely linked to the success of the group [8]. In fact, the theory of motivation treats rewards, penalties and purposes as essential tools for success. One of the basic principles of cooperative learning is positive interdependence which entails understanding and students' feeling that their success or failure is determined by their work within the group. Thus, in order to achieve personal goals, students are further motivated to help their classmates in completing the joint result.

According to Damon (1984) [2], in the frames of the cognitive theory, the interaction between students aimed at solving appropriate tasks develops their critical concept. When students discuss and express their personal perspectives and views on the given problems, there is a higher level of understanding of the material that is taught, and the struggle to resolve the potential conflict over cooperation results in a higher level of understanding [8]. Johnson, Johnson and Holubec (1989), [4] have shown that cooperative learning provides a greater contribution to the development of students' elaborative thinking. They more often give and accept explanations leading to a deeper understanding of higher levels of thinking and durability of knowledge

Multiple educational benefits of cooperative learning have been confirmed in numerous studies [5], [4], [7], [9].

- Achievements / results - higher individual success, more intrinsic motivation, positive attitudes towards education, assessors and other staff, positive attitudes towards individual subjects.
- Critical thinking - increased frequency of higher levels of thinking, deeper understanding, endurance, increased flexibility in solving problems, understanding of concepts.

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<sup>3</sup>The theory of motivation emphasizes the student's incentive for learning.

<sup>4</sup>Cognitive theories emphasize the effect of cooperative work.

- Improvement of the cross-cultural relationships - greater stability for analyzing situations from another perspective; relations based on support and acceptance of their peers belonging to other ethnicity, religion and gender, as well as of those socially depressed; the ability to create a learning environment; and a higher level of trust and cohesiveness.
- Personal benefits - greater social support, psychological health, adjustment and well-being, increased self-esteem based on acceptance of self, greater social competences.

Interactive teaching is a form through which effective and efficient acquisition of knowledge and developing of skills can be ensured. It can be organized using a variety of approaches, methods, techniques, and tools. When it comes to interactive teaching through the use of programmed material, some theoretical determinations of programmed instruction certainly need to be clarified.

Although the early forms with elements of programmed instruction can be found in the pedagogical views of Socrates, and its psychological foundations are placed in "Theories of effects" most teachers link the occurrence of programmed instruction to the construction of the first learning machines. Skinner's linear model (B. F. Skinner) and Crowder's branching model (N. A. Crowder) of programmed programmes. It is generally accepted that the theoretical basis of programmed instruction is located in four psychological theories: theory of reliance, theory of gradual formation of mental work, theory of algorithms and cybernetic theory.

While teachers in the Republic of Macedonia often say that the use of programmed materials in teaching is useful in a number of ways (developing independence, cost in terms of time, learning the procedures for solving problems, differentiation and individualization in teaching), yet in practice it is very rarely present. The most common reasons for this situation are the lack of ready programmed learning materials, and teachers reluctantly undertake this obligation even though it is not such a complex procedure from the aspect of teaching methods. The process of development of programmed material intended for the realization of certain objectives of the curriculum involves several stages: 1) the content is boiled down to what is most important (according to the purpose of the teaching lesson); 2) it is structured into "minor" logical sections<sup>5</sup> arranged according to complexity; 3) giving assignments after each sequence / unit; 4) space to

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<sup>5</sup>Units which contain the "information" on the basis of which tasks that the student needs to address / find / execute are set. Students work mostly independently, according to their own pace and they gradually discover solutions.



perform the task; 5) feedback and guidance for further action (moving to the next step / sequence or, if the solution is inappropriate, reversing). The correct answer is actually the support for further work, and an incorrect response suggests repeated and closer study of the same or previous units.

According to the manner of sequencing the units, programmed material may be linear, branched, and algorithmic.

In the linear (Skinner's) programme units are arranged in a sequence, one after another. Student processes the units in a certain order and solves tasks. The correct answer allows the passage to the next unit. But if the answer is incorrect, the student re-reads the same unit and answers the question again.

In the branching (Crowder's) system a student passes from one unit to another only if he/she chooses the correct answer out of the several ones offered for the question in the previous unit. Otherwise he/she is referred to the unit in which he/she can gain additional explanation so as to properly perform the task or answer the question.

The algorithmic (Landin's) programme provides guiding the student towards the goal with precise instructions (algorithms).

### **3. INTERACTIVE WORKSHOP WITH THE AID OF PROGRAMMED LEARNING MATERIAL - STUDY PROGRAMME: FACULTY OF EDUCATIONAL SCIENCES – ELEMENTARY SCHOOL TEACHING)**

In the academic year 2011/2012, during the course of the teaching subject Fundamentals of natural sciences - the thematic unit *Physiology of plants* was processed using programmed material with mandatory inclusion of cooperative learning: small groups (3 students) and working in pairs (2X2). 3 workshops lasting two hours respectively were held.

Educational workshops are one of the efficient ways of organizing interactive teaching using programmed material from the subject Fundamentals of natural sciences.

The performance of these workshops progressed through several stages:

- Instruction for work (frontal)
- Example for making a task (teacher – frontal)
- Independent work of students (individual, then in pairs or in groups)
- Feedback (correct answers)
- Additional interesting tasks (for advanced students)
- Guideline for further work (frontal).

Under strict set of planning the workshops (model) and the development of programmed material, they were realized by one author and the other was in the role of an observer. Observation was of a systematic character in order to detect: the degree of interaction between individual students and the material being taught, the extent and benefits of group cooperation and pair work, the quality of the performed tasks, repetition / recalling of the previous sequence (mistakes).

We expected the programmed material to cause high intellectual engagement to each student individually, and that discussions and cooperation prior to discover in real solutions to tasks, problems, and questions would encourage them in their efforts to go for her through the content.

During the procedure of preparing programmed sequences we strictly kept to tried methodical approaches: Determining sequence targets; Determining specific sequence contents; Establishing logical connections and important concepts in the curriculum content and its distribution in the units; Experimental verification of sequences, their correction or improvement.

We designed the micro-articulation by determining the following: *Introductory* units contain contents students must already be familiar with; Units for *learning* that contain new content that students should learn; *Criterion* or final units given at the end, after learning the programmed material on basis of which the teacher concludes on the efficacy of the prepared program.

In our country a programmed textbook does not exist still, but teachers themselves can perform programming of certain program contents and bring elements of interaction into their realization, i.e. pedagogical workshops, and thus rationalize their work, modernize and give their contribution to modern schools of the future. For this purpose, the following model of an educative workshop was used with certain activities.

**Teaching subject: Basics of natural sciences**

**Teaching theme: Physiology of plants**

**Teaching unit: Photosynthesis**

**The aim of the educational workshop:** independent acquisition of knowledge about the processes of cell division.

**Workshop tasks:**

**a) Educational:** To empower students to define the terms cell, cell types, and cell organelles with special emphasis on chloroplasts.

**b) Pedagogical:** To encourage students interest for working in steps (units), to develop a sense and ability to independently solve problems and apply the gained knowledge in everyday life.

**c) Functional:** To develop the ability for observation and logical deduction, creating work related habits in students for independent work.

**Teaching methods:** Interactive learning with programmed teaching, verbal-textual method, method of students' independent work

**Teaching forms:** frontal, individual, pair work.

**Teaching aids:** computer, LCD-projector, instructional sheets (programmed), instructional feedback sheets, instructional sheets with additional interesting tasks.

**Workshop structure (steps in the course of work):**

1. Joint introductory activities (instruction for work) - 7 minutes.
2. An example of making a task – 4 minutes.
3. Independent work of students (individual, then in pairs) – 25 minutes.
4. Feedback – 5 minutes.
5. Instruction for further work – 4 minutes.

**Course of the lesson:**

**First step:** *Joint introductory activities*

Students are given the instruction for working with and using the programmed material.

Study independently the written material you have received! Using it you will get familiar with the terms plant cell, chloroplasts, and photosynthesis. The content is divided into smaller parts that we call units (steps) or simple tasks. Each unit or task contains a part of the knowledge that needs to be learnt. Each unit (task) has:

1. Information based on which the task should be solved;
2. Task;
3. Place for writing down the solution to the task;
4. Feedback;
5. Instruction for further work.

Work according to the sequence, task after task. Start working by reading the information. After that answers the set tasks. With your friend compare the responses or decisions, then check the correct answers with the help of feedback (correct answers are at the end of material, but do not look them up ahead).

If your answer is correct, go to the next task. If your answer is incorrect or incomplete, return to the information and once again read it carefully, and then answer not erasing the previous answer. Use the textbook during work.

**Second step:** *An example of a done task.*

Joint work of the teacher and students to solve the next task.

The cell is a basic, structural and functional unit of all living beings. Chloroplasts are organelles that are found only in plant cells. The process of photosynthesis is done in them.

**Third step:** *Students' independent work*

Teaching unit: "PHOTOSYNTHESIS"

Brainstorming

Onscreen questions:

- What is photosynthesis?
- What does a plant need for performing photosynthesis?

Material needed:

- "Basics of natural sciences", Internal lecture notes
- Computer with Internet
- Sheet of paper and a pen

#### 4. CONCLUSION

Beside traditional teaching methods, modern teaching of natural sciences especially emphasizes programmed teaching that enables activity of all students in all stages of the educational process, and in that way it allows their self-education and self-control.

Programmed teaching as a model of flexible differentiation implies the acceptance of individual ability and pace of work of each student. In the methodological sense it means the programming of teaching contents and the manner of their processing. In it, the contents are reduced to what is relevant, logically structured into smaller parts which are subordinated by their complexity and which each student independently and gradually learns, he/she controls the results and observes his/her progress with permanent feedback information.

From the methodical point of view, the essence of programmed instruction is that learning content is distributed into units and they provide "information" resulting in the tasks the students solve. Immediately after solving they receive information whether the result is correct or not. The correct answer is the foundation for further work and incorrect responses suggest a closer study of the same or previous units.

Success in programmed instruction is influenced by the quality of the programmed material organized in sequences. A programmed sequence is a part of the programme for a didactically shaped member. A sequence can have more or fewer units.

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