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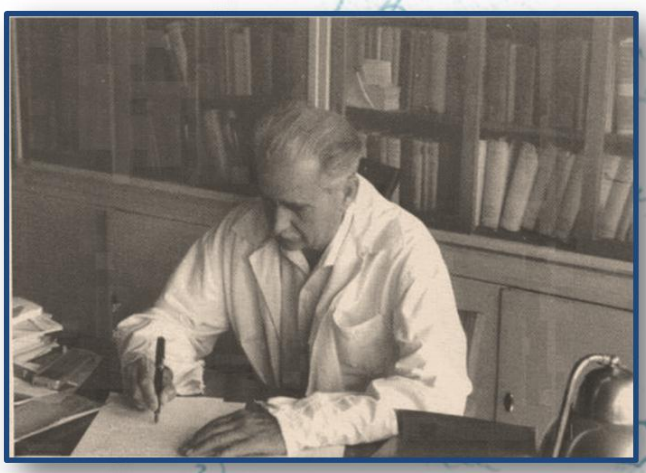

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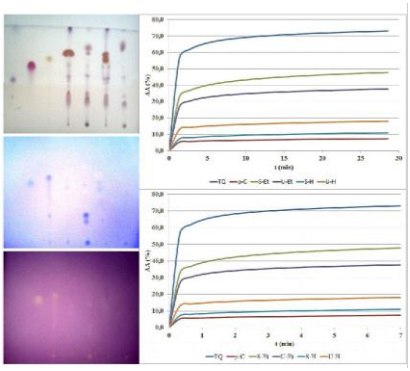
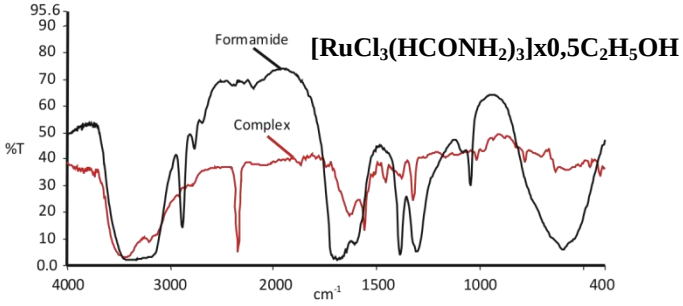
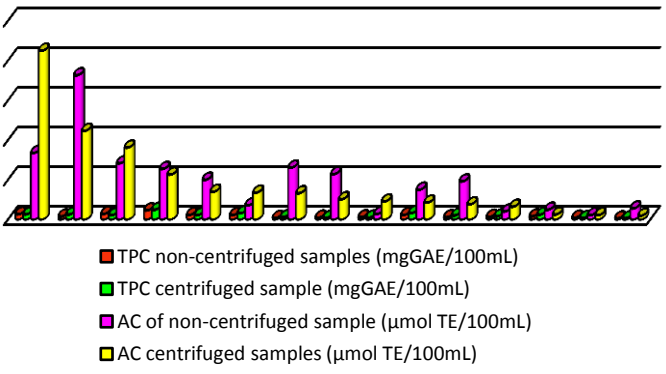
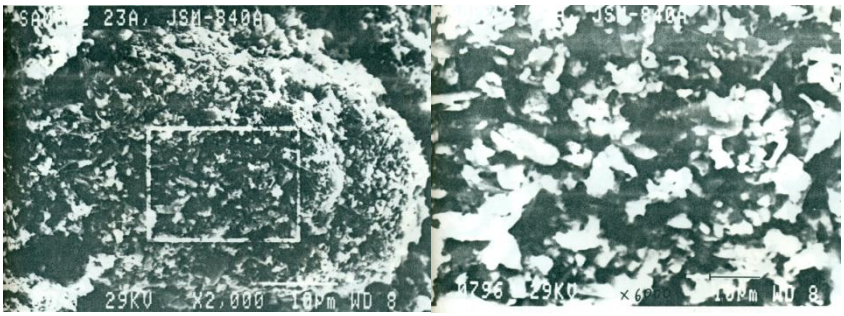
CONTENT / SADRŽAJ

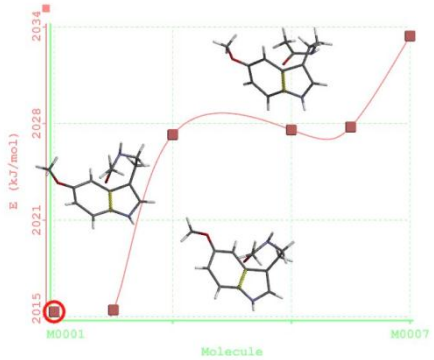
C(UAc) (g/ml) in the soil	A (Bq/kg) U-238	A (Bq/kg) U-235	A (Bq/kg) K-40
0.00	1.20	< 29	1819.98
0.03	20.10	< 22	1822.00
1.00	80.60 +/- 54.3	8.24 +/- 5.51	1881.35
2.00	152.58+/- 58.05	13.87 +/-11.40	1665.02



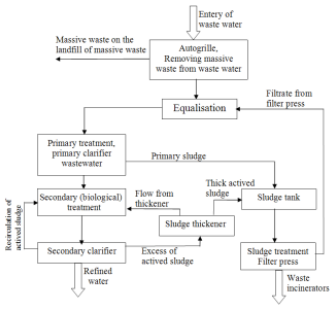








TECHNICAL PAPERS



Ovaj broj časopisa je posvećen pokretaču hemije u Bosni i Hercegovini, akademiku Mladenu Deželiću.

U mnogo čemu je akademik Deželić bio začetnik: osnivanje Katedre za hemiju na Filozofskom fakultetu, matičar Prirodno-matematičkog fakulteta u Sarajevu, osnivač Društva hemičara i tehnologa BiH, prvi Glavni urednik časopisa Glasnik hemičara i tehnologa BiH, jedan od osnivača Akademije nauka i umjetnosti BiH, i mnogo toga.

To je sve počelo još 1949. godine na poziv vlasti Bosne i Hercegovine da dođe u Sarajevo i utemelji univerzitetsku nastavu iz hemije. Njegovom inicijativom i zalaganjem projektiran je i izgrađen Institut za hemiju i fiziku u Sarajevu, a nakon osnivanja Prirodno-matematičkog fakulteta postaje voditeljem Odsjeka za hemiju i direktorom Hemijskog instituta.

Za Mladena Deželića se može kazati da je otac hemijske znanosti u BiH.

U svojem naučnom radu bavio se organskom sintezom i istraživanjem prirodnih spojeva. Istraživao je hemiju pirola, porfirina i hemina, proučavao nikotin i njegove derivate, kumarinske derivate i njihovo antikoagulacijsko djelovanje, te glikozidne spojeve. Bavio se i problemom stabilizacije vitamina C u otopini. U području fizičke hemije bavio se termohemijom, polarografijom i spektrofotometrijom. Rad na proučavanju ravnoteže smjese teške vode D_2O i obične vode H_2O dao je rezultate koji se i danas navode u priručnicima s tablicama fizikalnih i hemijskih podataka o teškoj vodi.

Bibliografija naučnih radova dr. Mladena Deželića obuhvata veliki broj naučnih radova u časopisima i naučnim zbornicima, knjiga, radova iz historije nauke i kulture, patenata, stručnih i enciklopedijskih članka.

Na Prirodno-matematičkom fakultetu u Sarajevu je nedavno organiziran „Dan akademika Mladena Deželića“ na kojem je prezentiran njegov život i rad.

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1-6

One-Shot Chemiluminescence Biosensor for Determination of Glucose in Soft Drinks

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Abstract: The preparation of a new biosensor for glucose was based on the fact that glucose can be determined by its enzymatic oxidation to gluconic acid with simultaneous formation of hydrogen peroxide in the presence of glucose oxidase. Hydrogen peroxide formed in previous reaction further reacts with luminol in presence of cobalt as catalyst producing chemiluminescence signal. This biosensor was made of three layers. The first layer contained luminol, sodium phosphate, sodium lauryl sulphate as a surfactant and a polymer, hydroxyethyl cellulose as a carrier applied to the support. The second was an aqueous solution of Co^{2+} as a catalyst, and the third layer was an aqueous solution of glucose oxidase. After applying the sample solution (glucose) by micropipette onto the sensor, glucose reacted with glucose oxidase and hydrogen peroxide was formed. Hydrogen peroxide diffused towards the polymeric layer containing luminol and produced chemiluminescence reaction. The detection limit for the new glucose biosensor (3σ) was found to be 19 mg L^{-1} glucose (σ from 5 determinations of 30 mg L^{-1}). A relative standard deviation of 7.6 % was recorded for 10 measurements of 50 mg L^{-1} standard glucose solution, and 6.8 % for 10 measurements of 500 mg L^{-1} standard glucose solutions. The glucose biosensor was used for the determination of glucose in soft drinks (mainly apple juices). The results obtained with the chemiluminescence sensors and commercial glucometer (as the reference method) are in good agreement. The corresponding recovery rates were between 93 and 105 %.

INTRODUCTION

Professor Clark has been known as the father of "Biosensor concept" since he published his definitive paper on the oxygen electrode in 1956 (Clark, 1956). Later, Clark and Lyons coined the term enzyme electrode, which was followed by Updike and Hicks (1967) when they experimentally detailed the fabrication of a functional enzyme electrode for glucose (Clark and Lyons, 1967). This enzyme biosensor was based on detecting the decrease of oxygen, which was the co-substrate for the conversion of glucose by the enzyme glucose oxidase (GOX). Later, the oxidation of glucose was also followed by the increase of hydrogen peroxide.

Of all biosensors, the glucose biosensor has been studied most. In 1994 almost 2000 articles describing glucose electrodes and glucose sensors have been published.

The first commercial biosensor for estimation of glucose was launched by Yellow Springs Instrument Company (Ohio) in 1975. This was based on the amperometric detection of H_2O_2.

The glucose analysers commercialized by Medi Sense (Abbott Laboratories, USA) in 1987 and Boehringer Mannheim and Bayer are also mediated biosensor. National Physical Laboratory, India has patented a technology based on mediated electron transfer for glucose estimation (Asha and Malhotra, 2002).

A lot of biosensors for the determination of glucose have been developing in last years for different purposes. Some of them are: Pan and coworkers prepared amperometric glucose biosensor based on immobilization of glucose oxidase in electropolymerized *o*-aminophenol film at copper-modified gold electrode. This biosensor has detection limit of 0.01 mM, high sensitivity (12.6 mA $M^{-1}cm^{-2}$) due to existence of Cu nanoparticles. Also, it exhibits good selectivity, large response current, fast amperometric response, good reproducibility and excellent stability (Pan *et al.*, 2005).

Benamin P. Corgier with coworkers developed screen-printed electrode microarray for electrochemiluminescent (CL) measurement of glucose and lactate. A microarray of nine screen-printed graphite electrodes was used to develop multi-parametric electroluminescent biochip. The whole biochip is based on the ECL detection of enzymatically generated H_2O_2. The intrinsic performances of this electrode array were evaluated through the cyclic-voltammetric experiments in 0.1M ferricyanide. Detection limit for glucose was 10 μM (Corgier, Marquette and Blum, 2005).

An interference-free implantable glucose microbiosensor based on use of a polymeric analyte-regulating membrane was developed by Xie *et al*. Two polymers, poly (4-vinyl pyridine), PVP and poly 4-vinyl pyridine-co-acrylic acid, PVP-PAC were investigated. The biosensor with PVP-PAC showed excellent selectivity to glucose against interferents like oxygen and ascorbic acid. The dynamic range is from 0-30 mM. The response time in amperometric measurement was less than 10 sec (Xie, Tan and Gao, 2005).

Biosensor for the determination of glucose in fruit juice by flow-injection analysis was developed by Guémas (Guémas, Boujtita and El Murr, 2000).

A glucose oxidase amperometric electrode was developed by Bacon and Hall. A "sandwich" bielectrode system is described for a glucose oxidase amperometric electrode that uses outer scavenger electrode to remove ascorbic acid interference from the measurement of enzyme generated hydrogen peroxide. In this work, it is shown that the scavenger electrode is able to remove about 80% of the ascorbate. This system is tested with samples of lemon juice (Bacon and Hall, 1999).

New biosensor for the determination glucose based on immobilized glucose oxidase based on homogeneous chemiluminescence detection by flow-injection system was developed. This biosensor was applied to fruit juices and biological fluid, human urine. The dynamic working range from 2.5×10^{-6} to 1.9×10^{-3} mol L^{-1} was obtained. The detection limit was 8.6×10^{-7} mol L^{-1} (at the 3σ) glucose for the Co(II)-luminol system (Manera *et al*., 2004).

Glucose biosensor for the quantitative detection of glucose in the physiological range (0-450 mg/dL, 0-25 mM) with 3-days stability based on surface-enhanced Raman scattering (SERS) was developed (Yonzon *et al*., 2004).

The aim of this work was to develop a one-shot chemiluminescence biosensor for determination of glucose based on coupling of two reactions: enzymatic oxidation of glucose and chemiluminescence reaction of luminol with hydrogen peroxide in presence of cobalt ions as catalyst. Chemiluminescence reagens: luminol, sodium phosphate and sodium lauryl sulphate are incorporated into polymeric matrix of hydroxyethyl cellulose (HEC). Second layer of the biosensor contained cobalt salt as a catalyst while third layer contained glucose oxidase. Microplate luminometer and a home-made luminometer were used for optimization of experimental parameters and construction of a calibration curve. The biosensor was used in the determination of glucose in soft drinks and obtained results were compared with commercial glucometer.

EXPERIMENTAL

Instrument

Measurements were performed with the *home made luminometer* (Moderegger, 2003) and with a *microtiterplate luminometer* Lucy1. *A commercial* Glucometer GlucoMen (GlucoMen, GlycÓ, A. Menarini Industrie, Farmaceutische Riunite S.E.L. Diagnostic Division, Firenze Italy) was used for the determination of glucose in the samples as a reference method.

Reagents

The hydrogen peroxide stock solution (10 000 mg L^{-1}) was prepared daily by diluting 1 g of 30% solution with water. It was stored at 4°C in the dark. Further dilutions were made immediately before use.

The sodium salt of luminol (100 mg) and $Na_3PO_4 \cdot 12H_2O$ (250 mg) were dissolved in water (10 mL). This stock solution was stored at 4°C in the dark. The freshly prepared solution was allowed to stand for 48 hours before use.

The stock solution of $CoCl_2 \cdot 6H_2O$ (0.1 mol L^{-1}) was prepared by dissolving $CoCl_2 \cdot 6H_2O$ (1190 mg) in water (50 mL).

A stock solution of glucose (10000 mg L^{-1}) was prepared by dissolving of 0.5 g of glucose in 50 mL of water. It was left to stand for 48 hours before use to facilitate α-β mutarotation, as it was recommended (Zhu, Li and Zhu, 2002; Lindfors, Lähdesmäki and Ivaska, 1996) and stored at 4°C when not in use. Solutions of lower concentration were prepared immediately before use.

The stock solution of surfactant, sodium lauryl sulfate (SLS) was prepared by dissolving it (100 mg) in (10 mL) water.

A stock solution of glucose oxidase was prepared by dissolving 6 mg of glucose oxidase in 0.5 mL water and then diluting it with water with ratio 1:3. The solution was stored in the freezer at -20°C.

Trisodium phosphate $Na_3PO_4 \cdot 12H_2O$ (1 g) was dissolved in 10 mL water.

A stock solutions of the interferent (ascorbic acid, citric acid monohydrate, D-(-)-fructose, saccharose, lactose, oxalic acid, L (+)- tartaric acid, D-(-)-quinic acid, fumaric acid, D-(+)-galacturonic acid) were prepared by dissolving of 0.1 g in 10 mL water on the same day of use. Solutions of lower concentration were prepared immediately before use.

The solutions of interfering compounds with concentration of 0.5 ppm (only for ascorbic acid), 5, 50 and 500 ppm in the sample solution of 50 ppm of glucose were prepared just before measuring. They were stored in the refrigerator at 4°C.

Samples

The glucose biosensor was used for the determination of glucose in soft drinks, mainly apple juices (Table 1).

Preparation of glucose biosensor

The biosensor was prepared by drop-coating technique. The concentration of substances, which were used for preparing sensor layers were: 0.15 % HEC (m/m), luminol

(0.11 mmol L^{-1}), sodium phosphate (5.26 mmol L^{-1}), sodium lauryl sulfate (60 mg L^{-1}), cobalt chloride (0.05 mmol L^{-1}), and water solution of glucose oxidase.

Table 1: Soft drinks analyzed with the new glucose biosensor.

#	Brand	Type
1.	Apfel saft, 100%, Spar	Apple juice
2.	Apfelsaft, 100%, Happy Day	Apple juice
3.	Apfelsaft, 100%, Clever	Apple juice
4.	Apfelsaft, 50%, Clever	Apple juice
5.	Apfelsaft, 100%, Obi	Apple juice
6.	Indian Tonic Water, Schweppes	Tonic water

Biosensor I was made by three layers: first: HEC, SLS, luminol and phosphate; the second: Co, HEC and SLS and the third: glucose oxidase.

Biosensor II was made by fourth layers, one layer more than Biosensor I with layer of HEC and SLS in order to separate Co and luminol layers, and Biosensor III, with the second layer made of water solution of cobalt chloride.

After preparing the solutions for the biosensor, it was applied on microscope cover glass, dry in the oven at 70° C for 2 hours. After that it was cooled in the desiccator. The next layers were made using the same procedure: applied volume of each layer was 10 µL. The last layer was glucose oxidase. A stock solution of this enzyme was applied (5 µL) and after drying in desiccator it was stored at 4°C in the fridge. Scheme of the biosensor preparation is given in Figure 1.

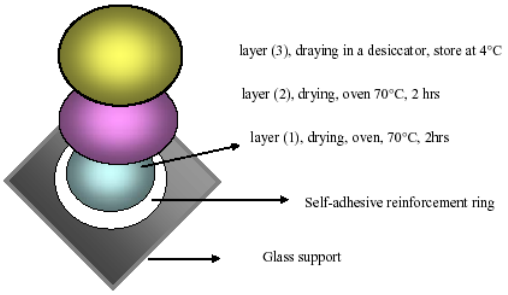

Figure 1: Preparation of glucose biosensor by drop-coating technique.

Results

After applying sample solution (glucose) by micropipette in the photodiode luminometer, glucose reacted with glucose oxidase and hydrogen peroxide was formed. Than H_2O_2 reacted with luminol in basic solution and in the presence of Co as catalyst emitting the light that can be detected by photodiode (Figure 2).

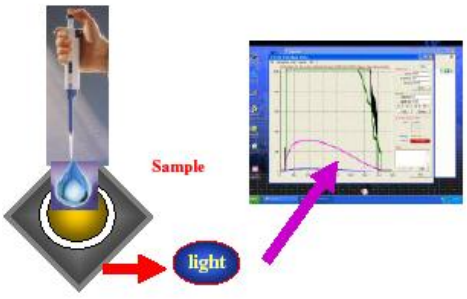

Figure 2: Chemiluminescence signal produced by glucose biosensor.

RESULTS AND DISCUSSION

Preliminary investigation of a biosensor preparation

Our investigations of a preparation of a new biosensor for glucose is based on a fact that glucose can be enzymatically determined by its enzymatic oxidation to gluconic acid and hydrogen peroxide in the presence of glucose oxidase. CL detection of hydrogen peroxide relies on its well-known CL reaction with luminol (Panoutsou and Economou, 2005) according to Scheme 1.

$$\beta\text{-D-glucose} + O_2 + H_2O \xrightarrow{\text{GOD}} \text{D-gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Luminol} \xrightarrow[\text{catalyst}]{\text{base}} \text{3-aminophtalate} + h\nu$$

Scheme 1

For the preliminary investigation we prepared three kinds of biosensors to achieve the best results, marked as Biosensor I, II and III (Figure 3). The biosensors were prepared by drop-coating technique. It consisted of layers applied on a microscopic cover glass by micropipette.

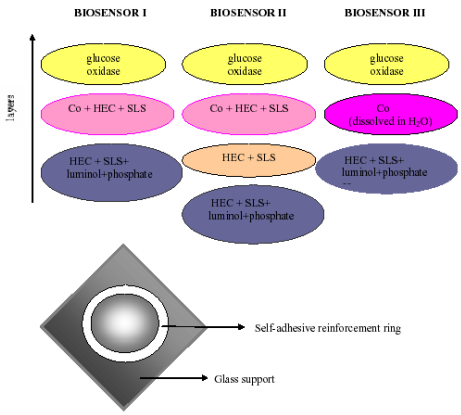

Figure 3: Preliminary investigations of glucose biosensor preparation

Biosensor III showed the best results, the highest chemiluminescence intensity and it was taken for the further investigations, while biosensor I and II showed weak chemiluminescence intensity or no signal was observed. The reason could be existence of a many sensor layers not allowing reactants to break in and get in contact with active surface.

Optimization of glucose oxidase concentration

Optimization of the glucose oxidase concentration was done with the microtiterplate luminometer. The results are shown in the Figure 4 and Table 2. The investigated solutions were prepared by dilution of stock solution of glucose oxidase, which were prepared by dissolving of 6 mg of glucose oxidase in 0.5 mL (2308 units/mL) water. Absolute concentration of glucose oxidase applied per biosensor was in the range 0.2 µg – 60 µg of enzyme.

Highest chemiluminescence intensity was achieved with a dilution of glucose oxidase stock solution in a range 1:3, which means that each biosensor contained 6.7 units of glucose oxidase. Smaller amounts of the enzyme gave lower signals, but also higher amounts of enzyme showed

increased signal but to a lesser extent. The reason for this behavior is unclear, but could be explained by surface blocked with too much enzyme, or binding of the catalyst to the protein.

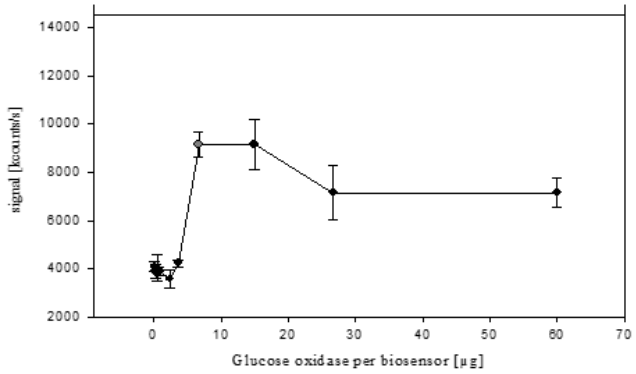

Figure 4: Dependence of the CL on the amount of GOX obtained with the microtiter luminometer. Membranes made by drop-coating, first layer (10 µL): 0.15 % HEC (m/m), trisodium phosphate (5.26 mmol L^{-1}), luminol (0.11 mmol L^{-1}) and sodium lauryl sulfate (60 mg L^{-1}), the second layer (10 µL) Co^{+2} (0.05 mmol L^{-1}), the third layer glucose oxidase (5 µL, 6.7 µg per biosensor), sample concentration: 500 mg L^{-1} glucose: the layers were applied to each well of a microtiter plate; repetitions =5.

Table 2. Chemiluminescence intensity obtained with different amount of glucose oxidase.

#	Absolute amount of glucoseoxidase applied per biosensor [µg]	Signal [kcounts/s]
1.	60	7150 ± 620
2.	26.7	7200 ± 1100
3.	15	9100± 1000
4.	6.7	9150 ± 510
5.	3.7	4230 ± 140
6.	2.4	3570 ± 360
7.	0.9	3880 ± 190
8.	0.5	4050 ± 540
9.	0.3	3910 ± 270
10.	0.2	4090 ± 180

Stability of the glucose biosensor

The long-term applicability of the biosensor was investigated. For that propose glucose biosensor was stored at room temperature, at 4°C in the refrigerator and at -18°C in a deep freeze for 18 days (Figure 5).

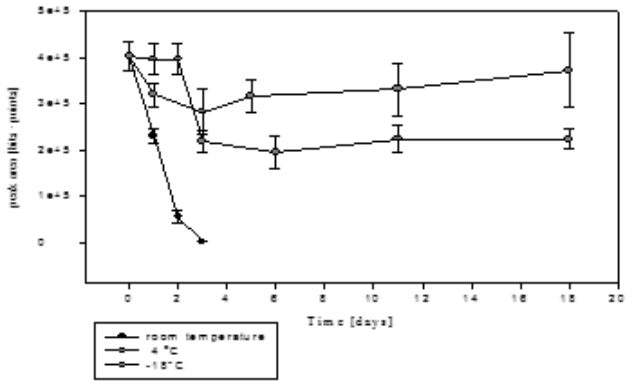

Figure 5: Stability of the glucose biosensor in dependence on the storage temperature. Membranes were made by drop-coating a solutions of: HEC 0.15 % (m/m), trisodium phosphate (5.26 mmol L^{-1}), luminol (0.11 mmol L^{-1}), Co^{+2} (0.05 mmol L^{-1}, SLS (60 mg L^{-1}), sample concentration: 100 µg L^{-1}; sample volume 10 µL, repetitions =5.

The biosensor gave a rather stable response after 2 days of storage in the refrigerator at 4°C without changes in its chemiluminescence intensity. Then the signal dropped for about 50% and stayed like that during the next two weeks. Further investigations were done within the first 2 days after preparation of the biosensors.

When stored at room temperature, the chemiluminescence intensity rapidly decreased, and after 3 days no signal was observed. When the biosensor was stored in a deep freeze (-18°C), the chemiluminescence signal dropped to one third of its initial value, and after that the signal was rather stable. But biosensor stored under these conditions showed very high standard deviations. Probably freezing destroys somehow the mechanical structure of the polymeric backbone (cellulose contains significant amounts of water) that deteriorates the analytical performances of the sensor.

Calibration curve

The calibration curve of the glucose biosensor is shown in Figure 6. In the investigated concentration range (20-1200 mg L^{-1}) two quasi-linear ranges were found, i.e., 20-100 mg L^{-1} (r^2=0.9992) and 100-900 mg L^{-1} (r^2=0.9994) glucose.

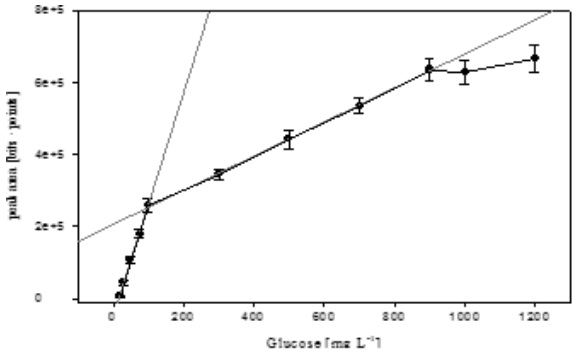

Figure 6: Calibration curve of glucose; membranes made by drop-coating from 10 µL of a solutions made of: 0.15 % HEC (m/m), trisodium phosphate (5.26 mmol L^{-1}), luminol (0.11 mmol L^{-1}) and sodium lauryl sulfate (60 mg L^{-1}), the second layer (10 µL), Co^{+2} (0.05 mmol L^{-1}), the third layer glucose oxidase (5 µL), sample concentration: 20-1200 mg L^{-1} 10 µL dispersed polymer solution on a glass lamella, sample volume 10 µL, repetitions =5.

The detection limit (3σ) was found to be 19 mg L^{-1} glucose (σ from 5 determinations of 30 mg L^{-1}). A relative standard deviation of 7.6 % was recorded for 10 measurements of 50 mg L^{-1} standard glucose solution, and 6.8 % for 10 measurements of 500 mg L^{-1} standard glucose solutions.

Interferences

Glucose is an important component in many alcoholic and soft drinks and often occurs together with many others organic species (acids and sugars), which might interfere with glucose. The main potential interferences are saccharides, metal cations, organic acids and reducing agents (Manera *et al*., 2004). The results are summarized in Table 3.

From the data it can be seen that practically all interferents, except ascorbic acid, interfere even at higher concentration only to a limited extent, i.e., below 20% change of the signal. As the experimental uncertainty is a bit less than 10% already, this seems acceptable for a simple portable device.

Ascorbic acid does strongly interfere even in a concentration of 5 ppm or higher, added to the glucose concentration of 50 ppm. Concentrations of 0.5 ppm or

lower in the glucose solution do not show any significant deviation of the glucose response.

Table 3: Investigated interferences on the determination of glucose; sample solution contains 50 ppm glucose and interferent.

Interferent	Change of signal (%) mg L^{-1}			
	0.5	5	50	500
Fructose		5.5	+3.1	+7.3
Saccharose		+9.2	-16.3	-17.1
Lactose		+8.6	-11.6	-12.8
Mannose		+3.5	-7.9	-10.6
Ascorbic acid	- 2	-94	-100	-100
Citric acid		+10.7	-9.9	-11.5
Oxalic acid		-8.9	+5.0	-5.1
Tartaric acid		-5.0	+12.9	+18.1
Quinic acid		-5.9	-6.9	+7.2
Fumaric acid		-6.1	-6.8	-15.5
Malic acid		-7.5	+6.7	+20.5
Galacturonic acid		+5.1	-4.6	+10.4

Thus, with real samples attention must be paid that the concentration of vitamin C does not exceed the given value in order to avoid erroneous results. With soft drinks it was found that dilution of the sample (100 to 200 fold) usually meets this criterion. If the ascorbic acid concentrations are too high either pretreatment of the sample is necessary, e.g., selective destruction of vitamin C by ascorbate oxidase and catalase, or interference-free sensor must be designed (protective layers).

One of the features of the proposed method is that it allows extensive dilution of the sample so that interferences due to the presence of possible interferences are alleviated.

Samples

The glucose biosensor was used for the determination of glucose in soft drinks.

The drinks were diluted 1:100 or 1:200 prior to analyses; the results obtained with the photodiode luminometer and a commercial glucometer as a reference are summarized in Table 4.

Table 4: Glucose concentrations in the investigated samples.

#	Glucose [g L^{-1}] *Photodiode Luminometer*	Glucose [g L^{-1}] *Glucometer*	Recovery (%)
1.	31.1 ± 3.0	29.9 ± 1.4	104.0
2.	29.7 ± 0.6	31.9 ± 1.1	93.1
3.	22.5 ± 2.6	23.5 ± 1.3	95.7
4.	16.2 ± 1.7	16.8 ± 0.8	96.4
5.	27.5 ± 2.1	28.0 ±1.6	98.2
6.	66.8 ± 2.0	63.7 ± 1.9	104.9

From the Table 4 it can be seen that results obtained by two methods agree very well. A recovery rates were between 93 and 105 %. This seems very acceptable for a simple portable device.

CONCLUSIONS

After preliminary investigations with a microtiterplate luminometer, and development of a sensor for the determination of hydrogen peroxide that was applicable to rainwater samples, a new biosensor was developed for the determination of glucose. The method is based on the determination of chemiluminescence given out by the cobalt-catalyzed reaction of luminol with hydrogen peroxide, which is produced by the reaction of glucose oxidase with glucose.

The biosensor was characterized with respect to storage, temperature and interferences, and its applicability for the determination of glucose in some soft drinks was proven.

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Summary/Sažetak

Pripremanje novog biosenzora za određivanje glukoze je bazirano na činjenici da se glukoza može oksidirati do glukonske kiseline uz pomoć enzima glukoza oksidaze. Biosenzor je napravljen od tri sloja. Prvi sloj sadrži luminol, natrijum fosfat, natrijum lauril sulfat kao surfaktant, i polimer hidroksietil celulozu, kao nosač. Drugi sloj je vodeni rastvor Co^{2+} kao katalizator, i treći sloj je vodeni rastvor glukoza oksidaze. Nakon apliciranja uzorka na biosenzor, glukoza reaguje sa glukoza oksidazom, i formira se hidrogen peroksid. Nagrađeni hidrogen peroksid prolazi do sloja koji sadrži luminol i javlja se kemiluminescentna reakcija. Limit detekcije novog biosenzora za glukozu (3σ) iznosi 19 mg L^{-1} glukoze (σ za 5 mjerenja koncentracije glukoze od 30 mg L^{-1}). Relativna standardna devijacija iznosi 7.6 % za 10 mjerenja standardnog rastvora glukoze koncentracije 50 mg L^{-1} i 6.8 % za 10 mjerenja standardnog rastvora glukoze koncentracije 500 mg L^{-1}. Novi biosensor je korišten za određivanje glukoze u bezalkoholnim pićima, većinom sokovima od jabuke. Rezultati dobiveni kemiluminescentnim biosenzorom i komercijalnim glukometrom kao referentnom metodom su pokazali jako dobro slaganje. Recovery vrijednost je bila između 93 i 105 %.

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Variation of PM_{10}, NO_2, NO and O_3 in City of Mostar, Bosnia and Herzegovina

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Abstract: Pollutants such as particulate matter PM, nitrogen oxides and troposferic ozone are harmful to a human health. Study of pollutant variation and its relationship is of importance not only for environmental protection but also for the benefit of public at large. The aim of this study was to analyze seasonal and daily variation of PM_{10}, NO_2, NO and O_3 in a residential part of an urban area. The study was conducted from January 1 till December 31, 2011 in the City of Mostar using the following methods: absorption of beta radiation, chemiluminescence and UV photometry. The results presented in this article, show the dependence of air pollution levels upon traffic density and seasons. Considering the level of air pollution relative to the regulated limited and tolerated values, the measured 24-hour concentrations of all studied pollutants did not exceeded the limited values and tolerated values.

INTRODUCTION

Air pollution is the introduction of chemicals, particulate matter, or biological materials that cause harm or discomfort to humans or other living organisms, or cause damage to the natural or built environment, into the atmosphere. According to the WHO (*World Health Organization*) air pollution is a significant risk factor for multiple health conditions including respiratory infections, heart disease, and lung cancer. Air pollution is always a public concern, especially in urban areas. Study of pollutant variation is of importance not only for environmental protection but also for the public at large. Air pollution can be natural or human-made.

Particulate matter is the term for solid or liquid particles found in the air. Because particles originate from a variety of mobile and stationary sources (traffic, industrial processes, combustion plants, *etc*.), their chemical and physical compositions widely vary. In 1987., EPA (*Environmental Protection Agency*) replaced the earlier Total Suspended Particulate (TSP) air quality standard with the PM_{10} standard.

The PM_{10} standard includes particles with a diameter of 10 micrometers or less. Because of their small size, particles PM_{10} can penetrate the deepest part of the lungs such as the bronchioles or alveoli. Larger particles are generally filtered in the nose and throat via cilia and mucus, but particulate matter smaller than about 10 micrometers, referred to as PM_{10}, can settle in the bronchi and lungs and cause health problems. Major concerns for human health from exposure to PM_{10} include: effects on breathing and respiratory systems, damage to lung tissue, cancer, and premature death (Li-Shun Lu et al. 2006).

The term »nitrogen oxides» NO_X refers to a group of oxides formed in the combustion process, the principal constituents of which are nitrogen monoxide (NO) and nitrogen dioxide (NO_2) are among the common atmospheric pollution. The principal sources of NO_X are motor vehicles and fuel combustion process and local heating. The majority of nitrogen oxides emitted from vehicle exhausts are in the form of NO. This gas can react with unburned hydrocarbonates, also present in the exhaust, to form NO_2. Nitric oxide is not considered harmful at ambient concentrations. Nitric dioxide ,NO_2 is a reactive pollutant

formed by oxidation of atmospheric nitrogen during fuel combustion at high temperature and a key component for the rise of secondary toxic pollutant (nitric acid, the nitrate part of secondary inorganic aerosols and photo-oxidants including ozone). Oxides of nitrogen have adverse effects on human beings, plants and animals. For human beings, NO_X can, at high concentrations (10-30 ppm), cause nose and eye irritation, pulmonary edema, bronchitis and even pneumonia. Fortunately, the levels even in the polluted areas are not quite that high. The effects on plants are more severe (necrosis and growth retardation) (Yuan Gao *et al.* 2011). In addition to potentially damaging human health, nitrogen oxides are precursors to ozone (O_3) formation, which can harm human health and vegetation. Finally, nitrogen oxides contribute to acid deposition, which damages vegetation and aquatic ecosystems (Hariri M.H., 1994; Lelieveld J. and Dentener F. J., 2000).

Ozone is the most important photochemical oxidant in the troposphere and it is the key ingredient of so-called summer smog, the main pollution problem in almost all big cities worldwide. It is a secondary pollutant since it is not emitted directly. The majority of tropospheric ozone formation occurs when nitrogen oxides (NO_X), carbon monoxide (CO) and volatile organic compounds (VOCs), react in the atmosphere in the presence of sunlight. NO_X, CO, and VOCs are called ozone precursors. Motor vehicle exhaust, industrial emissions, and chemical solvents are the major anthropogenic sources of these chemicals. Ozone has harmful effects on vegetation and human health. Long-term effects of ozone on human health include an increased incidence of asthma and lung cancer, impaired pulmonary function, *etc*. In addition, ozone is a significant greenhouse gas, particularly in the cold upper troposphere (Logan,1985).

Table 1. Limit and tolerance values of air pollutants – National Standards

Pollutant	Limit value c, μg m^{-3}	Maximum margin of tolerance c, μg m^{-3}	Averaging period	Permitted exceedences each year
NO_2	200	225	1 h	18
NO_2	85	125	24 h	/
PM_{10}	50	75	24 h	35
O_3	120	/	8 h	25*

* Averaged over 3 years

The European Union has developed an extensive body of legislation which establishes health based standards (limited and tolerated values) and objectives for a number of pollutants in air. These standards for measured pollutants in this study are summarized in the Table 1.

In the present study, seasonal and daily variation of PM_{10}, NO_2, NO and O_3 was analyzed in a residential part of an urban area.

EXPERIMENTAL

Measuring site and period

The study was conducted from January 1 till December 31, 2011 in the City of Mostar with 111 116 inhabitants (evaluation as per 2008). Station for monitoring (tracking) air quality Mostar-1 is placed in the western, residential part of the City of Mostar (coordinates: N 43° 20´ 42,6´´ i E 017° 45´ 34,7´´; 64 m above the sea level), at >6 m distance from a main street with high traffic intensity.

Sampling and measuring methods

Particulate matter PM_{10} was measured by the method of beta radiation absorption on a Verewa Beta-Dust Monitor F-701-20. The Beta Dust Meter measures the dust concentration in μg dust/cubic meters of gas. The sample gas is drawn through a glass fiber filter tape and the volumetric flow of the gas is recorded by the system. The dust particles are then trapped on the filter tape and radiometrically measured. The radiometric measurement is achieved using a Betaemitter (C-14) and a Geiger-Müller counter.

NO_2 and NO were measured by the method of chemiluminescence on NO_X–Analyzer, Horiba Model APNA–370. The APNA-370 uses a combination of the dual cross flow modulation type chemiluminescence principle and the referential calculation method. This gives it the advantages of the single-detector method plus the ability to do continuous measurements of NOx, NO, and NO_2. The design gives great stability and extremely high sensitivity.

O_3 was measured by the method of UV photometry on O_3– Analyzer Horiba Model APOA–370. The APOA–370 is using the non-dispersive ultraviolet absorption (NDUV) method as its operating principle. The ultra-violet-absorption method works on the principle that ozone absorbs ultra-violet rays in the area of 254 nm.

RESULTS AND DISCUSSION

During 2011, the measured concentrations of airborne pollutants showed quite a regular pattern. During the winter period (Oct-Mar), the highest average 24-hour a month air concentrations were recorded for the following pollutants: PM_{10} (17.21 – 29.99 μg m^{-3}), NO_2 (14.24 – 21.51 μg m^{-3}) and NO (2.38 – 10.90 μg m^{-3}).

In the summer period (Apr-Sep), these pollutants showed lowest 24- hour concentrations: PM_{10} (12.60 – 23.08 μg m^{-3}) NO_2 (7.70 – 14.96 μg m^{-3}), and NO (0.12 – 1.16 μg m^{-3}).

The results of seasonal variation in 24-hour concentrations of PM_{10}, NO_2 and NO, indicating higher values in winter, could be explained by air pollution from two emission sources: higher traffic intensity and a number of public institutions boiler-rooms. The higher values of PM_{10} concentration in the summer period was probably consequential to the growing number of resuspended particles due to dry weather and wildfires in this areas *(*Toth I. *et al*. 2011).

An inverse pattern was only observed for ozone (O_3), as lowest concentrations of this pollutant were recorded in the winter period (41.79 – 74.43 μg m^{-3}) and highest in the summer (85.28 – 94.53μg m^{-3}) (Table 2.).

In Figure 1 are presented the 24-hour concentrations of pollutants during 2011. The pollutants showed maximum values in late autumn and winter: PM_{10} (max value 61.83 μg m^{-3}, peak on 03/12/2011), NO_2 (max value 50.55 μg m^{-3}, peak on 12/02/2011) and NO (max value 47.78 μg m^{-3}, peak on 14/01/2011), while O_3 reach its maximum in spring and summer (max value 121.94 μg m^{-3}, peak on 28/09/2011).

Table 2. Monthly variation of 24-hour pollutant concentrations in Mostar, 2011.

	Pollutant/24-hour concentrations (μg m^{-3})					
	Particullate matter PM_{10}			Nitrogen dioxide NO_2		
	Min.	Max.	$\overline{X}$	Min.	Max.	$\overline{X}$
Jan	4.26	61.13	**24.58**	7.63	50.55	**20.64**
Feb	10.50	55.83	**29.99**	7.69	47.61	**21.51**
Mar	5.67	58.75	**23.79**	5.96	38.58	**18.52**
Apr	9.67	39.71	**17.86**	5.97	27.01	**14.96**
May	5.29	26.76	**15.47**	4.39	24.14	**10.28**
Jun	8.13	37.98	**17.65**	4.57	16.79	**9.28**
Jul	5.62	29.47	**12.60**	4.52	15.04	**8.75**
Aug	6.95	24.00	**22.08**	4.69	14.67	**7.70**
Sep	7.77	37.30	**23.08**	7.02	21.33	**12.93**
Oct	4.18	35.28	**17.21**	3.65	32.54	**14.24**
Nov	11.88	46.65	**23.60**	6.15	35.55	**20.24**
Dec	4.17	61.83	**17.80**	3.78	44.39	**18.21**

	Nitrogen monoxide NO			**Ozone O_3**		
	Min.	Max.	$\overline{X}$	Min.	Max.	$\overline{X}$
Jan	0.42	44.78	**10.90**	7.98	82.81	**43.69**
Feb	0.04	21.87	**4.92**	17.84	92.37	**63.55**
Mar	0.03	13.10	**2.38**	34.82	99.96	**74.43**
Apr	0.06	6.54	**0.90**	46.67	114.38	**85.28**
May	0.02	1.15	**0.12**	54.23	115.02	**92.54**
Jun	0.03	1.08	**0.52**	73.41	115.94	**94.53**
Jul	0.46	1.64	**0.97**	59.59	117.13	**90.23**
Aug	0.56	1.67	**1.04**	63.31	108.97	**90.90**
Sep	0.58	2.72	**1.16**	40.92	121.94	**91.30**
Oct	0.69	21.13	**4.60**	15.25	105.14	**54.73**
Nov	0.76	17.79	**7.06**	23.24	75.77	**44.06**
Dec	0.60	43.57	**10.31**	5.69	71.41	**41.79**

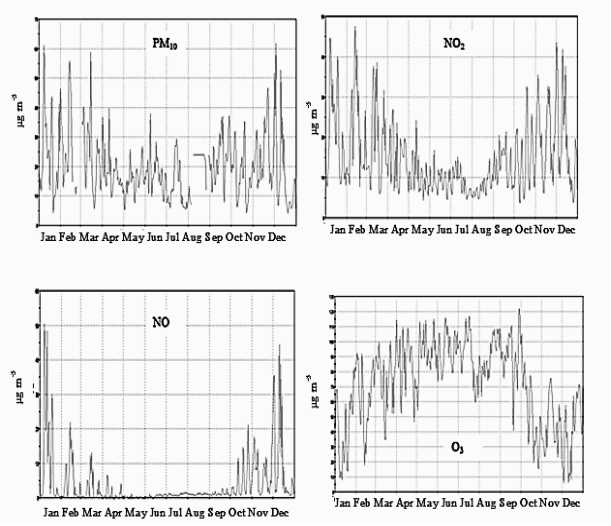

Figure 1. Variation of 24-hour pollutant concentrations during 2011.

In Figure 2 the curves showed a similar pattern of intra diurnal distribution of the mean pollutant concentrations PM_{10}, NO_2 and NO during work-days (Mon-Fri) and weekend (Sat- Sun) throughout 2011. Air concentrations of pollutants were higher on workdays.

The regular diurnal pattern of 24-hour PM_{10}, NO_2 and NO concentrations observed during the week and of intradiurnal concentrations was directly related to traffic intensity. It was indicated by elevated concentrations towards working days and lower values at weekends (WBG 1998).

Ozone is influenced by seasonal and intra diurnal variation. The concentrations decreased towards autumn, to be lowest during winter. Also, higher levels of nitrogen oxides were associated with lower ozone concentrations and *vice versa* because ozone is formed primarly by nitrogen oxides in the presence of sunlight (Figure 3).

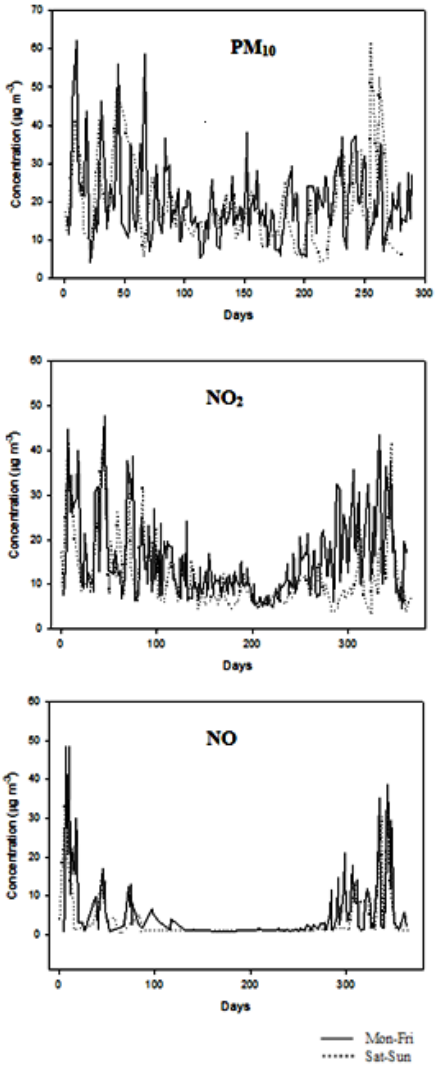

Figure 2. Intradiurnal hourly concentrations of PM_{10}, NO_2, NO and during working and weekend days (2011).

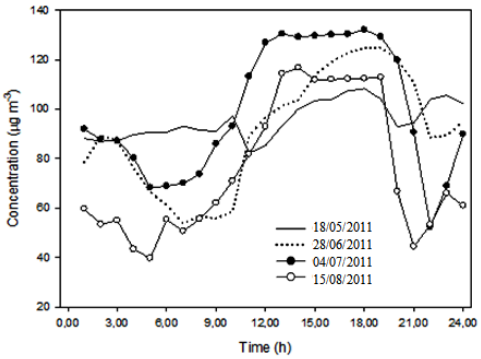

Figure 3. Hourly concentrations of O_3 (2011).

In Figure 4. can be seen that lower ozone concentrations were observed during early morning hours and high ozone levels were typically found in the afternoon. Those days were randomly chosen during summer time to see intradiurnal behavior variation in concentration of O_3.

Similar results have been reported in other study (Yuan Gao *et al.*, 2011, Lelieveld J. and Dentener F. J., 2000).

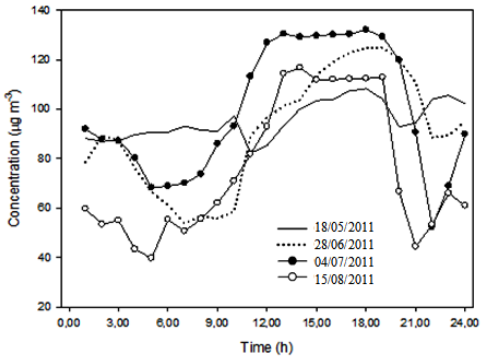

Figure 4. Hourly concentrations of NO_2 and O_3 (2011).

CONCLUSIONS

During the winter, the highest 24-hour air concentrations were recorded for the following pollutants: PM_{10}, NO_2 and NO. An inverse pattern was observed for ozone O_3.

The curves showed a distribution of the mean pollutant PM_{10}, NO_2 and NO concentrations during workdays (Mon-Fri: highest peaks) and weekend (Sat-Sun:lowest peaks). Air concentrations were higher on workdays.

Lower ozone concentrations were generally observed in winter and during early morning hours, while high ozone levels were typically found in summer and in the afternoon.

In the urban area in the City of Mostar the air quality is relatively good with occasionally higher concentration of ozone in the summer time, which did not exceeded limited and tolerated values.

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Summary/Sažetak

Zagađujuće tvari kao što su lebdeće čestice, dušikovi oksidi i prizemni ozon štetni su za ljudsko zdravlje. Svrha ovog rada je analizirati sezonske , dnevne i satne varijacije PM_{10}, NO_2, NO i O_3 na urbanom području grada Mostara. Istraživanje se provodilo u razdoblju od 1. siječnja do 31. prosinca 2011. godine u Gradu Mostaru. Koristile su se sljedeće metode: apsorpcija beta radijacije, kemiluminiscencija i UV fotometrija. Rezultati prezentirani u ovom radu pokazuju povezanost razina onečišćujućih tvari s gustoćom prometa i sezonskim uvjetima. 24 satne koncentracije svih mjerenih onečišćujućih tvari u zraku nisu prekoračile granične i tolerantne vrijednosti definirane zakonskom regulativom.

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Determination of Radionuclide Activity of U-238 in Wheat using Gamma Spectrometric Method

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Abstract: Migration and accumulation of contaminants in soil is complex and involves different processes such as leaching, capillary movement, sorption, nutrient resuspension in roots and into the atmosphere. Speciation of radionuclides in ecosystems depends on the source and layoffs, the distance from the source, dispersion processes and deposition conditions. Resuspension of radionuclides from the soil surface to the outer portions of the plants occurs due to the action of rain and wind. Significant variations in contamination can be expected depending on the type of plant, plant growth conditions and methods of its preparation before consumption. In this study we determined the activity of radionuclides U-238 in the aerial part of wheat that was sown on a sample of soil from Hadzic, and as an added contaminant different uranyl acetate concentrations were used. Gamma spectrometric measurements were carried out in a vertical coaxial HPGe detector. It is concluded that, regardless of the concentration of the contaminant, if the plant has a sufficient amount of essential elements, in this case potassium, it will not take an element that is harmful to its development, such as the uranium-238, which belongs to the toxic and unnecessary elements in plant nutrition.

INTRODUCTION

Due to differences in physico-chemical properties of the radionuclides and soil matrix in which the radionuclides are present, the different chemical forms of radionuclides present in the soil will be available for different plants. The process of migration of radionuclides in the soil-plant system and the behavior of radionuclides in soil and plants, and some certainly affect the capability of the plant. In order to quantify the transport process of radionuclides from soil to plants term plant / soil concentration ratio has been introduced often referred to as transfer factor (TF) (Mortverdt, 1994) The transfer factor (TF) is defined as a factor used to evaluate the transport of radionuclides and other elements of interest through the food chain. Shepard *et al*., defined it as a factor that describes the amount of an element which is expected to be from the substrate to enter the plant in terms of balance. Knowledge of transfer factors can theoretically allow the calculation of radionuclide activity in the plant and animal products on the basis of the measured activity in the soil, and the study of transfer still in the early radioecology been a frequent topic of research (Ng YC *et.al.*, 1982).

Contamination of plants is carried out in two ways:
- depostion of radionuclides on a plant surface (surface, foliar contamination); and
- radionuclide transfer from soil to plants via the root system.

Bettencourt *et al*.,(1988) have found that various factors such as soil characteristics, climate, plants and their parts, the physical and chemical form of radionuclides and the effect of competing organisms (organisms that compete with plants for food) may affect the value of transfer factors.

Understanding the behavior of uranium, thorium and artificially produced transuranic elements in the food chain

is important because of its long half-life, the fact that alpha emitters as well as their presence in the environment (Zovko, Pujić, 2003) . One of the very important questions is whether the uranium, if present in the soil in high concentrations of natural representation, can incorporate into biomass and above-ground part of the plant.

The task of this work was to investigate the possibility of transfer of uranium contamination on the surface of the plant from the part of the soil, which leads to the development of the primary plant species, and where there is the possibility of soil erosion, dependence on the type of soil.

For this purpose, wheat was selected and the transfer of uranium from the soil in wheat during the early stages of vegetative growth has been monitored. Reason to use this plant is its distribution in the dietary habits of the population in this region and beyond. Monitoring was carried out at an early stage of the vegetative period for practical reasons, and the scientific assumption that if uranium reached the overground part of the plant, there is a likelihood that they will be placed in the final stage of growth, that is, the fruit of the plant.(Radović-Rajević, 2011).

EXPERIMENTAL

In the experiment we used vegetable crops wheat seeds such as „Winter wheat" variety "Renaissance", the category C-1. General features of these seeds are that they have a good resistance to winter, it is very drought tolerant and resistant to powdery mildew, number of 1.000 grains weight s 40-45kg, very good milling and baking properties etc. This type of wheat has a very high yield at favorable conditions, but also in the conditions of stress caused by the drough .This put it in the group of plant varieties that can be grown in very heterogeneous agroecological regions.

The samples were taken from the soil from Hadzic area. Four wheat samples were weighed out .Additionaly, sample of the soil were packed in containers for three different uranyl acetate concentrations and one for zero concentration of UAc. The sown seeds were abundantly watered to maximum swelling, before the appearance of the first sprouts on grains. This was followed by a period of drying out of the soil in the pots, so the soil could accept a solution of the contaminant and not leak out of the pot. After that, three different concentrations of the solution of uranyl acetate were prepared: 0.03 g/cm^3, 1.00 g/cm^3 and 2.00 g/cm^3.

Samples were spiked with contaminants in small portions, making sure that all contaminant remains in the container with soil. In this way, the simulated contamination on the surface layer and maximum availability enabled contaminant to enter the root system of plants. To monitor the transfer of uranium from the soil in biomass it was necessary to monitor its presence in the soil, root system, and in the aerial part of the plant, but only after it is established radioactive equilibrium (six half-lives).

For this purpose, the separation was carried out above ground portion of the plant root system by cutting it to a height of 1 cm from the surface of the soil, to obtain a pure sample from the aboveground part of the plant. All four samples were dried at room temperature to a stage when the plant breaks down to hey and process of annealing can be performed.

Given that the experimental conditions did not allow a large yield of plant material above ground part of the plant, preparation phase annealing was not performed, because in this case the mass of the sample for measurement was extremely small. Prepared samples are weighted and packed in plastic boxes, and then the measurements were performed.

Gamma spectrometric measurements were performed on a vertical coaxial HPGe detector POP-TOP p-type, manufacturer "ORTEC" model "GEM 30P4" with relative efficiency of 30% and a resolution of 1.85 keV-MeV and at 1.33. Activity of this radionuclide (^{238}U) was measured from its gamma gamma lines and lines of his descendants. The specific activities of ^{238}U was calculated from ^{234}Th to 63 keV energy and the energy ^{234}Pa 1001 keV's.

RESULTS AND DISCUSSION

Since the task was to determine whether there is a transfer of uranium from the soil in the overground part of the plant, the experimental setup used was for areal soil specific chemical composition (Table 1).

Table 1: The chemical composition of the used sample soil.

Parameters	Sample
pH (H_2O)	7.46
pH (1 M KCl)	7.21
$CaCO_3$ (%)	2.04
K_2O (mg/100 g soil)	56.78
Cu (mg/kg)	42.0
Fe (%)	3.38
Ca (%)	0.25
Mg (%)	0.69

It was expected that such a chemical composition will have a quite an impact on the yield of plant species. And will transfer uranium from the soil in biomass. Which according to the classification of plant nutrient belongs to the group of toxic elements? Given the laboratory setting conditions of the experiment, eliminating the sensitivity of plant species to agroecological heterogeneity. We used the highly resistant wheat variety, which is easily adaptable and dry and moist soils and diversity of the chemical composition of the soil. The growth of plants was monitored during 26 days and then samples were prepared for measurement. The experiment was performed three times measuring of the height of the plant above the ground and data are presented in tabular and graphical form.

Table 2: Results of radionuclide activity in the overground part wheat.

C(UAc) (g/ml) in the soil	A (Bq/kg) U-238	A (Bq/kg) U-235	A (Bq/kg) K-40
0.00	1.20	< 29	1819.98
0.03	20.10	< 22	1822.00
1.00	80.60 +/- 54.3	8.24 +/- 5.51	1881.35
2.00	152.58+/- 58.05	13.87 +/-11.40	1665.02

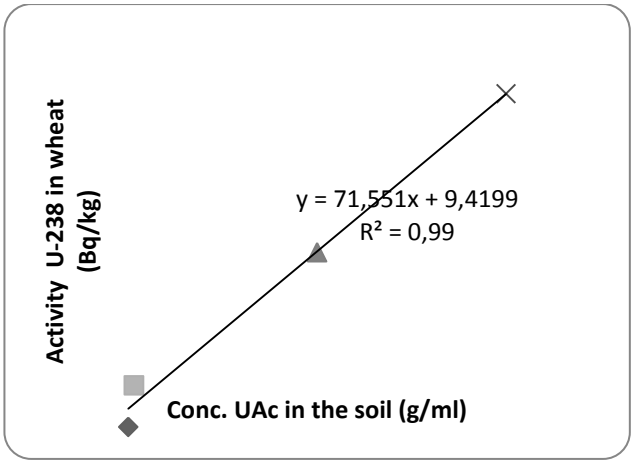

Diagram 1: Distribution of U-238 in the overground part wheat.

Table 3: The mass of absorbed radionuclides in the overground part wheat.

C(UAc) (g/ml) in the soil	0.00	0.03	1.00	2.00
NUCLIDE	**U-238**	**U-238**	**U-238**	**U-238**
Mass (µg) in the overground part wheat	1.05	2.8	6.53	12.35

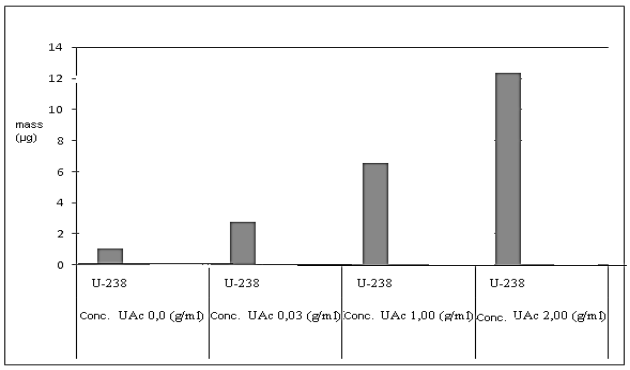

Diagram 2: Graphical representation of radionuclide mass depends on the concentration of the contaminant.

The results of measurements of activity of nuclides in the soil from the site Hadzici (Tables 2 and Diagram 1). The uranium activity in the soil increased proportionally increasing the concentration of the contaminant, and potassium content remained constant.

Aboveground part of plants were characterized by a sudden increase in the content of potassium, slightly below 2000 Bq/kg, and a sudden drop in uranium content. Therefore, plants have had quite enough source of potassium, which is incorporated in the overhead part of the plant, while in the same part of the plant. The uranium content is very low.

From obtained values for nuclides activity and using data on the specific activity (As) U-238 (1g U-238 = 12 350 Bq) we obtained results presented in Table 3 that shows a mass in micrograms absorbed radionuclides U-238 in surface parts of plants developed during the experiment.

The resulting values are shown in the diagram 2, from which it is evident that the amount of absorbed U-238 is measured in micrograms.

CONCLUSIONS

Following the development of the above-ground plant species in the course of 25 days, and measuring the height of plants on day 12, 19 and 25 from the time of seeding, it can be concluded that regardless of the concentration of the contaminant, the development of plant species flowed freely, and is directly proportionate to the amount of potassium- 40, present in the soil in which the plants are sown. Looking at the distribution of uranium-238 and potassium-40 in the system soil - plant, we concluded that regardless of the concentration of the contaminant, if the plant has a sufficient amount of essential elements, in this case potassium, it will not take an element that is harmful to its development, as it is uranium-238, which belongs to the unnecessary and toxic elements in food plants. But in all the works, and so in this experiment can be performed that every living organism, including the plant in the process of self-preservation, perform the selection of micro and macro elements from the soil, and takes the most necessary elements for optimal development.

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Summary/Sažetak

Migracija i akumulacija kontaminanata u tlu je kompleksna i uključuje različite procese kao što su ispiranje, kapilarno kretanje, sorpciju, unošenje korjenom i resuspenziju u atmosferu. Specijacija radionuklida u ekosistemima zavisi od izvora i načina otpuštanja, udaljenosti od izvora, disperzionim procesima i uslovima depozicije. Resuspenzija radionuklida s površine tla na vanjske dijelove biljaka dešava se usljed djelovanja kiše i vjetra. Značajne varijacije u kontaminaciji mogu se očekivati u zavisnosti od vrste biljke, uvjeta rasta biljke i načina njene pripreme prije konzumiranja. U ovom radu određivana je aktivnost radionuklida U-238 u nadzemnom dijelu pšenice koja je zasijana na uzorku zemlje iz Hadžića, a kao kontaminant je adiran uranil-acetat različite koncentracije. Gamaspektrometrijsko mjerenje vršeno je na vertikalnom koaksijalnom HPGe detektoru. Zaključeno je, da se bez obzira na koncentraciju kontaminanta, ukoliko biljka ima dovoljnu količinu esencijalnog elementa, u ovom slučaju kalija, ona neće uzimati element koji šteti njenom razvoju, kao što je to uranij-238 koji spada u toksične i nepotrebne elemente u ishrani biljke.

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Original Scientific Article

Phenolic Compounds and Antioxidant Activity of Extracts of *Nigella sativa* L.*

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Abstract: *Nigella sativa* L. (Black cumin) is an annual herbaceous plant which belongs to family Ranunculaceae. The plant commonly grows in the Middle East, Eastern Europe and Western and Central Asia. This plant has been extensively investigated in recent years, due to its notable pharmacological properties. This work presents the investigation of phenolic content and antioxidant activity in extracts obtained from seeds of *N. sativa*, using Soxhlet and ultrasound extraction techniques. Total phenolics content was measured using the Folin-Ciocalteu method, and they varied from 11.867±0.338 to 31.148±0.293 mg/g GAE. Radical scavenging activity of the samples was examined using two methods, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid), (ABTS) test, and reducing power of these samples was examined by reducing ferric, and molybdenum cations. All examined samples showed prominent antioxidant activity, except *p*-cymene. Thymoquinone and ethanolic extracts revealed the best results among six investigated samples.

INTRODUCTION

Plants are natural factories for the production of chemical compounds, many of which are used to promote health and fight diseases and some of them are marketed as food or herbal medicines. Herbal medicines have long been viewed as a source of curative remedy based on religious and cultural traditions (Ghosheh, Houdi, and Crooks, 1999). There is increasing evidence that consumption of a variety of phenolic compounds present in natural foods may lower the risk of serious health disorders because of the antioxidant activity of these compounds. Since ancient times, herbs and spices have been added to different types of food to improve the flavor and organoleptic properties. Especially popular today is the concept of food that combines nutritional and medicinal benefits, especially antioxidant activity.

Reactive oxygen species (ROS) are often generated as by products of biological reactions or from exogenous factors. These reactive species exert oxidative damaging effects by reacting with nearly every molecules found in living cells including DNA, if excess ROS are not eliminated by antioxidant system (Krötz, Sohn, Gloe, *et al*., 2002).

Nigella sativa L. (Black cumin) is an annual herbaceous plant which belongs to family Ranunculaceae. The plant commonly grows in the Middle East, Eastern Europe and Western and Central Asia. This plant has been have been extensively investigated in recent years, due to its notable pharmacological properties (Dubick, 1986). It tastes slightly bitter and peppery with a crunchy texture. Seeds are angular, of generally small size (1–5 mg), dark grey of black colour.

Seed oil of *N. sativa* is considered as health beneficial one among newer sources of edible oils, thanks to its important role in human nutrition
and health. This seed oil has been reported to possess antitumor activity (Worthen, Ghosheh, and Crooks, 1998), antioxidant activity (Burits, and Bucar, 2000), anti-inflammatory activity (Houghton, Zarka, de la Heras, and Hoult, 1995), antibacterial activity (Morsi, 2000) and astimulatory effect on the immune system (Salem, and Hossain, 2000).

*Parts of this paper have been presented as poster presentations at the IX Meeting of Young Chemical Engineers, February 16-17 2012, Zagreb, Croatia.

This investigation was undertaken to obtain information about the phenolic composition of seeds of *Nigella sativa* L. from market in Sarajevo, Bosnia and Herzegovina, and to determine antioxidant activity of isolated extracts.

EXPERIMENTAL

Isolation

The seeds of *Nigella sativa* L. were grounded and weighted in two portions of 20.0 g. Each portion has been used for successive Soxhlet extraction, and ultrasound extraction, using *n*-hexane and 96% ethanol as solvents (Fig. 1). The solvents were evaporated using rotary evaporator and crude extracts were dissolved in dimethyl sulfoxide in concentrations 0.10-20.0 mg/mL.

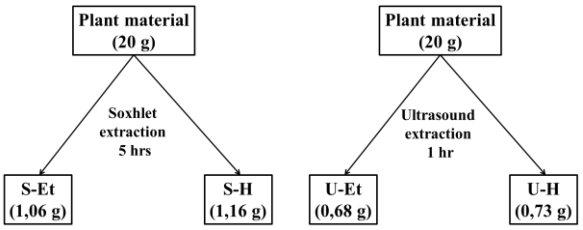

Figure 1: Extraction procedure.
S-Et, etanolic extraxt obtained by Soxhlet extraction; S-H, *n*-hexane extract obtained by Soxhlet extraction; U-Et, etanolic extraxt obtained by ultrasound extraction; U-H, *n*-hexane extract obtained by ultrasound extraction.

TLC preliminary investigation

Preliminary investigation of extracts composition were done by thin layer chromatography in toluene:ethyl acetate (93:7), and chloroform-acetone-formic acid (75:15:5) systems, for terpenoids and phenolics, respectively. Detection of sample components was done using vanillin-sulfuric acid, Folin-Ciocalteu and DPPH (1,1-diphenyl-2-picrylhydrazyl) reagents, and UV light. Thymoquinone, thymol and *p*-cymene were used as standards.

Determination of phenolics

Total phenolic content was measured using Folin-Ciocalteu spectrophotometric method (Singleton, and Rossi, 1965), using gallic acid for calibration curve. Total flavone and flavonol content has been measured by spectrophotometric method using aluminum chloride as chromophore reagent (Woisky, and Salatino, 1998), using quercetine for calibration curve. Total flavonone content was measured using colorimetric method with 2,4-dinitrophenylhydrazine as specific chromophore for carbonyl compounds (Nagi, and Grancai, 1996), and naringenin was used for calibration curve.

Antioxidant activity

Radical scavenging activity of these samples was examined using two methods, 1,1-diphenyl-2-picrylhydrazyl (DPPH), (Ćavar, Maksimović, Šolić, *et. al.*, 2008), and 2,2'-azino-*bis*(3-ethylbenzthiazoline-6-sulphonic acid), (ABTS) test (Ćavar, Maksimović, Vidic, *et* al., 2012).

Reducing power of these samples was examined by reducing ferric (Ćavar, Maksimović, Vidic, *et* al., 2012), and molybdenum (Pisoschi, Cheregi, Danet, 2009) cations.

All tests were performed in triplicates, and results are presented as IC_{50} values that indicate the concentration of extracts that reduces the 50% of radical, or transition metal. Thymoquinone and *p*-cymene were used as standard probes.

RESULTS AND DISCUSSION

Soxhlet and ultrasound extraction were employed for isolation of extracts *of N. sativa* seeds, with ethanol and n-hexane as solvents, and four extracts were obtained: S-Et (Soxhlet etanolic extract; yield: 5.30 %), S-H (Soxhlet *n*-hexane extract; yield: 5.38 %), U-Et (ultrasound ethanolic extract; yield: 3.38 %), and U-H (ultrasound *n*-hexane extract; yield: 3.64 %).

Preliminary investigation was done by thin layer chromatography (TLC) which has proved its worth as a simple, inexpensive method for the chemical and biological screening of plant extracts. Detection of natural products was done by spraying TLC plates with vanillin-sulfuric acid reagent, and for phenolic compounds using Folin-Ciocalteu reagent (Stahl, 1969). Positive detections were blue spots on white background (Fig. 2). The TLC plate with samples is developed with the elution solvent and dried. It is then sprayed with a DPPH solution. The plate is examined in daylight. Active (free-radical scavenging) compounds appear as yellow-white spots against a purple background (Marston, 2011).

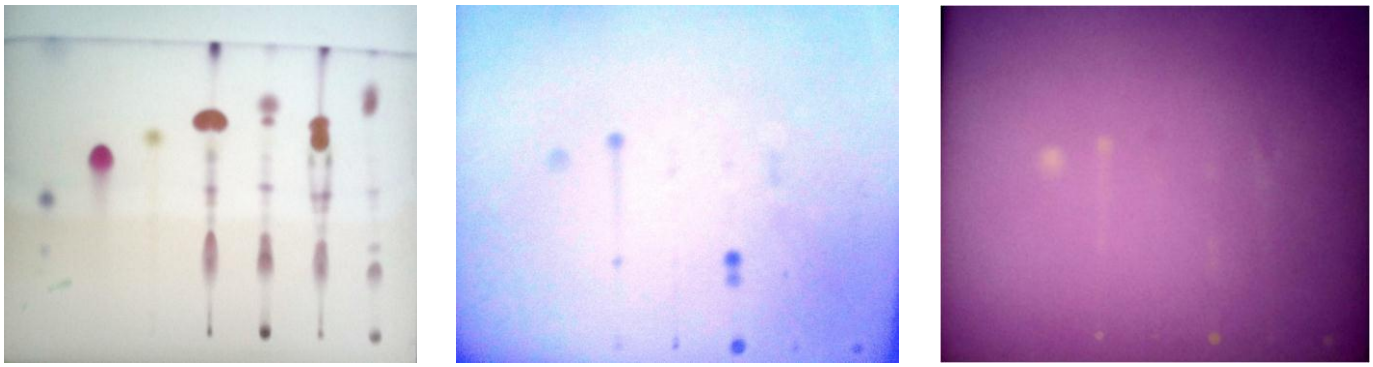

Figure 2: Thin-layer chromatograms of extracts of *N. sativa*.
(a) Vanilin-sulfuric acid reagent; (b) Folin-Ciocalteu reagent; (c) DPPH reagent; *p*-C, *p*-cymene; T, thymol; TQ, thymoquinone.

Results from the spectrophotometric determination of phenolic compounds were summarized in Table 1.

Table 1: The phenolic content of extracts of *N. sativa*.

Sample	TP mg GAE/g	TF-id mg GAE/g	TF-ol x 10^{-5} mg QE/g	TF-one x 10^{-3} mg NE/g
S-Et	**31.15±0.29**	**16.34±0.71**	6.86±2.34	3.64±0.46
S-H	5.58±0.31	6.35±1.40	**32.7±1.31**	1.52±0.16
U-Et	23.68±0.90	9.82±0.18	2.56±1.83	6.38±0.23
U-H	11.87±0.34	3.99±1.54	2.70±0.22	**8.09±0.65**

TP, total phenolics; TF-id, total flavonoids; TF-ol, total flavonols; TF-one, total flavonones.

Total phenolic content varied from 5.58 ± 0.31 to 31.15 ± 0.29 mg GAE/g (gallic acid equivalent), for Soxhlet etanolic and *n*-hexane extract, respectively, while total flavonoid content varied from 3.99 ± 1.54 to 16.34 ± 0.71 mg GAE/g, for ultrasound *n*-hexane and Soxhlet etanolic extract, respectively.

These results are expected due to the different polarity of used solvents. Ethanol is a polar solvent and extracts polar compounds. These results are consistent with the results Mariod *et al*. (2009), who also performed a determination of the total content of phenolic compounds in this plant. In comparison with results concerning *n*-hexane extracts, presented results are significantly lower than those published earlier (Martos, Mohamady, Fernández-Lòpez, *et al*., 2011). While, alcohol samples showed results comparable with those found in the literature (Tubesha, Iqbal, and Ismail, 2011).

Total flavone and flavonol content were ranged from (2.70 ± 0.22) x 10^{-5} to (32.7 ± 1.31) x 10^{-5} mg QE/g (quercetin equivalent). This result is consistent with these found in the literatue (Tubesha, Iqbal, and Ismail, 2011).

Total flavonone content varied from (1.52 ± 0.16) x 10^{-3} to (8.09 ± 0.65) x 10^{-3} mg NE/g (naringenin equivalent). To the best of our knowledge, there is no data concerning the the content of total flavanones in this plant species.

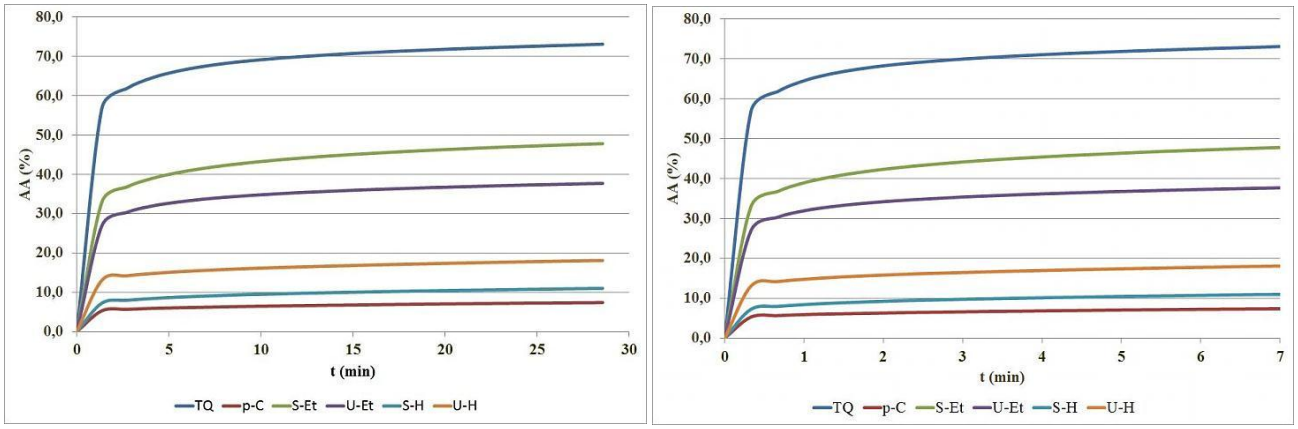

Figure 3: Progress of antioxidant activity of extracts of *N. sativa*.
(a) DPPH test; (b) ABTS test.

Antioxidant activity (Table 2) of isolated extracts as well as *p*-cymene and thymoquinone, as constituents of extracts, was examined by four different testing methods.

Figure 3 presents the progress of antioxidant activity of examined samples in concentration of 1.0 mg/mL tested by DPPH and ABTS methods.

The ability of samples to reduce stable DPPH radical, presented as IC_{50} values, were ranged from 0.70 ± 0.0.01 mg/mL, for thymoquinone, to 129.65 ± 1.67 mg/mL, for *p*-cymene. Moreover, thymoquionone showed the lowest IC_{50} values in the reduction of stable ABTS radical, while *p*-cymene showed the highest. Extracts of seeds of *N. sativa* revealed promient antioxidant activity in comparison with these two natural compounds.

However, the ability of samples to reduce molybdenum cations (Table 2), presented as IC_{50} values, were ranged from 12.90 ± 0.29 mg/mL, for U-Et, to 346.84 ± 8.57 mg/mL, for *p*-cymene. Moreover, thymoquionone showed the lowest IC_{50} value in reduction of ferric cations (20.23 ± 0.23 mg/mL), while again *p*-cymene revealed the lowest reduction potential (155.48 ± 9.48 mg/mL).

Table 2: Antioxidant activity of extracts of *N. sativa*.

Sample	DPPH IC_{50} (mg/mL)	ABTS IC_{50} (mg/mL)	RP-Fe IC_{50} (mg/mL)	RP-Mo IC_{50} (mg/mL)
S-Et	1.98±0.08	**14.02±0.62**	68.21±1.11	13.38 ±0.54
S-H	12.04±0.60	17.01±0.64	71.78±0.81	51.88±0.64
U-Et	**3.01±0.03**	16.79±1.05	**29.31±0.94**	**12.90±0.29**
U-H	8.177±0.11	18.67±1.54	73.32±3.91	25.45±0.59
p-C	129.65±1.67	165.65±16.43	155.48±9.48	346.84±8.57
TQ	0.70±0.01	6.07±0.57	20.23±0.23	39.98±2.12

In general, among examined estracts, ethanolic extracts revealed the lowest IC_{50} values that indicate the best antioxidant activity, compared with thymoquinone, an already known natural antioxidant (Milos, and Makota, 2012). This can be explained by the fact of high content of phenolic compounds found in these extracts.

Although DPPH and ABTS methods were based on the same principle; data obtained from ABTS assay are lower than those obtained from DPPH assay, but comparable. This is probably due to the steric factors that are one of the major factors for reducing of stable DPPH radical. Moreover, the IC_{50} values obtained from these two radical methods are much lower than IC_{50} values obtained from the methods of reduction of transition metals, iron and molybdenum.

The transition metal ions possess the ability to move single electrons by virtue of which it can allow the formation and propagation of many radical reactions even starting with relatively non-reactive radicals. The main strategy to avoid generation of reactive oxygen species is associated with redox active metal catalysis involves chelating of the metal ions.

However, presented results are in the agreement with those found in the literature (Khattak, Simpson, and Ihasnullah, 2008; Bourgou, Ksouri, Bellila, *et al*., 2008) and they suggest further analysis on chemical composition of the plant extracts in order to identify compounds with antioxidant properties.

CONCLUSIONS

To the best of our knowledge, this is the first study providing data on phenolic compounds and antioxidant activity of extracts of seeds of *Nigella sativa* L. found in the market in Sarajevo, Bosnia and Herzegovina. The samples obtained from investigated plant species are quite interesting from a pharmaceutical standpoint because of its prominent antioxidant properties.

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Summary/Sažetak

Nigella sativa L. je godišnja zeljasta biljka koja pripada obitelji Ranunculaceae. Biljka obično raste na Bliskom Istoku, Istočnoj Europi i zapadnoj i središnjoj Aziji. Zbog svojih značajnih farmakoloških svojstava ova biljna vrsta je intenzivno istraživana u posljednjih nekoliko godina. Ovaj rad predstavlja određivanje sadržaja fenolskih spojeva i antioksidacijske aktivnosti u ekstraktima dobivenih iz sjemenki *N. sativa*, koristeći Soxhlet i ultrazvučnu ekstrakciju. Ukupan sadržaj fenolskih spojeva određen je spektrofotometrijskom Folin-Ciocalteau metodom, i on varira od 11.867 ± 0.338 do 31.148 ± 0.293 mg GAE/g. Antioksidacijska aktivnost ekstrakata ispitana je pomoću četiri spektrofotometrijske metode. Dvije metode su bazirane na reduciranju slobodnih radikala, 1,1-difenil-2-pikrilhidrazil i 2,2'-azino-*bis*(3-ethylbenzthiazoline-6-sulfonska kiselina), a dvije na reduciranju prelaznih metala, željeza i molibdena. Svi ispitivani uzorci su pokazali značajnu antioksidativnu aktivnost, osim *p*-cimena. Timokinon i etanolni ekstrakti su pokazali najbolje rezultate.

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21-23

Synthesis and Characterization of Novel Chloro-Ru(III) complex with Formamide

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Abstract: A new neutral complex has been synthesized in reaction of $[RuCl_6]^{3-}$ with formamide in mixed solvent ethanol-water (10:1). Obtained olive green substance was characterized by mass spectrometry, CHN elemental analysis, IR and UV-VIS spectroscopy. Based on experimental data, compound was formulated as $[RuCl_3(HCONH_2)_3]x0{,}5C_2H_5OH$. Relative molecular mass of synthesized compound was determined by MALDI-TOF mass spectrometry as adduct with K^+ ion to have value of 382.8485. IR spectrum of formamide, indicates coordination through the amide carbonyl oxygen, which is indicated by shift of carbonyl absorption from 1681 cm^{-1} in free formamide to 1636 cm^{-1} in synthesized compound. UV-VIS spectrum of synthesized compound in water shows LMCT absorption centered around 300 nm. Hydrolytic profile indicates that compound hydrolyses with fast exchange of first chloride ion with water molecule.

INTRODUCTION

In recent decades, complexes of Ru(III) are being intensively studied because of their potential use as antitumor (Keppler *et al*, 2003.) and antibacterial agents (Bolhuisa *et al* 2011.), their electrontransfer mediating properties (Turkusic *et al*, 2012), which qualifies them for the design of new electrochemical sensors, and because of their catalytic properties (Kahrović *et al*, 2003.). Ru(III) complexes with simple amides are poorly explored group of compounds, since the $RuCl_3x3H_2O$ catalytically cleaves amides, and only two crystal structures are reported (Judd *et al*, 1995; Levason *et al*, 1997). A number of other metal complexes with simple amides are known and they are neutral adducts obtained from metal salt in direct reaction with formamide (Yilmaz, and Topcu, 1997). Formamide as ligand has potential two donor atoms, but coordination through carbonyl oxygen is preferred. There are some reports in which the formamide acts as bridging ligand through carbonyl oxygen (Betz and Bino, 1988.).

The study of Ru(III) complexes with amides is of special interest, considering that amides contain peptide link in their structure, and study their interactions with the Ru(III) may give insight into the interaction of Ru(III) with proteins. Due the facts named above, Ru(III) complex with formamide was synthesized from hexachlororuthenate(III), in order to override catalytic effect of $RuCl_3x3H_2O$ on cleavage of amides.

EXPERIMENTAL

Materials

All chemicals are purchased from commercial sources and used without any purification with exception of formamide which is purified by distillation.

Preparation of the complex

Complex was prepared in reaction of $[RuCl_6]^{3-}$ with formamide in molar ratio of 1:10, in mixed solvent ethanol – water (10:1)

Starting compound $[(CH_3)_2NH_2]_3[RuCl_6]x[(CH_3)_2NH_2Cl$, which contains $[RuCl_6]^{3-}$ ion, was synthesized by literature procedure (Kahrović *et al*, 2003.) and used for synthesis without any purification.

Reaction was carried out by heating the reaction mixture under reflux at temperature 60 – 70 °C for several hours. Olive green product of synthesis was filtered out after cooling the reaction mixture to the room temperature.

The amount of 0.2 g (0.38 mmol) of $[(CH_3)_2NH_2]_3[RuCl_6]x(CH_3)_2NH_2Cl$ was suspended in 10 mL of absolute ethanol and mixture was refluxed for 2 hours at 60 – 70 °C. Volume of 0.15 mL (3.8 mmol) of redistilled formamide was added to the suspension and mixture was further refluxed for 3 hours at same temperature, afterwards 1 mL of water was added with additional reflux for 2 hours. System was cooled to the ambient temperature and olive green solid was filtered out. Yield 21,6%.

Instrumental methods

Synthesized compound was characterized by MALDI-TOF mass spectrometry, CHN analysis, IR and UV-VIS spectrometry. Mass spectrum was recorded on MALDI-TOF/TOF mass spectrometer (4800 Plus MALDI TOF/TOF analyzer, Applied Biosystems Inc.) equipped with Nd:YAG laser operating at 355 nm with firing rate 200 Hz in the negative ion reflector mode. CHN analysis was performed on Perkin Elmer 2400 Series II CHNS analyzer. The infrared spectra were recorded as KBr pellets on a Perkin Elmer spectrum BX FTIR System in the region 4000-400 cm^{-1}. UV/Vis spectra and hydrolysis were recorded on Perkin Elmer lambda 35 spectrophotometer.

RESULTS AND DISCUSSION

Novel neutral olive green complex was formulated as $[RuCl_3(HCONH_2)_3]x0,5C_2H_5OH$ based on CNH analysis and mass spectrometry results.

The synthesis was carried out in order to examine the possibility of obtaining a defined product in reaction of $[RuCl_6]^{3-}$ with formamide. Isolation of synthesized non ionic compound was triggered by addition of water molecules, and even though, the yield was pretty poor. Further investigation of the reaction between hexachlororuthenate(III) and formamide also has to improve yield of the product.

IR spectra of complex compared to IR spetra of formamide (Figure 1) showes shift of the stretching frequency ν(C=O) from 1681 cm^{-1} in free formamide to 1634 cm^{-1} in complex which indicates coordination of formamide to Ru(III) through carbonyl oxygen atom.

This assumption is also confirmed by shift of C–N bond stretching frequency ν(C–N) towards higher values of wave numbers, from 1311 cm^{-1} in free formamide to 1327 cm^{-1} in complex and δ(NH_2) shift from 1606 cm^{-1} in formamide to 1564 cm^{-1} in complex. Weak isolated absorption at 3737 cm^{-1} in spectrum of complex is assigned to isolated hydroxyl group which confirms that complex is isolated as ethanol solvate.

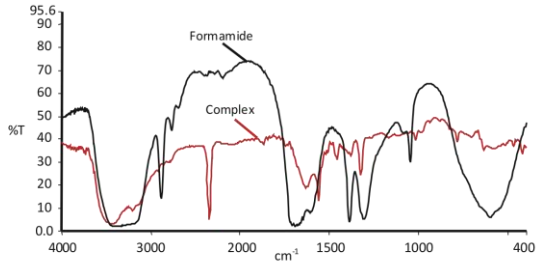

Figure 1: Comparative IR spectra of $[RuCl_3(HCONH_2)_3]x0,5C_2H_5OH$ and formamide.

UV-VIS spectrum of synthesized complex in water shows Cl→Ru charge transfer absorption at 301 nm, which is in accordance with literature data for chloro-Ru(III) complexes. Hydrolytic profile (Figure 2) shows that synthesized complex rapidly hydrolyses with fast loss of first chloride.

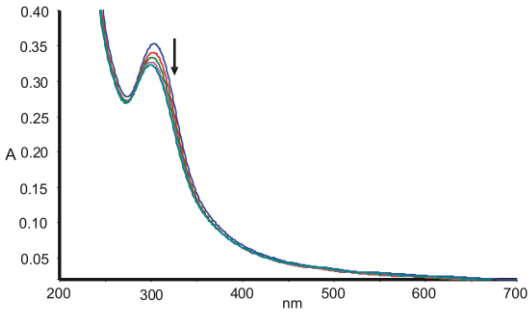

Figure 2: Hydrolytic profile of $[RuCl_3(HCONH_2)_3]x0,5C_2H_5OH$ in 20 minutes.

Hydrolytic profile of $[RuCl_3(HCONH_2)_3]x0,5C_2H_5OH$ does not show significant resistance towards hydrolysis compared with hydrolytic profile of $[RuCl_6]^{3-}$ which is in agreement with the fact that formamide is not ligand with strong coordination abilities, and stabilization of $[RuCl_6]^{3-}$ is moderate.

Analytical and spectral characteristics of the novel compound are given below.

Trichlorotriformamideruthenium(III): MALDI-TOF (*m/z*): $[M+K]^+$ calcd for $C_3H_9Cl_3N_3O_3Ru$, 382.8367; found, 382.8485.; Anal. calcd for $[RuCl_3(HCONH_2)_3]x0,5C_2H_5OH$: C 12.67, H 3.52, N 11.08. Found: C 13.14, H 3.31, N 11.49.; IR data for formamide (KBr, cm^{-1}) 1681 s [ν(C=O)], 1311 [ν(C–N)], 1606 [δ(NH_2)]; IR data for $[RuCl_3(HCONH_2)_3]x0,5C_2H_5OH$ (KBr, cm^{-1}) 1634 s [ν(C=O)], 1327 [ν(C–N)], 1564 [δ(NH_2)]; UV-VIS (H_2O) LMCT l_{max} 301 nm

CONCLUSIONS

Novel neutral complex of Ru(III) with formamide was synthesized from $[RuCl_6]^{3-}$ as starting compound. Based on analytical data complex was formulated as $[RuCl_3(HCONH_2)_3]x0,5C_2H_5OH$. Formamide is coordinated to Ru(III) through carbonyl oxygen. New synthetic pathway in which formamide complex with Ru(III) is synthesized from anionic octahedral complex

$[RuCl_6]^{3-}$ is significant and shows that $[RuCl_6]^{3-}$ can be used as good starting material for synthesis of Ru(III) complexes with amides in order to override catalytic effect of $RuCl_3x3H_2O$, as the most used starting compound for synthesis of Ru(III) complexes, on cleavage of amides.

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Summary/Sažetak

Sintetiziran je novi neutralni kompleks u reakciji $[RuCl_6]^{3-}$ sa formamidom u mješovitom rastvaraču etanol-voda (10:1). Dobivena maslinasto zelena supstanca karakterizirana je masenom spektrometrijom, CHN analizom, IR i UV-VIS spektroskopijom. Na osnovu eksperimentalnih podataka spoj je formuliran kao $[RuCl_3x(HCONH_2)_3]x0,5C_2H_5OH$. Relativna molekulska masa sintetiziranog spoja određema je MALDI-TOF masenom spektrometrijom kao adukt sa K^+ jonom i ima vrijednost 382.8285. IR spektar sintetiziranog spoja, u poređenju sa IR spektom formamida, ukazuje na koordinaciju preko karbonilnog kisika iz formamida, što je potvrđeno pomakom karbonilne apsorpcije sa 1681 cm^{-1} u formamidu na 1636 cm^{-1} u sintetiziranom spoju. UV-VIS spektar sintetiziranog spoja u vodi pokazuje LMCT apsorpciju centriranu oko 300 nm. Hidrolitički profil ukazuje da spoj podliježe hidrolizi uz brzu zamjenu prvog hloridnog jona molekulom vode.

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Total Phenolic Content and Antioxidant Capacity of Fruit Juices

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Abstract: The interest in polyphenolic antioxidants has increased remarkably in the last decades due to of their elevated capacity in scavenging free radicals associated with various human diseases. Previously, some fruits were shown to contain high antioxidant activities. Fifteen fruit juices were analyzed for total phenolic content and antioxidant capacity (oxygen radical absorbance capacity, ORAC). The total phenolic content (TPC) was measured by Folin-Ciocalteu assay and gallic acid used as standard. TPC varied from 7.3 mg GAE/100 mL for aloe vera juice to 71.8 mg GAE/100 mL for cranberry juice. The value of antioxidant capacity was determined by ORAC test, using 2,2'-azobis(2-amidino-propane) dihydrochloride as reactive species and Trolox as a standard. Obtained values were from 27.1 μmol TE/100 mL for aloe vera juice to 1271.8 μmol TE/100 mL for black currant juice. Results from the present study suggest further analysis on chemical composition of samples in order to identify compounds that might be responsible for antioxidant activity.

INTRODUCTION

Phenols are aromatic compounds containing one or several hydroxyl groups directly attached to the benzene ring. According to the number of hydroxyl groups, phenols are classified as dihydric, trihydric and polyhydric. By the year 2005, thousands of polyphenolic compounds have been isolated from plants (Prior, 1995). There are many spectrophotometric methods for the quantification of phenolic compounds in plant materials. Based on different principles, these methods are used to determine various structural groups present in the phenolic compounds. Spectrophotometric methods enable either the quantification of all extracted phenolics as a group (Swain, and Hillis, 1959; Price, and Butler, 1977; Earp et al., 1981), or the quantification of specific phenolic substances such as sinapine (Tzagoloff, 1963) or the sinapic acid (Naczk, et al., 1992). Spectrophotometric methods are also used in the quantification of a whole class of phenols such as phenolic acids (Price, et al, 1978; Mole, and Waterman 1987; Naczk, and Shahidi, 1989; Brune, et al, 1991).

Some of the most commonly used assay methods for phenolic compounds include the modified vanillin test (Price, et al., 1978), the Folin-Denis assay (Swain, and Hillis, 1959), the Prussian blue test (Price, and Butler, 1977) and the Folin-Ciocalteu assay (Maxson, and Rooney, 1972; Hoff, and Singleton, 1977; Earp et al, 1981; Deshpande, and Cheryan, 1987). The antioxidant capacity can be measured in pure substances as well as in mixtures of different samples of herbal and animal origin, such as plasma, blood, tissues homogenates of fruits and vegetables, juices and other foods. There are many methods

for measuring of total antioxidant capacity (AC), but in literature the most often cited are the following three: FRAP - Ferric Reducing Antioxidant Power (Benzie, and Strain, 1996), ORAC - Oxygen Radical Absorbance Capacity (Cao, and Prior, 1999), and TEAC - Trolox Equivalent Antioxidant Capacity (Rice-Evans, and Miller, 1994). Based on the reaction mechanism involved, major antioxidant capacity assays can be roughly divided into two categories (Huang, et al., 2005): hydrogen atom transfer (HAT) and single electron transfer (ET) reaction based assays. Most HAT-based assays monitor competitive reaction kinetics and the quantification is derived from the kinetic curves. Generally, these assays are composed of a synthetic free radical generator, an oxidizable molecular probe and an antioxidant. The aim of this study is to quantify the total phenolic content (TPC) and the antioxidant capacity (AC) in fresh juice of the following fruits: blueberry (*Vaccinum myrtillus* L.), cranberry (*Vaccinum macrocarpon* L.), black currant (*Ribes nigrum*), red currant (*Ribes rubrum*), red and white grapes (*Vitis vinifera* L .), red orange (*Citrus sinensis* L.), lemon (*Citrus limonia* L.), lime (*Citrus aurantifolia* L.), grapefruit (*Citrus paradisi* L.) kumquats (*Fortunella*), black chokeberry (*Aronia melanocarpa* L.), aloe vera (*Aloe vera* L.), apple (*Malus pumila*) and pomegranate (*Punica granatum*).

EXPERIMENTAL

Samples

Samples of blueberries, cranberry, black currant, red currant, red grape, white grape, red orange, lemon, lime, grapefruit, cumquat, commercial chokeberry, commercial aloe vera and pomegranate,were purchased from local markets.

Sample preparation

One mL of fresh juice from samples was diluted up to the volume of 25 mL. Part of solution was centrifuged at 15000 rpm for 20 minutes at 4°C. Supernatant solution was used for analysis. Also, non-centrifuged juices were analyzed.

Determination of total phenolic content

The total phenolic content (TPC) was determined by spectrophotometry, using gallic acid as a standard, according the method described by Singleton and Rossi (1965). Briefly, 0.2 mL of the diluted sample extract was transferred in tubes containing 1.0 mL of a 1/10 dilution of Folin-Ciocalteu's reagent in water. After waiting for 10 minutes, 0.8 mL of a sodium carbonate solution (7.5% w/v) was added to the sample. The tubes were then allowed to stand at room temperature for 30 min before absorbance at 743 nm was measured. The TPC was expressed as gallic acid equivalents (GAE) in mg/100 mL of fruit juice. The concentration of polyphenols in samples was derived from a standard curve of gallic acid ranging from 0.2 to 4 mg/L.

Oxygen Radical Absorbance Capacity (ORAC) Assay

The Oxygen Radical Absorbance Capacity (ORAC) assay measures the antioxidant scavenging function against peroxyl radical induced by 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH). Fluorescein is used as a fluorescent probe. The loss of fluorescence of fluorescein is an indication of the extent of damage from its reaction with the peroxyl radical (Cao, and Prior, 1999). The total reaction mixture of 100 μL of diluted supernatants of juices, 50μL solution of fluorescein (0.32 μM), and 1650 μL of water was incubated at 37 °C for 15 min. After the incubation, 200 μL of AAPH (320 mM) was added rapidly to start the reaction. The fluorescence was recorded every 5 min until relative fluorescence intensity of fluorescein was near to zero. Calibration of solutions of (±)-6-hydroxy 2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) (0.1; 0.25; 0.5; 0.75, and 1 μM) were carried out. The final ORAC values were calculated using a linear equation from calibrated curve. ORAC values were expressed as μmol Trolox equivalents (TE) per 100 mL of fruit juice.

RESULTS AND DISCUSSION

Total phenol content (TPC) in fruit juices was determined spectrophotometrically according to the Folin-Ciocalteu colorimetric method (Singleton & Rossi, 1965) using gallic acid as the standard. Maximum wavelength for blue colored complex was at 743 nm. After determination of the λ_{max} of colored complex the absorbances of all standards were taken to construct a calibration curve (Fig. 1).

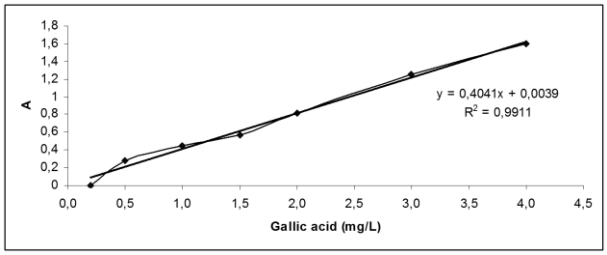

Figure 1: Calibration curve for gallic acid.

TPC was determined in 15 different fruit samples, first in non-centrifuged and then in centrifuged samples according to previously written procedure. As shown in Table 1 values for TPC varied from 6.16 to 71.76 mg GAE/100 mL. The highest TPC was in non-centrifuged (66.1 mg GAE/100 mL) and centrifuged sample (71.76 mg GAE/100 mL) of cranberry. The lowest TPC was in non-centrifuged (6.16 mg GAE/100 mL) and centrifuged (7.32 mg GAE/100 mL) sample of aloe vera.

Blueberries are a rich source of phenolic compounds such as phenolic acids and flavonoids. Literature value for TPC in blueberries ranges from 430 to 1990 mg GAE/kg of fresh fruit (Ehlenfeldt, and Prior, 2001). TPC values in blueberries measured in this work were 30.89 and 30.94 mg GAE/100 mL. For statistical analysis of the data t-test was used. The t-test showed statistically lower mean TPC values in non-centrifuged samples of nine fruits (cranberry, lemon, grapefruit, red orange, black chokeberry, black grapes, lime, apple and aloe vera) than in centrifuged samples ($p^{***}<0.001$). For the other six sorts of fruits (black and red currant, blueberry, kumquat, pomegranate and white grapes) the mean TPC values were higher in non-centrifuged than in centrifuged samples ($p^{*}<0.05$).

Measurement of antioxidant capacity (AC) was performed by manual ORAC method (Cao and Prior, 1999). Maximum of excitation (λ_{max}=485 nm) and emission (λ_{max}=520 nm) wavelengths were determined using trolox as a standard. After determination of wavelengths for excitation and

emission relative fluorescence intensity of all concentration of trolox was used to construct a calibration curve (Fig. 2).

Table 1: Total phenolic content in investigated samples.

Sample	***Non-centrifuged sample*** (mg GAE/100mL)	***Centrifuged sample*** (mg GAE/100mL)
Cranberry	**66.61**	**71.76**
Red currant	40.80	30.40
Black currant	37.20	36.72
Blueberry	32.89	30.94
Lemon	31.85	47.20
Grapefruit	30.60	45.12
Red orange	21.54	35.10
Black chokeberry	20.70	35.48
Black grapes	17.50	21.54
Kumquat	15.02	11.65
Lime	14.83	28.13
Pomegranate	12.23	10.22
White grapes	11.68	8.63
Apple	9.75	15.8
Aloe vera	**6.16**	**7.32**

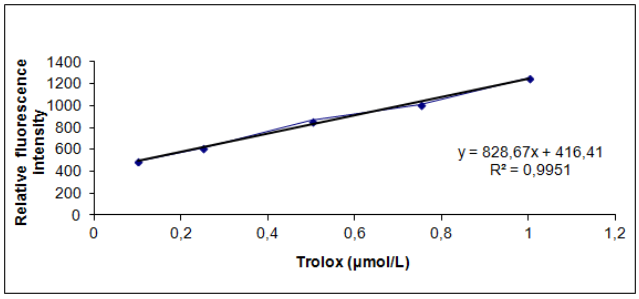

Figure 2: Calibration curve for trolox.

Determination of AC in 15 different fruit samples was carried out first for non-centrifuged and then for centrifuged samples with peroxyl radicals generated from AAPH and result are shown in Table 2. As shown in Table 2, there were big differences in antioxidant capacity between selected samples. The AC values varied from 27.07 to 1271.8 μmol TE/100 mL.

The highest value for AC was in black currant (1271.8 μmol TE/100 mL) and lowest in aloe vera (27.07 μmol TE/100 mL). For non-centrifuged sample the highest value for AC was in black chokeberry (1086.6 μmol TE/100 mL) and lowest in white grapes (30.82 μmol TE/100 mL). The t-test showed statistically significant higher mean AC values in non-centrifuged than in centrifuged samples of nine sorts of fruits (black chokeberry, apple, cranberry, pomegranate, blueberry, lime, lemon, aloe vera and red orange ($p^{**}<0.01$). For other six sorts of fruits (red and black currant, black and white grapes, kumquat, and grapefruit) the mean AC values were higher in centrifuged than in non-centrifuged samples, but no stastically significant difference was evident ($p>0.05$).

In a living system, phenolic compounds, some enzymes, peptides and vitamins serve as protection agents against oxidative damage caused by free radicals and are called antioxidants. Consumption of foods rich in this type of compounds have resulted in an increase in total antioxidant capacity (AC) in the blood plasma of people (Cao, & Prior, 1999; Sofić et al., 2005). As one potential source, plant phenols have primary antioxidant activity (Shahidi & Wanasundara 1992).

Table 2: Antioxydant capacity in investigated samples

Sample	***Non-centrifuged samples*** ORAC (μmol TE/100 mL)	***Centrifuged samples*** ORAC (μmol TE/100 mL)
Black chokeberry	**1086.60**	666.60
Black currant	500.80	**1271.80**
Red currant	422.60	540.50
Apple	389.40	196.40
Cranberry	379.70	336.60
Pomegranate	340.50	151.40
Blueberry	297.80	206.80
Lime	285.10	111.90
Lemon	223.10	125.90
Grapefruit	105.04	200.30
Aloe vera	81.35	**27.07**
Red orange	71.72	35.68
Black grapes	58.60	94.30
Kumquat	38.16	133.50
White grapes	**30.82**	31.10

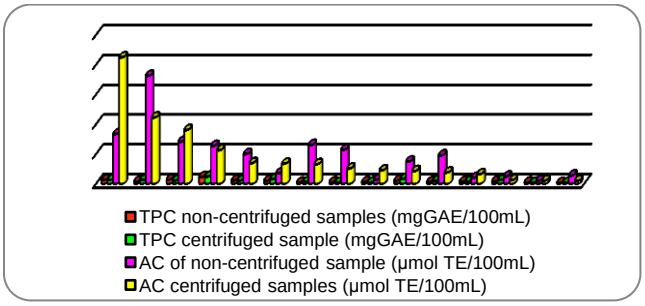

Figure 3: Total phenolic content and antioxidant capacity of fruit juices.

In summary, TPC and AC of 15 samples of fruit juices that is non-centrifuged and centrifuged was determined by the Folin-Ciocalteu method and ORAC assay with peroxyl radical generator. Black currant and black chokeberry showed the highest value of an antioxidant capacity but they have lower content of TPC than cranberry. Among all of this samples aloe vera showed lowest content of TPC and lowest value for AC.

CONCLUSIONS

Proteins residing in solutions of non-centrifuged samples increased the antioxidant capacity of those fruits. The influence of the preparation procedure on the total phenolic content and antioxidant capacity for each sample remains a subject for further research.

There is no linear corelation between the total phenol content and antioxidant capacity in neither the centrifuged nor in non-centrifuged samples. Our findings suggest that further studies should be conducted with non-centrifuged samples as such produce higher AC values.

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Summary/Sažetak

Interes za antioksidante polifenolske prirode se povećao zadnjih decenija zbog njihove sposobnosti hvatanja slobodnih radikala, povezanih sa različitim bolestima. Od ranije je već poznato da voće posjeduje visok sadržaj antioksidanata. Petnaest različitih voćnih sokova je analizirano na sadržaj ukupnih fenola i antioksidativnu aktivnost. Ukupni fenolski sadržaj određen je Folin-Ciocalteu metodom, uz upotrebu galne kiseline kao standarda. Vrijednosti variraju od 7.3 mg GAE/100 ml za sok od aloe vera do 71.8 mg GAE/100 ml za sok brusnice. Vrijednost antioksidativnog kapaciteta određena je ORAC testom, koristeći 2,2 '-azobis (2-amidino-propan) dihidrohlorid kao reaktivnu vrstu i troloks kao standard. Dobivene vrijednosti su od 27.1 μmol TE/100 ml za sok od aloe vera do 1271.8 μmol TE/100 ml za sok crne ribizle. Rezultati ovog istraživanja ukazuju na daljnju analizu hemijskog sastava uzoraka kako bi se identificirali spojevi koji bi mogli biti odgovorni za antioksidativnu aktivnost.

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The Impact of the Content of Lead Oxide on the Porosity and Volume of the Pores, Paste and Active Mass of the Positive Electrodes-Pasted Flat Plates of Lead-Acid Batteries

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Abstract: Porosity and the volume of the pores of active mass of lead-acid layout battery are one of the key factors that determine the capacity and their lifetime. Increase of pore diameter enables flow of sufficient quantitiy of electrolyte into the bulk active mass and, as a consequence, enables its maximum conversion. Smaller diameter of the pores in the same volume of active mass provides a larger surface area which has again a consenquence of higher capacity for the ephemeral dischargings, but also faster filling in of the pores with the compenents of electrochemical reaction, which in turn reduces their lifespan. The aim of this work was to consider the impact of different content of the lead oxide in lead powder as the main component in the production of these batteries regardings this phenomenon.

INTRODUCTION

Basic characteristics due to which lead baterry surpasses other chemical sources of electricity are high energy performances and relatively simple and developed technology of production. These high energy characteristics are influenced by the adequate structure and composition of the active mass that provides water supply and shunt of the electricity on each point of platinum. The last processes take place in two stages.Charge transport in solid porous phase is achieved by electron movement; in the pores at the plate surface, charge transport is maintained by ions of the electrolyte. Good organization of these processes requires the necessary presence of an optimal relationship between the solid phase and its pores. During filling in and emptying, the solid porous mass is changing due to its composition and weight, and the pores are doing the same due to its volume and diameter.

In order to have a long battery life time, the structure of active mass has been improved, which ensured strength and electrical conductivity of the porous mass, availability of ions of the solution in each point of platinum and reversibility of the process of charge-discharge (Pavlov 2011). There is a series of operations through which the production of positive electrodes of the lead battery goes, starting from the choice of its granulation and its phase composition, preparation of the paste with a certain phase composition and density, ripening of the paste and finally building the structure of the active mass and organization of the components duting the formation.

During the production of electrodes there are certain tolerances defined by technology in terms of quality of powdera, pastes, ripening and formation of the active mass. All this can significantly affect the quality of the battery and especially if is in use one or the other limit of tolerance in regarding parameters.

Active mass represents very porous electrode which is formed by precisely defined conditions from the various phases of the initial paste. Having in mind that different components composing the initial paste which are characterized by different physical attributes (shape, diameter of the particles, stoichiometric composition, electrical conductivity, oxidation rate), it is normal to

expect different physicochemical attributes of the active mass.

On the basis of electronic-microscopic observations of the active mass (Pavlov, Bashtavelova, 1984) the structure of the active mass is determined. The smallest element are separated PbO2 crystals and they are grouped into porous agglomerates consisting of a sequences of single crystals. Through grouping of crystals the pores are formed and the combination of crystals and micropores in one agglomerate builds microstructure of positive active mass, which represents the first structural level. Agglomerates of different shapes of diameter and microstructures are associating with each other in a spatial skeleton. It contains macropores that form channels which interupt whole cross section of the platinum. The combination of skeleton of the macropores formes microstructure of positive active mass, which forms a second structural level. The idea is that macropores found in skeleton level structure serve as a major transportation system for the movement of ions between the electrolyte volume and agglomerate inside of the platinum. Micropores constitute the main part of the area on which cirulates the electrochemical reactions, and the macrostructure serves as one mechanical skeleton that conduct electricity and form ionic transport system. (Pavlov, 1984; Pavlov, St. Ilija, Papazov, Bashtavelova, 1984)

The structure of the active mass made with lead powder changes during exploitation and, after more than 25% of its life span, the content of large pores is increased. Acording to some authors, this increase is consequence of oxygen evolution at the PbO_2 surface due to the overfilling. Platinums made of synthetic 4PbOPbSO4 when passes 25% of the life span does not contain such deep pores and are quite compact and on its surface the swelling is observed.

The structure of the positive active mass (Pavlov, Papazov, 1986) after the formation is similar to the structure of the initial paste.

Many authors have studied the processes that occur during the preparation of pastes, method of mixing of lead dust, acid and water, the duration of the mixing process, the temperature at which this process takes place, and drying processes and the formation of platinum, with a range of presented facts about ways of running of chemical reactions (chemistry) during these processes and factors that influence the formation of the structure of active mass. As during the tests examined in this work, all the active mass have been simultaneously treated and were subjected to the same parameters during the preparation of differences midst discrepancies of mentioned influenced are eliminated and omitted.

EXPERIMENTAL

The amount of 2 kg of the paste was prepared in the laboratory mixer (Scheme 1). In the mixer the leaded dust is first added (Table 1) and then a solutions of sulfuric acid and water, alternately in equal installments. The recipe for paste with its characteristics was given in Table 2. Lattice lubricated with paste (5.5% Sb, As 0.14%) lead alloy capacity of 36Ah were subjected to standard regime of ripening, drying and aftewards, the forming. Determination of total volumes of the pores and their distribution is made on the mercury pores meter Autopores 9200. Probations of active mass were observed by scanning electron microscope Joel T200.

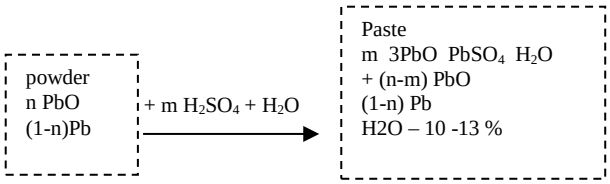
Table 1: Gradings analysis of lead powder.

	63%PbO			67%PbO			72%PbO			75%PbO			79%PbO		
	Tg	(%)	∑%	Tg	(%)	∑%	Tg	%	∑%	Tg	(%)	∑%	Tg	(%)	∑%
+125	0.12	2.4	2.40	0.10	2.0	2.0	0.20	4.0	4.0	0.26	5.20	5.20	2.60	2.60	2.60
-125 +90	0.08	1.6	4.00	0.10	2.0	4.0	0.14	2.8	6.8	0.09	1.80	7.00	1.80	1.80	4.40
-90 +63	0.25	5.0	9.00	0.16	3.20	7.2	0.26	5.2	12.0	0.12	2.40	9.40	3.40	3.40	7.80
-63 +40	0.98	19.6	28.6	0.67	13.4	20.6	0.61	12.2	22.2	1.55	31.1	40.4	24.2	24.20	32.0
-40 +32	0.19	3.8	32.4	0.06	1.20	21.8	0.24	4.80	27.0	0.10	2.00	42.4	0.60	0.60	32.6
-32 +0	3.38	67.6	100	3.91	78.2	100	3.55	73.0	100	2.12	57.6	100	67.4	67.4	100

The conditions of making of the paste are such, that all the sulfuric acid reacts with PbO and as the result 3PbOPbSO4H2O is formed during which process the part of non-reacted PbO and metallic lead remain in the paste which does not participate in the reaction. Phase composition of the paste - Scheme 1 (Ratio 3PbOPbSO4H2O, PbO, Pb) depends on the content of PbO in powder and quantities of used acid. (Pavlov, 1988).

Table 2: The recipe for positive paste with density and consistency.

% PbO	addid to 1 kg powder H_2SO_4 (g)	H_2O (ml.)	Paste densyti g/cm^3	Consist-ency circles
63	45.37	100	3.85	20
	60.49	88	3.63	20
67	45.37	83	3.95	20
	60.49	68	3.80	20
72	45.37	75	4.00	20
	60.49	55	3.81	20
75	45.37	80	3.98	20
	60.49	60	3.90	20
79	45.37	72	4.00	20
	60.49	58	4.03	20



Scheme 1: Phase composition of the paste.

The amount of water added to create a paste was within the limits of 10-13%. It determines density and consistency of paste. Paste consistency was maintained by constant (20 laps) changings of the amount of added water. In this way pastes with different density were obtained.

Table 3: Chemical analysis of paste and active mass.

Paste (%)				Active mass (%)				
PbO	Pb	PbO	$PbSO_4$	Pb	PbO	$PbSO_4$	PbO_2	Pb^{2+4+}
63	10.64	73.76	15.24	0.95	19.22	11.36	68.98	88.02
67	7.44	77.50	14.52	0.40	13.47	11.62	74.55	88.02
72	1.73	82.84	14.52	0.15	12.51	12.20	75.10	87.61
75	1.48	83.11	14.52	0.15	13.58	10.90	74.42	88.00
79	3.47	81.24	15.24	075	15.18	9.59	74.42	84.01

RESULTS AND DISCUSSION

As the paste was made with constant consistency, with the parameter that defines the relationships between the particles under dynamic conditions, the presented pastes have different densities. Density dependence of paste from the content of PbO in powder at constant consistency is given in Table 2. The table shows that when making pasta with a variety of PbO in order to maintain the consistency of paste constant with increasing % PbO in powder, the density of the paste has been increased. Also, from the same table is visible that in order to maintain the consistency of paste with an increase of content of acid, density of paste decreases. The results in the agreement with literary data listed in (Illiev, Pavlov 1974.)

Determination of total volume of pores volume and their distribution per diameter in the positive active mass was made on the mercury pores meter AUTOP 9200, Micrometritics.

The selected probe was placed in a small container, under vacuum filled with mercury and the pressure was gradually increase. During given amount of pressure certain quantity of mercury fills the pores of a given radius, the relationship between pressure (p) and radius (r) is given by the following equation:

$$r = \frac{2 \cdot \sigma - \cos\varphi}{p}$$

Where is: σ - surface tension of mercury, a cos φ its wetting angle. Volume of the pores of a given radius is determined by the amount of mercury that fills the pores. These measurements make it possible to measure the total porosity of the sample expressed in (cm3 / g) materials, the total surface of the pores (cm2 / g) and distribution of the pores per radius. In the calculations applies cylindrical model of the pore. The accuracy of the method is 5-8%.

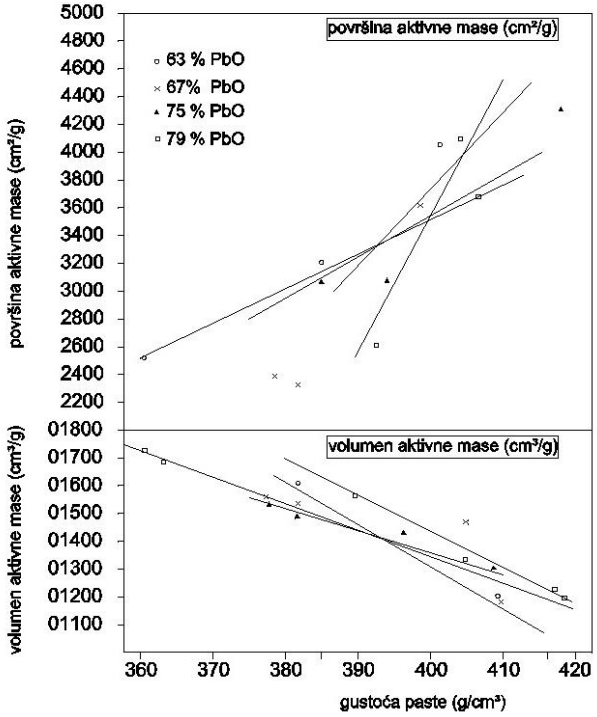


Figure 1: Surface area and volume of the paste, depending on the density of pastes.

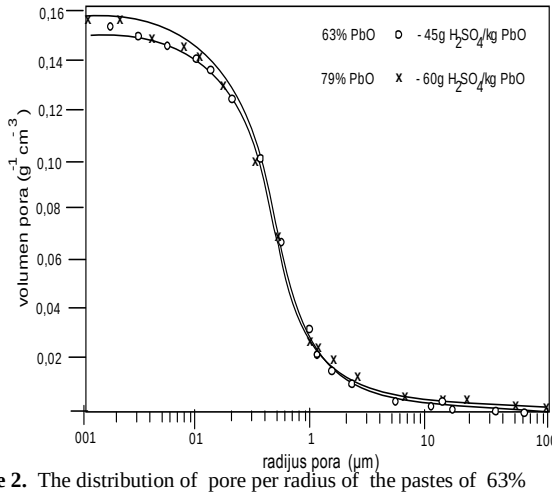


Figure 2. The distribution of pore per radius of the pastes of 63% and 79% PbO in powder with 45 and 60g H_2SO_4/kgPbO.

This independence can be explained through this - the diameter of PbSO4 crystals $3PbO \cdot PbSO_4 \cdot H_2O$ is almost the same as the PbO particle, so increasing the content of three-basic sulfate at the expense of decreasing of the content of PbO does not affect the volume and surface of the pores. These parameters are determined mainly with density of the paste. In Picture 1 the dependence of the surface and volume of the pores volume from density of the obtained pastes given in Table 3 is given

From the Figure 1 it is visible that with increasing the density of paste, there is a general tendency of decrease of the volume of pores and increase of the total surface of pores of the paste. This can be explained by the fact that with increasing the density of the paste relative amount of small pores increases and the surface is defined precisely by the quantity of small pores. In the field of density technology, the volume of pores is within the limits from 0.12 to 0.13 g/cm3.

In Figure 2 the distribution of the volume of pores per radius, for pastes prepared with 63 and 79% PbO in powder with 45 and 60g H2SO4/kg PbO is given. It can be seen that for the pastes made from various oxides there is no difference in the distribution of the pores per radius and total volume of pores of the paste. The curve shows that the quantity of pores is very small with a radius larger than 10μm and basic pores have a radius in the range 0.1 - 2μm. Inflexed point of curve of the distribution gives the middle radius of the pores that are located, in the tested paste, in the range from 0.4 to 0.5 um.

Poregrammes of other tested pastes are not different from poregrammes presented in figure 2.

All pastes in the tested range of composition, reagarding lead oxide powder and sulfuric acid, crystallise as $3PbO{\cdot}PbSO_4{\cdot}H_2O$, but do not have a clearly defined crystalline form (iow. create crystals without forms). These small crystals are visible in Figure 3. Lengths are 2.3 width are about 2 -0.5 μm and are grouped into the crystalline agglomerates. Among crystals of the paste pores are created with average diameter of 0.5 μm which correspondes to data obtained by analysis through pore-meters, Figure 2.

Agglomerates among themselves create large pores, as shown in Picture 4. Their length is about 10μm, and their volume is about 0.005 cm3 / g (Figure 3). So, the total porosity is determined by small pores, which volume is of over 80%, and not the big ones, which means that they are within much smaller quantity.

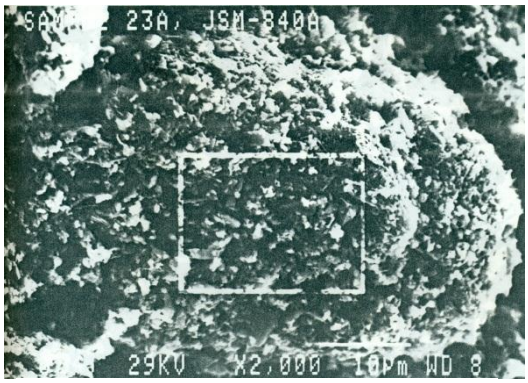


Figure 3: Structure paste made from 63% PbO in powder and 45 g H_2SO_4/kg PbO.

Tests have shown that changes in the content of PbO in powder makes no major changes in the characteristics of the paste, and paste made in such wide range interval of under the rust treatment and leaded dust, can be used in the technology manufacture of batteries.

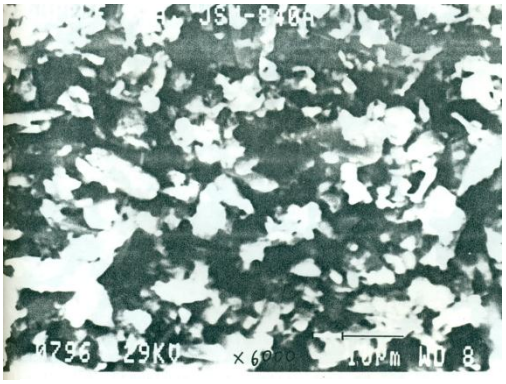


Figure 4: Structure paste made from 79% PbO in powder and 60g H_2SO_4/kg PbO.

Poregrammes of established active mass are given in Figure 5. Poregrammes are given only for two active mass, as all the others are very similar. These poregrammes talks about a small reduction in overall volume of the pores. The increase of the middle radius of the pores from 0.4 -0.5 μm of the paste and on 0.8 - 0.9 μm for the active mass was also observed.

Figure 6 shows the dependence of the volume of the pores and the pore surface of the active mass in regards the density of the paste.

There is no visible certain dependence of the pore surface of the active mass in regards to the density of pastes, and there is a tendency of reduction of the pores volume of the active mass with increase of density of the paste. The results of measurements of the volume and the surface area of pores of all pastes says that there is no specific dependence of these parameters on the content of PbO in powder and the amount of sulfuric acid used for making paste.

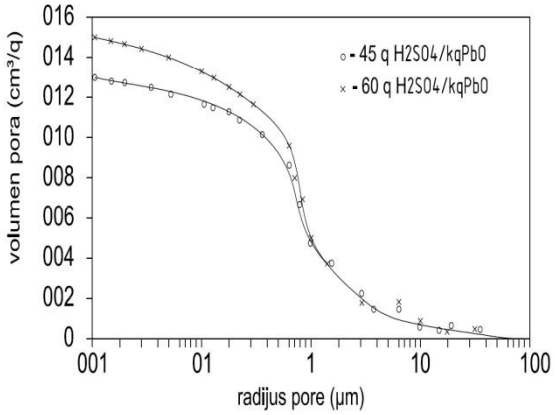


Figure 5: The distribution of pore per radius of the active mass made of 63% and 79% PbO in powder with 45 and 60g H_2SO_4/kgPbO.

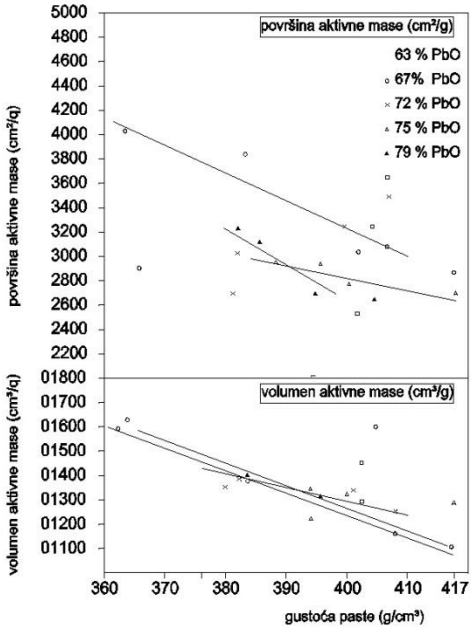


Figure 6: Surface area and volume of the active mass, depending on the density of pastes.

That independence can be explained by the fact, as proved by the results of other researchers, that thediamatres of the particles of PbO and $3PbO \cdot PbSO_4 \cdot H_2O$ are approximately the same and changing relations between the two phases of the paste does not affect the change of porosity.

CONCLUSIONS

These results suggest that changes in the content of PbO within leaded powder during the testing interval (63% - 79%) does not affect the structure and porosity of the paste, regardless the change of the phase composition of the paste. Structure and porosity of the paste compounds by shape and diameter are approximately equal and thus changes of the relationship of these two phases, which means changes in the phase composition of the paste, shows no effect on the structure and porosity of various paste.

The structure and porosity of the active mass does not change with the changing content of PbO in powder. As shown by other studies the structure of the active mass determines mainly the structure of the starting paste.

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Summary/Sažetak

Porozitet i volumen pora aktivne mase olovnih namaznih akumulatora jedan su od ključnih faktora koji određuju kapacitet i njihov životni vijek. Povećanjem dijametra pora ostvaruje se mogućnost pristupa dovoljne količine elektrolita u unutrašnjost aktivne mase a time i njeno maksimalno iskorištenje. Manji dijametar pora za isti volumen aktivne mase obezbjeđuje veću površinu što je posledica opet veći kapacitet za kratkotrajna pražnjenja, ali i brže popunjavanje pora komponentama elektrokemijske reakcije, što opet smanjuje njihov životni vijek. Razmotrimo utjecaj različitog sadržaja olovnog oksida u olovnom prahu kao osnovne komponente u proizvodnji ovih baterija na navedene pojave.

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Effect of Plant Nutrients on Antiradical Activity of *In Vitro* Cultivated Broccoli (*Brassica oleracea* L. var. *italica* Plenk.)

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antioxidative activity

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Abstract: Environmental conditions may have impact on plant metabolism, especially on secondary metabolism. As a result of different stress circumstance, plants have developed different protective mechanisms and major one is production of secondary metabolites. Plant growth conditions could be controlled and modified in *in vitro* plant culture, which usually results in higher or lower contents of secondary metabolites. We have established a rapid protocol for *in vitro* germination and cultivation of *Brassica oleracea* L. var. *italica* Plank. Three, ten, twenty and thirty days old seedlings, cultivated on three different Murashige-Skoog (MS) media, as well as two types of spontaneously induced calli were used for extraction. Ethanolic plant extracts were tested for their antioxidative potential using 2,2'-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging method. Extracts from three days old seedling demonstrated the highest antioxidative potential. On the other hand, extract of broccoli seedlings cultivated on basal MS medium have shown prooxidative properties that can be contribute to prooxidative properties of some unknown component in the presence of free transition metal ions, the type of oxidizable substrate in use, as well as to the biological environment in which they act.

INTRODUCTION

Oxidative stress, caused by reactive oxygen species (ROS), is one of the main cause of many pathological conditions of the human organism. At the molecular level, ROS induce several types of DNA damage (Halliwell and Aruoma, 1993). There are several ways to protect the organism from the harmful effects of "active" metabolic oxygen and one of them include antioxidant components. Antioxidant compounds represent a group of protective agents and defense mechanisms, whose role is regulation of cell redox state. Although almost all organisms possess antioxidant mechanisms to prevent and repair oxidative damage, often these endogenous mechanisms are insufficient for the prevention of oxidative stress and the diet should be supplemented with additional quantities of exogenous antioxidants. In this connection it is noteworthy that many plant species contain various components that possess antioxidant activity, such as vitamins C and E, β-carotene and polyphenols (Diplock, Charleux, Crozier-Willi *et al.*, 1998). For all these reasons, plant species are very important in the human diet. Epidemiological studies point out that consuming large amounts of antioxidants from fruits and vegetables could prevent carcinogenesis in many human and animal tissues (Bonnesen, Eggleston, Hayes, 2001). Also it has been shown that aqueous and ethanolic extracts of *Brassica oleracea* L. var. *italica* Plenk. (Brassicaceae), have strong antioxidant properties (Čakar, Parić, Maksimović *et al.*, 2011), and are very effective in scavenging superoxide anion and hydrogen peroxide (Gülcini, Sat, Bezdemir, *et al.*, 2004; Eberhardt, Kobira, Keck *et al.*, 2005). Elicitation of plant tissues in *in vitro* conditions is a valuable technique to study secondary metabolite production. Elicitation is useful technique for

induced production of secondary metabolites and therefore plant growth and development will be suppressed (primary metabolism-growth and development is decreased when secondary metabolism is induced). Secondary, metabolites are known to have a prominent antioxidant activity. Consequently, modification of plant growth conditions could affect antioxidative capacity of plant extracts.

The objective of our study was to investigate the influence of plant growth regulators on morphogenesis and antiradical activity of broccoli extracts. Concerning this, particular highlights to the influence of the type and concentration of plant growth regulators on plant development and antioxidative activity should be made.

EXPERIMENTAL

In vitro culture of broccoli

To establish *in vitro* tissue culture of broccoli, commercially purchased seed (*SEMENTI Franchi SpA Grassobbio-BG–Italy*) has been used. Sterilization of seeds was carried out in aseptic conditions, in absolute alcohol for one minute and 20 minutes in 15% sodium hypochlorite. Seeds were then rinsed in sterile distilled water and placed on MS (Murashige and Skoog (Murashige and Skoog, 1962) medium supplemented with appropriate combinations and concentrations of plant growth regulators. Three types of MS media was used. The first MS media used was basal MS medium (WH). Second (H) was MS medium supplemented with 0.1 mg/L 6-benzylaminopurine (BAP) and 0.1 mg/L indol butyric acid (IBA). The third one (3H) was MS medium with 0.5 mgL BAP, 0.2 mg/L IBA and 0.1 mg/L gibberellic acid (GA_3). Seedlings collected at third, tenth, 20th and 30th day, cultivated on three MS media, were utilized for extraction. Additionally, spontaneously formed calli on H and 3H media were also used for extraction.

Prepartion of extracts

Plant material (2 g) was subjected separately to Soxhlet extraction with 96% ethanol. Each extracts were filtered and concentrated in rotary evaporator at approximately 40°C. Finally, extracts were sterilized filtrating throughout syringe filters with 0.20 μm and stored at +4°C.

Antioxidative activity measuring

Antioxidative activity of extracts was determined using slightly modified 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) scavening radical method (Kulisic, Radonic, Katalinic *et al.*, 2003). Extracts were diluted to the three different concentrations (1:1, 1:3, 1:5) and aliquot of each extract solutions (100 μL) was mixed with 3 mL of 0.001% $DPPH^{\bullet}$ dissolved in absolute ethanol. The reaction of scavenging $DPPH^{\bullet}$ was carried out at room temperature in the dark for 30 min. Blank was a 100% ethanol and thymol was used as a positive control. The radical-scavenging activity of the tested samples, expressed as percentage inhibition of DPPH, was calculated according to Yen and Duh (1994).

$$\%Inhibition = \frac{(A_0 - A_t)}{A_0} \cdot 100$$

where A_0 is absorbance of the control at t = 0 min, A_t absorbance of the antioxidant at t = 30 min.

Statistical analysis

Experimental results were represented as the mean value of the three replicates with standard deviation (SD). Shapiro-Wilk test showed that data were not normally distributed, so Kruskal-Wallis test was used for testing differences between means of antioxidative activity of broccoli extracts cultivated at different media types and at different growth stage. One sample *t*-test was used for testing differences between means of antioxidative activity of broccoli extracts and positive control. Differences were considered significant at $p < 0.05$. For all analysis MedCalc® Version 10.4.0.0 software was used.

RESULTS AND DISCUSSION

In vitro culture of broccoli

Germination of *B. oleracea* var. *italica* shoots was achieved in all three types of MS media. After 20 days some differences in plant growth and development of *in vitro* seedlings have been observed, which could be correlated with different combinations and concentrations of exogenously added phytohormones. Explants cultivated on the WH and 3H media were significantly longer than shoots cultivated on H medium (Figure 1). A large number of lateral shoots were recorded on the explants cultivated at all three media. Many authors emphasize the influence of proper cytokinin/auxin combination on the formation of adventitious shoots in *in vitro* conditions (Lazzeri and Dunwell, 1986; Msikita and Skirvin, 1989). As an optimal combination for the lateral shoot formation in broccoli, authors indicate combination of 0.1 mg/L BAP and 0.1 IBA mg/L. This combination was favourable for lateral branching in our study also. Calli were induced on plantlets roots cultivated at the media with the addition of growth regulators (H and 3H). It is well known that the presence of cytokinins and auxins in the medium often caused callus formation (Widiyanto and Erytrina, 2001). Rhizogenesis was successfully obtained when shoots were cultivated in all three types of MS media. The roots were visible after twenty days.

Figure 1. Effect of different combinations and concentrations of plant growth regulators on growth of broccoli; WH – MS medium without hormones, H – MS medium with 0.1 mg/L BAP and 0.1 IBA mg/L and 3H – MS medium with 0.5 mgL BAP, 0.2 mg/L IBA and 0.1 mg/L GA_3.

Antiradical activity of broccoli

It is well known that plant growth regulators affect growth and development, and therefore they have an impact on the production of secondary metabolites. Research studies suggest that the addition of auxins in medium could increase the production of glucosinolates in broccoli, which have strong bioactive properties [Pasquali, Goddijn, De Waal *et al.*, 1992; Zhang, Li, Tang, 2005). Three days old broccoli sprouts exhibit the strongest antiradical activity regardless of the MS media type (Figure 2).

These extracts showed significantly stronger antiradical activity than thymol (t_{WH} = 6.315, p = 0.0032; t_H = 4.53, p = 0.011; t_{3H} = 5.364, p = 0.006). On the other hand, extracts of broccoli seedlings cultivated for ten days on H medium (428.57 mg/mL) and twenty days on H3 medium (434.48 mg/mL) showed very low antioxidant potential. Statistical analysis reported that the three-day-old seedlings shown significantly higher activity than plantlets cultivated for a longer period (H = 10.92, p = 0012). Antioxidant potential of broccoli extracts was published in several papers, and has shown that broccoli extracts have a strong potential for free radicals scavenging. Gülcini, Sat, Bezdemir, *et al.* (2004) examined the antioxidant properties of aqueous and ethanolic extracts from broccoli flowers. These authors reported that both types of extracts showed high antioxidant potential. Piao et al. (Piao, Kim, Yokozawa *et al*., 2005) isolated two active compounds (1.2-disinapoylgentiobiose and 1-sinapoyl-2-feruloylgentiobiose) from broccoli extracts. Our results showed that antiradical activity of both compounds is lower (5.18 mg/mL and 7.52 mg/mL, respectively) compared to antiradical activity of three days old broccoli obtained in our study. Antioxidant potential of broccoli may be directly related to the total amount of phenolics and flavonoids presented in the extracts (Dong-Jiann, Chun-Der, Hsien-Jung, *et al*., 2004; Moreno, Carvajal, Lopez-Berenguer, *et al*., 2006).

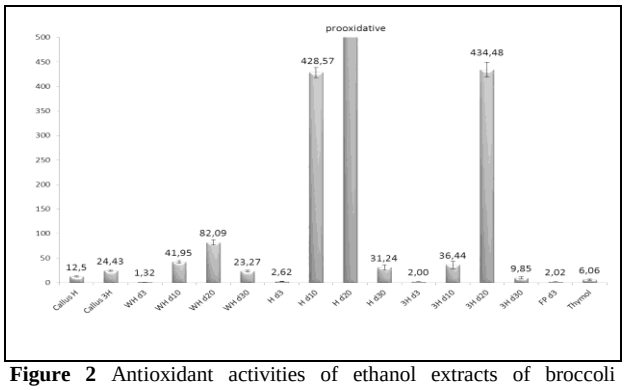

Figure 2 Antioxidant activities of ethanol extracts of broccoli cultivated at three different types of MS media.

In accordance with our results, it can be considered that the amount of phenolic content is higher in younger broccoli shoots. On the other hand, no statistically significant differences were recorded in the antioxidant potential of shoot extracts which origin from different types of MS media (H = 0.63, p = 0.83). Antiradical potential of broccoli extracts measured by DPPH method colud not be attributed to the various combinations of phytohormones. Ethanolic extract form 20 days old broccoli sprouts from H medium, showed prooxidative activity. Prooxidants increased the concentration of active oxygen and free radicals and encourage the growth of neoplastic cells. Hu, Zhang, Kitts (2000) suggest that the prooxidative activity of extracts can be due to the effects of plant phenolics in the presence of some transition metal ions. Similarly, Azuma, Ippoushi, Ito *et al.* (1999) showed that prooxidative properties of some extracts may be attributed to ascorbic acid, which in the presence of transition metal ions generates free radicals. Also the type of substrate used for oxidation, as well as the biological environment in which they act could alter oxidant/prooxidant activity of plant extracts (McGorum, Fry, Wallace *et al.*, 2000).

CONCLUSIONS

We have demonstrated in this study the effects of growth regulators on morphogenic response of broccoli cultivated *in vitro*. Appropriate combinations and concentrations of plant hormones result in higher yields of plant biomass, that is of great importance in plant mass propagation. Modification of growth conditions often results in changes of secondary metabolites production. In our work correlation between growth regulators and antiradical activity of extracts of broccoli has not been established. On the other hand, the radical DPPH$^{\bullet}$ test showed strong antiradical activity of three days old broccoli seedlings, regardless of the medium type. Presented results are preliminary in character, and further studies could be directed on the identification of specific bioactive compounds in broccoli extracts by HPLC-DAD-MS analyses.

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Summary/Sažetak

Uslovi spoljašnje sredine modificiraju matabolizam biljaka, pogotovo produkciju sekundarnih metabolita. Kao odgvor na stres izazvan promjenama u spoljašnjoj sredini biljke su razvile različite zaštitne mehanizme, a jedan od glavnih je produkcija sekundarnih metabolita. U kontrolisanim *in vitro* uslovima moguće je manipulisati rastom i razvićem biljaka, što najčešće rezultira povećanjem ili smanjenjem proizvodnje sekundarnih metabolita. U provodenom istraživanju, uspostavili smo brzi protokol za isklijavanje i uzgoj izdanaka vrste *Brassica oleracea* L. var. *italica* Plank. u *in vitro* uslovima. Tri, deset, dvadeset i trideset dana stari izdanci, kultivirani na tri različite Murashige-Skoog (MS) podloge te dvije vrste kalusa su korišteni za ekstrakciju. Za određivanje antioksidativnog potencijala petnaest etanolnih ekstrakata korištena je 2,2 '-difenil-1-pikrilhidrazil (DPPH$^{\bullet}$) radikalaska metoda. Ekstrakti tri dana starih izdanaka su imali najveći antioksidativni potencijal. S druge strane, ekstrakti izdanaka kultiviranih na osnovnoj MS podlozi su imali prooksidativnu aktivnost, koja bi se mogla pripisati prooksidativnim svojstvima nekih komponenti u prisustvu slobodnih iona prelaznih metala, tipa korištenog supstrata ali i biološkim uslovima u kojima dolazi do inteakcija.

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Theoretical Studies of Structures and Thermodynamic Parameters of Melatonin and its Metabolites: N1-Acetyl-N2-formyl-5-metoxy kynuramine and N1-Acetyl-5-metoxykynuramine

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Abstract: Melatonin, a neurohormone is well known regulator of a number of physiological processes. In addition, numerous studies both *in vitro* and *in vivo* showed a strong antioxidant and free radical scavenging activity usually considered as a result of direct melatonin reactivity. However, two major metabolites of melatonin, N^1-acetyl-N^2-formyl-5-metoxy kynuramine (AMFK) and N^1-acetyl-5-metoxykyunaramine (AMK) also showed strong free radical scavenging activity, but towards different biological free radicals. Melatonin oxidation mechanism in a complex biological environment has been studied at different conditions but still is partially understood. Reactivity of the molecule is always governed but its electronic properties and kinetic and thermodynamic stability. Thus, we performed theoretical calculations using Density Functional Theory (DFT) at with B3LYP/6-31G* basis set to calculate geometries, atomic charges and thermodynamic parameters for all three molecules. Semi-empirical calculations at PM1 level are also performed and compared with DFT data. Calculated atomic charges showed that nitrogen atoms as the most possible sites for interactions with electrophilic species such as free radicals. Oxygen atom in metoxy group also shows pronounced negative atomic charge. The most stable molecule is AMFK, followed by AMK and melatonin respectively. This trend can partially explain high melatonin reactivity and its fast decomposition in biological systems**.** Obtained values calculated at semi-empirical and *ab inition* levels are significantly different implying that conclusions based on calculations done at lower levels of theory can not be used as reliable when explaining experimental data.

INTRODUCTION

Melatonin is a neurohormone synthesized from the amino acid tryptophan and secreted by the pineal gland in the brain (Gastel, Roseboom, Rinaldi, 1998). It is involved in a number of biological and physiological regulatory mechanisms including circadian rhythm, ovarian physiology, blood pressure regulation, retinal physiology, seasonal reproduction, and immunity (Claustrat, Brun, Chazot, 2005, Jonas, Garfinkel, Zisapel, *et al*., 2003). Its synthesis and release are stimulated by darkness and suppressed by light (Zeman, Dulkova, Bada, *et al.*, 2005 Brzezinski, 1997, Pangerl, Pangerl, Reiter, 1990). Melatonin is considered to be a potent anti inflammatory reagent in both in vivo and in vitro (Ochoa, Vilchez, Palacios, et al., 2003). Resent data showed the inhibitor activity of melatonin on peroxidases catalyzed formation of hypohalous acids, responsible for host tissue injury. (Galijasevic, Abdulhamid, Abu-Soud, 2008, Lu, Galijasević, Abu-Soud,.2008). Antioxidative ability of melatonin is based on its role as a scavenger of reactive oxygen species including hydroxyl radical, superoxide ion,

peroxy radicals, singlet oxygen, nitric oxide, peroxynitrate and its metabolites.(Reiter, Guerrero, Garcia, et al., 1998) Ximenes, Silva, Rodrigues, et al., 2005) It plays an important role in protecting cell membranes from lipid peroxidation, neutralizing hydroxyl radicals and may bind to DNA, promoting further protection beyond antioxidant activity. The oxidized form of melatonin, N^1-acetyl-N^2-formyl-5-methoxynuramine (AFMK), is too free radical scavenger (Ximenes, Silva, Rodrigues, et al.,2005). Besides it beneficiary protective role, recent data showed that melatonin exhibits pro-inflammatory role at early phase of inflammation but switches to an antioxidant activity in a chronic inflammatory phase. (Radogna, F., Diederich,M., Ghibelli, L. 2010).

Number of studies compared reactivity of melatonin, AMFK and AMK showing a different susceptibility towards different free radicals. Despite the known general mechanism of melatonin catabolic pathway, different catabolic products and amounts have been detected in a different organs and tissues. Besides the availability of enzymatic and nonenzymatic reactants involved in formation of AMFK and AMK, usually considered to be major melatonin metabolites, stability and electronic properties of these compounds should play a major role in their activity. There is a possibility that free radical scavenging activity of melatonin molecule is due to the fast and complete formation of AMFK and AMK and their activity rather then the reactivity of the melatonin molecule itself. Thus, we performed theoretical study for all three molecules at the highest level of computational theory calculating energy, enthalpy, and total entropy for most stable structures of melatonin, AMFK, and AMK. Some earlier studies showed several of these parameters for melatonin and AMFK but for different molecule geometries. Also, calculations at semi-empirical and *ab initio* levels are compared in terms of energy trends for all three molecules.

EXPERIMENTAL

The geometry optimization of melatonin, AMFK, and AMK were performed using Density functional theory (DFT) method with B3LYP nonlocal exchange functionals and the 6-31-G(d) basis set as implemented in Spartan Software 08 (Wavefunction Inc. CA) First, seven different optimized melatonin conformational structures computed using RHF level using 6-31G(d) basis set are initially obtained and explored. One with a lowest energy was used for further optimization studies. The optimized structures, atomic charges and thermodynamic properties at DFT level were calculated. Additionally vibrational frequencies were calculated for the optimized structures with a same basis set used for geometry optimizations, in order to confirm structure minima.

RESULTS AND DISCUSSION

Geometries. A large number of melatonin conformers have been initially explored, and the most stable structure was chosen for further studies. Figure 1 shows a plot of energies of six structures with the lowest calculated energie. Only three structures are shown here due to the clarity of the graph. The bond angle C-O in the metoxy group differs for all three proposed structures. The side chain attached to the indole structure does not differ in two structures with the lowest energies being twisted toward the plane of the indole while a third structure, with a higher energy has a side chain being almost perpendicular to the plane of indole ring . In Figure 2, optimized most stable structures for all three molecules are shown with calculated dipole moment and its orientation in a molecule. Twisted geometry of the melatonin molecule visibly forms a globular structure efficiently exposing all the reactive sites towards other reactive molecules.

That conformational structure allows a molecule to get in closer contact with small reactive molecules, but in the same time has the ability to interact with conformationaly restricted sites of larger molecules such as enzymes. Thus, melatonin inhibitory activities towards peroxidases enzymes can be explained in part by this feature.

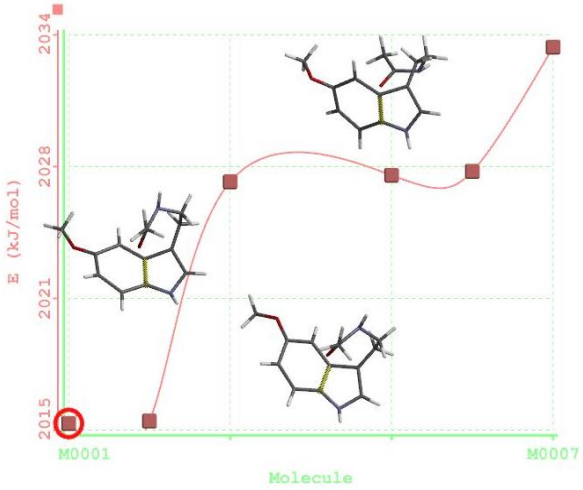

Figure1. Molecule plot of most stable conformers of melatonin molecule. Only three most stable structures has been shown here.

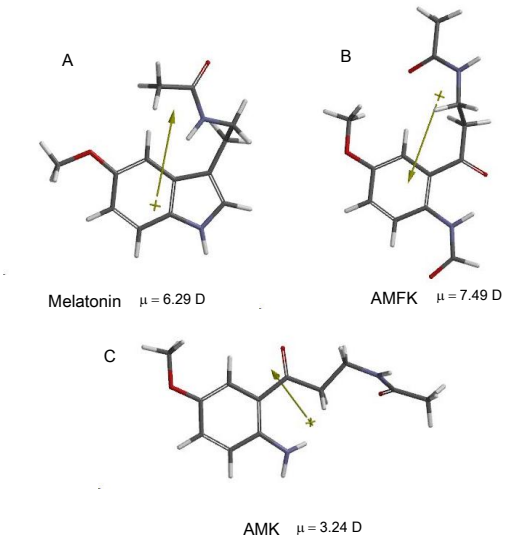

Figure 2. The optimized structures and dipole moment (in debye) of melatonin (A), AMFK (B) and AMK (C). Optimization was performed using Density Functional Theory at B3LYP/6-31G* level. The dipole moment magnitude and vector orientations are shown for each structure.

Atomic charges. Atomic charges using Mulliken theory were calculated and presented in Table 1. Calculations at lower level of theory using basis set HF/6-31G* assigned more negative charges on selected atoms in all structures,

namely oxygen and nitrogen. When compared to DTF calculations done using B3LYP/6-31G* basis set it is apparent that lower level of theory will give significantly lower charges on nitrogen atoms, while the difference in atomic charges of oxygen atoms in all structures is much smaller. Collectively, these values indicate possible sites of interactions with electron poor molecules, with nitrogen atoms been the most possible sites for interactions with electrophilic species such as free radicals. This is in accordance with experimental data and proposed mechanism of melatonin oxidations with hydroxyl radical forming indolyl cation radical.

However, this does not exclude interactions of other sites with high negative atomic charges. At higher level of theory the differences between atomic charges on oxygen and nitrogen are much smaller making interactions of electron poor species with oxygen atoms almost equally possible as the interactions with nitrogen atoms. As a result, several of mechanistic pathways of melatonin oxidations should be considered. Calculated atomic charges of AMFK and AMK also show high electron densities and as such are expected to have a high reactivity towards electron poor groups or species once they are formed in standard catabolitic melatonin mechanism. In addition, presented data clearly shows that charge densities are highly dependable on the selected level of theory used for calculations and atoms in question.

Table 1. Atomic charges of nitrogen and oxygen atoms of melatonin, AMFK, and AMK computed at HF/6-31G* and B3LYP/6-31G* levels of theory.

	Melatonin		AFMK		AMK	
Atom	HF/6-31G*	B3LYP/6-31G*	HF/6-31G*	B3LYP/6-31G*	HF/6-31G*	B3LYP/6-31G*
N (amine)					-0.90	-0.82
N (pyrrole)	-0.85	-0.69	-0.90	-0.68		
N (-CONH)	-0.79	-0.59	-0.80	-0.58	-0.81	-0.60
O (metoxy)	-0.66	-0.52	-0.66	-0.52	-0.66	-0.52
O (-CONH)	-0.68	-0.51	-0.63	-0.52	-0.62	-0.52
O (-HCONH)			-0.56	-0.45		
O (C=O)			-0.60	-0.52	-0.57	-0.49

Table 2. Energies of melatonin, AMFK, and AMK computed at semi-empirical (PM1) and *ab initio* (HF/6-31G* and B3LYP/6-31G*) levels of theory.

Energy (kcal/mol)	Base	Structure		
		Melatonin	AFMK	AMK
Semi-empirical				
Total energy	PM1	-62232.40	-75781.25	-66278.29
Core repulsion energy	PM1	349933.96	31628.80	355783.82
Electronic energy	PM1	-412166.36	-75781.25	-66278.29
Ab initio				
Total energy	HF/6-31G*	-477053.53	-571021.78	-500267.03
	B3LYP/6-31G*	-480032.06	-57448.16	-503319.68
ZPE	HF/6-31G*	185.00	191.59	183.64
	B3LYP/6-31G*	172.77	178.38	171.14
Nuclear repulsion energy	HF/6-31G*	750765.413	893163.63	732265.38
	B3LYP/6-31G*	741239.15	890363.54	73270.98

Thermodynamic parameters. Calculated thermodynamic parameters at semi-empirical level using PM1 basis set and *ab inition* calculations using two different basis sets, HF/6-31G* and B3LYP/6-31G* are presented in Table1 and Table 2. Calculations clearly show tenfold difference in calculated total energies at different levels of energy. It is obvious that any calculations done at PM1 level should not be used for a large molecule and can be used only as initial method to save on computing time rather than to give sound conclusions about reactivity of the molecule in question. Thus, we took into account only data calculated at *ab-initio* level. The stability of a molecule is determined by the total energy of the molecule denoting the kinetic energies of all particles forming the molecule and the potential energies of all their interactions. According to our calculations AMFK is the most stable molecule, followed by AMK and finally melatonin molecule. Using B3LYP/6-31G* basis set, changes in total energy are observed, lowering a differences in stabilities of all three molecules but following the same trend.

Zero-point energy is the lowest possible energy that a quantum mechanical system may have. The molecule with

the here is highest ZPE is AMFKA, followed with melatonin and AMK. Calculations at the higher level of energy improved values for AMK molecule the most when compared to other two molecules. Calculated values for standard enthalpy and entropy for all three molecules are given in Table 3. Direct comparison of the standard enthalpy values for nonisomeric compounds is not meaningful rather bond dissociation energies are considered better descriptors of a stability of the system. Experimental data for these parameters are not known but several calculated values for melatonin and AMFK have been reported. However authors did not stated basis set used for calculations or choice of conformer, for either compound thus any comparison with mentioned data can be reported. Reported experimental value for the standard molar enthalpy of formation in the gas phase for the indole compounds was 120.0 kJ x mol^{-1} (da Silva MA, Cabral JI, Gomes JR, 2008) being close to the calculated values for melatonin and its metabolites.

Table 3. Calculated changes in total enthalpy and entropy of melatonin, AMFK, and AMK at room temperature computed at *ab initio* (HF/6-31G* and B3LYP/6-31G*) levels of theory.

Parameters	(cal/mol) Base	Structure		
		Melatonin	AFMK	AMK
ΔH°	HF/6-31G*	195520	203647	194877
	B3LYP/6-31G*	183688	190806	182816
Total entropy	HF/6-31G*	132.69	145.13	138.00
	B3LYP/6-31G*	132.81	143.67	139.05

CONCLUSIONS

DFT calculations of melatonin and its metabolites, AMFK and AMK have been performed in order to obtain improved stable geometries and atomic charges and thermodynamic parameters. Calculations at HF level were carried out and compared to data obtained with DFT. Since all three molecules can exist in complex biological environment in the same time showing pronounced reactivity towards free radicals, their stability is an important factor contributing to the overall reactivity. Atomic charges, that can not be determined experimentally, were calculated showing areas of high electron density as possible sites of interactions with electrophilic species such as oxygen free radicals. Some previous molecula mechanics computational studies related to melatonin reactivity produced results in discrepancies with experimental data. *Ab initio* calculations showed significantly different values proving that force filed implemented in MM calculations can not accurately take into account electronic properties and atom interactions for molecular structures like these ones. Thus more accurate computational calculations should be carried out even for mechanistic pathways of melatonin oxidations and subsequent activity of its metabolites.

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Summary/Sažetak

Melatonin, neurohormone je poznat regulator brojnih fizioloških procesa. Osim toga, brojna istraživanja *in vitro* i *in vivo* su pokazala jaku antioksidativnu aktivnost te rekaktivnost prema slobodnim radikalima koja je obično smatrana kao posljedica direktne reaktivnosti molekule melatonina. Međutim, dva glavna metaboliti melatonina, N1-acetil-N2-formil-5-metoksi kinuramine (AMFK) i N1-acetil-5-metoxykinuaramine (AMK) također su pokazali veliku reaktivnost ali prema različitim biološkim slobodnim radikalima u poređenju sa melatoninom . Mehanizam oksidacije melatonina u složenom biološkom okruženju je ispitivan u različitim uslovima, ali jos uvijek nije u potpunosti definisan Elektronska svojstva te kinetička i termodinamička stabilnost molekule uvijek uslovljavaju molekularnu reaktivnost. Iz tog razloga teorijsko izračunavanje koristeći Density Functional teoriju (DFT) sa B3LYP/6-31G * parametrima je urađeno pri čemu su geometrije, atomska naboji i termodinamički parametri izračunati za za sve tri molekule. Podaci dobiveni sa Semi-empirical izračunavanjima koristeći PM1 parametre su upoređeni sa DFT rezultatima . Izračunati atomski naboji pokazuju da atomi nitrogena su moguća mjesta za interakcije sa elektrofilnim vrstama poput slobodnih radikala. Kisikov atom iz metoksi grupe također pokazuje izrazit negativan atomski naboj. Prema termodinamičkim parametrima najstabilnije molekula je AMFK, nakon koje slijedi AMK te melatonin. Ovaj trend djelomično može objasniti visoku reaktivnost melatonina i njegovu brzo razlaganje u biološkim sistemima. Dobivene vrijednosti izračunate koristeći semi-empirical i *ab inition* nivoe su znatno drugačije što znači da zaključci na temelju rezultata izračuna koristeći niže nivoe teorije se ne mogu koristiti kao pouzdani u analiziranju eksperimentalnih podataka.

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The Cellulose and Paper Industry Wastewater Treatment

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Abstract: A large amount of water is used in the cellulose and paper industry, which causes the production and release of the industrial wastewater which can, due to the amount and loads of contaminants, significantly affect the quality of the water environment if the adequate meassures for the rational use and purification of the same are not implemented and continuously applied. These wastewaters have a large organic contamination (BOD5 and COD), a large sulphite concentration, phenol and tannin (lignin) and chemicals that are used in the process of cellulose and paper production. The treatment of the wastewater from the cellulose and paper production in "Natron–Hayat" Maglaj after the realization of the wastewater disposal project done in 2007 is analyzed in this paper. This company has accomplished the project of recovery and modernization of the wastewater purification system towards the rational use and efficient purification of the industrial waters. With the device efficiency analysis for the wastewater treatment it was concluded that the purification efficiency level is acceptable according to the emissions standards issued by the regulations for the terms of wastewater release into the natural recipients and public sewerage system. Thereby, this company has significantly contributed to the water resources protection, ie river Bosna, which is a recipient of the wastewaters released from this company's plant. However, it would be good to analyze the possibility of the optimization of this device in oreder of exploiting the biogas as a potential fuel, adn sludge as a potential fuel and for other purposes.

INTRODUCTION

A large amount of water is used in the cellulose and paper industry, because of the technological process' nature. This causes the production and release of the industrial wastewaters which can significantly affect the quality of the water envirnoment if adequate meassures for rational use and purification of the same are not implemented and continuously applied.

Wastewaters from the cellulose and paper industry have a large organic contamination (BOD_5 and COD), a large hazardous substances concentration: sulphites, phenol and tannin (lignin) from the wood and therefore must be purified before released into the recipient, especially if the recipient are surface waters (Stanisavljevic, Krstic, Takic, et al., 2011).

Wastewaters from the cellulose and paper industry contain chemicals which are used in the process of cellulose and paper production, small parts of tree crust and wood, cellulose fibers and dissolved lignin from the wood. The wastewater often has a dark brow colour and odor from the organic sulfur compuonds (mercaptan) and compounds added for wood protection (Tuhtar, 1990).

The treatment of the wastewater from the cellulose and paper production in „Natron – Hayat" Maglaj after the realization of the wastewater disposal project done in 2007 is analyzed in this paper. This company has accomplished the project of recovery and modernization of the waste water purification system towards the rational use and efficient purification of the industrial waters. Thereby, this company has significantly contributed to the water resources protection, ie river Bosna, which is a recipient of the wastewaters released from this company's plant.

THE WASTEWATER CHARACTERISTICS IN THE CELLULOSE AND PAPER PRODUCTION PLANT OF THE „NATRON – HAYAT“ MAGLAJ COMPANY

The so called black wastewaters are produced during the cellulose production in the „Natron – Hayat“ Maglaj plant and the so called white wastewaters are produced during the paper production. Characteristics of these waters are given in Table 1.

Table 1: Industrial waste water characteristics.

Wastewater characteristics	Black wastewaters	White wastewaters	Characteristics of all wastewaters
Amount	18 000 m^3/day	22 000 m^3/day	40 000 m^3/day
Total BOD_5	7 000 - 12 000 kg/day	3 000 - 6 000 kg/day	10 000 - 18 000 kg/day
Total COD	14 000 - 35 000 kg/day	6 000 - 13 000 kg/day	20 000 - 48 000 kg/day
Total SM	3 500 - 7 000 kg/day	6 000 - 13 000 kg/day	9 500 - 20 000 kg/day
Maximal BOD_5 concentration	700 mg/l	350 mg/l	
Maximal COD concentration	2 000 mg/l	750 mg/l	
Maximal SM concentration	400 mg/l	750 mg/l	

All of the listed waste waters are led to the wastewater treatment plant, which characteristics are analyzed in this paper.

THE WASTEWATER TREATMENT DEVICES' CHARACTERISTICS IN THE „NATRON – HAYAT“ MAGLAJ

The wastewater treatment plant is concived as a physical, chemical and biological system for the waste water purification, that are again used in the technological pocesses (circular system), which provides a significant save and rational water use. The wastewater treatment process is shown in the Table 2 and in Figure 1.

Table 2:The operations of the wastewater purification device in the „Natron-Hayat“ Maglaj.

Previous treatment	Primary treatment	Secundary treatment	Tertiary treatment	Sludge treatment
Grille Equalisation	Dispersed substances removal: settling	Biodegradable substances removal: activated sludge procedure	Desinfection	Thickening and treatment on the belt presses

A large solid waste separated on the grille is disposed into the containers and the wastewater goes into the equlization tank where a mixing of wastewater with aluminum sulfate ($Al_2(SO_4)_3$ x $18H_2O$) is done. Aluminum sulfate affects the coagulation and settling. Also, a pH value adjustment is done in the equalization tank, ie a neutralization by automaticaly dispensing the NaOH or H_2SO_4. After that but before the primary clarifier an anionic polyelectrolyte is metered for faster settling in the primary clarifier.

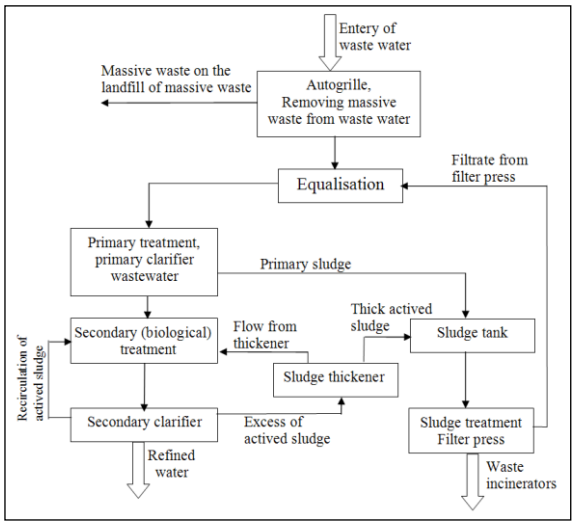

Figure 1: The wastewater treatment chart.

In the primary treatment, apropos in the primary clarifier the dispersed substances are removed. The antifoaming agents and sodium hypochlorite are added if needed, when a layer of foam starts to block the exchange of oxygen between the medium and air, therefore reducing the bio processes' efficiency. The water retention time is 2,4 hours. Around 500 m^3/day of sludge with the dry matter content around 4% is separated in this phase. The primary sludge is transported into the sludge tank via pumps. The primary clarifier waste waters go into the distribute pane where the needed chemicals are added (nutrients, antifoaming agents and ferric sulphate). The wastewater primary is shown treatment's efficiency is shown in the Table 3.

Table 3: The effluent characteristics after the primary clarifier.

Wastewater characteristics	Amount	Reduction
Biological oxygen demand (BOD_5)	10 000 kg/day	44.4%
Chemical oxygen demand (COD)	30 000 kg/day	37.5%
Suspended matters (SM)	4 000 kg/day	80%

The primary clarifier wastewaters go into three aeration tanks in which the biological treatment is done by the activated sludge procedure. Oxygen is introduced into the tanks with simultaneous water mixing. Thereby, preventing the settling and accelerating the microorganisms' contact with the food, ie incoming content in terms of BOD, COD and SM. The mass of microorganisms goes with the wastewater into the additional settling tank. A part of microorganisms' mass from settled sludge (activated sludge) is brought back into the biological tank (return sludge). Rest of the sludge goes into treatment before the final disposal.

The oxygen supply is done by 3 compresors of total capacity of 19260 kg/day and 20 spiral aerators in the first aeration tank of total instaled power of 370 kW (20 pcs. x 18,5 kW), and also by membrane diffusor system in the second and third tank. The total capacity of

oxygen influx is 28140 kgO_2/day. The disc type membrane diffusor (total 1800 pcs.) is used for the fine air bubble production to increase the oxygen transfer efficiency. The propeler mixers are instaled in the second and third aeration tank to prevent the forming of anaerobic conditions. This equipment works automaticaly depending on the meassured disolved oxygen concentration in the aeration tanks. The aeration tanks' oxygen concentration is monitored by three controllers (oximeter). In case of negative oxygen value one of the aerators or a compresor is activated to provide the oxygen.

The aeration tanks' wastewater goes into 3 secondary clarifiers through the distributional chamber. The secondary clarifiers' total volume is 6600 m^3. The secondary clarifiers purpose is to provide the microorganisms' settling which have envolved in the aeration tanks. For the microorganisms' evolvnig process a content in terms of BOD_5, COD and SM was used. The water retention time is 4 hours. In that time, the activated sludge is settling on the bottom of the tank and is collected with the chain scrapers. A part of the settled sludge is pumped on the begining of the biological process as a recirculated sludge, while the excess sludge is transported into the sludge thickener and then into the sludge tank. The amount of excess sludge is 1000 m^3/day.

After the biological treatment, the treated water has a following quality:

- BOD_5 < 25 mg/l,
- COD < 125 mg/l,
- SM <75 mg/l.

The wastewater testing before and after the biological phase is done in August 2012 to determin the biological treatment's efficiency. Obtained BOD_5, COD and SM values are given in the Table 4.

The table 4 data shows that all of the examined parameter values are lower than the defined limiting values of the qualitiy parameter emissions for the industrial wastewaters that are released into the surface waters. These emissions are issued by the regulations for the terms of wastewater release into the natural recipients and public sewerage system (c).

The BOD_5 is done every 5 days, thus it's value was meassured 4 times in the month of August, while COD and BOD were meassured 22 times which is the number of woking days in the month of August.

The BOD_5 average value before the biological treatment was 97,25 mg/l and after the biological treatment 19,125 mg/l.

The statistical efficiency of the BOD_5 removal is determined based on the ratio of the contamination indicators before and after the biological treatment (Imamovic, Goletic, and Ekinovic, 2010). Based on these two values the average degree of the BOD_5 removal efficiency is calculated:

$$\eta = \frac{97{,}25 - 19{,}125}{97{,}25} = 0{,}803$$

It follows that the BOD_5 removal efficiency is 80%, which is slightly lower than the values given by the BAT, which is 85–98 % (BAT).

Table 4: The wastewater analysis results before and after the biological treatment.

Date	**BOD_5, mg/l**		**COD, mg/l**		**SM, mg/l**	
	Before biological treatment	After biological treatment	Before biological treatment	After biological treatment	Before biological treatment	After biological treatment
1.8.12			507	122	172	16
2.8.12			530	118	118	22
3.8.12			653	115	72	17
6.8.12	105	18	609	116	80	10
7.8.12			629	115	92	12
8.8.12			571	114	44	12
9.8.12			611	123	89	14
10.8.12			700	120	96	12
13.8.12			840	120	20	16
14.8.12			617	117	88	12
15.8.12	85	18,5	600	120	108	12
16.8.12			555	117	80	10
17.8.12			560	112	140	12
21.8.12			584	110	80	12
22.8.12	112	22	557	119	132	16
23.8.12			517	117	136	16
24.8.12			598	114	92	16
27.8.12			480	116	116	20
28.8.12			416	118	28	10
29.8.12	87	18	403	113	24	12
30.8.12			380	117	32	10
31.8.12			403	105	40	10
Average values	97.25	19.125	560	116.27	85.4	13.76

There are some other data in the references about the BOD_5 removal efficiency which varies between 70–90 % (Tadeschi, 1997).

The average value of COD before the bioogical treatment was 560 mg/l, and after the biological treatment 116,27 mg/l. Based on these two values a degree of the COD contamination removal efficiency is calculated:

$$\eta = \frac{560 - 116{,}27}{560} = 0{,}79$$

It follows that the degree of the COD removal efficiency is 79%.

The degree of the COD removal efficiency after the activated sludge procedure is 75% (Tadeschi, 1997) or 60 – 80% (BAT).

The SM average value before the biological treatment is 85,4 mg/l and after the biological treatment 16,59 mg/l. Based on these two values a degree of the SM removal efficiency is calculated:

$$\eta = \frac{85{,}4 - 13{,}59}{85{,}4} = 0{,}84$$

It follows that the degree of the SM removal efficiency in the bioogical treatment is 84%.

The SM removal efficiency after the biological treatment is 85–90 % (BAT, 1997). Also it is considered that activated sludge wastewater treatment has a SM

reduction efficiency of 70–90 % (Simicic, 2002) or up to 95% (Tuhtar, 1990).

To perform the purification procedure efficiently, it is necessary to provide the food for the microorganisms, apropos the organic content in terms of BOD_5 or COD. There is a large chance of degradation at a lower organic matter concentration in the water, while the microorganisms activity is limited in case of a very high organic matter concentration in the water. Because of that it is important to establish the sludge content parameter which represents the ratio of the organic content and suspended matter and it is expressed by this equation

$$\frac{F}{M} = \frac{kgBPK_5}{kgSM}$$

The organic content to suspended matter ratio in particular case is F/M = 0,19–0,34 $kgBOD_5$/kgSM.

The references state the following data about the organic content and suspended matter ratio (Glancer-Soljan, 2001):

F/M = 0,5 – 5,0 – highly loaded activated sludge
F/M = 0,2 – 0,4 – medium loaded activated sludge
F/M = 0,1 - 0,2 - low loaded activated sludge.

With the comparative analysis of the organic content and suspended matter ratio in the wastewaters from the "Natron-Hayat" Maglaj comany's plant with the references data it can be concluded that this sludge is medium loaded.

The medium loaded sludge (F/M = 0,2 – 0,4) can achive a large BOD_5 removal efficiency up to 95% (BAT).

According to the BAT the sludge load of F/M = 0,15 with the water retention time in the aeration tanks from 14 hours up to two days is considered an optimal menegament of the activated sludge procedure. A lower F/M ratio gives a better BOD_5 removal efficiency, but at the same time if this ratio is too low there can be certain problems like defloculation and dispersal, apropos the sludge expansion inside the secondary clarifiers and its flow with the efluent (Simicic, 2002).

This ratio can be corrected and maintained constant by performing the higher or lower return of the activated sludge from the additional clarifiers to the aeration tanks after the biological treatment.

Other parameters of the successful management of the activated sludge procedure, that are in direct relation with the sludge content are sludge settleability, sludge volume index and sludge age.

The sludge settleability in the analyzed device varies within the limits of 250 - 500 ml/l. This parameter depends on the primary wastewater treatment. The sludge settling has no optimal value but it varies from plant to plant, but the most important property of this patameter is sludge stability, because a slight change (like slidge rising) can cause some problems in the wastewater treatment technology.

The sludge volume index in the analyzed device is around 150 ml/g. According to the references, sludge index varies within the following limits (BAT):

- SVI ≈ 100 ml/g good quality sludge
- SVI = 80–140 ml/g fair quality sludge SVI > 150 ml/g poor quality sludge.

This parameter is also important in terms of sludge settleability.

Sludge age in the analyzed device is around 6–11 days. The sludge age is inversly proportioned to the F/M content. Larger F/M content gives a lower sludge age and vise vrsa. The optimization of these factors (sludge content, settleability, sludge volume index and sludge age) affects the wastewater treatment efficiency.

Addition to the above, for a efficient wastewater biological treatment process it is necessary to provide an optimal ratio of the nutrients for the system performances improvement and the micronutrients (copper, manganese, cobalt, selenium), which are not necessary.

The data of the obtained wastewaters' biological treatment efficiency degree values from the "Natron–Hayat" Maglaj, and the values recommended by the BAT and the references (Tuhtar, 1990; Tadeschi, 1997; Simicic, 2002; Glancer-Soljan, 2001), are given in the Table 5.

Table 5: The comparative wastewater's biological treatment efficiency analysis.

Parameter	BOD_5	COD	SM
Natron-Hayat Maglaj	80 %	79 %	84 %
BAT	85–98 %	75–90 %	85–90 %
References	70–96 % to 95 % 70–90 %	> 75 %	70–90 % to 95 % 70–90 %

From the data given in the previous table it can be seen that BOD_5 removal efficiency is slightly lower than the BAT values but it is within the limits stated in the references. The COD and SM removal efficiency is within the limits stated in the BAT and in the references.

The primary and biological sludge is collected into the sludge tank from where it is transported to the dryin process via belt filter presses capacity of 70 m^3/h, 1400 kgSM/h. The presses filtrate goes into the equalisation tank for the inclusion into the purification process. To increasse the sludge drying process efficiency, the cation polyelectrolyte is introduced into the sludge. The dried sludge is now transported to the municipal landfills but the attempts are made to use it as an energy product for the industrial boiler or for other purposes.

The activated sludge procedure is widely used in the cellulose and paper production industry and it is used in 60–75% of the existing cellulose and paper production plants around the world (BAT).

The adctivated sludge procedure advantages are relatively high wastewater purification efficiency, possibility of process control (especially oxygen consumption control) and relatively small space demands. Disadvantages of this process are relatively high disturbance sensitivity during the process, high sludge production and relatively high operation expencess.

The tertiary treatment includes the effluent desinfection before its release into the river Bosna, which is the final recipient of the wastewaters from the mentioned company. Desinfection removes the pathogenic microorganisms.

The most economically justified way to treat the wastewaters with the high biodegradable organic content, as are the cellulose and paper industry wastewaters, is to treat them in two phases: anaerobic and aerobic, with the biogas production as a potential fuel. The anaerobic biological wastewater purification is a process which is energetically favorable, because it generates high energy value methane which can be used as the most environmentaly suitable fuel (Zarkovic, Krgovic, and Rajakovic, 2004; Avdic & Goletic, 2012).

CONCLUSIONS

The device efficiency analysis of the wastewater treatmen from the cellulose and paper production plant in the „Natron – Hayat“ Maglaj company led to conclusion that the purification efficiency degree is acceptable compared to the emissions standard issued by the regulations for the terms of the wastewater release into the natural recipients and public sewerage system.

The wastewater contaminating matter removal efficiency is satisfying. However, it would be good to analyze the possibility of the optimization of this device in order of exploiting the biogas as a potential fuel and sludge as a potential fuel and for other purposes.

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Summary/Sažetak

U industriji celuloze i papira koristi se velika količina vode, što uzrokuje nastanak i ispuštanje tehnoloških otpadnih voda koje zbog količine i opterećenja zagađujućim tvarima mogu značajno uticati na kvalitet vodnog okoliša ukoliko se ne primjene i kontinuirano ne provode adekvatne mjere za racionalno korištenje i prečišćavanje istih. Ove otpadne vode imaju veliko organsko zagađenje (BPK_5 i KPK), velike koncentracije sulfita, fenola i tanina (lignina) i kemikalije koje se upotrebljavaju u procesu dobijanja celuloze i papira. U ovom radu analizirana je obrada tehnoloških otpadnih voda iz proizvodnje celuloze i papira “Natron-Hayat“ Maglaj nakon realizacije projekta zbrinjavanja otpadnih voda izvedene 2007. godine. Ova kompanija je realizovala projekat sanacije i modernizacije sistema prečišćavanja otpadnih voda u cilju racionalnog korištenja i efikasnog prečišćavanja tehnoloških voda. Analizom efikasnosti uređaja za obradu otpadnih voda konstatovano je da je stepen efikasnosti prečišćavanja prihvatljiv u odnosu na emisione standarde propisane Uredbom o uvjetima ispuštanja otpadnih voda u prirodne recipijente i sistem javne kanalizacije. Time je ova kompanija značajno doprinijela zaštiti vodnih resursa, odnosno rijeke Bosne, koja predstavlja recipijent otpadnih voda ispuštenih iz pogona ove kompanije. Međutim, dobro bi bilo analizirati mogućnost optimizacije ovog uređaja u svrhu iskorištavanja bioplia kao potencijalnog goriva, te mulja kao potencijalnog goriva ili za druge svrhe.

Promocija diplomanata i magistranata fakulteta i akademija Univerziteta u Sarajevu

Svečana promocija diplomanata i magistranata fakulteta i akademija Univerziteta u Sarajevu održana je u subotu 10. novembra 2012. godine u Olimpijskoj dvorani „Juan Antonio Samaranch“.

Na ovoj svečanosti prof. dr Muharem Avdispahić, rektor Univerziteta u Sarajevu, prorektori Univerziteta u Sarajevu i dekani fakulteta i akademija promovirali su 6.209 diplomanata i magistranata fakulteta i akademija i pridruženih članica Univerziteta u Sarajevu.

Rektor Avdispahić je izrazio nadu da će Univerzitet upravo sa novim promoviranim diplomantima i magistrantima ojačati ulogu vodeće institucije u državi u oblasti visokog obrazovanja, naučnih i umjetničkih istraživanja i tržišno valorizabilnih ekspertskih znanja.

Prilikom svečanosti su 25 najuspješnijih diplomanata i 17 najuspješnijih magistranata primili Zlatnu značku Univerziteta u Sarajevu i prigodne novčane nagrade koje je osigurao Univerzitet u Sarajevu. Priznanja i nagrade uručili su im prof. dr. Muharem Avdispahić, rektor Univerziteta u Sarajevu, i gospodin Damir Mašić, ministar obrazovanja i nauke Federacije BiH.

Istaknuto je da su Adnan Zahirović, diplomant Prirodno-matematičkog fakulteta, i Alem Memić, magistrant Prirodno-matematičkog fakulteta, studij završili sa ukupnom prosječnom ocjenom 10, te da će obojica ponijeti naziv najuspješnijeg na Univerzitetu u Sarajevu za 2012. godinu.

Na Prirodno-matematičkom fakultetu u Sarajevu promovirano je 271 diplomant i magistrant.

Prvi put u dugoj historiji postojanja Odsjeka za hemiju na PMF-u imamo diplomanta, ADNANA ZAHIROVIĆA, koji je sve ispite položio sa ocjenom 10.

Redakcija Glasnika čestita Adnanu na postignutom uspjehu.

Dan Mladena Deželića

Na Odsjeku za kemiju Prirodno–matematičkog fakulteta u Sarajevu, 03.11.2012. godine obilježen je kao „Dan Mladena Deželića“. Svečanim otkrivanjem počasne ploče Kemijski amfiteatar PMF-a proglašen je imenom „Amfiteatar Mladena Deželića“. Zasluge za ovaj čin leže u činjenici da je ime akademika Mladena Deželića velikim slovima ugrađeno u temelje Univerziteta u Sarajevu, Prirodno-matematičkog fakulteta, Instituta za hemiju i fiziku u Sarajevu, te kemije kao nauke u Bosni i Hercegovini.

Svečanoj akademiji prisustvovali su njegovi bivši studenti i saradnici, studenti i osoblje PMF-a, predstavnici Medicinskog i Farmaceutskog fakulteta, obitelj akademika Mladena Deželića, sin Đuro, kćerka Dora Sečić i unuk Branko, te mnogi gosti.

Uvodni pozdravni govor održali su: šef Odsjeka za kemiju doc. dr. Mustafa Memić, dekan PMF-a prof. dr. Rifat Škrijelj, rektor Univerziteta u Sarajevu prof. dr. Muharem Avdispahić, a ispred Akademije nauka i umjetnosti BiH akademik prof. dr. Sulejman Redžić, nadahnuto, biranim riječima obratili se auditorijumu.

Prof. dr. Meliha Lekić dala je prilog, prisjetila se udjela u radu i doprinosu profesora Deželića u razvoju Medicinskog fakulteta.

Na kraju se, pred punim Amfiteatrom, obratio prisutnima sin akademika Mladena Deželića, dr. Đuro Dežélić. Vidno uzbuđen, lijepim riječima zahvalio se svima, počev od pokretača inicijative, do realizacije ovog izuzetnog društvenog događaja. Naglasio je da je ukazana velika čast njegovom ocu i cijeloj obitelji. Ponosni su i duboko dirnuti kada su se uvjerili kako se mlade generacije sjećaju, poštuju vrijednosti prethodnika, svojih

učitelja, te nadahnjujući se njihovim djelima koja im služe kao putokaz, idu ka svojim radnim i životnim ciljevima, što je bio i moto i želja njegovog oca.

O životu i radu akademika Mladena Deželića, govorila je dr. Marija Janković prezentirajući izdvojene dijelove iz svoje knjige: „Mladen Deželić život i djelo“. Kazano je:

Mladen Deželić bio je po profesiji naučnik, po naravi i ljudskim osobinama rođeni pedagog, po životnim stavovima neovisni intelektualac, a po svjetonazorima kozmopolita. Bio je kemičar, univerzitetski profesor kemije u Zagrebu i Sarajevu, redovni član Akademije nauka i umjetnosti BiH.

Svoj naučni rad započeo je kao prirodoslovac i kemičar, završio je Filozofski fakultet Sveučilišta u Zagrebu - kemiju sa fizikom i matematikom. Doktorirao je 1928. godine. Ljubav prema organskoj kemiji vodi ga u München gdje je u periodu 1932.–1939. godine, tokom ljetnih i zimskih dopusta radio kao asistent-volonter na Tehničkoj visokoj školi kod prof.dr. Hansa Fischera, nosioca Nobelove nagrade za kemiju 1930.godine. S njim specijalizira organsku kemiju i objavljuje radove u znanstvenim časopisima: *Zeitschrift für Physikalische Chemie*, *Liebigs Annalen* der *Chemie*, *Monatshefte für Chemie*...

Na istoj visokoj školi specijalizira i fizikalnu hemiju. Upoznaje se, sluša predavanja najpoznatijih kemičara toga doba, laureata za Nobelovu nagradu: P. Debay-a, R. Kuhna, O. Hahna, A. Butenandta, C. Ramana, i dr. Sve vrijeme radi najprije kao asistent, kasnije nastavnik Fizikalne kemije na Filozofskom fakultetu, a potom kao redovni profesor na Katedri za Opću i anorgansku kemiju, na Farmaceutskom fakultetu Sveučilišta u Zagrebu. Uspostavlja kontakte sa Leopoldom (Lavoslavom) Ružičkom (Zürich), (dobitnik Nobelove nagrade za kemiju 1939. godine, rođen u Vukovaru), te Vladimirom Prelogom (dobitnik Nobelove nagrade 1975.godine, rođen u Sarajevu).

Život i rad u Sarajevu

Poslije Drugog svjetskog rata u Sarajevu se krenulo sa formiranjem Univerziteta, otvaranjem fakulteta, kadrova je nedostajalo. Poziv Mladenu Deželiću da dođe u Sarajevo uputio je dr. Aleksandar Sabovljev, tadašnji dekan Medicinskog fakulteta i prof. Pavao Štern. Predsjednik Komiteta za fakultete i visoke škole i naučne ustanove NRBiH dr. Ante Babić zamolio ga je na pristanak uz obećanje: „da će se u Sarajevu osnovati Filozofski fakultet sa grupom kemija i da je za njega predviđeno mjesto redovnog profesora, te da će mu biti omogućen razvoj ne samo pedagoškog, nego i naučnoistraživačkog rada“. U svojim memoarima akademik Mladen Deželić o tom životnom trenutku piše: „Moram priznati da me je taj poziv zanimao i oduševio“. Odlučio se i školske godine 1949/1950. stupio na dužnost redovnog profesora kemije na Medicinskom fakultetu i šefa Kemijskog instituta istog fakulteta.

Na Sarajevo, u koje je došao da utemelji visokoškolsku nastavu i naučna istraživanja u području kemije, akademik Mladen Deželić gledao je kao na budući univerzitetski centar, što se najbolje vidi iz teme njegovog prvog predavanja prvoj generaciji studenata kemije u BiH, održanoj 15.10.1950. godine u predavaonici Medicinskog fakulteta u Sarajevu (jer još uvijek nije bila završena adaptacija prve kemijske predavaonice na Filozofskom fakultetu, u dvorištvu bivše Medrese): „ O važnosti studija kemije i potrebi kemičara u NRBiH".

Kako je planirano, početkom 1950. godine, osnovan je Filozofski fakultet u Sarajevu i tom prilikom utemeljen Prirodno-matematički odsjek sa grupama 1. kemija, 2. geografija. Prof. dr. Mladen Deželić imenovan je za jednog od matičara novog fakulteta, za redovnog profesora kemije za predmet Organska kemija i šefa Katedre za kemiju na Filozofskom fakultetu.

A zatim je profesor Deželić punih 10 godina vodio borbu za izgradnju zdanja Prirodno-matematičkog fakulteta i Instituta za hemiju i fiziku.

Na zasjedanju Narodne Skupštine BiH 1950.godine, obrazložio je taj zahtjev riječima da bi time: „u budućnosti bilo moguće normalno razvijanje nastave i znanstvenog rada iz kemije na Univerzitetu u Sarajevu. Novi Institut za kemiju sa suvremeno uređenim predavaonicama, laboratorijama, bibliotekom i pratećim uređajima i opremom, ukoliko bi bio centralni, poslužio bi i ostalim fakultetima na kojima se predaje kemija. Postao bi središte kemijske nauke u NRBiH, a poslužio bi i razvoju industrije u NRBiH, koja je u izgradnji..."

Tek 16.01.1959. godine Narodni odbor opštine Novo Sarajevo dodijelio je lokaciju. Izvedbeni projekat prof. arh. Juraja Neidhardta je krenuo, uz danonoćne korisne sugestije prof. dr. Mladena Deželića.

Posjedovao je ogromno iskustvo na polju organizacije praktične nastave iz kemije jer je sam radio, odnosno poznavao vrhunske sveučilišne institucije svojega doba. Prije projektovanja i izgradnje novog instituta obišao je novogradnje, kemijskih instituta u Zapadnoj Njemačkoj i Švajcarskoj, proučio obimnu literaturu, a vlastito iskustvo stekao kao glavni stručni nosilac uređenja kemijskih laboratorija Duhanskog instituta u Zagrebu i Mostaru.

Samo čovjek velikog znanja, sposobnosti, snage i entuzijazma mogao je prevladati takve poteškoće kao što su uvjeravanja, nabavke novčanih i materijalnih sredstava, opsežne rasprave za znanim i neznanim. Uspio je. Početak gradnje vezan je za 1962. godinu, a 1964. godine osvanulo je novo, moderno arhitektonsko zdanje sa funkcionalnim unutrašnjim rasporedom prostora.

U času svog dolaska u Sarajevo, akademik Mladen Deželić bio je kemičar međunarodnog renomea, svojim imenom i ličnim vezama otvorio je budućim bosanskohercegovačkim kemičarima kontakte sa evropskim naučnim centrima.

Prof. dr. Mladen Deželić bio je redovni član Naučnog društva BiH (1956.) i član njegovog Nadzornog odbora. Na dužnosti sekretara Naučnog društva (1963.) i u saradnji s akademikom Edhemom Čamom, izradio je statut Akademije nauka BiH. Kad je 1966. godine naučno društvo preraslo u Akademiju nauka i umjetnosti BiH, postao je član njenog Predsjedništva.

Odgoj kemijskih kadrova u BiH smatrao je prioritetnim djelatnim procesom. Bio je izvrstan profesor-pedagog. Sa mnogo znanja i pažnje prilazio je svakom studentu, diplomcu. Brinuo se da praktična nastava bude temeljita, naročito zbog zamašnih zadataka koji su tada postavljeni pred kemičare u industriji i naučno-istraživačkim ustanovama. Zato je naročitu pažnju posvjećivao uređenju i opremanju laboratorija.

Mnogo je teže bilo organizovati naučna istraživanja iz kemije u BiH, jer su takva istraživanja praktično bila na nuli, nije bilo niti opreme, niti kadrova. U početku se oslanjao na svoje asistente kojima je davao teme za disertacije. Više od 30 diplomiranih kemičara i farmaceuta „iz njegove radionice“ stekli su naučni stepen doktora kemijskih nauka. Oni su postali nosioci važnih zadataka razvoja kemijskog istraživanja i kemijske industrije u BiH. Na Medicinskom, Prirodno-matematičkom, kasnije Farmaceutskom fakultetu u Sarajevu najbolji među njima zauzeli su mjesta nastavnika kojima je profesor Deželić velikodušno i dostojanstveno predao dužnost.

Naučni opus prof. dr. Mladena Deželića sadrži više od 150 radova i patenata. Oblast njegovog istraživanja je organska kemija, kemija pirola, pirazolona, porfirina, kumarina, glikozida, alkaloida...Radovi mu se i danas, nakon toliko godina objavljivanja citiraju u naučnim časopisima koji prate najselektivnije baze podataka. Citiramo: rad „ O ledištu smjese teške vode D_2O i H_2O“ štampan 1935. godine, Zeitschrift für anorganische und allgemeine Chemie (Leipzig) još uvijek se citira u svjetskoj kemijskoj literaturi a rezultati su ušli u priručnike sa tabelama fizičkih i kemijskih podataka o teškoj vodi.

Želeći podići standard kemijskih istraživanja u BiH, najviše je objavljivao u Glasniku hemičara i tehnologa BiH, koji je on utemeljio i bio njegov urednik od 1952 -1966. godine. Časopis je bio citiran u međunarodnoj bazi *Chemical Abstract*.

Za svoj pedagoški i naučni rad dobio je sljedeće nagrade i priznanja:

- Zaslužni član Saveza hemičara i tehnologa BiH, 1964.
- Zaslužni član Saveza hemičara i tehnologa Jugoslavije, 1965.
- Orden rada sa crvenom zvijezdom za naročite zasluge stečene dugogodišnjim radom na polju nauke, kulture i prosvjete i za postignute uspjehe na uzdizanju stručnih i naučnih kadrova, 1966.
- Republička nagrada za naučni rad BiH, 1966.
- Počasni član Saveza hemičara i tehnologa Jugoslavije, 1968.
- Orden zasluga za narod sa srebrenim zracima, 1971.

Akademik Mladen Deželić bio je izuzetna osoba. Odmjeren, skroman i vješt predati znanje na metodičan način, znao je saslušati sagovornike. Studenti koji nisu zadovoljili na ispitu nisu odlazili srditi, jer su znali da njihov profesor prilazi ispitu „ocjeniti koliko student zna, a ne koliko nezna".

Obrazovan, kulturan, sa estetskim jezičkim pristupom sagovorniku, smion, razborit, kakav je bio profesor Deželić ostavio je neizbrisiv trag, te i danas njegov duh živi na Katedri za organsku kemiju i biokemiju.

Treba napomenuti da akademik Mladen Deželić potiče iz ugledne zagrebačke obitelji. Rođen je 03.01.1900. godine u Zagrebu. Njegov djed po ocu Đuro Stjepan Deželić bio je istaknuti djelatnik, književnik i publicista, dugogodišnji popularni senator grada Zagreba. Otac dr. Velimir stariji, književnik, bibliotekar, leksikograf sa doktoratom iz biologije. Majka Antonija, glazbeno nadarena studirala je solo pjevanje u Beču.

Iz skladnog braka sa Sofijom Eder, koncertnom pijanisticom, direktoricom muzičke škole u Sarajevu, imao je dvoje djece, sina Đuru, doktora kemijskih nauka i kćerku Doru Sečić, doktora informatičkih nauka.

U mirovinu je otišao 1968. godine. Umro je 28.11.1989. u Krapinskim Toplicama.

Ono što je Sveučilište u Zagrebu izgubilo odlaskom profesora Mladena Deželića dobila je naučna misao u Bosni i Hercegovini. Na Bosnu i Hercegovinu nikad nije gledao kao „susjednu državu". Sarajevu je poklonio sebe, u njemu je prepoznao potencijale naučnog središta Bosne i Hercegovine, što se i obistinilo.

Inicijativa Odsjeka za kemiju da se Kemijski amfiteatar nazove imenom „Amfiteatar Mladena Deželića" realizirala se uz znak zahvalnosti čovjeku, naučniku, graditelju.

Dr. Marija Janković

IN MEMORIAM

Jela Vasić Grujić

(1923-2009)

Rođena je u Foči 1923. godine, Gimnaziju završila u Sarajevu 1941. godine. Diplomirala na Farmaceutskom fakultetu Sveučilišta u Zagrebu 1947. godine. Nakon završenih studija radila u tvornici lijekova ˝Pliva˝ Zagreb, a od 1949. godine kao asistent Hemijskog instituta Medicinskog fakulteta u Sarajevu. Svoj naučni opus nastavlja tako što je doktorirala 1960. godine na Filozofskom fakultetu (sada Prirodno-matematičkom fakultetu) sa doktorskom disertacijom ˝Glukozidi izolirani iz jasena iz okoline Sarajeva i njihovi derivati˝. 1961. godine izabrana za docenta na Medicinskom fakultetu na predmetu Hemija. 1968. godine izabrana je za vanrednog profesora Medicinskog fakulteta na predmetu Hemija, a 1975. godine za redovnog profesora Medicinskog fakuteta na predmetu Hemija. Iste godine izabrana je u zvanje redovnog profesora Farmaceutskog fakulteta u Sarajevu na predmetu Fakmakognozija i hemija droga. 1984. godine izabrana je za dopisnog članа Akademije nauka i umjetnosti BiH, a 1995. godine za redovnog članа Akademije nauka i umjetnosti BiH. Za emeritiranog profesora izabrana je 2004. godine. Kao asistent, a zatim docent i profesor učestvovala je u edukaciji studenata na Medicinskom fakultetu, Stomatološkom fakultetu, Farmaceutskom fakultetu, Višoj medicinskoj školi, Prirodno-matematičkom fakultetu, Veterinarskom fakultetu Univerziteta u Sarajevu. U svojoj dugogodišnjoj, izuzetno plodnoj karijeri obavljala je niz visokopozicioniranih aktivnosti kao što je: od 1976. do 1985. godine bila je šef Katedre za Medicinsku hemiju Medicinskog fakulteta u Sarajevu, te Šef Katedre za farmakognoziju i hemiju droga na

Farmaceutskom fakultetu u Sarajevu od 1975. godine do penzionisanja; Dekan Farmaceutskog fakulteta u Sarajevu od 1982. do 1988. godine, te Direktor Radne organizacije Farmaceutske discipline.

U toku svog višedecenijskog djelovanja u području hemije, te farmakognozije i hemije droga boravila je na različitim vidovima usavršavanja i specijalizacija u Beču, Bonnu, Saarbrickenu, Minhenu, Moskvi i Pragu. Njen naučni opus bi se mogao opisati sa 195 naučno stručnih radova od toga publicirano 80 u naučnim časopisima, 116 kongresnih saopštenja, autorom jednog univerzitetskog udžbenika i dva priručnika, koautorom jednog udžbenika i dva priručnika, učesnika u 14 naučno-istraživačkih projekata, a od toga 7 kao nosioca projekta. Svojim radom i angažmanom u nauci doprinijela je kao mentor izradi dvije doktorske disertacije, 6 magisterija i 5 specijalizacija.

Njen naučni i stručni opus kretao se u području istraživanja u vezi sa analitikom alkaloida, glukozida i vitamina koristeći savremene metode detekcije, sinteze i analitike od kojih posebno polarografske i kromatografske metode.

Njena izuzetna radna angažovanost i predanost povjerenim poslovima zahtijevala je odricanja u njenom privatnom životu, tako da je za nju radni dan iznosio često i po 20 sati. Disciplinirranost u poslu je prenosila i na svoje saradnike koji su imali obavezu da je slijede u naučno-nastavnoj aktivnosti. Njena često izrečena izreka je bila: ˝Ko se u kolo `fata, u noge se uzda˝. Poznata je anegdota, prepričana od studenata Farmaceutskog fakulteta, iz vremena kada je kao saradnike imala pretežno žensku radnu snagu fertilnog doba. Česta odsustvovanja najbližih saradnika po osnovu porodiljskog odsustva remetili su njenu izuzetnu radnu disciplinu, pa bi na pitanje o tome kako se snalazi bez odsutnih saradnika odgovorila : ˝Ubi me fertilno doba!˝

Svoje posljednje godine života posvetila je Akademiji nauka i umjetnosti BiH, gdje je kao redovan član, učestvovala u aktivnostima Sekcije i Akademije.

Doc. dr Zlatan Rimpapa
Šef Katedre za medicinsku hemiju
Medicinskog fakulteta u Sarajevu

INSTRUCTIONS FOR AUTHORS

GENERAL INFORMATION

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina (Glasnik hemičara i tehnologa Bosne i Hercegovine) is a semiannual international journal publishing papers from all fields of chemistry and related disciplines.

Categories of Contributions

1. *Original Scientific Papers* – (about 10 typewritten pages) report original research which has not been published previously, except in a preliminary form. The paper should contain all the necessary information to enable reproducibility of the described work.
2. *Short Communications* – (about 5 typewritten pages) describing work that may be of a preliminary nature but which merits immediate publication.
3. *Notes* – (about 3 typewritten pages) report unpublished results of short, but complete, original research or describe original laboratory techniques.
4. *Reviews* – (about 30 typewritten pages) present a concise and critical survey of a specific research area. Generally, these are prepared by the invitation of the Editor.
5. *Book and Web Site Reviews* – (about 2 typewritten pages).
6. *Extended Abstracts* – (about 2 typewritten pages) of Lectures given at international meetings.
7. *Technical Papers* – (about 10 typewritten pages) report on applications of an already described innovation. Typically, technical articles are not based on new experiments.

Reviewing the Manuscript

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1. ***Melting and boiling points***:
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   Abbreviations: mp, melting point; bp, boiling point; lit., literature value; dec, decomposition.
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   $[a]^{23}_{D}$ –222 (*c* 0.35, MeOH).
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   ^{1}H NMR (500 MHz, DMSO-d_6) d 0.85 (s, 3H, CH_3), 1.28–1.65 (m, 8H, 4´CH_2), 4.36–4.55 (m, 2H, H-1 and H-2), 7.41 (d, *J* 8.2 Hz, 1H, ArH), 7.76 (dd, *J* 6.0, 8.2 Hz, 1H, H-1'), 8.09 (br s, 1H, NH).
   ^{13}C NMR (125 MHz, $CDCl_3$) d 12.0, 14.4, 23.7, 26.0, 30.2, 32.5, 40.6 (C-3), 47.4 (C-2'), 79.9, 82.1, 120.0 (C-7), 123.7 (C-5), 126.2 (C-4).
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   IR (KBr) n 3236, 2957, 2924, 1666, 1528, 1348, 1097, 743 cm^{-1}.
   Abbreviation: n, wavenumber of maximum absorption peaks in reciprocal centimetres.
5. ***Mass Spectrometry***:
   MS *m/z* (relative intensity): 305 (M^+H, 100), 128 (25).
   HRMS–FAB (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{38}N_4O_6$, 442.2791; found, 442.2782.
   Abbreviations: *m/z*, mass-to-charge ratio; M, molecular weight of the molecule itself; M^+, molecular ion; HRMS, high-resolution mass spectrometry; FAB, fast atom bombardment.
6. ***UV–Visible Spectroscopy***:
   UV (CH_3OH) l_{max} (log e) 220 (3.10), 425 nm (3.26).
   Abbreviations: l_{max}, wavelength of maximum absorption in nanometres; e, extinction coefficient.
7. ***Quantitative analysis***:
   Anal. calcd for $C_{17}H_{24}N_2O_3$: C 67.08, H 7.95, N 9.20. Found: C 66.82, H 7.83, N 9.16. All values are given in percentages.
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