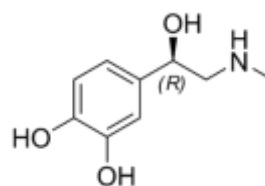
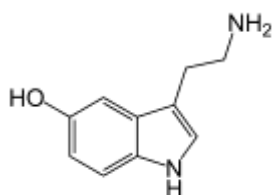


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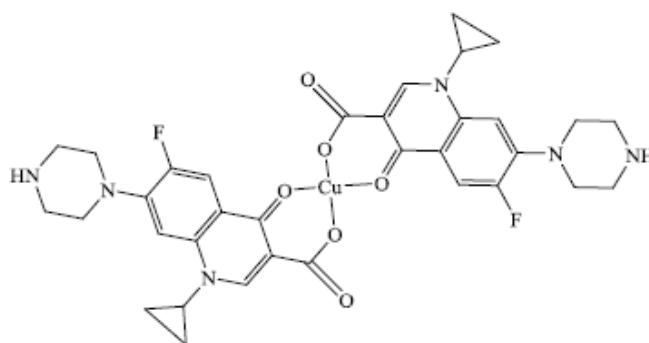


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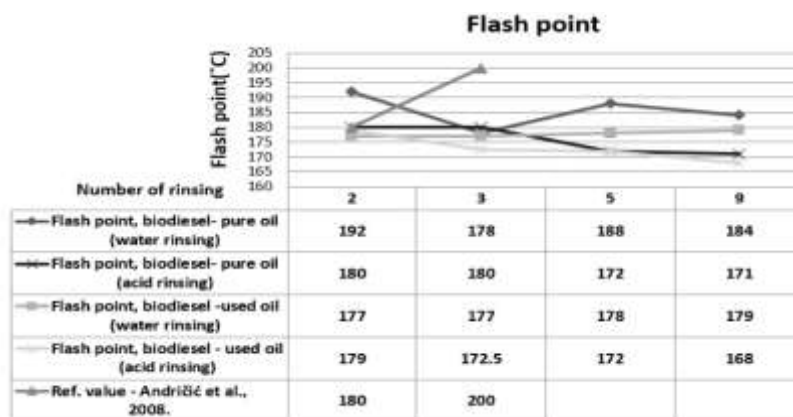
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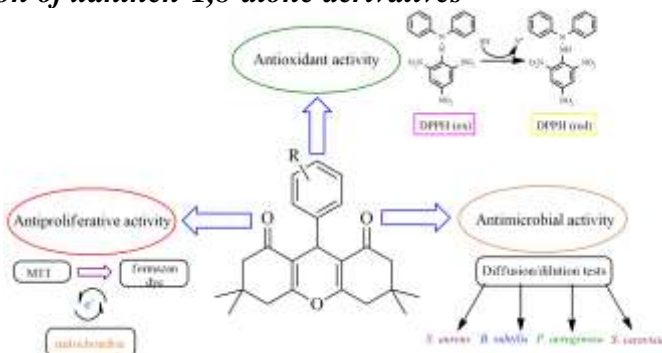
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		vocational	65.65			
2	Text/image materials	high	70.75	2329.5	-0.261	.794
		vocational	69.07			
3	Digital materials	high	73.20	2140.0	-1.136	.256
		vocational	66.02			
4	Audiovisual materials	high	69.24	2328.5	-0.273	.785
		vocational	70.94			
5	Audio-materials	high	70.47	2350.0	-0.182	.856
		vocational	69.41			

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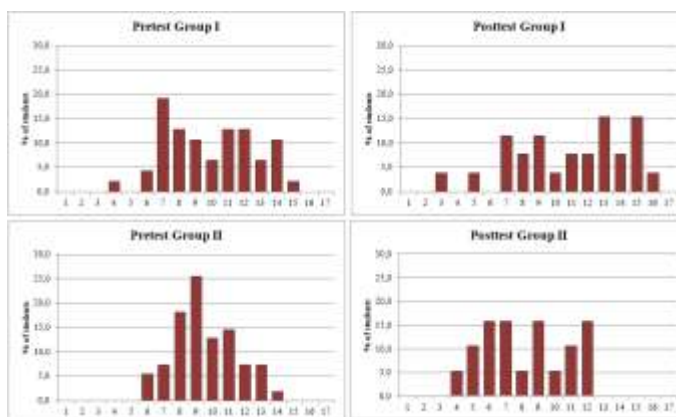
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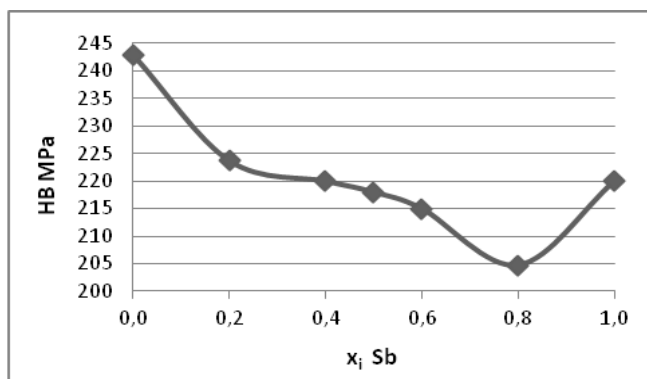
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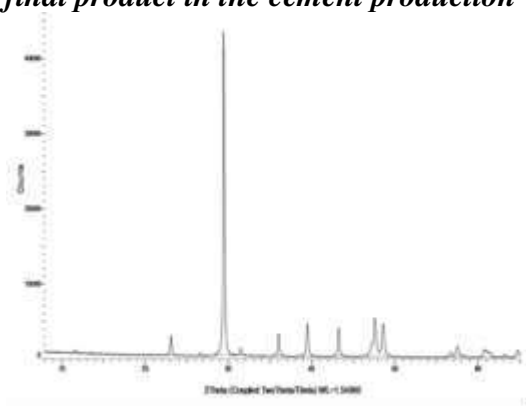


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## **Editorial**

### **EVOLUTION OF ENZYMES: *Benefit for humankind***

Diversity of life revealed the power of evolution. Since the first seeds of life arose around 3.7 billion years ago, almost every crevice on Earth has filled with different organisms. Forms of life are present in every part of Earth because evolution has solved a number of chemical problems. Proteins or as they are commonly called “chemical tools of life” have been optimized, modified and involved in different aspects of life’s diversity.

This year’s Nobel Laureates in Chemistry have been inspired by the power of evolution and used the principles – genetic change and selection – to develop proteins that solve mankind’s chemical problems. The 2018 Nobel Prize in Chemistry was awarded partially to Frances H. Arnold, fifth women to receive this outstanding award. She was the pioneer in conducting research on direct evolution of enzymes. Directed evolution is an iterative process scientists use to design biological molecules like enzymes. It requires inducing some randomness in the target enzyme within an organism like bacteria. The resulting mutated bacteria are screened to see which ones do the intended job the best. The winners are then cultured, and from their offspring, the best are selected, and then cultured, and so on. Enzymes produced through directed evolution are used to manufacture everything from bio-fuels to pharmaceuticals. Antibodies evolved using a method called phage display can combat autoimmune diseases and in some cases cure metastatic cancer. Other half of Nobel Prize in Chemistry is awarded to George P. Smith and Sir Gregory P. Winter for the development of the phage display - where a bacteriophage – a virus that infects bacteria – can be used to evolve new proteins. Phage display was used for the directed evolution of antibodies, with the aim of producing new pharmaceuticals. The first one based on this method, adalimumab, was approved in 2002 and is used for rheumatoid arthritis, psoriasis and inflammatory bowel diseases. Since then, phage display has produced anti-bodies that can neutralise toxins, counteract autoimmune diseases and cure metastatic cancer. Today, many phage display were used to produce different antibodies that are capable of neutralizing wide range of toxins, counteract autoimmune diseases and cure metastatic cancer.

Incredible fields of new therapy approaches for cancer therapies are now available because of the methods created and modified by this year Nobel Prize winners. We are currently living in the era of great evolution of enzymes with outstanding benefit for humankind.

**Editors**

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## Synthesis, characterization and *in vitro* antimicrobial activity of the Cu(II) and Fe(III) complexes with 1-cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid

Horozić, E.<sup>a\*</sup>, Cipurković, A.<sup>b</sup>, Ademović, Z.<sup>a</sup>, Kolarević, L.<sup>c</sup>, Bjelošević, D.<sup>c</sup>,  
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**Abstract:** 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (Ciprofloxacin, CFL) is a drug that belongs to the second generation of fluoroquinolone antibiotics with a wide range of effects on Gram-positive and Gram-negative bacteria. The bactericidal action of ciprofloxacin results from the inhibition of the enzymes topoisomerase II (DNA gyrase) and topoisomerase IV. In organism there is a possibility of interaction of CFL with biogenic elements in the blood, which could lead to the formation of complexes. This can cause change in the activity of antibiotics towards pathogenic microorganisms. The aim of this work was to investigate the interaction of CFL as ligand with the biological cations Cu(II) and Fe(III) in physiological condition. Synthesized complexes were characterized using IR spectroscopy and stereo-microscopy. Antimicrobial screening was performed on bacterial strains of *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Salmonella Enteritidis*. The results of IR spectroscopy showed that the Cu(II) and Fe(III) complexes with CFL were formed through the oxygen donor atoms of carboxyl and carbonyl group of the ligand. The color and size changes of the crystal of the ligand and complexes were also clearly seen. Antimicrobial screening has shown that CFL and CFL complexes have similar antimicrobial activity against all tested strains. The Cu(CFL)<sub>2</sub> complex showed better antimicrobial activity compared to the Fe(CFL)<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub> complex.

## INTRODUCTION

1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (Ciprofloxacin, CFL) is a fluoroquinolone antimicrobial commercially available as the monohydrate phase of its hydrochloride. It is formulated for oral and ophthalmic use, while intravenous administration is in the form of lactate salt

negative microorganisms. The structure of CFL is shown in Figure 1. The mechanism of action of CFL is based on the inhibition of the activity of enzymes of DNA gyrase (topoisomerase II) and topoisomerase IV which are required for bacterial DNA replication, transcription, repair, strand supercoiling repair, and recombination. The CFL is one of the most frequently prescribed antimicrobial drugs (19th WHO Model List of Essential Medicines, April 2015) and is the first fluoroquinolone

that has shown significant activity outside the urinary tract (Correia *et al.*, 2017). The reason for its widespread is the susceptibility of multi-resistant pathogens to ciprofloxacin. Additionally, drug efficacy of CFL is clinically proved in the treatment of nosocomial infections, such as those of the respiratory and urinary tract, skin infections and sexually transmitted diseases (Sharma *et al.*, 2010). Ciprofloxacin is the most active quinolone against *Pseudomonas aeruginosa* (LeBel, 1988).

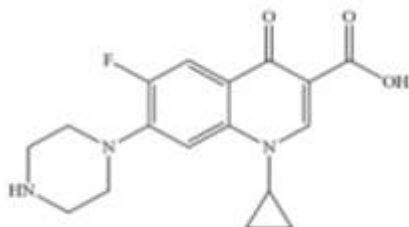


Figure 1. The structure of CFL

The analysis of the interaction of biogenic metal M(II) cations with O, N, S-donor atoms of ligands often used in the treatment of a wide spectrum of diseases is important for monitoring of distribution, pharmacokinetics, excretion, drug efficacy and adverse effects (Cipurković *et al.*, 2017).

The copper is present in the organism in the composition of cupro-enzymes and regulators of expression, of which the most famous are lysyl oxidase - involved in the crosslinking of collagen and elastin, tyrosinase - essential for the synthesis of melanin and superoxide-dismutase - responsible for defense against free radicals (Solioz, 2018). The most important characteristics of the active copper associated with proteins are its function in the metabolism of O<sub>2</sub> or N/O compounds and a frequent association with oxidative organic and inorganic radicals (Wolfgang and Jochen, 1996). Biological functions of copper are mainly related to its role as ligand for metallocenes and the biochemical role of copper is primarily catalytic (Hefnawy and El-Khaiat, 2015). Copper is in serum present in two main forms; bound to plasma-ceruloplasmin protein and to albumin (Angelova *et al.*, 2011). Human gastrointestinal system can absorb 30-40% of copper introduced by ingestion. Minerals with similar chemical properties can reduce copper absorption, while proteins, soluble carbohydrates and organic acids, except for ascorbic, produce a positive effect in terms of increased solubility and copper flow in the intestines, which improves absorption and bioavailability (Wapnir, 1998).

Iron as an essential bioelement in contact with oxygen forms oxides, which are highly unstable and therefore not readily available for absorption in organisms (Abbaspour, Hurrell and Kelishadi, 2014). Iron is an integral part of proteins, such as hemoproteins, which are involved in various crucial biological processes, such as reversible oxygen binding for hemoglobin and myoglobin and is responsible for transport and storage of oxygen (Souza, 2005). In an adult male body the iron content is 3000-4000 mg, and the adult female body contains only 2000-3000 mg. The difference in iron content in men and women is attributed to lower iron

reserves in women, lower hemoglobin concentrations and lower vascular volume than men (Arora and Kapoor, 2012). The most important forms of iron storage are ferritin and hemosiderin and are stored in the bone marrow and liver.

## EXPERIMENTAL

### Synthesis of the complex

A mixture of ethanol and water in a ratio of 50/50 (v/v) was used as a solvent for the ligand and Cu (II) and Fe (III) salts. The metals (M) and the ligand (L) were mixed in a 1:2 molar ratio (n/n). The solutions of the metal (10 mL) and ligand (10 mL) were mixed in a glass and stirred on a magnetic stirrer without heating. The pH value of the solution was adjusted with 1 M NaOH. The optimum pH value for testing the Cu (II)-CFL system is 5.6 and for the Fe (III)-CFL system is 7.3. The prepared solutions were mixed on a magnetic stirrer for 30 minute, then left to stand in a darkened area for two weeks in order to precipitate the complex. The resulting products were filtered through a blue strip filter paper and then dried at room temperature, after which their characterization was performed.

### FTIR characterization

In order to determine the structure of the complex Nicolet iS10 FT-IR spectrophotometer - Thermo Fisher Scientific was used.

### Morphological characterization

In order to compare color, texture and ligand particle size, the synthesized solid complexes were treated with DMSO and subjected to microscopic analysis. Analysis was performed on the binocular microscope Leica DM 2500P in polarized light with and without crossed Nicole (XPL and PPL).

### In vitro antimicrobial activity

Antimicrobial activity was studied by a diffusion method on reference bacterial strains (from ATCC collection) from gram positive group (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 51299) and gram negative bacteria (*Escherichia coli* ATCC 25922 and *Salmonella Enteritidis* ATCC 13076) by procedure form Clinical and Laboratory Standards Institute, 2009. From the bacterial strains of overnight cultures, suspensions of 0.5 McFarland turbidity were prepared (density 107-108 CFU/mL, depending on soy). The strains were then placed on the surface of the nutrient substrate-Mueller-Hinton agar (MH), dispersed in sterile Petri dishes. Substrate thickness was 4 mm. In the agar sterile drill-shaped holes were made ("wells"), into which 50 µL of CFL and Cu (II) and Fe (III) complexes solution in concentration of 1 mg/ml were added. After the plates were left at room temperature for 15 minutes, the substance was diffused into agar, incubated at 37°C/24 h. After the incubation period, the size of the inhibitory zone was measured and the sensitivity of the microorganisms was expressed as follows: if the zone for inhibition of microorganism growth was greater than 20 mm, it was labeled with three pluses (+++), representing the highest sensitivity of



the microorganisms. If the inhibitory zone ranged from 16 to 20 mm, it was marked with two pluses (++). Very weak sensitivity is marked with a plus (+) if the inhibitory zone is 10-15 mm in diameter. For the inhibitory zone less than 10 mm or if absent, the minus (-) has been used (Pirvu *et al.*, 2014).

## RESULTS AND DISCUSSION

### Structure of the complex

Due to the presence of oxygen donor atoms and one nitrogen atom, CFL can act as a ligand in the reactions with metals ion. Spectral studies suggest that ciprofloxacin behaves as a bidentate ligand in these complexes, the carboxylic oxygen and carbonyl oxygen atoms participating in these bonds. In this case the results obtained indicate the formation of complexes like:  $[M(CFL)_2(H_2O)_2]$ , where  $M = Fe^{3+}$ , and  $[M(CFL)_2]$ , where  $M = Cu^{2+}$ . The proposed structures of metal complexes CFL with  $Cu^{2+}$  and  $Fe^{3+}$  are shown in Figure 2. These structures show that the complex with  $Fe^{3+}$  contains two molecules of CFL per unit metal atom and each molecule is directly coordinated to the  $Fe^{3+}$  ion along with two water molecules giving an octahedral complex, similar to the molecular structures of the complexes of the other fluoroquinolone (Gao *et al.*, 1995). Based on the IR spectra of the Cu(II) complex with CFL, its square-planar configuration is proposed (Chohan, Supuran and Scozzafava, 2005).

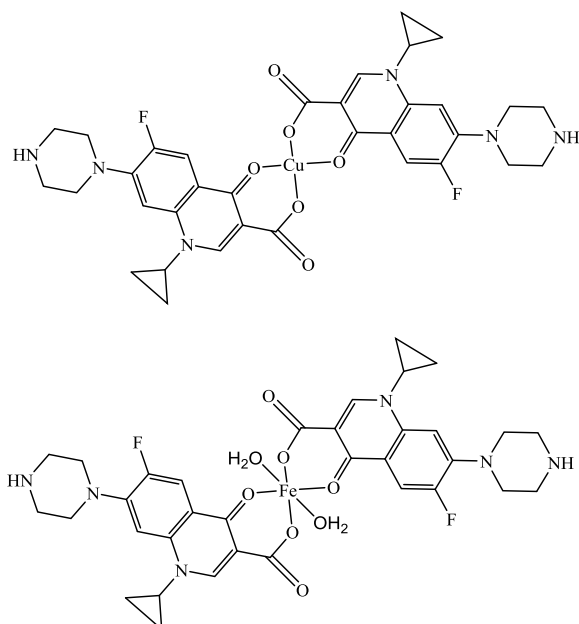


Figure 2. Proposed structure of  $Cu(CFL)_2$  and  $Fe(CFL)_2(H_2O)_2$  complexes

Synthesized complexes differ in color from the parent ligand. The resulting complex  $Fe(CFL)_2(H_2O)_2$  is orange, while the complex  $Cu(CFL)_2$  is colored light blue.

### Spectral characterization

Figure 3 shows the FTIR spectra of CFL and CFL complexes with Fe(III) and Cu(II).

At the IR spectrum of ciprofloxacin, the strong, sharp peak was detected at  $1703.2\text{ cm}^{-1}$ , indicating the vibration of the carbonyl group from CFL.

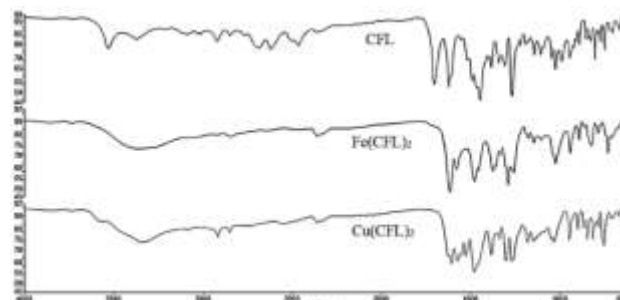


Figure 3. FTIR spectra of CFL and complexes  $Fe(CFL)_2$  and  $Cu(CFL)_2$

At  $3376.4\text{ cm}^{-1}$ , a wide peak was detected which corresponds to the N-H bond vibrations. Also, the wide peak of the O-H group is present at  $3529\text{ cm}^{-1}$  in the ligand spectrum. The strong peaks at  $1622.8$  and  $1443.8\text{ cm}^{-1}$  confirm the presence of a double bond C=C. Vibrations of the C-F bond cause the appearance of a strong, sharp peak at  $1023.7\text{ cm}^{-1}$  and the weak peak at the interval of  $3085.8\text{--}3013.2\text{ cm}^{-1}$  is most likely due to the vibration of the Ar-H bond. Peaks of medium to low intensity were observed at  $1106.3$  and  $1143.6\text{ cm}^{-1}$ , which were caused by stretching of the C-N bond. Due to the appearance of a strong intensity peak at  $1268.4\text{ cm}^{-1}$ , the presence of the C-O group was confirmed.

Comparing FTIR spectra of the parent ligand with the spectra of synthesized complexes, some differences were registered. Due to the absence of strong bands at  $\sim 1700\text{ cm}^{-1}$  characteristic of C=O and  $\sim 3500\text{ cm}^{-1}$  from O-H group, it was concluded that the bonds between metal and ligand were realized via the oxygen donor atoms of the carbonyl and carboxyl groups of the ligand. The characteristic vibrations of the ligand and complexes are shown in Table 1.

Table 1. Characteristic vibrations of the ligand and complexes ( $\text{cm}^{-1}$ )

Functional group	Ligand/Complex		
	CFL	$Cu(CFL)_2$	$Fe(CFL)_2(H_2O)_2$
C=O	1703.2	-	-
C-O	1268.4	1268.9	1291.2
O-H	3529	3511.2	-
N-H	3376.4	3337.3	3351.5
C=C	1622.8	1606.2	1615.4
C-F	1023.7	1030.5	1022.9
C-N	1143.6	1339.2	1103.2
Ar-H	3085.8	-	-

### Morphological characterization

The morphology of CFL crystals and its complexes with Cu(II) and Fe(III) are shown in Figures 4-6.

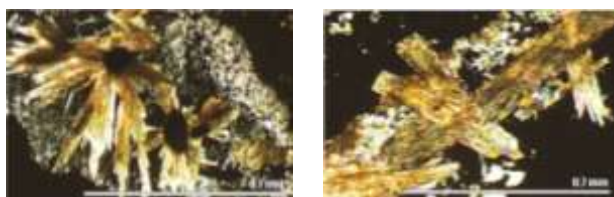


Figure 4. Morphology of CFL crystals

Regarding the size of the crystals of the ligand, they are divided into small crystals (below 1 mm) and medium crystals (1-10 mm). Interferential colors are live of the first order. These crystals are characterized with radially shape originated from the same center (Figure 4 - left) and prismatic forms (Figure 4 - right).

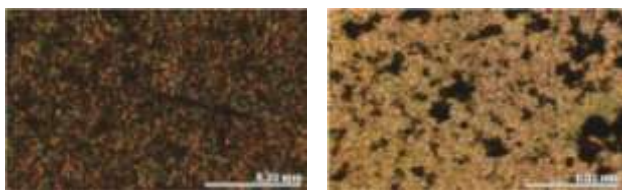


Figure 5. Morphology of  $\text{Fe}(\text{CFL})_2(\text{H}_2\text{O})_2$  crystals

Regarding the size of the  $\text{Fe}(\text{CFL})_2(\text{H}_2\text{O})_2$  complex crystals presented at Figure 5 they are very small crystals called microcrystals (below 1 mm). Interferential colors are live of the first order.

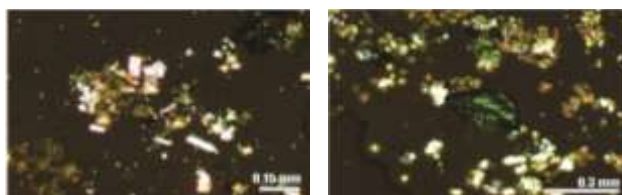


Figure 6. Morphology of  $\text{Cu}(\text{CFL})_2$  crystals

Figure 6 shows  $\text{Cu}(\text{CFL})_2$  complex crystals. Crystals are small (below 1 mm). Interferential colors are poorly expressed and crystals are mostly transparent. The morphology of crystals points to prismatic forms (Figure 6 - left). Crystals with pronounced interferential colors appear in a different way (Figure 6 - right). Also, at Figure 6 - right a crystal that could be classified as euohedral (idiomorphic) and indicates an irregular hexagram. The color of the crystal indicates presence of Cu and is probably a consequence of the thickness of the crystal.

#### Antimicrobial activity

Results of *in vitro* antimicrobial activity on selected bacterial strains are shown in Table 2. As already mentioned, the mechanism of CFL action is based on the inhibition of DNA enzyme activity (topoisomerase II) and topoisomerase IV, which are necessary for DNA replication. Before binding to these enzymes, CFL is chelated with metal ions (most commonly  $\text{Mg}^{2+}$ ). Only after the chelation, CFL can bind to any of these two enzymes and block DNA chains synthesis.

Table 2. Antimicrobial activities of CFL and  $\text{M}(\text{CFL})_2$  complexes

Microorganism	Inhibition zone of L/ML <sub>2</sub> [mm]		
	CFL	$\text{Cu}(\text{CFL})_2$	$\text{Fe}(\text{CFL})_2(\text{H}_2\text{O})_2$
<i>Escherichia coli</i>	40	40	40
<i>Staphylococcus aureus</i>	37	35	34
<i>Salmonella Enteritidis</i>	39	39	36
<i>Enterococcus faecalis</i>	27.5	26	23

In this regard, the results of antimicrobial *in vitro* screening test show that the tested  $\text{Cu}(\text{CFL})_2$  and  $\text{Fe}(\text{CFL})_2(\text{H}_2\text{O})_2$  complexes have almost the same antimicrobial activity as the antibiotic itself. It is very important that formation of copper and iron complexes does not reduce antimicrobial effect of CFL, since the forming of complexes with some of biometals is an indispensable process of antibiotic.

#### CONCLUSIONS

The analysis has shown that in physiological conditions, the selected bioelements interact with CFL through the oxygen donor atoms of carbonyl and carboxyl groups. The morphology of the ligand and complexes are significantly different, primarily in the  $\text{Fe}(\text{CFL})_2(\text{H}_2\text{O})_2$  complex. Relative to the parent ligand,  $\text{Cu}(\text{CFL})_2$  and  $\text{Fe}(\text{CFL})_2(\text{H}_2\text{O})_2$  complexes shows similar antimicrobial activity as the CFL alone. Forming of complexes does not disturb the mechanism of action of antibiotics in the bacterial cell.

#### ACKNOWLEDGMENT

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### Summary/Sažetak

1-ciklopropil-6-fluoro-4-okso-7-(piperazin-1-il)-1,4-dihidrokinolin-3-karboksilna kiselina (Ciprofloksacin, CFL) je lijek koji pripada drugoj generaciji fluorokinolonskih antibiotika, a ima širok spektar efekata na Gram-pozitivne i Gram-negativne bakterije. CFL sprečava diobu bakterijskih ćelija, inhibiranjem DNA giraze, topoizomeraze tipa II, te topoizomeraze IV. U organizmu postoji mogućnost interakcije CFL sa biogenim elementima iz krvi, što vodi nastanku kompleksa. Ovo može uzrokovati promjenu aktivnosti antibiotika prema patogenim mikroorganizmima. Cilj ovog rada je istražiti interakciju CFL sa biološkim kationima koji su inače prisutni u krvi, Cu(II) i Fe(III), u približno fiziološkim uslovima. Sintetizirani kompleksi su karakterizirani upotrebom IR spektroskopije i stereo-mikroskopije. Antimikrobni skrining je obavljen na bakterijskim sojevima: *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* i *Salmonella Enteritidis*. Rezultati IR spektroskopije su pokazali da su kompleksi Fe(III) i Cu(II) sa CFL formirani preko O-donorskih atoma karbonilne i karboksilne grupe liganda. Takođe, promjene boje i veličine kristala matičnog liganda i kompleksa su jasno vidljive. Antimikrobni skrining je pokazao da CFL i kompleksi imaju sličnu antimikrobnu aktivnost u slučaju svih bakterijskih sojeva, pri čemu kompleks  $\text{Cu(CFL)}_2$  pokazuje bolju antimikrobnu aktivnost u poređenju sa kompleksom  $\text{Fe(CFL)}_2(\text{H}_2\text{O})_2$ .





## **Influence of tested parameters on biodiesel quality obtained from used and unused vegetable oil**

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**Abstract:** The aim of this study was to obtain biodiesel, synthesized by transesterification from used (waste) and unused (industrial) sunflower vegetable oil, purify with following solvents: distilled water, 4% H<sub>3</sub>PO<sub>4</sub> and 4% HCl, determine biodiesel quality parameters, and finally analyze the influence of the selected parameters and used solvents for purification on the content of soap in biodiesel. Rinsing of biodiesel for each used solvent was carried out 2, 3, 5, 7 and 9 times with a certain amount of above-mentioned solvents. Differences in the values of selected parameters (indicators) of biodiesel quality as well as the concentration of soap in the purified biodiesel between single rinses for the used solvents were observed, and the values of the examined parameters between different purifiers with the same rinse number were compared. In this study, the estimated effects of washing methods on biodiesel density, kinematic viscosity, acid number, peroxide number, flash point, and biodiesel synthesis yield were determined. It was found that there were no significant changes and deviations of the obtained values of the examined parameters, when purifying biodiesel with different solvents (for both type of used oils), except for the value of the peroxide number for acid washing. Based on the results obtained in this work, acid solution is better for soap removal.

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## **INTRODUCTION**

Recently, biodegradable biofuels replace traditional fossil fuels, and most researchers talk about biofuels as a perfect renewable source of energy. Its main advantages are biodegradability, non-toxicity by its nature, low emission profile, which reduces global warming and is therefore environmentally friendly (Krawczyk, 1996; Mittelbach and Tritthart, 1988; Srivastava and Prasad, 2000; Dorado *et al.*, 2003; Tashtoush, Al-Widyan and Al-Shyoukh, 2003; Labeckas and Slavinskas, 2006). It is chemically defined as fatty acid methyl ester. Its production is of significance because it represents an environmentally acceptable alternative fuel for diesel engines. Biodiesel can be produced from a variety of raw materials such as edible vegetable oils, inedible vegetable oils, animal fats, recycled fats and oils, waste edible oils, waste materials (fat from oil and fat separators), by-products from edible

oil production processes etc. (Demirbas, 2003; Frohlich and Rice, 2005; Bhati *et al.*, 2008; Issariyakul *et al.*, 2008; Singh and Singh, 2009; Schinas *et al.*, 2009; Hameed *et al.*, 2009). Secondary raw materials, i.e. those that remain as waste from other industries and households, which were also used in this paper, were found very useful. Most of today's mass-production of biodiesel is based on the chemical transesterification reaction of triglyceride with an alcohol (usually methanol) using a base catalyst (Ferella *et al.*, 2010; Leung *et al.*, 2010). The transesterification process itself depends on the parameters such as temperature, pressure, reaction time, mixing rate, type and molar ratio of the used alcohol, type and concentration of the used catalyst, moisture concentration, the amount of free fatty acids used for the synthesis of biodiesel. The optimum values of the above parameters to achieve maximum conversion depend largely on the physical and chemical properties

of the raw material (Banerjee and Chakraborty, 2009). Once produced mixture of fatty acid methyl ester (crude biodiesel) should be subjected to the purification process to meet the specifications specified by appropriate standards such as EN 14214 or ASTM D6751. The performance of biodiesel as an alternative fuel for diesel engines depends on its purity (Banga and Varshney, 2010). Impurities reduce biodiesel quality and affect engine performance through a number of negative effects (Atadashi *et al.*, 2011; Berrios and Skelton, 2008). The purification of crude biodiesel is usually achieved via two notable techniques; wet and dry washings. This process is necessary to remove various types of contaminants such as unconverted triacylglycerols, diacylglycerols, free fatty acids, glycerol, water, catalyst, soaps, etc. from crude biodiesel, thus preventing any significant eventually diesel engine damage. The quality of biodiesel fuel is determined by a set of indicators, such as the kinematic viscosity, density, oxidation stability, lubricity, quality of fuel ignition expressed in cetane number, etc (Tai Shung, 2007, Sharma & Singh, 2008, Demirbas, 2009). The aim of this study was to obtain biodiesel, synthesized by transesterification from used and unused sunflower vegetable oil, purify with solvents: distilled water, 4% H<sub>3</sub>PO<sub>4</sub> and HCl, determine the quality parameters of biodiesel and finally analyze the influence of the selected parameters and the used solvents for purification on the content of soap in biodiesel.

## EXPERIMENTAL

All chemicals used in this study were of the high purity grade.

**Sample preparation:** For synthesis of biodiesel two types of vegetable oil were used: pure "BIMAL FINO" oil, which is a mixture of sunflower, rapeseed and soybean oil, produced by BIMAL d.d. Brčko and used, waste oil from the working restaurant. The waste oil collected during one week from the restaurant was mainly used for frying meat and vegetables. It was first decanted to remove the largest food residues and then purified by vacuum filtration in order to eliminate the presence of mechanical contamination (food residues). After filtration, both types of oil were heated up to 110-120°C to remove any water present.

**Synthesis and purification of biodiesel:** Conventional synthesis of biodiesel was carried out at 60-65°C with constant mixing (magnetic stirrer) during 120-150 min. Cooling and separation of glycerol from biodiesel was carried out during 4h. Methanol:oil molar ratio was 6:1 (Banerjee and Chakraborty, 2009; Stojkovic *et al.*, 2014). A total of 6 syntheses were carried out, 3 for used and 3 for unused (pure) sunflower oil. Purification of the obtained biodiesel was done by a wet process and consisted of 2, 3, 5, 7 and 9 washings using distilled water, 4% H<sub>3</sub>PO<sub>4</sub> and 4% HCl.

**Characterization:** Product characterization, after purification, was done after each wash (2, 3, 5, 7, 9 rinsing) by viscosity measurement (Ostwald), density

measurement (pycnometer), flash point measurement (Marcuson), yield calculation (Vatrenjak-Velagić, 1997). The same quality parameters were determined for used and unused oil before the production of biodiesel. The content of soap in obtained biodiesel was determined by the titrimetric method.

## RESULTS AND DISCUSSION

The characterization of used (waste) and unused (pure) oils was carried out prior to the synthesis of biodiesel. The requirements for edible oils indicate that the value of their acid number should not be higher than 0.6 mg KOH/g, and the peroxide number may be up to 5.0 mmol/kg. Based on the obtained results (Table 1) it can be concluded that the density, viscosity and acid number for pure and used oil are very similar, i.e. there are no significant differences, but it cannot be observed for the peroxide number. Compared to value of peroxide number obtained for pure oil (2.30) and the used oil (3.927), a greater difference can be observed with respect to other parameters. And compared to reference values the difference is even greater. Autooxidation is considered as the main cause of difference between the reference values and obtained values of peroxide number, since the waste oil used in this study was exposed to high temperatures, oxygen from the air, and it also contained a water from the food. Andričić, Kovačić and Čagalj (2008) have also used the waste and pure edible sunflower oil, and obtained the following values for density, acid number and peroxide number for pure oil: 0.915 g/mL, 0.163 mgKOH/g and 9.8 mmol/kg. While for the waste oil (in the same order) values were: 0.915 g/mL, 0.416 mg KOH/g and 21.2 mmol/kg.

**Table 1.** Quality parameters of oils used for the synthesis of biodiesel.

Parameter	Acid number (mgKOH/g)	Peroxide number (mmol/kg)	Density (g/cm <sup>-3</sup> )	Viscosity (E°) at 20°C
Pure oil	0.112	2.30	0.919	8.76
Used (waste) oil	0.149	3.927	0.922	8.66
Reference for pure oil	0.163	9.80	0.915	/
Reference for used (waste) oil	0.416	21.2	0.915	/

### Synthesis of biodiesel

A total of 6 syntheses was carried out, of which for 3 was used pure vegetable oil, and for 3 was used oil from the restaurant, previously processed. In Table 2 and 3 are given the amounts of the substances for synthesis and obtained yields for rinsing with distilled water. Based on the obtained yields (Table 2 and 3) for synthesized biodiesel, it can be concluded that these are high values in comparison to Andričić, Kovačić and Čagalj (2008). In this study (Andričić, Kovačić and Čagalj 2008) transesterification was carried out in a reactor with a stirrer at 60°C with methanol and KOH as a catalyst.

Obtained yield for biodiesel produced from pure oil was 83% and the yield for biodiesel produced from used oil was 81%. The yield was calculated as in this paper, i.e. relative to triglyceride olein, since the oleic acid esters are most commonly present in edible oils.

**Table 2.** Amounts of substances used for the synthesis of biodiesel from pure oil and the resulting yields for washing with distilled water.

Synthesis	Mass of oil (g)	Mass of KOH (g)	Volume of methanol (mL)	Mass of biodiesel (g)	Yield (%)
1.	500.29	5.01	137.30	467.79	93.50
2.	500.26	5.08	137.38	461.34	92.22
3.	500.48	5.04	137.44	455.34	90.48

**Table 3.** Amounts of substances used for the synthesis of biodiesel from waste oil and resulting yields for washing with distilled water.

Synthesis	Mass of oil (g)	Mass of KOH (g)	Volume of methanol (mL)	Mass of biodiesel (g)	Yield (%)
1.	500.09	5.00	137.33	477.86	95.55
2.	500.16	5.02	137.35	466.77	93.32
3.	500.15	4.99	137.35	467.57	93.48

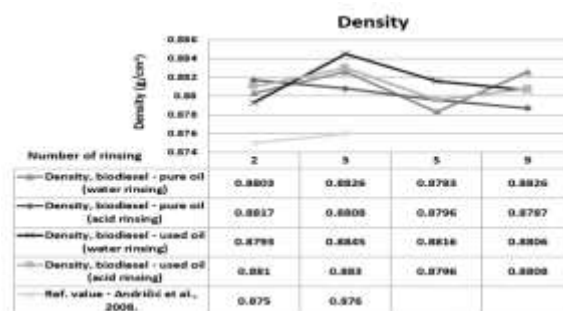
### Purification of synthesized biodiesel and determination of quality parameters

Distilled water, 4%  $H_3PO_4$  and 5% HCl were used as the solvents for the purification of the synthesized biodiesel. For all three solvents, the rinsing process was the same as the rinsing time, as well as the amount of used rinsing solvent. Characterization of biodiesel after rinsing with any of the three mentioned solvents was carried out after 2, 3, 5, 7 and 9 rinsing.

### Determination of biodiesel density after purification

Density values of biodiesels, purified by water washing, were between 0.8783-0.8826  $g/cm^3$  (biodiesel obtained from pure oil), and 0.8793-0.8845  $g/cm^3$  (biodiesel obtained from used oil), complying the EN 14214 standard limits. For biodiesel purified by acid washing, obtained density values were between 0.8787-0.8817  $g/cm^3$  (biodiesel obtained from pure oil), and between 0.8796-0.8830  $g/cm^3$  (biodiesel obtained from used oil), complying the EN 14214 standard limits. According to EN 14214, biodiesel density values should be within the recommended limits of 0.860 to 0.890  $g/mL$ . Obtained results from this study (Figure 1) are also confirmed by the literature data (Ljupković, 2014) where the value of density was 0.8884  $g/mL$ , while the value of biodiesel density in the work (Lučić, 2015) was 0.8868  $g/mL$ . Gligoričević *et al.* (2010) conducted experiments with aim to compare the density of diesel and biodiesel fuel, density of diesel fuel was 0.84  $g/mL$ , and density of

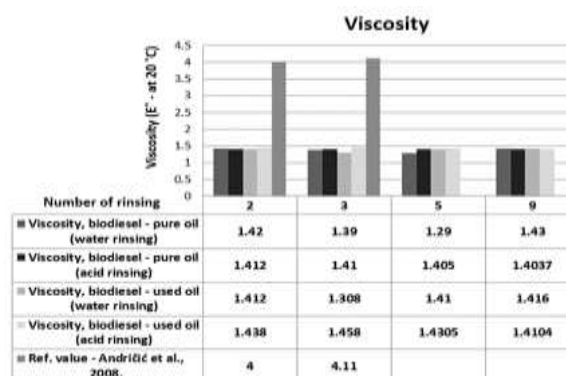
biodiesel was 0.88  $g/mL$ , so it is noticeable, biodiesel fuel has a higher density than diesel fuel.



**Figure 1.** Dependence of biodiesel density from the solvents for purification

### Determination of biodiesel viscosity after purification

According to EN 14214, the values of biodiesel viscosity should be within the recommended limits of 1.263-1.394 $^{\circ}E$ , while according to American Standard ASTM D6751, the recommended viscosity values for biodiesel are 1.1195-1.482 $^{\circ}E$ . Based on the obtained results for viscosity of biodiesel, all obtained values are within the recommended limits (for biodiesel obtained from pure and used oil). The results from this paper are also confirmed by the literature data (Ljupković, 2014) where the value of biodiesel viscosity was 1.342 $^{\circ}E$ , while the value of biodiesel viscosity in the work (Kovač, Saravan and Šikuljak, 2012) was 1.359 $^{\circ}E$ .



**Figure 2.** Dependence of biodiesel viscosity from the solvents for purification

### Determination of acid number of biodiesel after purification

Acid numbers of biodiesels purified by water washing were between 0.0547-0.0652  $mgKOH/g$  (biodiesel obtained from pure oil), and 0.0652-0.0745  $mgKOH/g$  (biodiesel obtained from used oil). Acid numbers of biodiesels purified by acid washing were 0.0418-0.0559  $mgKOH/g$  (biodiesel obtained from pure oil), and 0.0604-0.0671  $mgKOH/g$  (biodiesel obtained from used oil). According to EN 14214, value of the acid number of biodiesel shouldn't exceed the recommended upper limit of 0.50  $mgKOH/g$ , while according to American standard ASTM D6571-02, recommended acid number is 0.80  $mgKOH/g$ . The results from this work are also confirmed by the literature data (Andričić, Kovačić and Čagalj, 2008) and (Ljupković, 2014).

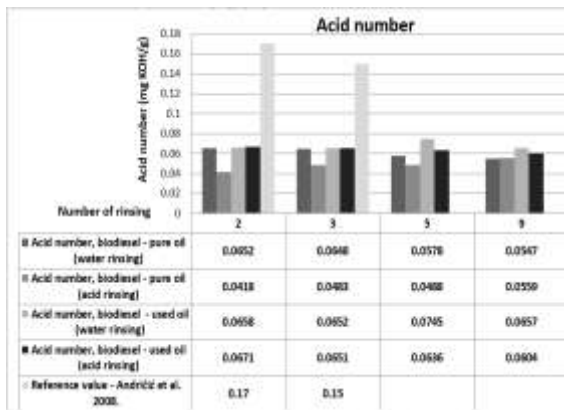


Figure 3. Dependence of biodiesel acid number from the solvents for purification

### Determination of peroxide number of biodiesel after purification

According to EN ISO 3960, the value of the peroxide number of biodiesel should not exceed the recommended upper limit of 10 mmol/kg. From Figure 4, it can be seen that, using acid washing to purify crude biodiesel results in decrease of the value of the peroxide number.

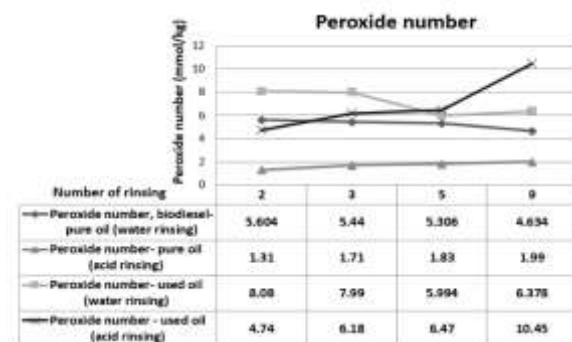


Figure 4. Dependence of biodiesel peroxide number from the solvents for purification

### Determination of flash point of biodiesel after purification

Flash points for most of synthesized and purified biodiesels are between 170-190°C, what is a satisfactory quality of biodiesel, regardless of the used purification agent. Flash point is generally reduced with an increase in the number of rinsing. According to EN 14214, flash points of biodiesels should not exceed the recommended limit below 120°C. From the obtained values in this study (Figure 5), it was found that all values obtained for the flash point of produced biodiesels are within the recommended limits. Obtained results are also confirmed by the literature data (Andričić, Kovačić and Čagalj, 2008) where the value of the flash points of biodiesels was 182-191°C.

### Determination of the soap concentration

Soap concentration for both, unused (pure) and used (waste) oil, decreases with an increase in the number of washings (Figure 6). Considering the results, efficiency of removing impurities in the form of soaps was better when washing was carried out with an acid than with water, regardless of the kind of used raw materials.

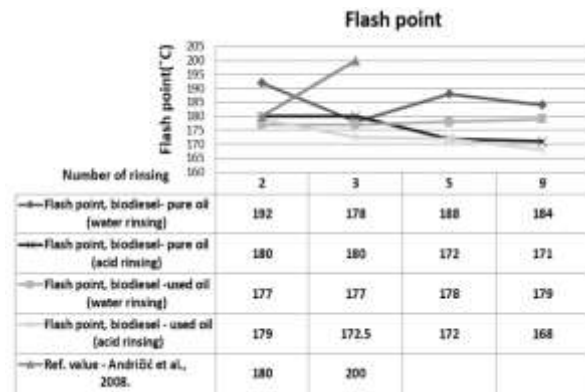


Figure 5. Dependence of biodiesel flash point number from the solvents for purification

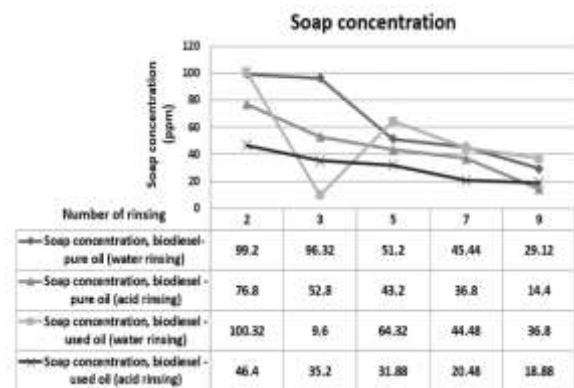


Figure 6. Dependence of soap concentration from the solvents for purification

In the study of Predojević *et al.* (2008) two solvents for purification of biodiesel were compared: hot distilled water and 5% phosphoric acid solution. Influence of wash methods on the biodiesel quality parameters as in this paper (kinematic viscosity, acid value, yield loss etc.) were measured and evaluated. There were no major changes between the two used solvents and investigated parameters. It was established that the phosphoric acid wash decreases the acid value of obtained biodiesel, and produced a higher yield compared to distilled water wash.

## CONCLUSIONS

The work has shown that the used solvents for purification of crude biodiesel do not greatly effect on the examined biodiesel quality parameters such as acid number, peroxide number, density, viscosity, and flash point. Washing with all three solvents have proven to be efficient purification techniques. The best results of biodiesel wet washing were with 4% chloride acid, which did not deviate much from the results obtained by distilled water. Analysis of synthesized biodiesels have shown that their quality parameters correspond to the requirements set by the European biodiesel standard. It was also found that the acid solution is better for removing of the soaps, *i.e.* fewer soap concentrations in biodiesel were observed with an increase in the rinsing rate with a 4% H<sub>3</sub>PO<sub>4</sub> solution.



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**Summary/Sažetak**

Cilj ovog rada je dobivanje biodizela, sintetiziran transesterifikacijom iz korištenog (otpadnog) i nekorištenog (industrijskog) suncokretovog biljnog ulja, prečišćen odabranim otapalima (destilovana voda, 4%-tna  $H_3PO_4$  i 4%-tna HCl), određivanje parametara kvalitete biodizela, te u konačnici analiziranje uticaja odabranih parametara i korištenih otapala za prečišćavanje na sadržaj sapuna u biodizelu. Ispiranje biodizela, za svako korišteno otapalo, vršeno je 2, 3, 5, 7 i 9 puta sa određenom količinom navedenih otapala. Praćena je razlika u vrijednostima odabranih parametara (pokazatelja) kvaliteta biodizela, kao i koncentracije sapuna u prečišćenom biodizelu između pojedinačnih ispiranja za korištena otapala, i upoređene su vrijednosti ispitivanih parametara između različitih otapala za prečišćavanje sa istim brojem ispiranja. U ovom istraživanju su procijenjeni učinci metode pranja na gustoću biodizela, kinematičku viskoznost, kiselinski broj, peroksidni broj, tačku paljenja i prinos sinteze biodizela. Utvrđeno je da nema nekih značajnih promjena i odstupanja dobijenih vrijednosti ispitivanih parametara, prilikom prečišćavanja biodizela različitim otapalima, kao i prilikom upotrebe otpadnog i čistog biljnog ulja za sintezu biodizela, osim za vrijednost peroksidnog broja pri ispiranju sa kiselinom za korišteno i čisto ulje, te je utvrđeno da otopina kiseline bolje za sobom povlači sapune, tj. zabilježene su manje koncentracije sapuna u biodizelu sa povećanjem broja ispiranja sa 4%-tnom otopinom  $H_3PO_4$ .

## Synthesis and biological evaluation of xanthen-1,8-dione derivatives

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**Abstract:** Fourteen previously synthesized 3,3,6,6-tetramethyl-9-aryl-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione derivatives were evaluated *in vitro* for their antimicrobial, antiproliferative and antioxidant activity. Also, in this work 3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione (**15**) was synthesized according to the same procedure and structure was confirmed by IR, NMR spectroscopy and mass spectrometry. Compounds were screened against Gram negative bacterium *Pseudomonas aeruginosa* and Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The antifungal activity of synthesized xanthene compounds was tested against fungus *Saccharomyces cerevisiae*. The cell lines HeLa, SW620, HEPG2, A549 and 3T3 were targets for antiproliferative effects of synthesized compounds. The results showed that the most potent, as antimicrobial and antiproliferative agent, was compound with two atoms of bromine substituted on aryl ring, and the most potent antioxidant agent was compound 3,3,6,6-tetramethyl-9-(2-methoxy-3-hydroxy-4-nitrophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione with IC<sub>50</sub> of 0.045 mM and 70.41% of inhibition DPPH.

## INTRODUCTION

Xanthene and their derivatives are important heterocyclic compounds with interesting biological activities, such as antibacterial (Lall, Hussein and Meyer, 2006), anti-inflammatory (Hafez *et al.*, 2008), antiviral (Ram *et al.*, 2000), antioxidant (Veljović *et al.*, 2015), and antitumor (Pinto, Sousa and Nascimento, 2005). Xanthenes were shown to function well as hydrogen donor and suppress the oxidation of linoleic acid in homogeneous solution. Unlike most other phenolic antioxidants, xanthenes react with peroxy radicals and their antioxidant activities were close to that of  $\alpha$ -tocopherol (Nishiyama, *et al.*, 1998). Xanthene derivatives have moderate to excellent activities against number of biological targets. With changing substituent on the xanthene nucleus, the biological targets vary from

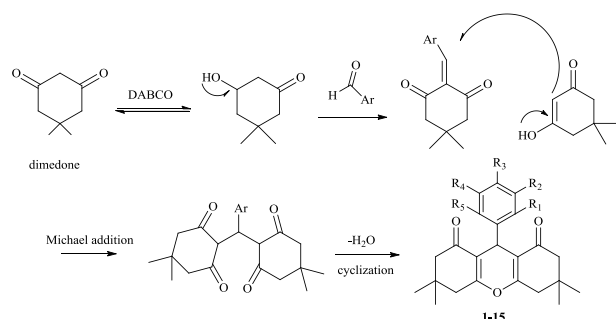
microbial diseases to viral problems, and variety of cancerous cells. Xanthene derivatives target different biological problems by interacting with enzymes and proteins (Gunjegaonkar *et al.*, 2018). Based on biological activities of xanthenes described in literature, we became interested in the evaluation of the obtained xanthen-1,8-dione derivatives as antioxidant, antimicrobial and antiproliferative agents.

## EXPERIMENTAL

### General Procedure for Synthesis

A mixture of 4-acetamidobenzaldehyde (1 mmol), 5,5-dimethylcyclohexane-1,3-dione (2 mmol) and DABCO (10 mmol%) in H<sub>2</sub>O (20 mL) was refluxed for 30 min (Figure 1). The progress of the reaction was monitored by TLC, using silica gel 60GF<sub>254</sub> plates, and as a mobile

phase dichloromethane:hexane=1:1. After completion of the reaction, the mixture was cooled to room temperature, and the solid was filtered off and washed with distilled water. The crude product (3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione) was purified by recrystallization from 95% ethanol (Paliwal *et al.*, 2013).



**Figure 1.** Synthetic route for xanthen-1,8-dione derivatives.

In our previous work (Veljović *et al.*, 2017) according to described procedure, we synthesized fourteen 3,3,6,6-tetramethyl-9-aryl-3,4,5,6,7,9-hexahydro-1*H*-xanthen-1,8(2*H*)-dione derivatives, that are structural analogs with different substituents on aryl ring (Table 1).

**Table 1.** Substituents of synthesized xanthenes aryl ring.

Compound	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>	R <sub>5</sub>
1	H	H	H	H	H
2	NO <sub>2</sub>	H	H	H	H
3	H	H	NO <sub>2</sub>	H	H
4	H	F	H	H	H
5	H	H	F	H	H
6	H	H	CF <sub>3</sub>	H	H
7	H	Cl	H	H	H
8	H	H	Cl	H	H
9	H	H	Br	H	H
10	H	Br	H	Br	H
11	OH	H	H	Br	H
12	H	OCH <sub>3</sub>	OCH <sub>3</sub>	H	H
13	H	OCH <sub>3</sub>	OH	NO <sub>2</sub>	H
14	H	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	H

### Instrumentation

All melting points (m.p.) are uncorrected and expressed in °C. Melting points of the compounds were determined using BÜCHI Melting Point B-545. IR spectra were recorded by Shimadzu IR Prestige 21 ID using KBr pellets. The <sup>1</sup>H and <sup>13</sup>C NMR spectra were recorded at 600 and 150 MHz, respectively, in CDCl<sub>3</sub> at 25°C using NMR spectrometer Bruker AV600, with tetramethylsilane (TMS) as internal reference. Elemental analyses of synthesized compounds were recorded by Vario EL III C, H, N, S/O Elemental Analyzer, Elementar Analysensysteme GmbH, Hanau-Germany. ESI-MS measurements were performed on a high performance liquid chromatography–mass spectrometry (HPLC-MS) triple quadrupole instrument equipped with an autosampler (Agilent Technologies, Palo Alto, CA, USA). The desolvation gas temperature was 300°C with flow rate of 6.0 L min<sup>-1</sup>. The fragmentor voltage

was 135 V and capillary voltage was 4.0 kV. Mobile phase was 0.1% formic acid in 50% methanol and a flow rate of mobile phase was 0.2 mL min<sup>-1</sup>.

### Antimicrobial activity

The antimicrobial activity of synthesized compounds was assessed against bacteria *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Pseudomonas aeruginosa* ATCC 15442 and fungi strain *Saccharomyces cerevisiae* ATCC 9763. Stock solutions were prepared by dissolving compounds in 99.5% dimethyl sulfoxide (DMSO) to obtain a 1 mg mL<sup>-1</sup>. Overnight cultures of bacteria were kept for 18 h at 37°C, while fungi strain was incubated for 48 h at 25°C. As nutrition bases for diffusion method Müller-Hinton and Sabouraud microbiological growth medium were used. The antibacterial activity of synthesized compounds, that showed good activity in diffusion method, was studied by employing a dilution method, using Casein soya bean digest broth (Tryptic soya bujon). The inoculum was prepared as described previously. The synthesized compounds were dissolved in DMSO and diluted with a culture broth to a concentration of 0.5–0.00024 mg mL<sup>-1</sup>. Chloramphenicol and fluconazole were used as referent compounds in concentration of 500 µg mL<sup>-1</sup>.

### Antiproliferative activity evaluation

The synthesized compounds were also evaluated for in vitro antiproliferative activity against HeLa (cervical carcinoma), SW620 (colorectal adenocarcinoma, metastatic), hepatocellular carcinoma (HEpG2), lung carcinoma cells (A549), and mouse embryo fibroblast cell line (3T3). For investigation 96-well microtiter plates was used and incubated at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air for 72 h. To all of the culture media, 100 µg mL<sup>-1</sup> penicillin, 100 µg mL<sup>-1</sup> streptomycin, 2 mM L-glutamine and 10% fetal bovine serum were added. In order to evaluate cell proliferation, the cells were treated with compounds at concentrations of 0.01 µM to 100 µM for 72 h. DMSO, which was used as solvent, was also tested for antiproliferative activity in the working concentration. In the present work, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay) was used to assess the antiproliferative activity of the synthesized compounds, while absorbance was measured at 570 nm. The results were presented as a cell percentage growth (PG) using the formulas proposed by NIH and described previously (Gazivoda *et al.*, 2008). The cell viability was determined with Trypan blue solution based on the quantification of the color intensity in each culture well by using an automatic cell counter (Countess, Invitrogen, USA), while morphology was determined under light microscope (Axio Vision-Zeiss, Germany). The IC<sub>50</sub> curves were plotted and the IC<sub>50</sub> and LC<sub>50</sub> values were calculated by using linear regression analysis.

### Evaluation of antioxidant activity by using DPPH method

The antioxidant activity of the synthesized xanthen derivatives was evaluated using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging assay. The

reducing abilities of the synthesized compounds were determined by their interaction with the free stable radical 1,1-diphenyl-2-picrylhydrazine (DPPH) at 0.2 mM concentrations for 30 min, by measuring absorbance at 517 nm. The antioxidant activity of tested compounds was determined by measuring the percentage of DPPH neutralization by them and compared to the standard antioxidant Trolox. The EC<sub>50</sub> (the concentration of antioxidant which eliminate 50% of DPPH radicals) was determined for each compound using calibrated curves. The inhibition percentage (%) of radical scavenging activity was calculated using the formula:

$$\% \text{ inhibition} = \frac{Ac(0) - AA(t)}{Ac(0)} \times 100 \quad (1)$$

where Ac(0) is the absorbance of control at t=0 and AA(t) is the absorbance of antioxidant at t=30 min. All measurements were done in triplicate (Burda and Oleszek, 2001).

## RESULTS AND DISCUSSION

According to described procedure in this work, we synthesized 3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1H-xanthen-1,8(2H)-dione (Figure 2). This compound was previously synthesized (Kaya, Demir and Bekci, 2013), but by different synthetic route. Its structure was confirmed by IR, NMR spectroscopy and mass spectrometry. Introducing acetamido group in structure of similar compounds increased antimicrobial activity (Sotirovaa *et al.*, 2014; Shah, 2017); therefore, we also evaluated this compound for antimicrobial, antiproliferative, and antioxidative activity along with 14 previously synthesized similar compounds (Figure 2).

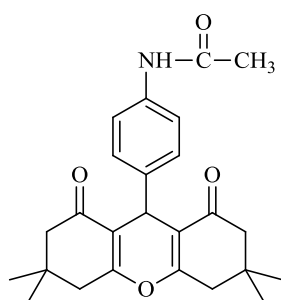


Figure 2. Structure of 3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1H-xanthen-1,8(2H)-dione (15).

3,3,6,6-tetramethyl-9-(4-acetamidophenyl)-3,4,5,6,7,9-hexahydro-1H-xanthen-1,8(2H)-dione (15): mp 170-171°C. IR (KBr)  $\nu$  3000, 1700, 1620, 1604, 1400, 1300, 1150 cm<sup>-1</sup>. <sup>1</sup>H NMR (600 MHz, CDCl<sub>3</sub>)  $\delta$  1.09 (s, 6H, H-14 and H-16), 1.21 (s, 6H, H-15 and H-17), 2.12 (s, 3H, H-8', NHCOCH<sub>3</sub>), 2.20-2.52 (m, 8H, H-2, H-4, H-5 and H-7), 5.48 (s, 1H, H-9), 7.01 (d,  $J_{2:3'}$  9.2 Hz, 2H, H-2' and H-6'), 7.40 (d, 2H, H-3' and H-5'), 7.56 (s, 1H, NHCOCH<sub>3</sub>). <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>)  $\delta$  24.45 (C-8', NHCOCH<sub>3</sub>), 27.31 (C-14, C-16), 29.55 (C-15, C-17), 31.34 (C-3, C-6), 32.30 (C-9), 46.37 (C-4, C-7), 47.00 (C-2, C-5), 115.50 (C-10, C-12), 119.56 (C-2',

C-6'), 127.27 (C-3', C-5'), 133.77 (C-4'), 135.79 (C-1'), 168.29 (C-7', NHCOCH<sub>3</sub>), 189.35 (C-11, C-13), 190.48 (C-1, C-8). MS (m/z) 426.3 (M+Na)<sup>+</sup>. Anal. Calcd. for C<sub>25</sub>H<sub>29</sub>O<sub>4</sub>N: C 73.68, H 7.17. Found: C 73.72, H 7.09.

## Antimicrobial activity evaluation

Microbiological activity was determined by diffusion and dilution method on three strains of bacteria and one strain of fungi. The tests were carried out on Gram negative bacterium *Pseudomonas aeruginosa* and Gram positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. The antifungal activity of synthesized xanthene compounds was tested against fungus *Saccharomyces cerevisiae*. Chloramphenicol (for antibacterial testing) and fluconazole (for the examination of antifungal activity) were used as standards.

The results of antimicrobial activity of synthesized compounds by diffusion method are shown in Table 2 and Table 3.

Table 2: Results of antimicrobial activity by diffusion method.

Compound	Zone of inhibition (mm)			
	<i>S. cerevisiae</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>B. subtilis</i>
1	14.5	12	14	15
2	16.5	14	12.5	15
3	17	16	18.5	17.5
4	18.5	16.5	17.5	20.5
5	16	14	10	14
6	13.5	14.5	18.5	14
7	16.5	12	12	19
8	17	12.5	10	14
9	18.5	15.5	14	18
10	19.5	18.5	20.5	21.5
11	-	12	21.5	14
12	14	14	15	18.5
13	19.5	17.5	20	19.5
14	15.5	13.5	16	18
15	14	11	10	14
Fluconazole	21	-	-	-
Chloramphenicol		20	24	22.5

Compound 6 with CF<sub>3</sub> group on *para* position on aryl ring, was most potent compound against *S. cerevisiae* in diffusion method. The new synthesized compound 15 with acetamido group in *para* position of aryl ring, showed the best activity against Gram negative bacterium *P. aeruginosa*, while compounds with substituents on *para* position of aryl ring (compounds 5, 8 and 15) were most potent against Gram positive bacteria *S. aureus* and *B. subtilis*.

In dilution method, compound 10 with bromine as substituent, was most potent. Biological comparative effects, as function of the nature of the substituents, reveals that the insertion of bromine atoms in the 3 and 5-position of the aryl ring of the xanthene derivatives, is an important factor on the antimicrobial activity.

Already published studies have shown that the introduction of bromine as a substituent, contributes to the antimicrobial activity (Shridhar *et al.*, 2009). Thus, the compound with bromine (compound 10), has shown a good activity against *S. cerevisiae* and *S. aureus* with MIC 0.313 mg mL<sup>-1</sup>, against *B. subtilis* with MIC of 0.626 mg mL<sup>-1</sup> while the activity against *P. aeruginosa*,

with MIC 1.25 mg mL<sup>-1</sup>, was significantly weaker. The results of antimicrobial activity by dilution method of synthesized compounds are presented in Table 3.

**Table 3:** Results of antimicrobial activity by dilution method.

Compound	MIC, mg/mL			
	S. cerevisiae	P. aeruginosa	S. aureus	B. subtilis
1	1.25	2.50	1.25	2.50
2	1.25	2.50	1.25	2.50
3	1.25	1.25	1.25	1.25
4	2.50	1.25	1.25	1.25
5	1.25	1.25	1.25	2.50
6	1.25	1.25	1.25	2.50
7	1.25	1.25	1.25	1.25
8	1.25	1.25	1.25	1.25
9	1.25	1.25	1.25	1.25
10	<b>0.313</b>	1.25	<b>0.313</b>	<b>0.625</b>
11	/	2.50	0.313	2.50
12	0.625	1.25	1.25	2.50
13	0.625	1.25	1.25	1.25
14	1.25	2.5	2.5	1.25
15	1.25	2.50	5.5	2.5
Fluconazole	0.313	-	-	-
Chloramphenicol	-	0.313	0.01	0.01

#### Antioxidant activity

The antioxidant activity was performed using DPPH radical scavenging method where Trolox was used as a positive control for comparison. The results of antioxidant activity of the compounds **1-15** are shown in Table 4. The results of the radical scavenging was expressed in terms of half-inhibition concentration (IC<sub>50</sub>) which denotes the concentration required to scavenge 50% of DPPH radicals.

**Table 4:** Results of antioxidant activity of synthesized compounds using DPPH method.

Compound	Absorbance (t=30 min)	% Inhibition	IC <sub>50</sub> , mM
1	0.456	66.76	0.09
2	0.478	65.16	0.1
3	0.558	59.33	0.14
4	0.504	63.27	0.12
5	0.544	60.35	0.131
6	0.631	54.01	0.195
7	0.574	58.16	0.165
8	0.580	57.73	0.18
9	0.603	56.05	0.17
10	0.667	51.38	0.193
11	0.621	54.74	0.19
12	0.431	68.59	0.16
13	0.406	<b>70.41</b>	<b>0.045</b>
14	0.485	64.65	0.13
15	0.575	58.10	0.16
Trolox	0.212	84.54	0.018

Compound **13**, with IC<sub>50</sub> of 0.045 mM and 70.41% of inhibition DPPH, seemed to be most active. It is assumed to be due to the presence of nitro substituent group in the molecule, as the presence of nitro group in any organic molecule in general, confers significant biological activity like in metronidazole (Al-Masoudi and Abbas, 2016), and nitrazepam (Gilli *et al.*, 1977).

Also, in literature, it is described that introduction of nitro group as substituent, significantly increases antioxidative activity, and compounds with halogen derivatives, and methoxy substituents also showed promising antioxidant activity (Karmaker *et al.*, 2018), which correlate well with results of our antioxidant study, while compound **13** possesses methoxy group at position C3 and nitro group at C5 on aryl ring of xanthene derivative.

#### Antiproliferative activity

The synthesized compounds **1-15** were tested for cytotoxicity in four human cancer cell lines, which contained human HeLa (cervical carcinoma), SW620 (colorectal adenocarcinoma, metastatic), hepatocellular carcinoma (HEpG2), lung carcinoma cells (A549), and mouse embryo fibroblast cell line (3T3). The IC<sub>50</sub> values of the xanthene derivatives for antiproliferative activity are listed in Table 5.

**Table 5:** Antiproliferative activity for xanthene derivatives.

Compound	Tumor cells				
	IC <sub>50</sub> , μg mL <sup>-1</sup>				
	SW620	HEpG2	3T3	HeLa	A549
1	>100	>100	86.1	>100	>100
2	>100	>100	0.02	>100	>100
3	>100	>100	11.9	>100	>100
4	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100
6	>100	>100	25.5	>100	>100
7	>100	>100	>100	>100	>100
8	>100	>100	<0.01	>100	>100
9	>100	>100	<0.01	>100	>100
10	<b>85.6</b>	>100	<0.01	<b>62.0</b>	>100
11	>100	>100	<0.01	62.2	>100
12	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100
15	>100	>100	0.05	>100	>100

The antiproliferative screening results show that synthesized xanthene-1,8-dione derivatives show weak antiproliferative activity against tested tumor cell lines.

The results revealed that compound **10** with two bromine atoms substituted on aryl ring of xanthene, exhibited the highest activity against HepG2 cell line with IC<sub>50</sub> 62.0 μg mL<sup>-1</sup> and IC<sub>50</sub> 85.6 μg mL<sup>-1</sup> against SW620 tumor cell lines, while other synthesized xanthene-1,8-dione derivatives had IC<sub>50</sub> more than 100 μg mL<sup>-1</sup> against all tested tumor cells.

#### CONCLUSIONS

A series of 3,3,6,6-tetramethyl-9-aryl-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione derivatives has been *in vitro* biological evaluated as potential antimicrobial, antiproliferative and antioxidant agents. The results showed that compound **10**, having two atoms of bromine substituted on aryl ring at positions C-3 and C-5 was the most potent as antimicrobial and antiproliferative agent. The results revealed that the most potent antioxidant agent was compound **13** (3,3,6,6-tetramethyl-9-(2-methoxy-3-hydroxy-4-nitrophenyl)-3,4,5,6,7,9-hexahydro-1*H*-xanthene-1,8(2*H*)-dione) with

IC<sub>50</sub> of 0.045 mM and 70.41 % of inhibition DPPH. Further experiments aimed at defining the target and the mechanisms of antimicrobial and antiproliferative activity showed by these molecules, are in progress and the results will be reported in a forthcoming paper.

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## Summary/Sažetak

U našem prethodnom radu sintetizirali smo četrnaest 3,3,6,6-tetrametil-9-aril-3,4,5,6,7,9-heksahidro-1*H*-ksanten-1,8(2*H*)-dion derivata, koji su evaluirani *in vitro* kao antimikrobni, antiproliferativni i antioksidativni agensi. Također, u ovom radu sintetiziran je i 3,3,6,6-tetrametil-9-(4-acetamidofenil)-3,4,5,6,7,9-heksahidro-1*H*-ksanten-1,8(2*H*)-dion, po istoj proceduri čija je struktura potvrđena IR, NMR spektroskopijom i masenom spektrometrijom. Spojevi su ispitivani kao antimikrobni agensi prema Gram negativnoj bakteriji *Pseudomonas aeruginosa* i Gram pozitivnim bakterijama *Staphylococcus aureus* i *Bacillus subtilis*. Antifungalna aktivnost sintetiziranih spojeva ispitivana je prema gljivici *Saccharomyces cerevisiae*. Stanične linije HeLa, SW620, HEPG2, A549 i 3T3 bile su meta za ispitivanje antiproliferativnog učinka sintetiziranih spojeva. Rezultati su pokazali da je najbolje antiproliferativno i antimikrobno djelovanje pokazao spoj koji u strukturi na arilnom prstenu ima supstituirana dva atoma broma, dok je najpotentniji antioksidativni agens 3,3,6,6-tetrametil-9-(2-metoksi-3-hidroksi-4-nitrofenil)-3,4,5,6,7,9-heksahidro-1*H*-ksanten-1,8(2*H*)-dion sa IC<sub>50</sub> 0.045 mM i 70.41% inhibicije DPPH reagensa.



## The use of chemistry learning materials within the pre-learning strategy: findings from a teachers' survey

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**Abstract:** The pre-learning strategy includes activities oriented at students' preparation prior to the class. Using such activities increases the pre-knowledge level which allows linking new information with the existing knowledge more efficiently and consequently reduces working memory overload. Research results indicate frequent use of various learning materials within the frame of the pre-learning strategy. The main purpose of this research is to determine the frequency of chemistry learning materials (CLMs) use within the frame of the pre-learning strategy in Croatian secondary schools. The quantitative descriptive survey research was conducted on a sample consisting of 77 high-school chemistry teachers and 62 vocational school chemistry teachers from all regions in Croatia. Data were collected through an online questionnaire and processed with descriptive and inferential statistics. According to the obtained results, it is most common that teachers use their own materials (Word, PowerPoint) and text/image chemistry learning materials for students' preparation prior to the chemistry class. Audio-materials (podcasts), audiovisual materials (screencasts) and digital materials are represented insufficiently. The frequency of use of chemistry learning materials does not differ between high-school and vocational school chemistry teachers'.

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## INTRODUCTION

The traditional pattern requires teachers to adjust a large amount of subject matter by introducing most of it in class, thus limiting the time available for active engagement. It is clear that there would be more time for active learning in class if more knowledge-level information could be moved out of class (Moravec, Williams and Aguilar-Roca, 2010). By providing students with some information prior to the class, the amount of new information presented during class time is reduced (Seery, 2012a). There is evidence that various pre-learning activities that introduce new material can increase student performance compared with traditional lectures (Seery, 2010).

In this paper, the term *pre-learning* refers to pre-class (Long, Logan and Waugh, 2016), pre-lecture (Kinsella,

Mahon and Lillis, 2017) or "to present lecture material in advance of class" (Day and Foley, 2005). According to Cognitive Load Theory (CLT), information processing and knowledge building are limited by the working memory capacity (Sweller, Van Merriënboer and Paas, 1998). Increasing the level of pre-knowledge by using pre-learning activities within the frame of the pre-learning strategy leads to efficient connecting of new information with the existing knowledge, as well as reducing working memory overload. The use of a pre-learning strategy has shown to be an efficient way of preparing students' minds because it helps them focus their attention on new relevant information and connect it with their pre-knowledge. This especially benefits the

students whose fundamental knowledge and experience are insufficient (Sirhan, Gray and Johnstone, 1999).

Pre-learning activities could take the form of reading a textbook excerpt or Word document, listening to a podcast (audio file), performing an online activity or completing a quiz. The key aspect is that pre-learning activities are integrated into the module design and into the class itself, so they are attributed with a sense of value by students and teachers (Seery, 2010). The pre-learning strategy is an opportunity to effectively include learning materials in the chemistry teaching in accordance with the teaching syllabus. Research results at an undergraduate level indicate frequent use of pre-learning worksheets and narrated PowerPoint videos (Moravec *et al.*, 2010), three types of videos and text-formatted materials (Long *et al.*, 2016), a combination of audio/video/PowerPoint materials (Collard, Girardot and Deutsch, 2002; Day and Foley, 2006), podcasts (audio files) (Seery, 2012b), and a short audiovisual material (Seery and Donnelly, 2012). In Alam, Zaman and Khan's study (2014), pre-lecture assignments were practiced to minimize the load on working memory at a secondary school level.

In the scientific literature, there is a lack of research analysing the use of pre-learning activities with the aim of reducing the cognitive load during classes in secondary chemistry education. This research is the first part of a more comprehensive study within a Ph.D. project regarding the implementation of the pre-learning strategy into secondary chemistry education. The main purpose of this research was to determine the frequency of using various chemistry learning materials (CLMs) within the frame of the pre-learning strategy in the secondary chemistry teacher population. Findings from this research should provide a direct insight into the actual practice of teachers and their priorities in the selection of certain CLMs in chemistry teaching, thus preparing the way for methodological intervention strategies.

This research shall answer the following questions:

1. How often do chemistry teachers use different types of CLMs within the frame of the pre-learning strategy?
2. Is there a difference in the frequency of using different types of CLMs for teaching chemistry in high schools and vocational schools?

## EXPERIMENTAL

This quantitative descriptive survey research enables the collection of data about the frequency of using CLMs with a self-administered online questionnaire. Once obtained, the responses are immediately stored in a database, which enables easy data handling and reduces the possibility of data errors. A nationwide questionnaire survey was administered to the population of secondary-school chemistry teachers in the Republic of Croatia.

### Research Participants

The sampling frame consisted of 573 secondary chemistry teachers from 21 regions in Croatia whose email addresses were obtained upon a request from the

education advisor database. A sample of 139 of the targeted chemistry teachers (response rate = 24.3 %) completed the online survey, but an ideal representation with regard to the number and gender of chemistry teachers in each region could not be achieved.

Most of them were in the City of Zagreb region (25.9 %) and the fewest in the Karlovac region (0.7 %). The data presented in Table 1 show that the majority of the participants were female (89.2 %). A total of 66.2 % of the participants had a master's degree in chemistry education whilst others were chemical engineers with additional pedagogical training. About a half of the participants (55.4 %) were high-school chemistry teachers.

**Table 1:** Demographic characteristics of the participants

School types	N	Gender		Teacher Education	
		Female	Male	Teacher	Engineer
High-school	77	68	9	59	18
Vocational school	62	56	6	33	29
Total	139	124	15	92	47

### Research Instrument

The Using Chemistry Learning Materials Questionnaire (UCLMQ) was constructed for the purpose of this research, based on the data obtained from a review of the existing literature, and it was made with the free web survey tool Google Docs. The questionnaire also included items that arose from the researcher's personal and professional experience. UCLMQ consisted of 10 items; the first four items were designed to gain insight into demographic characteristics of chemistry teachers (region, gender, teacher's education, and school type). The remaining five closed-ended items required participants to estimate the frequency of the use of various CLMs:

1. Teachers create learning materials themselves (Word, PowerPoint) for students' preparation prior to the class.
2. Students' preparation with text/image materials prior to the class.
3. Students' preparation with digital materials prior to the class.
4. Students' preparation with audiovisual materials prior to the class.
5. Students' preparation with audio-materials prior to the class.

The Likert six-point scale of frequency (1-never, 2-sometimes, 3-usually, 4-often, 5-very often, 6-always) was chosen (Burušić, 1999). In the last open-ended question, the teachers were asked to state whether they prepared students for classes using types of CLMs not mentioned above.

The credibility of the applied instrument was assured by considering test validity and reliability. The first version of UCLMQ was reviewed by two university chemistry professors and two high-school chemistry teachers.

UCLMQ was revised according to their recommendations. The next step in the development of the instrument was the pilot research (March 2017) focused on the quality control of the questionnaire and the gathering of data for its optimization (Figure 1). Five questionnaire items, which provide information about the frequency of the use of various CLMs, were used as a basis for determining the internal consistency. The internal consistency was calculated using the Cronbach's  $\alpha$  coefficient, which was found to be 0.755. The results indicated that the scale had an acceptable level of reliability.



Figure 1: The development of the Using Chemistry Learning Materials Questionnaire

### Data Collection

In the present research, an email invitation with a link to access UCLMQ was distributed to 573 secondary-school chemistry teachers at the same time. During June and July 2017, UCLMQ was completed online by 139 chemistry teachers. They completed the questionnaire at their own pace, chosen time and preferences. Prior to filling out UCLMQ, teachers had to read the introductory text in which the research purpose, result process, and instructions for completing UCLMQ were stated.

Teachers had to click on one of six response options, which took a maximum of five minutes for all items. The online questionnaire offered two solutions: one in which the participant could participate alone and only once, and the other one where responding was required to move on to the next question. The time frame for completing the web survey was not limited. The researcher was available via email for addressing any issues or comments regarding the survey questionnaire throughout the timeline of the research.

Participation in this survey research was completely voluntary. All participants were informed of the research purpose, research contents, and benefits such as their contribution to the advancement of the education research. Confidentiality and anonymity were a priority for the participants of the current research.

### Data Analysis

The collected data were analysed using the statistical package IBM SPSS Statistics 21.0 where descriptive and inferential analyses were employed. Descriptive statistics was employed to describe the general data of this research. For the purpose of revealing any differences between the frequency in the use of CLMs at high schools and vocational schools, inferential analyses such as the non-parametric two-tailed Mann-Whitney U-test was used. This test was chosen since it enabled the testing of hypotheses on small and asymmetrically distributed samples.

## RESULTS AND DISCUSSION

The descriptive statistics analysis was carried out in order to investigate the frequency of the use of CLMs in secondary chemistry education. The frequency distributions of teachers' responses, expressed in percentages, were calculated for the data collected with UCLMQ on the overall sample (Figure 2).



Figure 2. The percentage of the teachers' responses for claims of UCLMQ (N=139)

The majority (83.5 %) of the teachers who participated in the research indicated that either they did not use digital materials or that they used them sometimes, which implied once or twice per semester. Furthermore, 87.0 % of the participants indicated they used audiovisual materials either never or sometimes prior to the class. Audio-visual materials with key terms for the next class are used sometimes by 28.8 % of the participants, whereas 63.3 % of the participants never use them. Interestingly, one-third of teachers often to very often create learning materials themselves (Word, PowerPoint) for students' preparation prior to the class whereas 20.0 % of teachers create materials for each chemistry class. In addition, the teachers also had an opportunity to add their own comments about CLMs. There were no new suggestions for using learning materials. However, they most commonly expressed dissatisfaction with technical difficulties (e.g., "Working in a classroom without computer equipment") and being overloaded with the teaching syllabus (e.g., "Too lengthy and too detailed subject matter (I have 2 years to teach what high-school students learn in 4 years) for me to be able to have the time to introduce students with the terms from the following lesson").

For the evaluation of differences in the teachers' responses regarding the school types, the two-tailed non-parametric Mann-Whitney U-test was used for two independent groups: group 1 – high-school (N=77) and group 2 – vocational school (N=62). The results reported in Table 2 show the highest mean rank (MR) values for high-school teachers in designing their own materials (MR=73.50), and the use of digital materials (MR=73.20). The Mann-Whitney U test (N<sub>1</sub>=77, N<sub>2</sub>=62) indicates that the observed differences were not statistically significant.

**Table 2.** The results of the Mann–Whitney U test (two-tailed) of statistically significant differences

Item	Chemistry learning materials	Group	Mean Rank	U	Z	p
1	Teachers materials	high vocational	73.50 65.65	2117.5	-1.168	.243
2	Text/image materials	high vocational	70.75 69.07	2329.5	-0.261	.794
3	Digital materials	high vocational	73.20 66.02	2140.0	-1.136	.256
4	Audiovisual materials	high vocational	69.24 70.94	2328.5	-0.273	.785
5	Audio-materials	high vocational	70.47 69.41	2350.0	-0.182	.856

It can be noted from the obtained information that chemistry teachers use different types of learning materials which they have created themselves for students' preparation prior to the class, but they most commonly apply text/image materials (worksheets, PowerPoint) described in the previous research (Moravec *et al.*, 2010). On the other hand, the lowest frequencies in use appeared with the use of audio-materials although studies show that the use of audio files (podcasts) provides supporting resources to enhance student learning (Seery, 2012b). With our teachers the use of audiovisual materials (screencasts) is insignificantly higher than the use of audio-materials even though they have been mostly researched in the international scientific literature within the framework of the pre-learning strategy (Seery and Donnelly, 2012). According to the results, there is no statistically significant difference in the frequency of using CLMs between chemistry teachers in high schools and vocational schools. There were several limitations that should be taken into account when drawing conclusions from this research. Although the self-administered online questionnaire permits increased anonymity, there was no way to tell how truthful the participants were, they could have forgotten something or not think within the full context of statements of the questionnaire. The second limitation was the fact that the entire population was not sampled since the email addresses of 573 teachers were obtained on request from the education advisor database. The obtained sample of 139 teachers is not an accurate representation of the entire population of secondary chemistry teachers, and as such cannot be used as a basis for generalisations. However, by applying appropriate statistical tests, useful conclusions on the population could be extrapolated.

## CONCLUSIONS

This survey research was aimed at using CLMs for secondary chemistry teaching. UCLMQ was offered to get a direct insight into how often secondary chemistry teachers use CLMs in their classes. According to the research results, it can be concluded that teachers most commonly use their own materials (Word, PowerPoint) and text/image CLMs for students' preparation prior to the class. Audio-materials (podcasts), audiovisual

materials (screencasts) and digital materials are represented insufficiently in teaching chemistry in secondary schools. The frequency of high-school chemistry teachers' use of CLMs does not differ from vocational school chemistry teachers' use. Based on the research and the results obtained, it is possible to offer certain proposals for future research with the aim to determine the frequency of using learning materials in teaching natural sciences subjects, which includes larger sample sizes on the territory of the entire country.

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### Summary/Sažetak

Strategija predučenja uključuje aktivnosti usmjerene na pripremu učenika prije nastave. Primjena takvih aktivnosti povećava razinu predznanja što omogućuje učinkovitije povezivanje novih informacija s postojećim znanjem, a time i smanjenje opterećenja radne memorije. Rezultati istraživanja ukazuju na čestu uporabu različitih materijala za učenje u okviru strategije predučenja. Glavna svrha ovog istraživanja je utvrditi učestalost primjene materijala za učenje kemije u okviru strategije predučenja u hrvatskim srednjim školama. Kvantitativno deskriptivno anketno istraživanje provedeno je na uzorku od 77 gimnazijskih nastavnika kemije i 62 nastavnika kemije strukovnih škola iz svih hrvatskih županija. Podaci su prikupljeni pomoću online upitnika i obrađeni deskriptivnom i inferencijalnom statistikom. Prema dobivenim rezultatima, za pripremu učenika prije nastave kemije nastavnici najčešće koriste vlastite materijale (Word, PowerPoint) i tekstualno/slikovne materijale. Audiomaterijali, audiovizualni i digitalni materijali nedovoljno su zastupljeni. Učestalost primjene materijala za učenje kemije ne razlikuje se kod nastavnika u gimnazijama i strukovnim školama.





## Atmospheric Corrosion of Metals in Urban Area

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**Abstract:** Atmospheric corrosion rate depends on the content of the individual components in atmosphere, temperature of air, and refers primarily on the moisture content, the content of the various particles and the SO<sub>x</sub> and NO<sub>x</sub> gases. Washing out the pollutants from the atmosphere creates strong acids, bases and salts that can corrode various metal and non – metallic structures. The analysis of certain precipitation parameters, analysis of metal and alloy samples exposed to the atmosphere in Sarajevo and Kiseljak, and analysis of metal and alloy samples without atmospheric influence were carried out. The methods used for sample analysis are linear voltammetry and cyclic voltammetry. An iron plate, chrome coated iron plate with worn out surface, zinc coated iron plate with worn out surface and brass plate were used as a samples. It was observed that the corrosion of samples in Sarajevo is more intensive as compared to the corrosion of samples tested in the area of Kiseljak. Linear voltammetry measurements in a given range of potentials gave more pronounced corrosion currents in the samples from urban area. Cyclic voltammetry measurements gave pronounced reduction peak at -0.25 V for brass, and for chrome coated iron plate at -0.05 V. For the zinc coated iron plate sample, potentials were shifted to negative values that are characteristics of zinc pair. For chrome coated iron plate, reduction peak was detected at -0.75 V. The cyclic voltammogram for zinc coated iron plate with worn out surface is similar to the cyclic voltammogram for zinc coated iron plate. Urban area of Sarajevo is heavily polluted by different pollutants which can affect all aspects of life and environment. The occurrence of acid rains is intensified, which increases the corrosion of material of which the constructions are made.

## INTRODUCTION

Atmospheric corrosion is the degradation of materials under the chemical and electrochemical influence of the atmosphere.

This type of corrosion is mostly prevalent. The largest number of metal structures is corroded by moisture caused by condensation of water vapor when exposed to atmospheric air. The rate of atmospheric corrosion depends on the atmospheric conditions to which metal structures are exposed, atmospheric effects on the metal, the nature of the metal, and the state of the metal surface. (Milenković, Mladenović and Vučković, 1996)

When it comes to the content of individual atmospheric components that have a significant influence on corrosion, it is primarily about the content of moisture, various particles and SO<sub>x</sub> and NO<sub>x</sub> gases.

These pollutants are emitted into the atmosphere as a direct or indirect consequence of human activity. The distribution of pollutants depends directly on meteorological conditions. If there is no wind, the transmission of the pollutant away from their source is slow, so that the reduction in local concentrations of air pollutants emitted by air dilution is slow. The direction of the pollutants movement and their distribution on a local and global scale depends on the direction, intensity and length of wind currents. In addition, rinsing

pollutants from the atmosphere by precipitation decreases their concentration.

In recent years, the increase in SO<sub>2</sub> concentration has been recorded in Sarajevo due to temperature inversion resulting in the absence of airflow, the use of fuel of extremely poor quality, increased number of vehicles, unplanned construction, (Đuković, 2001).

Sulfur dioxide and a much more reactive product of its conversion, sulfuric acid is very destructive in the contact with metals, construction materials, and leather, paper, and textile materials.

The main sources of nitrogen oxides occurring in the air are natural sources, whose emissions are globally estimated at around 450×10<sup>6</sup> t/year and sources of anthropogenic origin, whose emission is estimated at 45×10<sup>6</sup> t/year. (Miyazaki, Eskes and Sudo, 2012).

Although the emissions of nitrogen oxides of anthropogenic origin make up about 1/10 of total emissions, it is very important for air pollution, especially urban and industrial areas, where nitrogen oxides production is mainly concentrated. Basic stationary sources are: steam generators with conventional fuels, power plants, domestic heating, incinerators, heating processes, emissions from other sources (chemical production). In addition to stationary nitrogen oxide sources, significant sources are motor vehicles. Nitrogen oxides in the combustion process are formed thermally - in the flames of the present nitrogen and oxygen and by the oxidation of nitrogen compounds containing fuel. The degree of conversion of nitrogen compounds from coal to nitrogen oxides depends on the fuel / air ratio and temperature, if standard burners are used. Increase in air volume leads to increased nitrogen oxide emissions. The amount of nitrogen oxide produced from fuels such as coal and oil derivatives can exceed 50%. The fraction of the nitrogen oxide is produced in some industrial processes, among which the processes of electric metal application are, metal cleaning, by nitric acid production, refineries, and similar process. The content of nitrogen oxides in urban areas is much higher than in non-urban areas. (Đuković, 2000).

The aim of this paper is to examine the corrosion behavior of metals, metal coatings and alloys, both visually and electrochemically. Our goal is to examine their behavior in different environments and compare them with different atmospheric constituents in those environments. (Presuel-Moreno *et al.*, 2008).

## EXPERIMENTAL

As samples an iron plate, chrome coated iron plate with worn out surface, zinc coated iron plate with worn out surface and brass plate were used.

The methods used were: visual examination, linear voltammetry and cyclic voltammetry (Geler *et al.*, 1997).

## RESULTS AND DISCUSSION

### Setting up a meteorological station

Due to the great influence of meteorological parameters on the content of individual components in the atmosphere and, consequently, on the processes of

atmospheric corrosion, the first meteorological station was installed at the Department of Chemistry for the purposes of this research. The meteorological station was used to measure parameters such as pressure, temperature, rainfall, humidity, wind speed and wind direction, and is equipped with data acquisition and storage. The weather station was set up to measure these parameters every 30 minutes.

### Sampling of precipitation

Sampling of precipitation was done on the roof of the Faculty of Science in Sarajevo and from the area of Kiseljak. Location of the measurement station on the roof of the Faculty is a typical urban area that is about 20 meters away from the main road. The location in Kiseljak represents an area that is much less urban sampling location. Precipitation patterns were used to test the pH value and electrical conductivity. Also, the sampling of precipitation was performed at a same time when the samples were exposed to the atmosphere.

### Determination of pH value and conductivity

The pH value represents the chemical quality indicator of atmospheric water. The pH value of a natural, unpolluted rainfall is between 5 and 6.

The following table shows the measured values of pH and electrical conductivity in precipitation samples.

**Table 1.** Measured values of pH and electrical conductivity in the area of Sarajevo

Sample	Date	pH	κ (mS/cm)
1.	27.11.2017.	4,93	0,0620
2.	3.12.2017.	5,02	0,0480
3.	9.12-10.12. 2017.	6,40	0,1480
4.	13.12.2017.	6,04	0,0630
5.	15.12 - 8.12. 2017.	5,28	0,0650
6.	29.12.2017.	7,55	0,0719
7.	3.1.2018.	6,60	0,0526
8.	10.1.2018.	6,20	0,0334
9.	14.1.2018.	6,35	0,0471
10.	17.1.2018.	5,92	0,0250
11.	21.1.2018.	6,50	0,0260
12.	23.1.2018.	6,60	0,0460
13.	3.2.2018.	6,50	0,0402
14.	7.2.2018.	6,70	0,0379

**Table 2.** Measured values of pH and electrical conductivity in the area of Kiseljak

Sample	Date	pH	κ (mS/cm)
1.	3. 11. 2017.	5,30	0,0102
2.	9. 12. 2017.	5,33	0,0278
3.	11. 12. 2017.	6,17	0,0376
4.	19. 12. 2017.	6,66	0,0458

The acidity of the rainwater ranges from 5 to 6.5 and there is no major deviation. Electrical conductivity represents an indirect measure for the total amount of dissolved substances in water. No significant changes were observed here also.



### Samples of metals and alloys and their exposure to the atmosphere

The thickness of the coating of chromium and zinc applied to the plates was determined by the x - ray diffraction method. Tables 3 and 4 show the results.

**Table 3.** Chromium, nickel and copper coating thickness

#	Sample	Cr ( $\mu\text{m}$ )	Ni ( $\mu\text{m}$ )	Cu ( $\mu\text{m}$ )
1	Chrome coated iron plate with worn out surface – desiccator	0,585	4,39	5,04
2	Chrome coated iron plate with worn out surface – Sarajevo	0,626	4,98	4,93
3	Chrome coated iron plate with worn out surface – Kiseljak	0,477	14,10	8,36

**Table 4.** Zinc coating thickness

#	Sample	Zn ( $\mu\text{m}$ )
1	Zinc coated iron plate with worn out surface - desiccator	5,32
2	Zinc coated iron plate – Sarajevo	6,03
3	Zinc coated iron plate with worn out surface – Sarajevo	6,02
4	Zinc coated iron plate with worn out surface – Kiseljak	5,81

The samples were placed in acetone, where there were no visible signs of corrosion and were placed in three different "atmospheres". The first set of samples was placed in a desiccator, so it was not exposed to external influences of the atmosphere. The second set of samples was set up on the roof of the Natural and Mathematical Faculty and exposed to the atmosphere. The third series of samples were placed in the area of Kiseljak. Samples were exposed to the atmosphere in period from 14.11.2017. to 26.3.2018.



Iron



Brass



Chrome coated iron plate with worn out surface



Zinc coated iron plate with worn out surface

**Figure 1.** Samples before exposure in Sarajevo area

### Analysis of samples

The analysis of the samples was performed after exposure to the outside environment in the period of time from 14.11.2017. to 26.3.2018. The methods used were: visual method and electrochemical methods.

### Visual method

After the samples were removed from the outside environment, a visual examination method was performed. The images show iron samples after removal from the outside.

As can be seen from the figures, the iron that was in the desiccator has no visible corrosion products. Also, one can notice the color difference of corrosion products between the iron samples exhibited in the Kiseljak area and the iron that was exposed to the atmospheric conditions in the area of Sarajevo. This difference can be attributed to the different content of the individual components of the atmosphere in which these samples were exposed. It is evident that the corrosion of objects in Sarajevo is more intense.

Figures show that the brass from the desiccator has no visible corrosion products. The sample of brass that was exposed to atmosphere in Kiseljak, showed corrosion. This result can be explained by the brass characteristics and its reaction to the environment. The difference between the corrosion products between the brass which was exposed in the area of Kiseljak and the brass that was exposed in the area of Sarajevo, when excluding the experimental error, cannot be visually determined. The possible reason for this is that patina can be formed in environments that are not burdened with pollutants.

Chrome coated iron plate with worn out surface exposed to the atmosphere: it can be seen that the sample has no visible signs of corrosion. The difference between chrome coated iron samples that were exposed in two different atmospheres can be seen in the color of corrosion products. Also, the chrome coated iron that was exposed in the area of Sarajevo was more intensely corroded on other parts of the plate as well as on the part where it was worn out. Since the compactness of the chrome coating has been damaged, iron corrosion has occurred since iron pair potential is more negative than chrome pair potential. Iron behaved as anode and oxidized in a given conditions.

The samples of zinc coated iron with worn out surface that were exposed to atmospheric effect are shown: from figures it can be seen that zinc coated iron from the desiccator has no visible signs of corrosion. The difference between the samples that were exposed in two different atmospheric environments is seen in the corrosion intensity and color of the products of corrosion. Even when the zinc-coated layer was worn out, corrosion was not detected because zinc had a good protective role.

Sample	Desiccator	Kiseljak	Sarajevo
Iron			
Brass			
Chrome coated iron plate with worn out surface			
Zinc coated iron plate with worn out surface			

Figure 2. Samples after exposure to the atmosphere

### Electrochemical methods

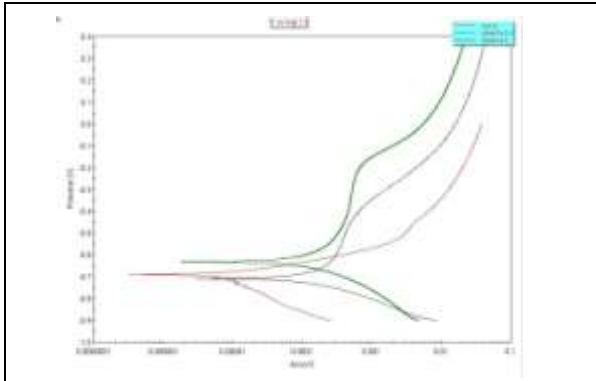
Electrochemical methods used for sample analysis were linear voltammetry and cyclic voltammetry. After the atmosphere exposure, the samples were prepared by isolating the surface of plate, except 1 cm<sup>2</sup> which was the active surface for electrochemical measurements. (Korać *et al.*, 2013)

### Linear voltammetry

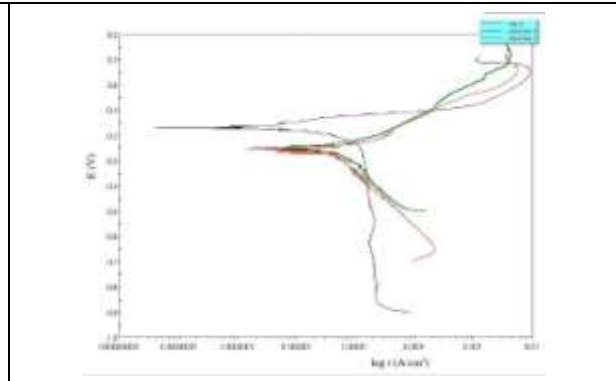
Potentiodynamic measurements were performed by linear voltammetry in a given range of potentials depending on the sample type at a scan rate of 0.166 mV/s and 1 mV/s. The measurements were performed in a three electrode system, where sample was working electrode, Ag/AgCl reference electrode, and platinum electrode as a counter electrode providing the current flow through the cell. As electrolyte, 3% NaCl solution was used. The cell is connected to the

Potentiostat/Galvanostat PAR 263A instrument with PowerCV controlled software (Korać *et al.*, 2012). Tafel extrapolation of voltammograms for each sample gave the values of Tafel constants  $\beta_a$  and  $\beta_c$ , corrosion potential  $E_{corr}$ , corrosion current  $i_{corr}$  and the value of the polarization resistance  $R_p$ . Based on the polarization resistance it is possible to calculate the rate of corrosion.

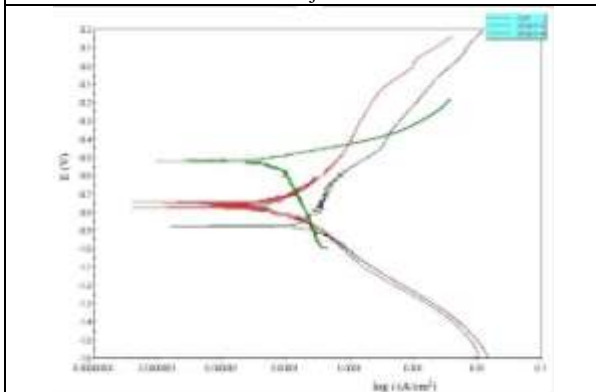
The obtained results of electrochemical methods are shown in the Tafel diagrams in Figures 3,4,5 and 6. From these curves the corrosion parameters shown in Table 5 are derived.



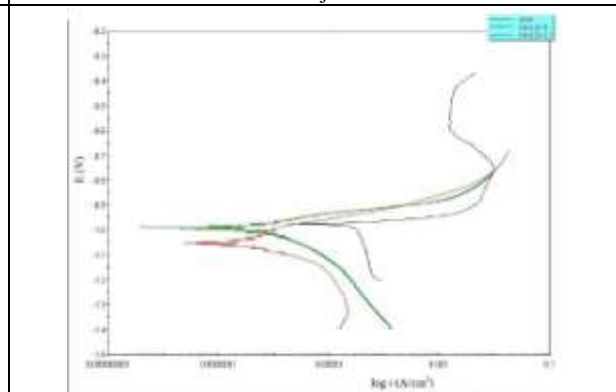
**Figure 3.** Tafel plots of iron samples from 3 different environments. Red – Desiccator, Green – Sarajevo, Black – Kiseljak.



**Figure 4.** Tafel plots of brass samples from 3 different environments. Black – Desiccator, Green – Sarajevo, Red – Kiseljak.



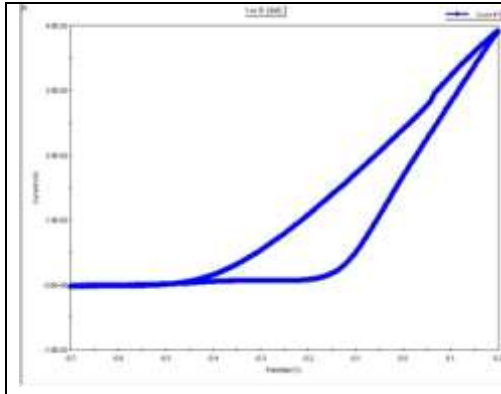
**Figure 5.** Tafel plots of chrome coated iron with worn out surface samples from 3 different environments. Green – Desiccator, Black – Sarajevo, Red – Kiseljak.



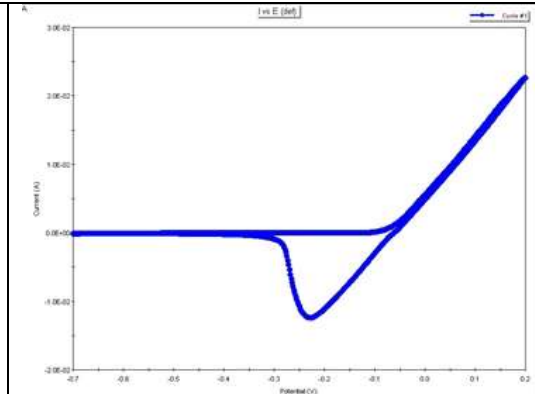
**Figure 6.** Tafel plots of zinc coated iron with worn out surface samples from 3 different environments. Black – Desiccator, Green – Sarajevo, Red – Kiseljak.

**Table 5.** Values of Corrosion Potential, Current, Tafels Constants and Polarization Resistance

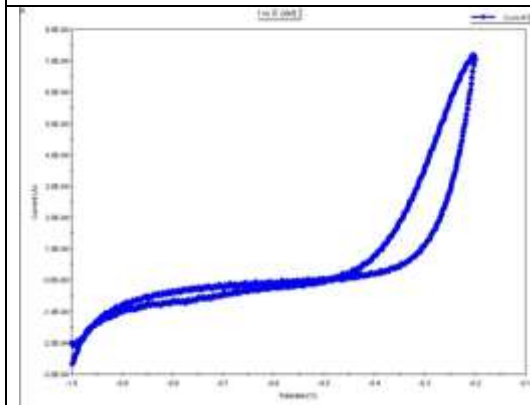
Sample	Atmosphere	$E_{corr}$ (mV)	$I_{corr}$ ( $\mu$ A)	$\beta_c$ (mV)	$\beta_a$ (mV)	$R_p$ ( $\Omega$ )
<b>Iron</b>	Desiccator	-695,288	7,802	161,095	60,768	3202,690
	Kiseljak	-705,605	24,750	99,223	130,618	818,570
	Sarajevo	-631,916	20,860	89,355	147,490	1542,990
<b>Brass</b>	Desiccator	-153,151	2,03	60,88	30,106	10950,97
	Kiseljak	-256,096	3,763	223,057	92,198	9689,935
	Sarajevo	-248,947	3,804	169,495	64,586	6510,677
<b>Chrome coated iron with worn out surface</b>	Desiccator	-517,563	4,356	190,274	62,609	20861,723
	Kiseljak	-756,595	9,859	264,045	277,250	4683,620
	Sarajevo	-854,572	6,240	236,306	660,776	3009,945
<b>Zinc coated iron with worn out surface</b>	Desiccator	-981,415	8,907	625,970	25,098	6947,269
	Kiseljak	-1053,536	1,050	60,747	89,264	10686,568
	Sarajevo	-982,829	2,100	102,552	46,956	18906,150



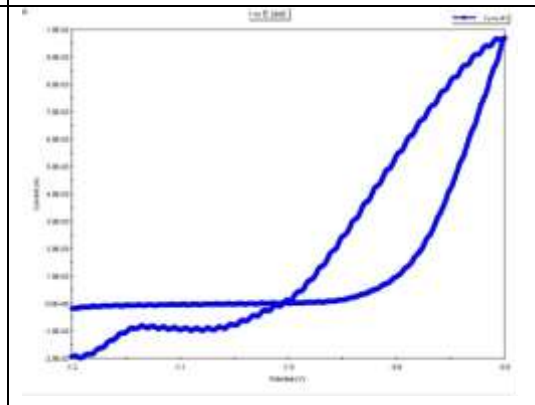
**Figure 7.** Cyclic voltammogram of iron sample from desiccator



**Figure 8.** Cyclic voltammogram of brass sample from desiccator



**Figure 9.** Cyclic voltammogram of chrome coated iron with worn out surface sample



**Figure 10.** Cyclic voltammogram zinc coated iron with worn out surface

**Table 6.** Values of pitting potential and repassivation potential

Sample	Location	$E_{pit}$ (mV)	$E_{rp}$ (mV)
Iron	Desiccator	-0,1938	-0,5098
Brass	Desiccator	-0,03139	-0,8467
Chrome coated iron with worn out surface	Desiccator	-0,3932	-0,4965
Zinc coated iron with worn out surface	Desiccator	-0,9393	-1,007

### Iron

First, a voltammogram of iron that was exposed in the desiccator was recorded at a scan rate of **0.166 mV/s** with a potential range between -0.95 and 0.4 V, and then samples of iron were exposed in Kiseljak and Sarajevo area were recorded at the scan rate of **0.166 mV/s** in the potential range between -0.95 and 0.4 V.

From the obtained values, it can be concluded that the sample from Kiseljak shows the lowest polarization resistance, and the highest value of the corrosion current. The iron that was exposed in the desiccator shows the highest value of polarization resistance, and the lowest corrosion current.

### Brass

Desiccator brass was recorded in a potential range between -0.9 and 0.2 V at a scan rate of 0.166 mV/s. Brass samples that were exposed in the area of Sarajevo and Kiseljak were recorded in the potential range between -0.7 and 0.2 V at a scan rate of 0.166 mV/s.

From the obtained values it can be concluded that the brass exposed in the area of Sarajevo has the lowest value of the polarization resistance and the highest value of the corrosion current. The Tafel diagrams show clearly the section of passivating properties of the corrosion products.

### Chrome coated iron whose surface is worn out

Samples of chrome coated iron whose surface was worn out were recorded in the potential range between -1.6 and 0.2 V. The sample from the desiccator was recorded at a scan rate of 0.166 mV/s, while the samples that were exposed in the area of Kiseljak and Sarajevo were recorded at a scan rate of 0.166 mV/s. From these obtained values it can be concluded that the chrome coated iron sample whose surface is worn out from the desiccator has the highest value of the polarization resistance and the lowest value of the corrosion current. The corrosion potential of the sample from the desiccator is most positive – it can be attributed to

chromium. The other two are those in which the iron oxidation process begins and slowly reaches the potential of iron, which is more negative.

#### Zinc coated iron whose surface is worn out

Samples of zinc coated iron whose surface was worn out were recorded in the range of potentials from -1.4 to -0.4 V. The sample from the desiccator was recorded at a scan rate of 0.166 mV/s, while the samples that were exposed in the area of Kiseljak and Sarajevo recorded at a scan rate of 0,166 mV/s. From the obtained values of corrosion potentials, it can be concluded that there are no significant changes in the corrosion parameters. There was no critical degradation of zinc, so it retained protector properties. Also, these samples show no significant changes in corrosion parameters.

#### Cyclic voltammetry

Samples for measuring this method were prepared in the same way as for linear voltammetry. This method was used to capture samples that were exposed in the desiccator.

The results obtained by this method are shown in the cyclovoltammograms in Figures 7, 8, 9 and 10. The corrosion parameters shown in Table 6 are derived from these voltammograms.

#### Iron

A sample of iron from the desiccator was recorded in the potential range of -0.7 - 0.2 V, at a scan rate of 1 mV/s. Cyclic voltammogram (CV) shows no peaks obtained in the selected range of potentials.

#### Brass

The brass sample from the desiccator was recorded in the potential range of -0.7 - 0.2 V at a scan rate of 1 mV/s. On voltammograms of brass, there is a defined reduction peak at about -0.25 V, which corresponds to processes related to the reduction of oxidized species of copper.

#### Chrome coated iron whit worn out surface

The CV of sample of chrome coated iron whit worn out surface is recorded in the potential range of -1.0 V to -0.1 V at a scan rate of 1 mV/s. On this voltammogram, peaks occur at potentials of around -0.75 V. This may suggest that chrome coating no longer protects iron. The compactness of the coating is disturbed, and iron, as a more negative one, gets the role of anode and corrodes.

#### Zinc coated iron whit worn out surface

A CV of a zinc coated iron sample whose surface is worn out is recorded in a potential range of -1.2 V to -0.8 V at a scan rate of 1 mV/s. The cyclic voltammogram of this sample is similar to the sample of a not worn out one. There was no disturbance in the zinc protective properties, even though it is worn out.

## CONCLUSIONS

An iron plate, chrome coated iron plate with worn out surface, zinc coated iron plate with worn out surface and brass plate were used as samples.

The analysis of the samples was carried out after exposure to the outside environment in the period between 14.November 2017 to 26.March 2018. The methods used are: visual method and electrochemical methods. Based on the obtained results, the following conclusions are:

- Iron in the desiccator has no visible corrosion products, while the samples from Sarajevo and Kiseljak show more corrosion
- The brass that was in the desiccator has no visible corrosion products. On brass sample, from both tested areas of Kiseljak and of Sarajevo, layer of patina was seen;
- The chromium-iron sample whose coating was frozen, has no visible signs of corrosion. The difference between samples of chromium iron exposed in two different atmospheres can be seen in the color of corrosion products;
- Galvanized iron with a sealed coat kept in desiccator has no visible signs of corrosion. Even when the zinc-coated layer was not corrosive, zinc had a good protective role.

Tafel extrapolation of the shown voltammograms for each sample determined the values of Tafel constants  $\beta_a$  and  $\beta_c$ , corrosion potential  $E_{\text{corr}}$ , corrosion current  $i_{\text{corr}}$  and the value of the polarization resistance  $R_p$ . Based on the polarization resistance it is possible to calculate the rate of corrosion.

- Iron sample from Kiseljak shows the lowest polarization resistance, and thus the highest value of corrosion. The iron that was kept in the desiccator shows the highest value of the polarization resistance, and thus the lowest corrosion current.
- From the obtained values, it can be concluded that the brass exposed in the area of Sarajevo has the lowest value of the polarization resistance and the highest value of the corrosion current. On the Tafel plots, it is possible to clearly see the sections showing the passivating properties of the products of corrosion.
- Chrome coated iron sample whose surface is worn out from the desiccator has the highest value of the polarization resistance and the highest value of the corrosion potential. The corrosion potential of the sample from the desiccator is the most positive - belongs to the chromium. The other two are those in which the iron oxidation process begins and slowly reaches the potential of iron, which is more negative.
- The zinc coated iron sample from the desiccator has the highest value of corrosion current and the lowest value of the polarization resistance. Also, in these samples there are no significant changes in corrosion parameters. There was no critical degradation of zinc, so it retained protective properties.

Cyclic voltammetry provides following data:

- CV of iron shows no detected peaks in the selected potential range.

- CV of brass shows a defined reduction peak at a value of about -0.25 V, which corresponds to processes related to the reduction of oxidized species of copper.
- CV of chrome coated iron shows the peak at a value of about -0.75V. This suggests that chrome coating no longer protects iron. The compactness of the coating is disturbed, and iron, as a more negative one, gets the role of anode and corrodes.
- CV of zinc coated iron with worn out surface is similar to that which has no worn out surface. There was no disturbance in the zinc protective properties, even though it is worn out.

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### Summary/Sažetak

Brzina atmosferske korozije je funkcija sadržaja pojedinih komponenata u atmosferi i temperature vazduha, prvenstveno se misli na sadržaj vlage, različitih čestica i gasova SO<sub>x</sub> i NO<sub>x</sub>. Ispiranjem polutanata nastaju jake kiseline, baze i soli koje mogu da nagrizaju različite metalne i nemetalne konstrukcije.

Izvršena je analiza određenih parametara kvaliteta padavina, analiza uzoraka metala i legura koji su bili izloženi atmosferi na području Sarajeva i Kiseljaka, te analiza uzoraka metala i legura bez atmosferskog uticaja. Metode analize uzoraka koje su korištene su linearna voltometrija i ciklična voltometrija. Kao uzorci su korištene pločice željeza, hromiranog željeza, pocinčanog željeza, hromiranog željeza čija je površina bila zaparana, pocinčanog željeza čija je površina bila zaparana i pločica mesinga. Vidljivo je da je intenzivnija korozija predmeta u Sarajevu. Nešto manje je u području Kiseljaka. Linearna voltometrija u određenom rasponu potencijala dala su izraženije korozione struje u urbanoj sredini.

Ciklična voltometrija kod mesinga je javlja izraženi redukциони pik na vrijednosti od oko -0,25 V. Kod hromiranog željeza se javlja na -0,05 V. Kod pocinčanog željeza potencijali su pomjereni prema negativnijim vrijednostima koji pripadaju cinku. Na ciklovoltamogramu kod zaparane hromne prevlake je na -0,75V. Ciklični voltamogram zaparanog cinka je sličan onom koji nije zaparan. Urbano područje Sarajeva, značajno opterećeno polutantima koji mogu uticati na sve aspekte života i okoline. Izražena je pojava kiselih kiša, što pojačava korozione osobine materijala od kojih su izgrađene konstrukcije.







## Physical Chemistry for Undergraduate Students: Sources of Students' Difficulties and Potential Solutions

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**Abstract:** Traditionally, university students struggle with physical chemistry courses at Faculty of Science. This is particularly evident in the mutual integration of knowledge in general chemistry, physics, and mathematics that are considered as fundamental to physical chemistry. This paper presents the results of research conducted with the 2<sup>nd</sup> year chemistry students at the Faculty of Science University of Sarajevo, with the main aim to find solutions to these difficulties that could lead to greater learning efficiency, and a successful continuation of their chemistry studies. The results indicated that students' knowledge of concepts relevant to physical chemistry is not at a satisfactory level. This is in line with the rather low students' grades in general chemistry, general physics and mathematics during the first year of the study. Students reported their most common difficulties: the lack of time for learning, the lack of literature recommended by the syllabus, and certain mathematical concepts they do not understand. To overcome them, it is important to direct students to use the available resources more efficiently, and to emphasize the significance of knowledge integration.

## INTRODUCTION

Education in the 21<sup>st</sup> century is mainly oriented towards gaining good, solid, and permanent knowledge that will serve as a fundament for lifelong learning (LLL). Science educators give priority to the quality of knowledge over its quantity, to the knowledge that can be used for solving problems. In order to prepare for active participation in EHEA (European Higher Education Area), teachers at University of Sarajevo, Bosnia and Herzegovina (B&H), are in a process of modernization of their teaching practices.

Chemistry students make up about 20% of total enrolled students at the Faculty of Science, University of Sarajevo. They generally believe that chemistry professionals have a perspective: some of them have their interest in this study thanks to the ability to find their place as future experts in chemical, pharmaceutical, and food industry.

The world around us integrates science. To understand the world and nature, we need to look at the world in an

integrated way (Hewitt, Lyons, Suchocki, *et al.*, 2007). Knowledge integration is a way to utilize knowledge and a core competency (Wang and Farn, 2012). It is defined as a process of "adding, distinguishing, evaluating, and sorting out phenomena, situations, and abstractions in science" (Linn, Davis, and Bell, 2004), as cited in Liu, Lee, Hofstetter, and Linn (2008). This presumes that knowledge integration needs to be one of the characteristics of university courses.

Traditionally, integration within science courses (physics and chemistry) at the freshman level is not very common, in spite of their fundamental similarities. Students encounter many, often confusing and conflicting ideas when they learn science (Linn, Lee, Tinker, *et al.*, 2006). One of the consequences is that students have difficulties with concepts that cannot be classified as purely physical, chemical or mathematical (Izatt, Harrell, and Nikles, 1996). Izatt *et al.* (1996) attempted to develop an integrated curriculum of physics and chemistry for freshmen students. They believe they have created a positive learning experience for their

students. Curriculum integration leads to standardized notation and the elimination of duplication of teaching content.

One of the important interdisciplinary fields of science is physical chemistry. It is based on mathematical, chemical and physical principles and is of a vital significance in medicine, pharmacy, agriculture, development and application of new materials, in monitoring and protection of a healthy environment. Physical chemistry spreads its boundaries and overlaps with other fundamental and applied sciences (Zielinski and Schwenz, 2004; Peric, 2009).

The importance of knowledge integration is highlighted in many research studies (Izatt *et al.*, 1996; Linn *et al.*, 2006, Liu *et al.*, 2008). It relies on a constructivist theory of learning that assumes that existing knowledge is the basis for a new, meaningful learning (Taber, 2008). However, Taber also pointed out that some students believe that teachers' expectations for the integrating chemical knowledge with the existing basis from physics are unreasonable to demand (Taber, 1998).

Preliminary research results, mostly diagnostic, pointed to many conceptual difficulties (Gojak, Galijašević, Hadžibegović, *et al.*, 2012), but also the lack of procedural knowledge originating in middle and high school. Insufficient integration of knowledge from chemistry, physics, and mathematics was observed, as well as the insufficient use of recommended literature, a low degree of permanent knowledge, mostly regarding general courses on the first year of study (Zejnlagić-Hajrić, Hadžibegović, Galijašević, *et al.*, 2010a). Students have problems that come from their earlier education: operating with units of measurement, SI units (also reported in Pitt, 2003; Ford and Gilbert, 2013), mathematical operations involving exponents, as well as knowledge of functional relationships between physical values (Zejnlagić-Hajrić, Hadžibegović, Galijašević, *et al.*, 2010b; Nuić, Zejnlagić-Hajrić, Hadžibegović, *et al.*, 2011).

Physical chemistry has been taught continuously at several faculties within the University of Sarajevo since the Chair of Physical Chemistry at the Department of Chemistry has been established in 1954. Traditionally, courses within this scientific discipline have been demanding and difficult for students.

According to the pre-Bologna curriculum, the domain of *Physical chemistry* was represented by four two-semester courses (Physical Chemistry I, Physical Chemistry II, Corrosion and Corrosion Protection, and Kinetics and Catalysis). They were taught during 2<sup>nd</sup> and 3<sup>rd</sup> year of a 4-year study programs *Chemistry* and *Chemistry Education*. The introduction of the Bologna model of study in 2005 resulted in transforming former Physical Chemistry I into two one-semester courses: Physical Chemistry I (PC I) and Physical Chemistry II (PC II), in 3<sup>rd</sup> and 4<sup>th</sup> semester, respectively. Teaching content was slightly changed, and the number of hours of lectures and exercises per week was reduced. Accordingly, Physical Chemistry II transformed into Physical Chemistry III (PC III) and Physical Chemistry IV (PC IV), both in 3<sup>rd</sup> year. The present model of study contains seven compulsory and three elective courses with a total of 48 ECTS points for the domain *Physical*

*chemistry* during the first cycle (total 240 ECTS). After the first cycle, students can enroll the second cycle (60 ECTS) and choose *Physical chemistry* as their major and earn total 20 ECTS for elective courses.

General Physics courses during 1<sup>st</sup> year of study comprise topics of mechanics, thermodynamics, electricity, and magnetism (1<sup>st</sup> semester), optics and quantum mechanics (2<sup>nd</sup> semester). Mathematics curriculum includes linear equations, matrices, operations with vectors (1<sup>st</sup> semester) derivation and integral calculus (2<sup>nd</sup> semester).

Course PC I consists mainly of chemical thermodynamics, while PC II includes chemical equilibrium, phase equilibrium, and chemical kinetics. The programs of the PC I and PC II courses assume that students have certain knowledge in general chemistry, physics and mathematics in order to learn new concepts by integrating previous and new knowledge. In the end, it facilitates passing the final exam, as one of the most important students' goals.

Laboratory and calculation exercises make a substantial part of the courses PC I and PC II. Students, supervised by teaching assistants and technicians, usually perform laboratory exercises in pairs. After measuring, collecting and analyzing data, students present their results to the teaching assistants for verification. Verification of the entire set of laboratory exercises is one of the prerequisites for taking the final exam.

## METHODOLOGY

### Research problem

In recent years, the number of students who had passed the final exam or the continuous partial exams during the semester was fairly low. The fact is that students experience difficulties in applying the appropriate learning techniques. The main aim of this research was to explore the sources for the observed low passing rate and potential ways for improving students' understanding of physical chemistry concepts.

### Participants and research instruments

The research described in this paper has been conducted at the Faculty of Science, Department of Chemistry, with 2<sup>nd</sup> year chemistry students in two subsequent academic years.

Research instruments included a test of knowledge (Gojak *et al.*, 2012) used as a pretest and posttest. Additionally, the initial questionnaire Q<sub>I</sub> aimed to explore students' grades in relevant courses, their satisfaction with the knowledge they acquired, and certain general characteristics of the learning process. Final questionnaire Q<sub>F</sub> aimed to explore students' perceptions on courses PC I and PC II, and most common difficulties they encountered during learning.

Test of knowledge contained 17 items, including basic mathematical calculations and their application into chemistry and physics, therefore all relevant to physical chemistry. Each item was given one point if true, 0 point if it is false. The data was confidential and had no impact on students' grades. The criterion for passing the test of knowledge was set to 55%, according to Bologna's model of study at the University of Sarajevo.

The analysis of the internal consistency of the test of knowledge resulted in lower values of Cronbach's alpha for the pretests and higher for the posttests (Table 1), indicating that teaching process affected the participants' knowledge regarding test items.

**Table 1.** Analysis of internal consistency for the test of knowledge

Group	Pretest		Posttest	
	I	II	I	II
N	47	55	26	19
Alpha	0.652	0.385	0.831	0.853

## RESULTS

### Students' knowledge on concepts relevant for Physical chemistry

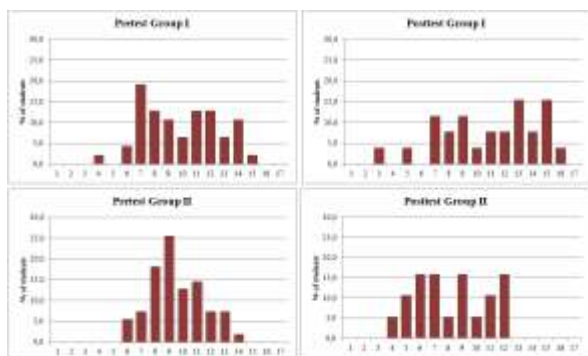
Table 2 represents the descriptive statistics for the test of knowledge aimed to explore students' knowledge of mathematical, chemical and physical concepts relevant to physical chemistry courses. It was administered as a pretest and as a posttest for two generations of students.

**Table 2.** Descriptive statistics for the test of knowledge

Group	N	M	SD	Pretest			
				SE	X <sub>Min</sub>	X <sub>Max</sub>	Mode
I	47	9.61	2.67	0.39	4.0	14.5	7.0 <sup>a</sup>
II	55	9.29	1.90	0.26	6.0	14.0	8.5
Group	N	M	SD	Posttest			
				SE	X <sub>Min</sub>	X <sub>Max</sub>	Mode
I	26	10.81	3.57	0.70	2.5	16.0	13.0
II	19	8.07	2.68	0.61	4.0	12.0	9.0 <sup>a</sup>

Legend: N - number of participants; M - arithmetic mean; SD - standard deviation; SE - standard error of the mean; X<sub>Min</sub> - lowest score on the test; X<sub>Max</sub> - highest score on the test; Mode - value that appears most often; <sup>a</sup> - multiple modes exist, the smallest value is shown

The maximum score that could be earned was set to 17. Means indicate that average students' achievements were around 50% of the maximum score, which is rather low for university students and this type of the test of knowledge addressing their previous knowledge relevant to physical chemistry.



**Figure 1.** Frequency distributions of students' total scores on tests of knowledge

Visual representation of students' total scores on tests of knowledge indicates that Group I progressed from pre- to posttest, but that was not the case for Group II students.

Table 3 represents students' average grades (with

standard deviations in parentheses) in *General Chemistry* (Ch), *General Physics* (Ph) and *Calculus/Mathematics* (M) courses during the first year of study, and their own grades that they believe they deserved, both on scale 6-10. The non-parametric Wilcoxon signed-rank test was performed to compare them.

**Table 3.** Students' grades and perceptions on the acquired knowledge

	Group I			Group II		
	Ch	Ph	M	Ch	Ph	M
Average grades	7.30 (0.72)	7.04 (0.65)	6.53 (0.72)	6.86 (0.67)	6.95 (0.75)	6.40 (0.56)
Assessment of acquired knowledge	7.85 (0.88)	7.15 (0.93)	7.00 (0.91)	7.64 (1.19)	7.15 (0.89)	7.14 (0.92)
Z	-3.556	-0.767	-3.429	-4.910	-1.592	-5.019
Asymp. Sig.	0.000	0.443	0.001	0.000	0.111	0.000

It is evident that students from both groups tend to evaluate their knowledge in *General Chemistry* and *Mathematics* with higher grades than their teachers, while in the case of *General Physics* the difference was not statistically significant.

### Students' perceptions on the first year of the study

Initial questionnaire Q<sub>1</sub> covered various topics such as students' assessment of the courses, and aspects considering preparation for exams.

**Table 4.** Students' satisfaction with the acquired knowledge

	Group I			Group II		
	Ch	Ph	M	Ch	Ph	M
M	3.32	3.11	3.06	3.69	3.34	3.29
SD	0.96	1.05	1.03	0.68	0.98	1.02
MR	2.17	2.02	1.81	2.25	1.86	1.89
$\chi^2$		<b>4.672</b>			<b>8.792</b>	
p		<b>0.097</b>			<b>0.012</b>	

Legend: M - arithmetic mean; SD - standard deviation; MR - mean rank;  $\chi^2$  - chi-square value (Friedman's test); p - significance level

Table 4 represents the data on students' assessment of the acquired knowledge in general chemistry, general physics and mathematics on scale 1-5. Means indicate that students are generally more satisfied with acquired knowledge in *General Chemistry*. The difference between students' assessment among courses was examined using Friedman's test which indicated statistically significant difference in case of Group II students ( $p = 0.012$ ). Wilcoxon signed-rank test<sup>1</sup> pointed to the difference between students' assessment of knowledge in favor of *General Chemistry* compared to both *General Physics* ( $p = 0.008$ ) and *Mathematics* ( $p = 0.006$ ).

**Table 5.** Selected data from the initial questionnaire Q<sub>1</sub>

Statement	Group I (%)	Group II (%)
I am motivated for continuing the study	21.3	20.3

<sup>1</sup> The Bonferroni correction was applied resulting in a significance level set at  $p < 0.017$

I plan to change the field of the study	19.1	18.6
I would recommend my friends to enroll Chemistry	63.8	71.2
I am able to integrate acquired knowledge	74.5	81.3
I own a laptop/computer at home	85.1	96.6
I have unlimited access to the Internet	63.8	74.6
I have the prerequisites for online communication with the teacher	87.2	93.2
My knowledge of English is at a satisfactory level	87.2	88.1
I use the recommended literature for preparing the exams	74.5	54.2

The data in Table 5 reflects interesting findings: only about 20% of students are motivated to continue the study, but they do not plan to change it and recommend it to their friends. Additionally, they believe they are able to integrate knowledge, but this is not consistent with the results of the knowledge tests. Analyzing the necessary prerequisites for learning and preparing for exams, students mostly have good conditions: a laptop or a computer, Internet access, while their knowledge of English is quite good. It is interesting that Group II students do not use the recommended literature for exams to the extent as the Group I students. Group II students rely on their lecture notes and PowerPoint presentations provided by teachers. The fact is that our libraries do not follow the modern trends and have deficiencies in the recommended literature due to financial obstacles, but useful sources can be found online from safe and trusted websites (universities, academic associations, free e-books, and scientific papers). This requires knowing foreign languages (mostly English) which apparently is not a problem for most students

These data suggest that online communication and English language skills with students can be used to a greater extent.

#### Students' perceptions on the second year of the study

The final questionnaire covered various topics related to the teaching process at the PC I and PC II courses.

The results revealed students' difficulties regarding some mathematical concepts required for PC I and PC II, such as deriving equations, differential, and integral calculus. These concepts make part of the syllabi of the courses Calculus I and Calculus II. Students experience difficulties in integrating knowledge, explaining, deriving a logical conclusion, solving problems.

Students' perceptions of supplementary classes in Physics and PC I are generally positive. They see benefits in clarifying some ambiguities and emphasizing the most important topics by the teacher but also pointing to the importance of previous learning activities.

One item in Q<sub>F</sub> asked students for specific themes within PC I and PC II they found to be most difficult. The most

common answer was thermodynamics, a broad area that requires solid previous knowledge, especially in mathematics.

**Table 6.** Selected data from the final questionnaire Q<sub>F</sub>

Statement	Group I (%)	Group II (%)
I have attended the supplementary classes in Physics	63.0	9.1
I have attended the supplementary classes in PC I	7.4	59.1
I have consulted the teacher regarding some topics in PC I	22.2	45.5
I have consulted the teaching assistant regarding some topics in PC I	85.2	40.9
I combine recommended literature with my notes in learning PC I	40.7	27.3
I am aware of the rules of taking exams and evaluation process in PC I	81.5	86.4
I find the oral exam in PC I to be transparent	48.1	50.0

#### Students' exam results

According to the syllabus of the PC I course, students' achievements are evaluated as follows: Attending lectures and engagement (5%); Physical chemistry calculation exercises (5%), Laboratory exercises (10%), two written exams during the course and combined (written and oral) final exam (80%).

In order to pass the course, a student needs a minimum of 55%. In case they fail, there are the possibilities to repeat the final exam at the end of the particular semester and at the end of the academic year (September). These facts are regulated by the Law of higher education at the University of Sarajevo.

Physical chemistry teacher's records show that 44.4% of Group I students passed the exam with average grade 7.43<sup>2</sup>. Group II students pass rate was higher (76.2%), but the average grade was only slightly higher (7.66).

#### DISCUSSION

Inadequate and insufficient previous knowledge of students could originate from various sources, such as secondary school education, inadequate curricula, the lack of motivation, etc. Students do not exploit resources provided by the institution, such as consultation with teachers and teaching assistants to resolve doubts and ambiguities they encountered during learning. Those students, who come for consultations, usually ask help regarding the lack of mathematical skills needed for required calculations.

Even though students were encouraged to talk about their conceptual difficulties, these consultations were more instructive and focused mainly on some specific

<sup>2</sup> Scale is defined according to the Law of Higher Education in Canton Sarajevo - Scale 6-10, grade 6 (E) being the lowest passing grade.

task rather than a discussion about some problem regarding the overall understanding of the concept.

The students showed a self-critical opinion regarding their individual work and stated some difficulties they struggle with: the lack of time for learning and preparing for exams, the lack of literature recommended by the syllabus, certain mathematical concepts they do not understand and difficulties when working on numerical assignments. Exam items requiring explaining and reasoning of given answer or problem solving were reported as too demanding. Definitely, *Chemistry* is a demanding study program and requires commitment not only in the exam period but during the entire semester. The mandatory, as well as most of the recommended literature are available at the library of the Faculty as well as at the National and University Library of Bosnia and Herzegovina.

From the teachers' perspective, a large number of students attending lectures (approx. 60) reduces the opportunity for a single student to approach the teacher, or for the teacher to treat students individually. This can be solved by consultations with the teacher, which students generally do not usually use.

We should also emphasize that, due to financial difficulties, our laboratory equipment is rather old and insufficient. Increased number of students in laboratory groups (up to 16, while labs are initially planned for a maximum 10 students) affects the quality of laboratory exercises. They are planned for individual students' engagement in order to take the responsibility (or even consequences) of the results obtained.

From students' exam results we got the impression that students learn only as much as they think it is enough to pass, and not to acquire permanent, integrated and usable knowledge. We need to emphasize to students that their main interest is to learn for their future, and the grades they receive are not as important. There is no use in forcing students to learn information they will remember during the exam period, but later they could not even remember them. After all, in our own research or teaching, we do not simply rely on our memory for even the simplest formula, but we check it in the appropriate textbook. The responsibility for students' results in learning and integrating knowledge is on students, their parents, but also on their teachers (Cooper, 2012).

## CONCLUSIONS

In order to properly understand physical chemistry concepts, it is clear that students need proper assistance with learning, mostly due to integration of previous knowledge. We need to motivate our students to solve the problems they have in their learning process through different ways of assistance they have the right under our law and regulations (to consult their teachers more frequently, to attend the lectures within additional teaching). The difficulties reported by students relate to the lack of certain resources. Although we admit that the situation is inconvenient to a certain extent, some assistance might help in resolving them. The main goal is for students to find adequate techniques of learning in order to make their achievements higher and the learning process more efficient. We need to direct our students

not to differentiate their knowledge by course programs but to integrate it among courses, levels of education, and with previous knowledge. In order to accomplish this, students need to increase their active participation.

Certain limitations of the study need to be acknowledged. The data collected did not affect students' grades in any way, but we cannot be sure whether students therefore put an effort in answering the test items or simply superficially choose certain options. The data gathered using questionnaires resulted in a certain number of (mostly open-ended) items without answer. We cannot be sure whether students considered the questionnaires in their full context or how truthful they were since some items required self-critical evaluation of their own learning process. For a complete image of students' knowledge and attitudes towards their study, these results need to be seen as a reflection of the current socio-economic situation in our country. There is a certain number of students who enroll „any“ study program in order to be eligible for financial support<sup>3</sup>. Additionally, Bosnia and Herzegovina's chemical industry had been seriously damaged during the war and still has not recuperated, which limits the potential employment for graduate students.

Since this study is a part of a longitudinal research, these results point to the fact that student's difficulties observed in our earlier research are common among our students and are not related to a single generation. We have explored their potential sources and we will try to address them during the teaching process in the following period, but we do expect students to take active role in this process. After all, we have the same goal – acquiring solid, applicable knowledge for future chemists.

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<sup>3</sup> Students who come from families of disabled war veterans or fallen soldiers have right to financial support as long as they are full-time students.

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## Summary/Sažetak

Tradicionalno, predmeti iz oblasti fizikalne hemije predstavljaju određene poteškoće za studente u postizanju dobrih rezultata učenja i polaganja ispita. Ovo je posebno uočljivo kroz nedovoljnu integraciju stečenog znanja u fizici, hemiji i matematici, za koje se smatra da su temeljni za fizikalnu hemiju. U ovom radu prikazani su rezultati istraživanja provedenog sa studentima druge godine studija hemije na Prirodno-matematičkom fakultetu Univerziteta u Sarajevu, s glavnom svrhom pronalazjenja mogućih rješenja za navedene teškoće, koja bi mogla dovesti do veće učinkovitosti učenja i uspješnije nastavljanje studija hemije. Prema dobivenim rezultatima, možemo zaključiti da znanje studenata o pojmovima relevantnim za fizikalnu hemiju nije na zadovoljavajućoj razini. To je u skladu s relativno niskim ocjenama iz predmeta iz oblasti opće hemije, opće fizike i matematike tokom prve godine studija. Najčešći problemi koje su studenti naveli su nedostatak vremena za učenje, nedostatak literature preporučene silabusom, te određeni matematički pojmovi koje ne razumiju. Kako bi ih prevazišli, važno je usmjeriti studente kako bi efikasnije koristili pružene resurse, kao i istaknuti važnost integriranja znanja.

## Hardness of the solder alloy Cu-In-Sb in dependence of the molar ratio of the components

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**Abstract:** An increasing concern for environmental protection led to the establishment of strict provisions regarding the use of alloys that could have a detrimental effect on the environment, which resulted in reduction of use lead solder materials. Copper-based alloys in particular represent a possible replacement for standard not-lead solder. Alloys on the basis of copper, indium, and antimony are important in production of new solder materials. Thus, it is desirable the knowledge about different ratios of mentioned elements in these alloys, related to their influence on the mechanical properties of alloy, for the development of new high quality solder materials. In this paper, the hardness of ternary system Cu-In-Sb was investigated, for three vertical cross sections with molar ratio: Sb:Cu = 1, Cu:In = 1, Sb:In = 1. Highest hardness has been found with 0,30 mol fraction of copper in Cu-InSb vertical cross section. Hardness continually decreases with the increase of molar percent of antimony. Indium has shown the least variation and influence on the hardness of these alloys. The most variation in hardness was during the change of the molar fraction of copper, which indicates that the copper is the most important element for the hardness of this system.

## INTRODUCTION

The industry uses large amounts of alloys based on lead due to its physical and chemical properties and low cost. Lead toxicity poses a major threat to the environment, and scientific research has begun to accelerate the industrial development of new ecological materials to completely eliminate the use of existing alloys based on lead (Smith, 2004). New solder materials, environmentally more acceptable, should not contain lead and that's mentioned in the European Union directives (EU Directive 2008/34/EZ). The development of unleaded alloys is one of the world's environmental trends. Alloys on the basis of copper, indium and antimony are important in production of new solder materials - possible replacement for traditional lead solder.

Indium is a silver soft metal with relatively low melting point. Same as sodium, indium can be cut with an ordinary knife. For a human organism, indium is not an essential element but also very little is known of its toxic properties (Oberndorff, 2001; Liu *et al.*, 2003). Elemental indium could have a significant effect on changes in the microstructure of the alloy, from the aspect of the formation of intermetallic compounds with copper (Liu *et al.*, 2002; Manasijević *et al.*, 2009). Indium is primarily used as a soldering material. Antimony is glossy gray metal with low-hardness. Four allotropic modifications of this element are known. Antimony is easily alloyed with a large number of metals, improving their mechanical properties. Its characteristic is that it is corrosion resistant and easily merged with other metals (Liu *et al.*, 2003). Antimony is considered strategic raw material (Gomidželović *et al.*,

2009). The effects of antimony and its compounds on human health and environment differ widely (Oberndorff, 2001). Surely, some antimony compounds are toxic. The copper is relatively soft metal, it can be shaped well. As an excellent conductor of heat and electric current he found many applications in the technique. Copper is very often part of many alloys, probably it is the most used metal in technology of alloys. Elemental copper in its basic state is not poisonous, but copper ions are very strong poisons for microorganism. Copper alloys are important technical materials with excellent mechanical properties. They are extremely resistant to corrosion and wear. In this sense, copper based alloys have good characteristics that are desirable for their application in the electro and mechanical engineering (Oberndorff, 2001; Smith, 2004; Liu *et al.*, 2000).

Cu-In and Cu-Sb alloys are well known binary system in engineering of alloys, and already have a usage in electro and mechanical industry (Liu *et al.*, 2002; Gierlotka and Jendrzeczyk-Handzlik, 2009; Liu *et al.*, 2000). These two systems, especially relation between Cu and In, are the reasons for the research of this Cu-In-Sb alloy. Indium and copper react and form intermetallic compound that is much harder and stiffer than the parent indium and copper. As well, this binary system, as contact material, is thermally very stable, and that is important in electro industry (Liu *et al.*, 2002). Ternary system Cu-In-Sb belongs to a group of new unleaded solder materials. Regardless of significant application there is insufficient data in the literature on the mechanical properties of this system. All this provides sufficient space for research in this field, in terms of contribution of knowledge about the mentioned ternary system, which besides the theoretical contribution and importance, should enable the technical application of this research. The system Cu-In-Sb, exactly its hardness, is the main goal of the research, so far not examined, which provides the possibility of research in this field.

Hardness presents one of the mechanical properties of the material. Hardness of material implies the resistance that the material show when a harder body penetrates through its surface. It is expressed by the ratio of force per unit of the surface of the imprint, or by the depth of the imprint in the material, expressed in the corresponding hardness units. In this study, the hardness of Cu-In-Sb alloy was determined, according to the Brinell method.

## EXPERIMENTAL

Hardness of material is the property of the material which enables it to resist plastic deformation, usually by penetration or by indentation. The Brinell hardness test procedure is governed by several standards depending on the type of material. This hardness testing procedure was suggested by the Swedish engineer Johan August Brinell in 1900, and it was the first widely accepted and standardized method of hardness testing in material science. Brinell method of measuring the hardness consists of hardened steel or tungsten-carbide ball against the smooth material surface under standard test condition. Standard test condition resumes diameter of

ball, load (force, pressure) and period. The dept of ball penetration depends on the type of material being tested.

### Sample preparation

The preparation of the samples was carried out with 99.99% purity metals (Alfa Aesar GmbH, Germany). The corresponding masses of copper, antimony and indium were weighed, crushed and mixed. The stirred samples were melted in a quartz furnace under vacuum (Becej, KP 03-30). Samples were gradually heated to a melting point and maintained at the same temperature for a further 30 min. The quartz tube with the dissolved sample was cooled to room temperature. The weight of the prepared alloys was 3g. The loss of antimony due to evaporation was up to 3%, so the antimony was added to excess of 3%, as compensation for weight loss during sample preparation.

The samples were brushed with abrasive papers made of SiC, Al<sub>2</sub>O<sub>3</sub>, Al<sub>2</sub>O<sub>3</sub> + Fe<sub>2</sub>O<sub>3</sub> and grain size 3 according to ASTM. After brushing, they were polished on several rotary discs that are coated with the felt. The speed of rotation of the disc can be changed, if necessary, but not exceed 15000 rpm. Fine polishing was done manually with alumina Al<sub>2</sub>O<sub>3</sub> suspension.

After completion of the polishing, the samples were washed with distilled water and alcohol and dried in a stream of warm air. The samples, thus prepared, are free from any visible irregularities and impurities in order to obtain more accurate results of hardness measurement (ASTM, 2011; Marković, 2013; Kraut, 2009).

### Conditions of test

The surface of the sample must be clean and polished. The thickness of the sample must be at least 8-10 times higher than the depth of imprint. No traces of deformation must be seen on the back of the sample after measurement. The distance between the center of the print and the edge of the sample should be cared, as well as the distance between the two adjacent prints during multiple measurements. The angle between the axis of the impeller and the surface of the sample must be 90°, which is achieved by surface treatment and the adjustment of the stand (SRPS JUS C.A4.030). The entire process of alloys testing was carried out at room temperature.

### Test procedure

The ball for imprinting (impeller) was placed in the carrier, the surface of the sample is lighted, prepared sample was placed on the stand and tightened with the impeller carrier. With the activation of device the injection was performed. After imprinting, the sample was released and the diameter of the print was measured. Diameter reading was performed using a microscope (1/100 mm accuracy). The accuracy must not be less than 0.25% of the diameter of the ball (SRPS JUS C.A4.030). The used force was 25.2 N for a period of 15 s and ball was made from hardened steel. The process testing was carried out at 23 °C.

The hardness, by the Brinell method (HB), is defined as the ratio of the force of the imprint and the surface of the print, formed by the ball that was imprinted in the surface of the test material. Brinell hardness is



calculated:  $HB = F / A$ .  $A$  is the surface of the imprint ( $\text{mm}^2$ ).  $F$  is the injection force (N).

### Apparatus

Hardness measurement was done on the Brinell apparatus of the manufacturer Plasmait GmbH (Lebring, Austria). Results were expressed as mean value  $\pm$  standard deviation (SD) of triple measurements. Unit is Brinell's hardness number HB that is in correlation to  $\text{MN}/\text{m}^2$  (MPa).

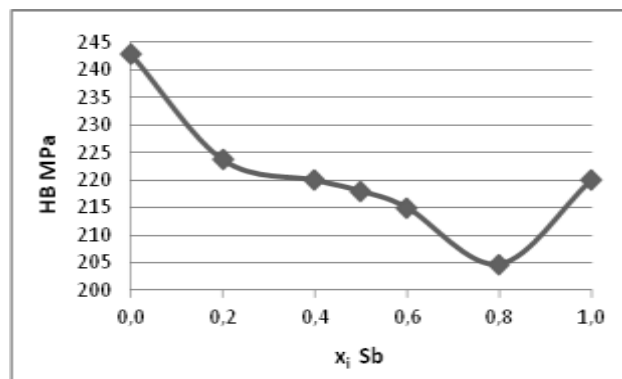
## RESULTS AND DISCUSSION

Various combination of molar fraction of ternary alloys (Cu-In-Sb system), from three vertical sections (Cu-InSb, In-CuSb and Sb-CuIn), were experimentally investigated on the hardness by Brinell method. All results of the measured hardness are given in the Table 1. The results are expressed as mean value of the triple measurements  $\pm$  standard deviation (SD). Function of hardness from molar fractions of the elements (quasi-binary cross sections) are graphically presented in Figures 1, 2 and 3.

**Table 1.** Brinell hardness (HB) of the alloys from the Cu-InSb, Sb-CuIn and In-CuSb vertical sections

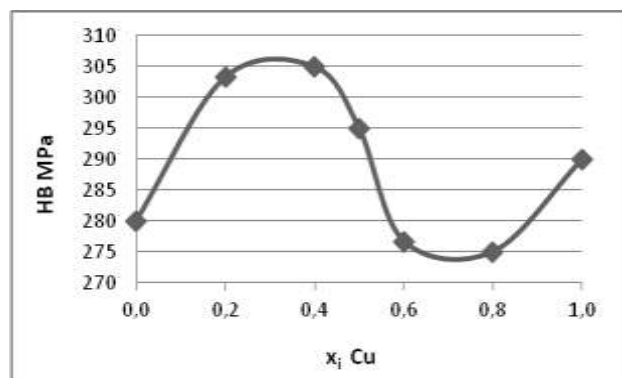
molar fraction $x_i$ (Cu,Sb,In)	HB Cu-InSb $\pm$ SD (MPa)	HB Sb-CuIn $\pm$ SD (MPa)	HB In-CuSb $\pm$ SD (MPa)
0.0	280.0 $\pm$ 10.0	243.0 $\pm$ 25.0	150.0 $\pm$ 18.0
0.2	303.3 $\pm$ 5.8	223.7 $\pm$ 36.6	135.0 $\pm$ 18.0
0.4	305.0 $\pm$ 15.0	220.0 $\pm$ 26.6	126.0 $\pm$ 23.3
0.5	295.0 $\pm$ 10.0	218.0 $\pm$ 35.4	143.3 $\pm$ 5.8
0.6	276.7 $\pm$ 12.6	215.0 $\pm$ 6.0	166.7 $\pm$ 12.6
0.8	275.0 $\pm$ 17.3	204.7 $\pm$ 16.7	138.0 $\pm$ 23.1
1.0	290.0 $\pm$ 0.0	220.0 $\pm$ 0.0	145.0 $\pm$ 0.0

Dependence of hardness in function of the molar fraction of the antimony (Sb-CuIn vertical section) is shown in Figure 1. There is decreasing of hardness with the increasing of Sb content to 80 %, and after 80 % there was enhancement of hardness. Main reason for decreasing of hardness on the Figure 1. is tight binary system of indium and copper, and addition of antimony disturbs this strong intermetallic connection and leads to reduction of hardness (Liu *et al.*, 2002). On the base of results, in order to increase hardness, it is assumed that antimony is used in smaller concentrations. This assumption certainly increases the ecological acceptability of the alloy regarding that some antimony compounds are toxic. The content of the antimony in the alloys usually ranges from 1 % to 20%, in solder alloys its usually about 5% (Ipser *et al.*, 2007).



**Figure 1.** Brinell hardness of alloys in the Sb-CuIn vertical section

The hardness of alloy vary with the contents of the copper (Cu-SbIn vertical section). The highest hardness was recorded for copper molar content of about 30 % in the Cu-SbIn vertical section. Alloys with copper content greater of 30 % have a declining hardness. Minimum of hardness was found for alloy with of 80 % of copper in the vertical sections Cu-SbIn (Figure 2). Copper is the most used element in alloying. Its content improves the mechanical properties of many technically important alloys, even in the small presence (Oberndorff, 2001). In this research copper proved to be the most influential element for the hardness of tested Cu-Sb-In ternary system.



**Figure 2.** Brinell hardness of alloys in the Cu-SbIn vertical section

The dependence of the hardness in function of the indium molar fraction, from In-CuSb vertical section, is given in Figure 3. Minimum of hardness was found for 40 % of indium molar fraction. From 40 to 60 % of indium content, hardness grows rapidly in the In-CuSb vertical section. The content of indium is the most significant from the aspect of the price of the alloy because its price on the market is still high, but with the tendency of significant fall in recent years (Kellya and Matos, 2014). On the other hand, more presence of indium generally reduces the melting point of the alloy and that is important for the solder material (Ipser *et al.*, 2007, Oberndorff, 2001). Regard of hardness, according to the vertical In-CuSb section, the most preferred amounts of indium are in the range of 40 - 60 %.

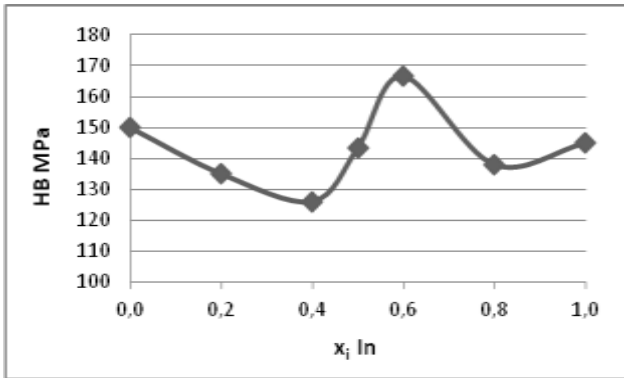


Figure 3. Brinell hardness of alloys in the In-CuSb vertical section

The content of the elements for the purpose of high hardness of the alloy should be at intervals of significant increase of hardness in the vertical sections for individual elements. High hardness of tested ternary system should be expected in alloys with low antimony content, indium in content of about 50 % of molar ratio and copper round 30%.

Melting point and electrical conductivity are significant properties of materials that used for soldering. The melting points of the various molar combinations of this ternary system range from 430 °C to 510 °C (Manasijević, 2009). In general, with the increase of the indium molar fraction, the melting point of the alloy decreases. Increasing the copper and antimony molar ratio generally increases the melting point of the alloy (Manasijević, 2009). The electrical conductivity of various molar combinations of this triple system ranges from 0,9 MS/m to 10 MS/m, which certainly meets the requirements of the solder material (Aljilji, 2010).

This ternary system has shown very good thermal stability, practically the same on the 25 °C and 400 °C, and that is also very important for solder material (Aljilji, 2010).

Finally, the presented results for new solder materials based on indium, antimony and copper are significant improvement compared with lead based alloys. The most important aspects of improvement are mechanical property (hardness), thermal stability and ecological properties.

## CONCLUSIONS

The low content of the antimony, in the tested Cu-In-Sb ternary system, is recommended for purpose of higher hardness, potentially less toxicity and environmental protection aspects. Copper is the most influential on the hardness of this system. The higher content of the indium lowers the melting point of the alloy, which is important for the solder material, but the greater content of the indium increases the price of alloy. The melting point is higher than many others solder materials, but on the other hand there is a very good thermal stability, and the hardness significantly exceeds most of the solder materials, so the possibility of cracking and physical damage to the soldering weld are lower. High hardness of the solder material can be very significant in some specific production needs.

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### **Summary/Sažetak**

Povećan interes za zaštitu životne sredine rezultirao je smanjenjem ili potpunim odstranjenjem iz upotrebe olovnih lemnih materijala. Legure na bazi bakra predstavljaju potencijalnu zamjenu za ovu vrstu olovnih legura. Legure koje, pored bakra, sadrže indijum i antimon pokazale su se kao dobar materijal za lemljenje, nove generacije. Svakako, poželjno je poznavati uticaj količinskih omjera pojedinih elemenata (Cu, In i Sb) na fizička svojstva ove legure. Ovim radom ispitivana je tvrdoća trojnog sistema Cu-In-Sb u ovisnosti od molarnog udjela komponenti. Najveća tvrdoća nađena je u 0.3 molarnoj frakciji bakra u vertikalnom presjeku Cu-InSb. Zaključeno je da tvrdoća opada sa porastom molarnog udjela antimona. Promjene molarnog udjela indijuma su pokazale najmanje varijacije i uticaj na tvrdoću ove legure. Najviše promjene u tvrdoći bilo je kod promjena molarnog udjela bakra pa se on pokazao kao najvažniji element za tvrdoću ovog trojnog sistema.



## Characterization of raw materials and final product in the cement production

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**Abstract:** Cement is a hydraulic binder formed by the grinding process of cement clinker, as intermediate product, which is produced by baking the lime-clay raw material mixture to the sintering temperature. This research paper describes mineralogical analysis technique of primary raw materials, auxiliary components for cement production, by-product clinker and final product, cement. Used technique is X-ray diffraction technique, which is one of the most modern instrumental techniques today. Obtained results are provided in the form of diffractogram, that is used to display the mineralogical phase of components. X-ray diffraction method confirmed the theoretical knowledge of the mineralogical components of tested raw materials, clinker and cement. As expected, the main component of limestone is mineral calcite, as active compound, fly ash and slag as amorphous substances and clinker contains clinker-minerals and gypsum contains calcium sulfate dihydrate in large percentage. Main components of cement are all minerals provided in clinker and raw materials. These experiments were carried out in the Holding Company Cement Plant in Lukavac, Bosnia and Herzegovina.

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## INTRODUCTION

The raw materials for cement production include raw materials of carbonate character and raw material of acid character. Corrective raw materials include corrective raw materials of silicate character and corrective raw materials with high content of iron oxide.

The basic raw materials for cement production are limestone and clay. The limestone represents the dominant component, most often 75%, clay and corrective raw materials remaining 25% of the raw blend (Kostić-Gvozdrenović et al., 1987). Contrary to artificially prepared raw materials, natural material containing 65-75% limestone ( $\text{CaCO}_3$ ) and 25% clay without corrective properties (Brzaković, 2000). The limestone is a sedimentary rock composed of calcite

minerals ( $\text{CaCO}_3$ ), but also contains chemical admixtures of iron, manganese, magnesium, clay, hydrargylite, sand, organic matter, etc. (Brzaković, 2000). In addition to calcite in limestone, mineral aragonite, also calcium carbonate ( $\text{CaCO}_3$ ), which crystallizes rhombically, can be found (Brzaković, 2000). Dolomite ( $\text{MgCO}_3 \cdot \text{CaCO}_3$ ) can be found in limestone rocks (Brzaković, 2000). Some studies had shown that substitution of zeolite require less amount of clinker, which results in producton growth and energy savings. Therefore it reduces air pollution with decreasing the  $\text{CO}_2$  emission and other gases as well (Canpolat et al, 2004).

Marlstone is a sedimentary rock, which is built of clay particles and calcium carbonate or dolomite (Hamzabegović, 2015). The clay content ranges from

20% to 80%, while the percentage of calcium carbonate in the form of mineral calcite ranges from 35% to 65% (Hamzabegović, 2015). The non-carbonate part in marlstone are clay minerals, such as montmorillonite, illit, kaolinite and others.

Clay is the most widespread geological material in nature formed by the sludge diagenesis that remained at the site of the decomposition of the primary material of Pelitian origin transported with water and deposited in the aquatic environment (Hamzabegović, 2015; Pofuk, 2012). The most important clay minerals are kaolinite, hydrolysis (illite), montmorillonite.

Corrective raw materials for the production of cement are: quartz sand ( $\text{SiO}_2$ ), tuffs, pyrite burns, gypsum, natural and artificial shells and melting stones. These auxiliary raw materials are added to improve cement binding properties. The auxiliary raw materials that are taken for analysis are plaster, fly ash and slag.

The main structural mineral of quartz sand is quartz,  $\text{SiO}_2$ , in interval of 90-99% (Brzaković, 2000). Quartz appears in two modifications, such as  $\alpha$ -quartz and  $\beta$ -quartz.

Flying ash is produced in high temperature zones (above 1000 °C), where softening and melting of indelible mineral particles occurs, which come in the lower temperature zone (about 250°C) (Brzaković, 2000). Because of these temperature changes, fly ash acquires a glass structure and form in the form of balls or particles of irregular shape due to the effect of surface stress (Brzaković, 2000). The chemical composition of flying ash consists of the following oxides:  $\text{SiO}_2$ ,  $\text{Al}_2\text{O}_3$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{CaO}$ ,  $\text{MgO}$ ,  $\text{SO}_3$ ,  $\text{Na}_2\text{O}$  and  $\text{K}_2\text{O}$  (Brzaković, 2000). As for the mineralogical composition of flying ash, the basic mineral component consists of glass components and mulite ( $2\text{SiO}_2 \cdot 3\text{Al}_2\text{O}_3$ ), with smaller quartz ( $\text{SiO}_2$ ), hematite ( $\text{Fe}_2\text{O}_3$ ), magnetite ( $\text{Fe}_2\text{O}_3$ ,  $\text{FeO}$ ), magnesite ( $\text{MgCO}_3$ ), getite ( $\text{HFeO}_2$ ), carbonaceous substances (Brzaković, 2000). Other minerals that can be found in the structure are given in Table 1.

**Table 1.** Mineral phases of fly ash (Brzaković, 2000)

Mineral phases	Chemical symbol
Corundum	$\text{Al}_2\text{O}_3$
Rutile	$\text{TiO}_2$
Wustite	$\text{FeO}$
Pyrite	$\text{FeS}_2$
Free CaO	$\text{CaO}$
Calcite	$\text{CaCO}_3$
Anhydrite	$\text{CaSO}_4$
Portlandite	$\text{Ca}(\text{OH})_2$
Periclase	$\text{MgO}$
Brucite	$\text{Mg}(\text{OH})_2$

The slag is also one of the corrective raw materials in portland cement production. It is a by-product obtained from the production of raw iron in high furnaces, and is formed by cooling the melting mixture of iron ore and limestone, which is added to reduce the melting temperature of iron ore.

The chemical composition of this raw material includes the Si, Al, Ca and Mg oxides. Minerals that are part of this raw material are: main mineral gelenite ( $2\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot \text{SiO}_2$ ), followed by minerals monocalcium silicate ( $\text{CaO} \cdot \text{SiO}_2$ ) and dicalcium silicate ( $2\text{CaO} \cdot \text{SiO}_2$ ) (Brzaković, 2000).

There are also crystals of olivine ( $\text{MgO} \cdot \text{FeO} \cdot \text{SiO}_2$ ), monticellite ( $\text{CaO} \cdot \text{MgO} \cdot \text{SiO}_2$ ), akermanite ( $2\text{CaO} \cdot \text{MgO} \cdot 2\text{SiO}_2$ ) and calcium sulphide ( $\text{CaS}$ ).

Gypsum ( $\text{CaSO}_4$ ) is used in cement technology as an active filler. It serves as a binding component and allows quick bonding of cement, reducing the time of cement binding. It is added in the amount of 3 to 5% (Kostić-Gvozdenović et al, 1987; Brzaković, 2000).

In the clinker structure, the most important minerals are: tricalcium silicate ( $3\text{CaO} \cdot \text{SiO}_2$ ), dicalcium silicate ( $2\text{CaO} \cdot \text{SiO}_2$ ), tricalcium aluminate ( $3\text{CaO} \cdot \text{Al}_2\text{O}_3$ ) and tetracalcium aluminoferrite ( $4\text{CaO} \cdot \text{Al}_2\text{O}_3 \cdot \text{Fe}_2\text{O}_3$ ) (Kostić-Gvozdenović et al, 1987; Brzaković, 2000). In addition to these four main minerals, mineral monocalcium aluminate ( $\text{CaO} \cdot \text{Al}_2\text{O}_3$ ) can also be found in the clinker structure. In order to facilitate the writing of these minerals, a system of abbreviations has been introduced, consisting of: A- $\text{Al}_2\text{O}_3$ , C-CaO, S- $\text{SiO}_2$ , F- $\text{Fe}_2\text{O}_3$ .

## EXPERIMENTAL

Mineralogical analysis of raw materials for cement production, analysis of clinker and analysis of portland cement as a final product were carried out with Bruker D4 Endeavor X-ray diffractometer in the Holding Plant Lukavac in Bosnia and Herzegovina. The device is very precise and serves for qualitative and quantitative determinations of all phases in one single sample, also for determining the crystal structure of the compound and for determining the phase transformations in the crystal. For this reason, it is used for various mineralogical tests, especially in the modern cement industry, where mineralogical components influence the quality and properties of cement. This device is based on the x-ray diffraction and follows Bragg's law accordingly.

This device analyzes samples in liquid, solid and powder form, whether in small quantities or small in size, whether they are of an irregular shape, that they are sensitive or that they are completely powdery. The D4 diffractometer uses a modern x-ray tube using a modern X-Ray cathode containing tungsten cathode and copper anode, a  $\text{CuK}\alpha = 1.5418 \text{ \AA}$  (Bruker, D4) wavelength, with an advanced LinxEye detector.

### Sampling and analysis

Lime, fly ash, slag, gypsum, clinker and final product, cement, CEM I 52.5N were analyzed. Sampling of the raw material was carried out as follows: A sample of 50 g of each raw material together with four cellulose tablets, which are used as a binder supplement, were placed in the Centaurus device (manufacturer FLSmidth).

This device is very advanced in the preparation of the sample hence it combines a grinding operation with a sample pressing operation, after which the device weighs

10 g of sample and makes a pressed tablet from it, is positioned at the appropriate position in the D4 Bruker XRD instrument.

**RESULTS AND DISCUSSION**

The result of the mineralogical analysis of the limestone is given in Figure 1. As shown from the diffractogram, the main component is mineral calcite, as in the theoretical part stated. In smaller amount are following minerals: quartz, dolomite, illite, chlorite and pyrite. The exact number of minerals and their amounts are given in Table 2.

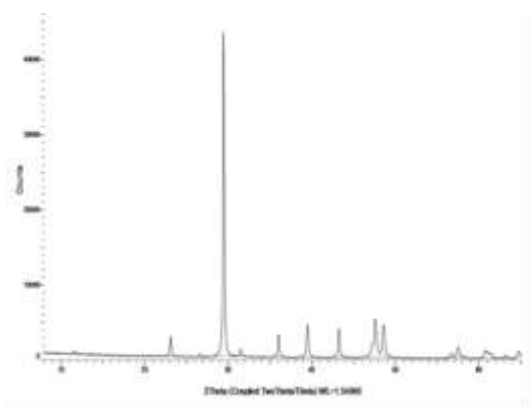


Figure 1. Limestone diffractogram

**Table 2.** Mineral phases of limestone (Vijenac, Tuzla)

Mineral phase	Amount (%)
Calcite	98.86
Dolomite	0.50
Illite	0.25
Chlorite	0.18
Quartz	0.17
Pyrite	0.03

The result of a mineralogical analysis of fly ash is given in Figure 2. Figure 2 shows that amorphous phase has largest part in this raw material, which was presumed, since this raw material does not belong to a group of rocky materials, therefore emphasize absence of crystal structure. Quartz, mullite, alite, magnetite, hematite, lime, and minor amounts of CaCO<sub>4</sub>-anhydrite, tenardite, periclase and rutile are present in the following minerals. The list of mineral components and their quantity are given in Table 3.

**Table 3.** Mineral phases of fly ash (Tuzla Termal Power Plant)

Mineral phases	Amount (%)
Amorphous phase	61.02
Quartz	18.08
Mullite	7.34
Alite	6.46
Magnetite	2.29
Hematite	2.24
Limestone	1.28
CaSO <sub>4</sub> -anhydrite	0.87
Tenardite	0.21
Periclase	0.14
Rutile	0.06

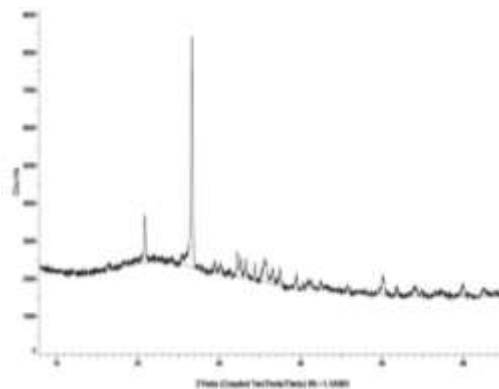


Figure 2. Fly ash diffractogram

The result of the mineralogical analysis of gypsum is given in Figure 3. Figure 3 shows main component of gypsum, calcium sulphate dihydrate, CaSO<sub>4</sub> • 2H<sub>2</sub>O. There is a small percentage of clay minerals, such as calcite, chlorite, illite, anhydrite, hemihydrate, magnesite and dolomite, orthoclas, quartz and thaumasite. The exact list of mineral components and mineral amounts in this gypsum sample, are given in Table 4.

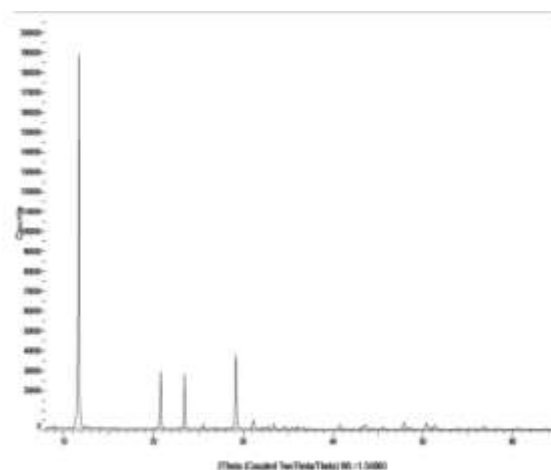


Figure 3. Gypsum diffractogram

**Table 4.** Mineral phases of gypsum (Gornji Vakuf)

Mineral phases	Amount (%)
Gypsum	92.81
Calcite	1.71
Hlorite	1.48
Illite	1.20
Anhydrite	0.59
Hemihydrate	0.58
Magnesite	0.54
Dolomite	0.51
Ortochclase	0.29
Quartz	0.15
Thaumasite	0.14

The result of a mineralogical analysis of slag is given in the next diffractogram.

The diffractogram confirmed the slag is an amorphous substance, such as fly ash (Figure 4). Minerals: bredigite, calcite and quartz are traced in this sample of slag. A list of mineral components and their respective amounts are given in Table 5.

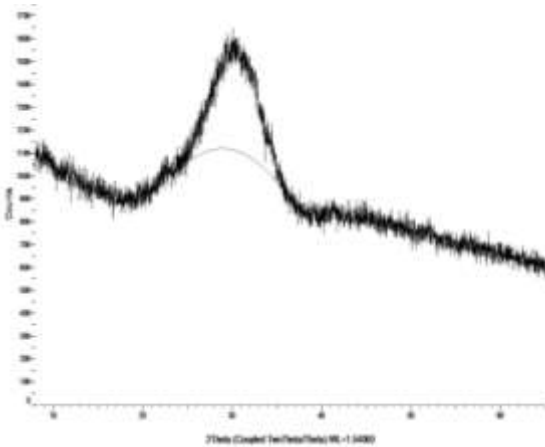


Figure 4. Slag diffractogram

Table 5. Mineral phases of slag (Zenica)

Mineral phases	Amount (%)
Amorphous phase	99.59
Bredigite	0.31
Quartz	0.06
Calcite	0.04

The result of mineralogical analysis of clinker is given in Figure 5.

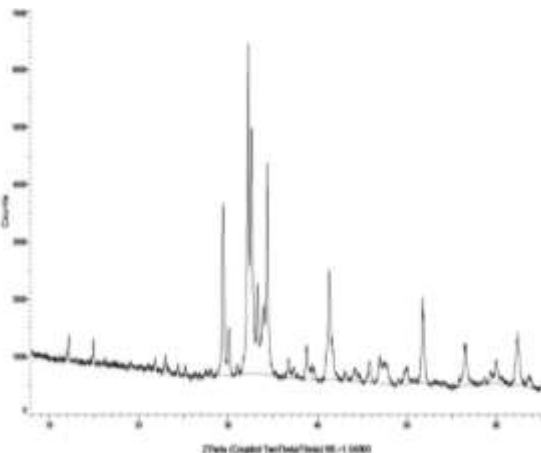


Figure 5. Clinker diffractogram

As was expected, the main components in the clinker are the main four clinker minerals: alite, belite, aluminate and ferrite. The main mineral phase is an alite with 60.86%, that 40-70% (Stutzman et al, 2004). Percentage of belite is 19.76% that corresponds to interval of 15-45%, stated to other cement studies. Figure 5 also shows amounts of aluminate and ferrite. Amount of ferrite in sample of clinker is 10.80% and amount of aluminate is 7.18%. The amounts of aluminate and ferrite corresponds to range stated in some cement studies (Stutzman et al, 2004). In this case, theoretical knowledge and research studies are confirmed. In minor amounts, other minerals are present: arcanite, free CaO, limestone, periclase, arcanite, aphtitalite and portlandite. A list of mineral components of clinker as well as their quantities are given in Table 6.

Table 6. Mineral phases of clinker (Holding Company Cement Plant Lukavac)

Mineral phases	Amount (%)
Alite	60.86
Belite	19.76
Ferrite	10.80
Aluminate	7.18
Arcanite	0.58
Free CaO	0.38
Limestone	0.32
Periclase	0.30
Aphtitalite	0.12
Portlandite	0.08

The result of a mineralogical analysis of cement is given in Figure 6. Figure 6 shows the final product, portland cement, contains in the largest amount of clinker minerals, hence cement is milled clinker. These minerals are in largest amount: alite, belite, ferrite and aluminate. In minor amounts, the following minerals are present: anhydrite, hemihydrate, arcanite, amorphous phase, portlandite, hematite, aphtitalite, quartz, magnetite and limestone. A list of mineral components and their quantities are given in Table 7.

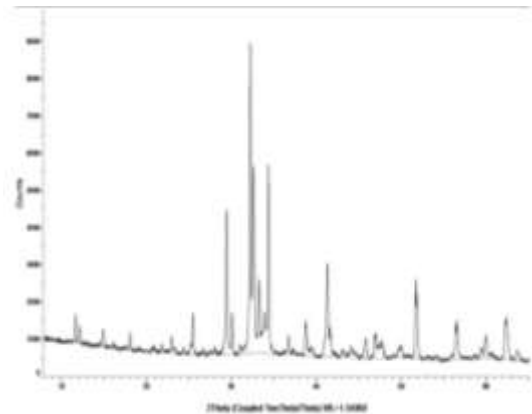


Figure 6. Cement diffractogram

Table 7. Mineral phases of cement CEM I 52.5 N (Holding Company Cement Plant Lukavac Bosnia and Herzegovina)

Mineral phases	Amount (%)
Alite	61.77
Belite	14.94
Ferrite	8.78
Aluminate	8.20
Anhydrite	1.31
Hemihydrate	1.04
Arcanite	0.79
Amorphous phase	0.72
Gypsum	0.71
Portlandite	0.57
Calcite	0.51
Free CaO	0.49
Hematite	0.39
Periclase	0.36
Aphtitalite	0.23
Quartz	0.19
Magnetite	0.14
Limestone	0.07



## CONCLUSIONS

The basic raw materials for cement production are limestone and clay in, though some cement plants, such as the Lukavac cement plant do not use clay as the basic raw material but only limestone. Corrective raw materials for the production of cement are fly ash, slag, quartz sand and gypsum. Based on the analysis and the obtained results, theoretical knowledge of the mineralogical composition of the raw materials as well as clinker and cement is confirmed. In limestone from the quarry Vijenac, the main mineral is dominated by the component calcite in the amount of 98.86%, then quantities dolomite 0.50%, illite 0.25%, chlorite 0.18%, quartz 0.17% and pyrite 0.03%.

In the fly ash from Tuzla Thermal Power Plant, the main component is the amorphous phase in the amount of 61.02%, because ash does not have a crystalline structure. The largest percentage of minerals is quartz, 18.08%. The following minerals are found in smaller quantities: mullite 7.34%, alite 6.46%, magnetite 2.29%, hematite 2.24%, limestone 1.28%, anhydrite 0.87%, tenardite 0.21% periclase 0.14% and rutile 0.06%.

Gypsum is added to strengthen the binding power of cement. Gypsum, or  $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ , is dominant in the structure of gypsum, in the amount of 92.81%. Other minerals found in gypsum are: calcite 1.81%, chlorite 1.48%, illite 1.20%, anhydrite 0.59%, hemihydrate 0.58%, magnesite 0.54%, dolomite 0.51%, orthoclase 0.29%, quartz 0.15% and thaumasite 0.14%.

Iron slag from Zenica as well as fly ash, is an amorphous material, in an amount of 99.59%. Other minerals that are present in slag are: bredigite 0.31%, quartz 0.06% and calcite 0.04%.

The main minerals in clinker are: alite in amount of 60.86%, followed by: belit 19.76%, ferrite 10.80% and aluminate 7.18%. Other mineral components present in clinker are: arcanite 0.58%, free CaO 0.38%, limestone 0.32%, periclase 0.30%, aphtitalite 0.12% and portlandite 0.08%.

In cement, as a final product there is a large number of minerals, which originate from raw materials and are similar in composition as in clinker. Minerals that dominate cement are clinker minerals: alite 61.77%, belite 14.94%, ferrite 8.78% and aluminate 8.20%.

Other cement minerals are: anhydrite 1,31%, hemihydrate 1.04%, arcanite 0,79%, gypsum 0.71%, portlandite 0,57%, calcite 0.51%, hematite 0,39%, periclase 0.36%, aphtitalite 0.23%, quartz 0.19% and magnetite 0.14%. In the traces there is a limestone 0.07%, a smaller proportion of the amorphous phase 0.72% and a free CaO in an amount of 0.49%.

By analyzing the results of the analysis, it was found that the use of other raw materials for the production of cement, such as limestone with corrective raw materials, leads to the same mineralogical composition of cement, that is to the determination of the main clinker minerals, which are also the most important mineral components of cement.

## ACKNOWLEDGEMENT

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**Summary/Sažetak**

Cement predstavlja hidraulično malterno vezivo koje nastaje procesom mljevenja cementnog klinkera, kao međuproizvoda, koji nastaje pečenjem sirovinske mješavine na bazi krečnjaka i gline do temperature sinterovanja. U ovom radu je opisana tehnika mineraloške analize kako glavnih, tako i korektivnih sirovina za proizvodnju cementa, međuproizvoda klinkera i konačnog proizvoda, tj. cementa. Korištena je jedna od najsavremenijih instrumentalnih tehnika i to tehnika savremene rentgenske difrakcije. Rezultati analize su priloženi u vidu difraktograma, analitičkog zapisa mineraloške analize. Postupkom rentgenske difrakcije potvrđeno je teoretsko saznanje o mineraloškim komponentama ispitivanih sirovina, klinkera i cementa. Kao što se i očekivalo glavna komponenta krečnjaka je mineral kalcit, elektrofilterski pepeo i troska su amorfna supstance, u klinkeru prevladavaju klinker minerali, gips sadrži dihidrat u velikom procentu. Glavne komponente u cementu su svi minerali koji su prisutni u sirovinama i klinkeru. Analize su vršene u laboratoriji Fabrike cementa Lukavac, Bosna i Hercegovina.

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