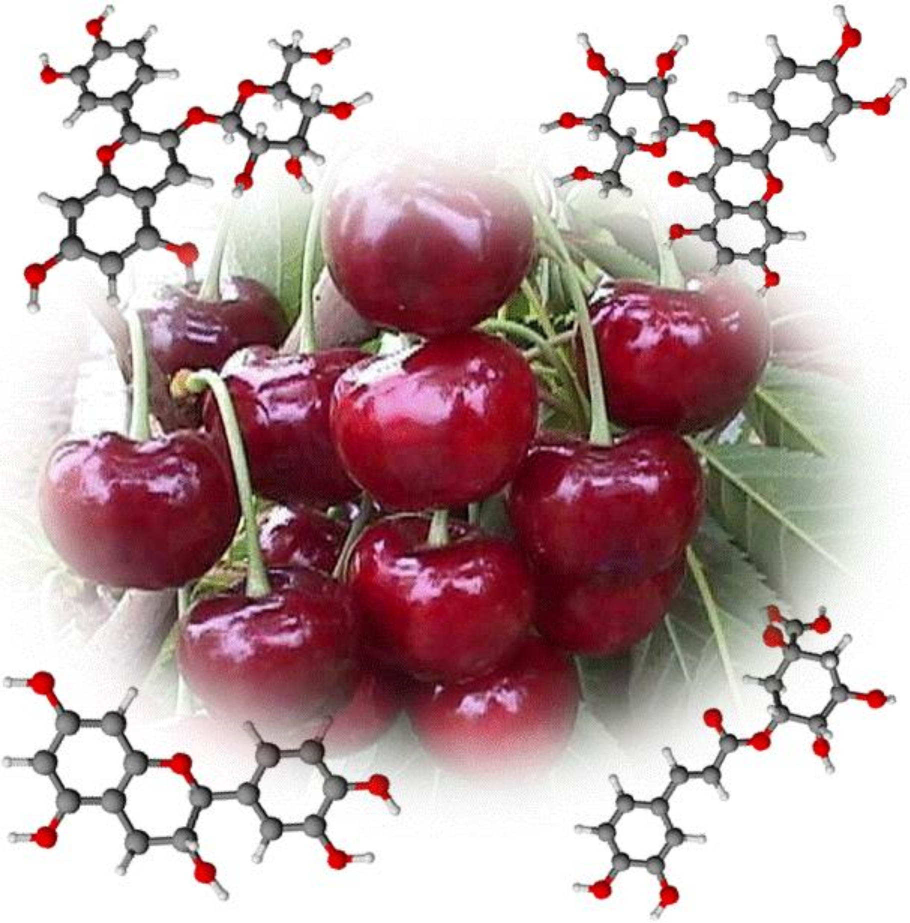
Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266



40

Juni 2013

Prirodno-matematički fakultet Sarajevo
Faculty of Science Sarajevo

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

40

Juni 2013

Prirodno-matematički fakultet Sarajevo
Faculty of Science Sarajevo

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Zmaja od Bosne 33-35, BA-Sarajevo
Bosnia and Herzegovina
Phone: +387-33-279-918
Fax: +387-33-649-359
E-mail: glasnik@pmf.unsa.ba
glasnikhtbh@gmail.com

Print ISSN: 0367-4444
Online ISSN: 2232-7266

REDAKCIJA / EDITORIAL BOARD

Advisory Editorial Board / Članovi redakcijkog savjeta

Lectors / Lektori

Administrative Assistants / Sekretari redakcije

Electronic Edition and Executive Editors / Elektronsko izdanje i izvršni redaktori

Časopis izlazi polugodišnje, a kompletna tekst verzija objavljenih radova je dostupna na http://www.pmf.unsa.ba/hemija/Glasnik-WEB/glasnik_bhs.html.

The journal is published semiannual, and full text version of the papers published are available free of cost at http://www.pmf.unsa.ba/hemija/Glasnik-WEB/glasnik_eng.html.

Citiran u *Chemical Abstracts Service*.

Cited by *Chemical Abstracts Service*.

www.cas.org

Glasnik hemičara i
tehnologa
Bosne i Hercegovine

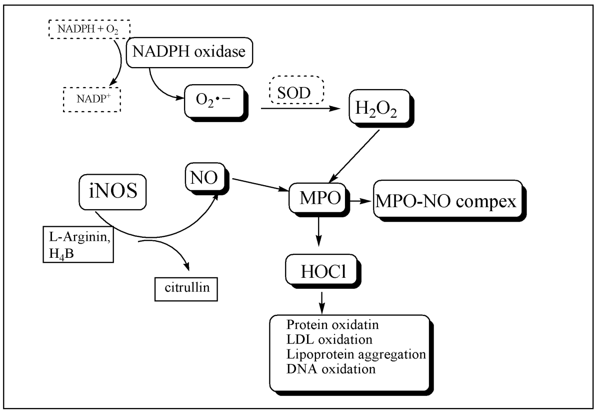
Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

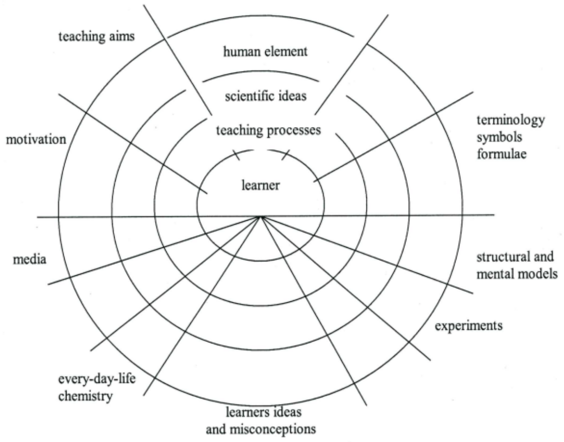
2013
Issue *40*

Print ISSN: 0367-4444
Online ISSN: 2232-7266

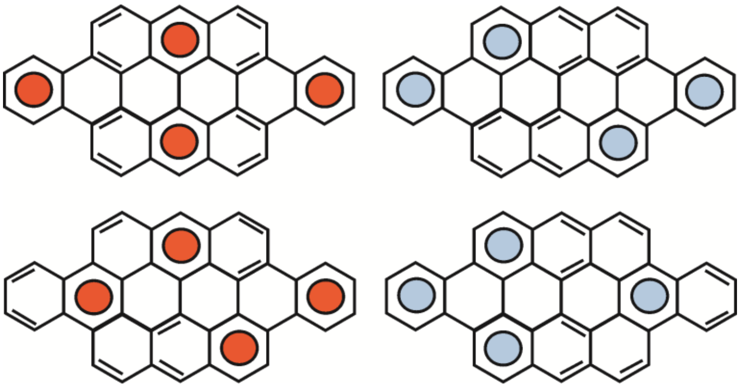
CONTENT / SADRŽAJ

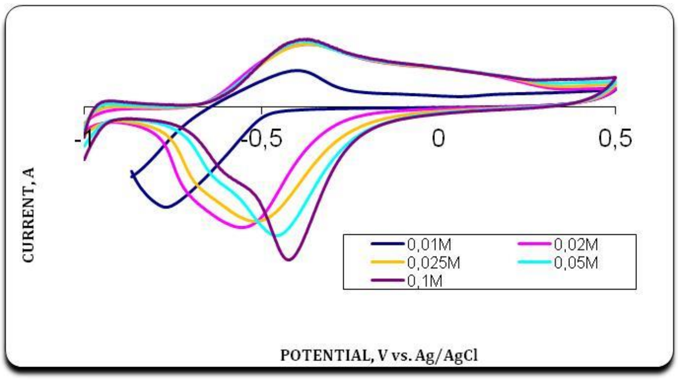
REVIEWS

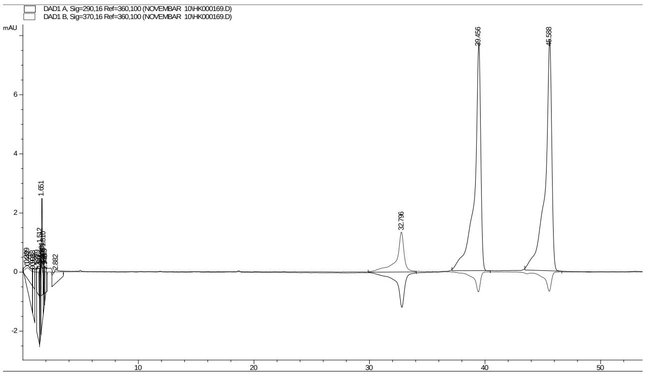




ORIGINAL SCIENTIFIC ARTICLES

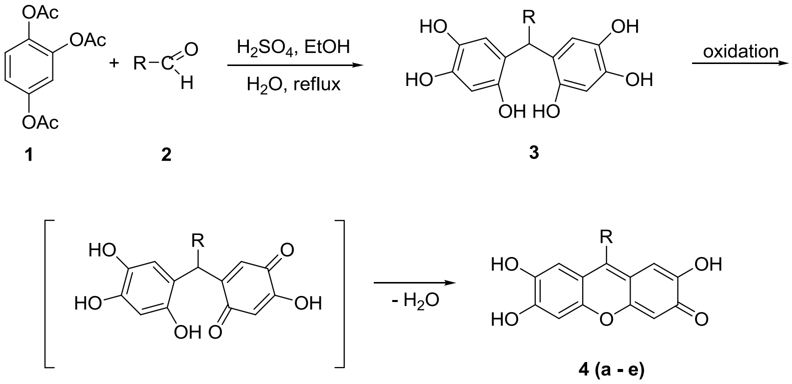






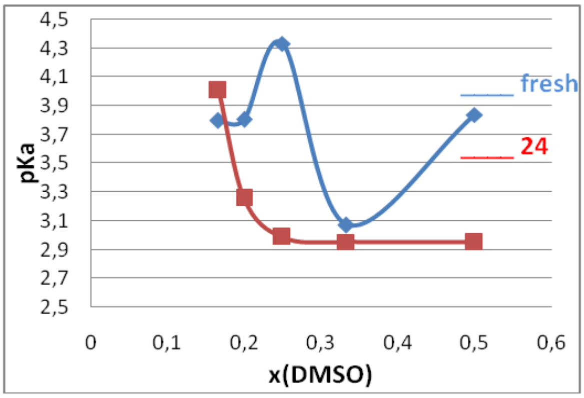
Thermal and crystallographic characteristics of synthesized xanthen-3-one derivatives 31-34

Davorka Završnik
Elma Veljovića
Adnan Bajrović
Martina Vrankić
Biserka Gržeta
Amar Osmanović
Selma Špirtović-Halilović
Samija Muratović



Change in Acidity of L-Ascorbic Acid in the Mixed Solvent DMSO - Water Followed by Conductometric Determination of Dissociation Constants 35-38

Zlatan Rimpapa
Amna Pleho-Kapić
Semira Galijašević
Aida Šapčanin
Fehim Korać



Energy consumption and environmental impact of the foundry industry 39-46

Nafija Šehić-Mušić

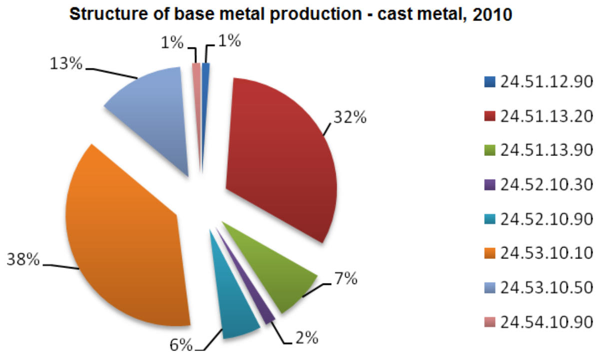
Šefket Goletić

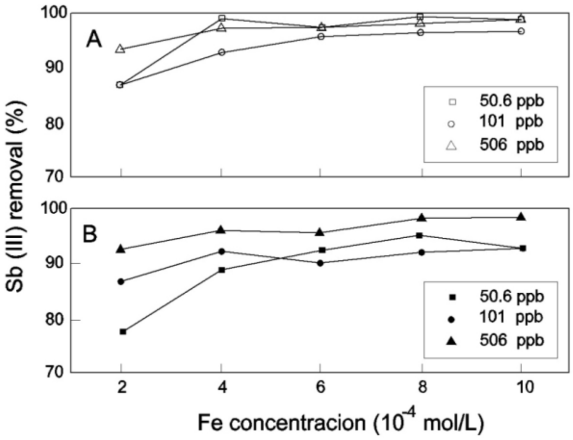
Derviš Pihura

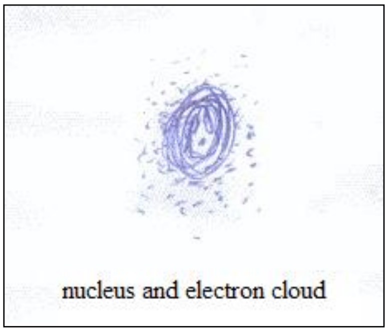
Lejla Mušić

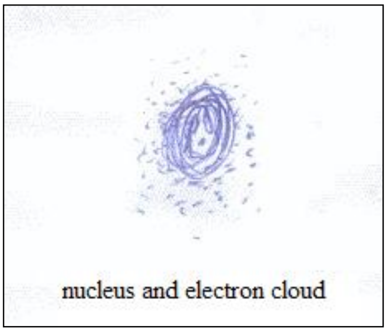
Kadrija Hasanović

Aida Kalajdžisalihović

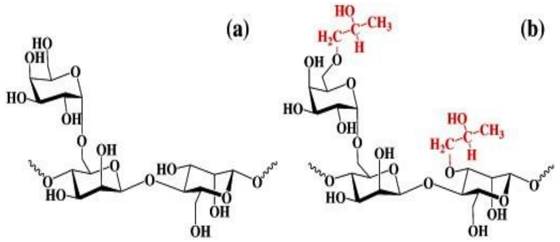








TECHNICAL PAPERS



Editorial

Subscribing to modern lifestyle, humans have directly and indirectly affected d environment in a positive and sometimes less positive way. A number of “so called modern age” diseases have a direct link to environmental changes. The World Health Organization estimates that about a quarter of the diseases facing mankind today occur due to prolonged exposure to environmental pollution. Apparently, there was a price to be paid for a commodity of a modern life. Inorganic and organic pollutants have found its way into a water streams, soli, and air disrupting complete eco systems, and affecting human health. At this time, the need for trained environmental chemist is not only necessary but urgently needed.

The field of environmental chemistry is both very broad, but core knowledge stems from basic chemical principles containing all the aspects of organic chemistry, analytical chemistry, physical chemistry and inorganic chemistry. Additional topics dealing with a specific aspect of applied chemistry in environment ads to a broad and interdisciplinary nature of a subject.

Environmental chemistry is a fairly a new science discipline that emerged only in the last decades of the 20th century. The benefits of having sound environmental chemistry program are immense and absolutely necessary in regards to a global environmental strategy outlined by international and regional organization. Thus, a new program at Faculty of Science, Department of Chemistry, focusing on monitoring and environmental protections with strong core knowledge in chemistry is exactly what is needed at this time. After all, we are morally obligated to consider the consequences of our acts and act upon it.

Editors

Glasnik hemičara i
tehnologa
Bosne i Hercegovine

Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

2013

40

1-8

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: ________________________
Review

Myeloperoxidase Interactions with Nitric Oxide: A Review of Mechanistic Pathways

Galijašević S.

University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35 Sarajevo,
Bosnia and Herzegovina

Article info
Received: 06/05/2013
Accepted: 31/05/2013

Keywords:
myeloperoxidase,
nitric oxide,
superoxide,
NOS

Corresponding author:
E-mail: *semira.galijasevic@gmail.com*
Tel: +387 033 279 917
Fax: +387 033 649 359

Abstract: The phagocytic enzyme myeloperoxidase (MPO) plays an essential role in the inflammatory response by catalyzing formation of reactive species involved in microbial killing by generating hypochlorous acid (HOCl) from H_2O_2 and physiological (≥100 mM) Cl^- concentrations.

However, increased MPO activity has been linked to a number of pathologies with compelling evidence in initiation and progression of inflammatory events. For example, leukocyte and serum MPO levels are elevated in patients with coronary artery disease and thus may be used as a marker for cardiovascular events. MPO-derived oxidants have been linked with neurodegenerative disorders, carcinogenesis, lung disease and respiratory damage, rheumatoid arthritis, kidney damage and atherosclerosis, respectively. Recent data showed the link between increase levels of MPO and development of diabetes, implicating the enzyme as a catalyst for oxidative reactions in the vascular wall. One of the important molecules directly modulated by MPO is nitric oxide, whose bioavailability plays the central role in the development of different pathophysiologies. Thus, we reviewed and analyzed available data and proposed the comprehensive reaction pathways connecting inflammatory action of MPO and bioavailability of NO resulting in a major disturbance of normal psychological functions.

INTRODUCTION

Myeloperoxidase (MPO), hemoprotein present in neutrophils and monocytes, has an essential role in immune surveillance and host defense mechanisms. Upon phagocyte activation in peripheral tissues and fluids, MPO is secreted into both the extracellular milieu and the phagolysosome where it uses hydrogen peroxide (H_2O_2) generated during a respiratory burst by activated NADPH as co-substrate. (Hurst, 1991) A kinetic model that describes the classic myeloperoxidase cycle is presented in Figure 1.

The ground state (secreted) form of the enzyme, MPO-Fe(III), reacts in a rapid and reversible manner with H_2O_2 to form Compound I, a two e^- oxidized intermediate with Fe(IV)=O center and a resonance-stabilized porphyrin π cation radical with reduction potential of ~ -1.1 Volts (Hurst, 1991). Compound I has the ability to oxidize chloride ion normally presented in biological systems (100mM) via a single two e^- oxidation forming hypochlorous acid (HOCl) (Harrison, and Schultz, 1976).

HOCl has a potent macrobicidal and viricidal activity by playing the key role in protective and inflammatory responses in the host defense reaction (Albrich, McCarthy, and Hurst, 1981). In addition, Compound I may oxidize multiple available substrates through two sequential one e^- steps forming Compound II and MPO-Fe(III), (ground state) respectively. Some of biologically available substrates include nitrite ion, tyrosine, ascorbate, urate, estrogens, serotonin, melatonin, catecholamine, polyphenols, nitric oxide and many more. The conversion of Compound II to MPO-Fe(III) is the rate limiting step of the catalytic cycle of peroxidases. Enhancement in peroxidase catalysis due to reduction of MPO-Compound II has been noted with a series of physiological reductants like superoxide ($O_2^{\bullet -}$), melatonin, tryptophan, nitric oxide (NO), and ascorbic acid (Kettle and Candaeis, 2000), (Allegra, Furtmuller, and Regelsberger *et al.*, 2001), (Kettle, and Winterbourn,1988), (Bolscher, and Wever, 1984), (Kettle, and Winterbourn, 19970), (Abu-Soud, and Hazen, 2000). Besides beneficial protective role that MPO has in host defense system, a number of pathophysiological conditions implicated MPO system as a sources of direct and indirect tissue injury (Daugherty *et al*., 1991), (Leeuwenburgh et al 1997).

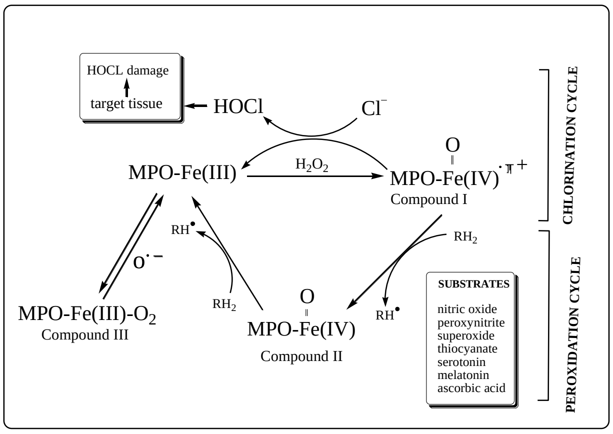


Figure 1. Classic MPO catalytic system.

A major product of MPO halogenations catalytic cycle, HOCl, has been involved in number oxidative reactions, modifying a range of biological targets such as lipids, DNA, lipoproteins and proteins (Daugherty *et al*., 1991, Leeuwenburgh *et al*., 1997, Hazell *et al.,* 1996).The end products of these reactions have been implicated in an initiation and development of many different diseases. In a Table 1, target molecules and end product of HOCl related to specific disease are shown.

HOCl induced target molecules oxidation and end products in relation to different diseases

Oxidizing species	Target Molecule	End product	Disease
$HOCl/Cl_2$	proteins	3-chlorotyrosins	cardiovascular disease
$HOCl/Cl_2$	DNA	5-chlorouracil	carcinogenesis
$HOCl/Cl_2$	unsaturatted faty acids, cholesterol	chlorohydrines	atheroslerosis
$HOCl/Cl_2$	LDL	oxidisedLDL	atherosclerosis
HOCl	NO bioavailabilty, Larginine	impared NO activity	endothelial dysfunction
HOCl/NO	proteins, lipids	chlorinated products	renal injury
HOCl	chondroitin sulfate, hyaluronic acid	degradation products	rheumatoid arthritis
HOCl	beta amyloid protein	increased ROS, structural changes	Alzheimer's disease
HOCl/MPO	mechanistic action	MPO oxidant generation NO bioavailbilty	diabetes

In addition to HOCl, MPO can generate variety of reactive oxidant species and consequently multiple distinct protein and lipid oxidation products, which have been identified in tissues associated with atherosclerosis and other inflammatory conditions (Tang, *et al*., 2006), (Daugherty *et al*., 1994), Hazell *et al.,1*997), (Leeuwenburgh,, Hardy, and Hazen, S 1997). MPO has been recently reported to be useful for identifying inflammation in the walls of coronary arteries, which in turn may indicate a risk for heart disease or heart attack (Shishehbor and Hazen, 2004). Increased levels of MPO have been detected in diabetes mellitus type 2 (Zhang, Yang, and Jennings, 2004) in joints synovial fluid of patients with developed rheumatoid arthritis(Sampaio , Fernandes, and da Silva 2012), in amyloid plaques isolated form Alzheimer type pathology tissues (Green, Mendez, and Jacob, 2004) and in kidney disease (Malle, Buch, Grone, 2003). Clearly, MPO system and its interactions with a a number of different available substrates and free radicals plays significant role in a number of pathophysiologies. Available data done *in vitro and in vivo* is extensive, but comprehensive molecular mechanism linking the reactions, inhibitory pathways, and substrate and enzymes activity under defined conditions has not been presented yet. Thus defining exact molecular mechanism of MPO activity in relationship with the surrounding system is of great importance especially when referring to a particular disease o molecular level. Thus, we analyzed available reaction pathways, our previous extended data describing the enzyme activity and kinetic of specific reactions and proposed mechanism. Apparently MPO activity is the main link between different pathways, showing the significant of ongoing inflammation in a number of pathophysiologies.

MYELOPEROXIDASE AND NITRIC OXIDE

Biological free radicals such as nitric oxide and superoxide are directly involved in MPO catalytic mechanism by a number of different pathways, thus modulating MPO activity and contributing to the detrimental effect of MPO system in the biological milieu at the sites of enzyme expression. Nitric oxide (NO), signaling molecule, plays essential bioregulatory roles in a wide range of processes, including vasodilatation, cell proliferation, nerve transmission, tumor surveillance, antimicrobial defense, and regulation of inflammatory responses (Ignarro, 1990), (Moncada, Palmer, and Higgs,1991) ,(Schmidt, and Walter, 1994), (Vincent, 1994). It is generated by a family of enzymes know as nitric oxide synthases (NOSs) which utilize molecular oxygen, NADPH, and tetrahydrobiopterin (H_4B) to convert L-arginine to NO and citrulline (Griffith, and Stuehr, 1995). Although there are three forms of NOS, only one is responsible for NO synthesis during inflammation, the cytokine inducible NOS (iNOS)(Weinberg, et al., 1999, Fang, 1997).Once expressed, it carries out continuous NO synthesis. In biological systems, enhancement of NO production by iNOS display beneficiary or harmful roles, depending on the rate of NO formation and diffusion, availability of factors that stimulate its overproduction, and local chemistry of NO degradation (Liu, et al., 1998, Gryglweski, Plamer, and Moncada, 1986).

Inducible nitric oxide synthase (iNOS) is associated with most diseases involving overproduction of NO (MacMicking, Xie, and Nathan, 1997, Nathan, 1997). Endothelial NOS (eNOS), whose expression is restricted to the vascular endothelium, is normally dormant and can generate NO upon Ca^{2+} calmoduling binding (Stuehr 1997). However, in diabetic pathology, eNOS is found to be source of superoxide radical that is directly related to the modulation of NO concentrations by forming another reactive species peroxynitrite (Gryglweski, Plamer, and Moncada, 1986). Role of MPO system on regulation of NO production has been detected in a number of *in vivo* and *in vitro* studies (Abu-Soud, Rousseau, and Stuehr, 1996, Wang *et al*., 1994). Interplay between MPO and NO can proceed via several molecular pathways. In all cases, both iNOS and eNOS can be affected structurally or kinetically, resulting in disturbed NO chemistry.

One of the possible pathways is called uncoupling of eNOS that occurs when either tetrahydrobiopterin of L-arginine concentrations are lowered or molecules are modified. As a result, eNOS switches to production of superoxide radical resulting in decreased NO generation Recent studies showed that in addition to substrate disturbance, MPO produced HOCl can induce uncoupling of eNOS by enzyme monomerisation and switch it to superoxide production ((Berka, *et al.,* 2004),(Figure 2).

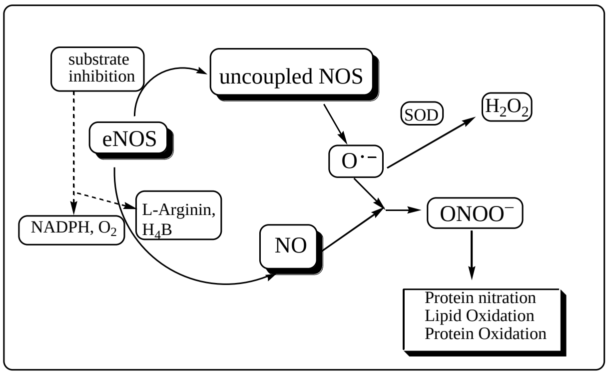


Figure 2. Uncoupling of NOS by HOCl as a source of superoxide radical.

Involvement of eNOS in superoxide radical production, and modulation of NO production caused by indirect action of MPO system is an additional pathway .directly linked to the control of inflammatory response. One of the most biologically significant molecular functions of NO is its ability to bind reversibly to many hemoproteins and nonhemoproteins thus acting either as activator or inhibitor of enzyme catalytic activity. For example, the activation of soluble guanylate cyclase takes place through the interaction with NO in vascular smooth muscle, by coordination to a heme associated with the enzyme, and resulting increase in cGMP-dependant responses induce smooth muscle relaxation associated with control of blood flow through vessels, and prevention of platelet aggregation and adherence. Several studies indicate NO activation of inter- or intracellular guanylate cyclase may play a role in many important physiologic processes, including regulation of blood pressure and coronary blood flow (Stone and Marletta, 1996, Ignarro, Wood, and Wolin, 1984, Ignarro, 1990).

Nitric oxide and NO releasing compounds have been implicated in inhibition of cytochrome P450 via the formation of an iron-nitrosyl complex that prevents access of O_2 to the catalytic site of the enzyme. Nitric oxide also mediates inhibition of mitochondrial cytochrome *c* oxidase and deenergizes mitochondria at low NO and O_2 concentrations. In the absence of L-Arg and H_4B, NO binds to the NOS heme iron at a near diffusion rate, and generates a five coordinate Fe(II)-NO complex that inhibits the catalytic activity of the enzyme (Galijasevic *et al.,* 2003) Structurally, Fe (II) is coordinated to four porphyrin nitrogens and one NO molecule, while the sixth coordinate site opposite to NO is free. Studies on MPO and its interactions with NO have demonstrated that NO modulates the catalytic activity of MPO by distinct mechanisms. Nitric oxide accelerates both the formation and decay of compound II, the rate-limiting step in the classic peroxidase cycle At higher levels of NO, reversible inhibition of MPO occurs through the formation of MPO-Fe(III)-NO complex Thus, NO serves as both a ligand and a substrate for MPO, and the overall effect of NO on the catalytic activity depends on the affinity of MPO for NO vs. H_2O_2 and their concentrations. It has been showed that NO bind to both ferric and ferrous forms of MPO, generating stable low-spin six-coordinate nitrosyl complexes. The rate of NO binding to ferrous MPO is slowed considerably with respect to ferric form, indicating that heme reduction limits the affinity of NO for the heme iron. This behavior is not typical for hemoproteins and has suggested that reduction of MPO-Fe(III) induces unusual structural, i.e., collapse or narrowing and/or electronic alterations in the heme pocket(Abu-Soud *et al.*,2002) as seen in Figure 3.

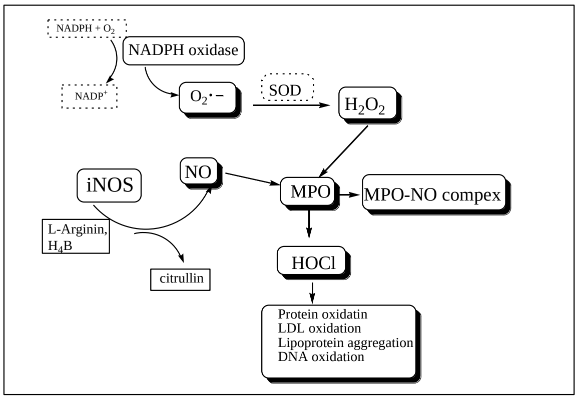


Figure 3. Interaction between MPO and NOS produced NO.

Previous studies have also indicated that heme reductions of MPO have different effects on the heme iron environment and suggest that conformational and/or electronic changes associated with heme reduction differentially affect the affinity of the heme group of mammalian heme peroxidases for diatomic ligands.

Another mechanism of interactions between MPO and iNOS suggest that the MPO system consumed NO released by iNOS during steady-state catalysis, thereby preventing the NO-induced inhibition attributed to the formation of the iNOS–nitrosyl complex. Thus, removal of NO from the iNOS milieu by the MPO system during steady-state catalysis causes a significant increase in iNOS catalytic activity, production of citrulline, and presumably production of NO. Myeloperoxidase, acting as a sink for NO efficiently activates iNOS preventing shutdown of the NO production system (Galijasevic *et al.,* 2003). However, MPO produced HOCl can react with L-arginine producing chlorinated product that can act as NOS inhibitors ultimately causing endothelial dysfunction. Number of studies confirmed that HOCL induced methylation of L-arginine inhibits NO production and contributes to the pathogenesis of inflammatory cardiovascular disease. Another reactive species, peroxynitrite $ONOO^-$, is a potent oxidant and an effective nitrating agent, promotes nitration of tyrosine residues, depletes lipid-soluble antioxidants, and initiates lipid peroxidation (van der Vliet, 1996, Lymar, , Jiang, and Hurst, 1996), Radi, 1991, Graham, 1993, Hazen, 2004). Wherever enhanced rates of NO and $O_2^{\bullet}$ production occur, peroxynitrite ($ONOO^-$) is formed in a reaction with near diffusion-controlled rate (6.7 X 10^9 M^{-1} s^{-1}), lowering the NO concentration achieved during the NOS reaction. The rate of $ONOO^-$ production exceeds the rate of $O_2^{\bullet -}$ removal by copper-zinc superoxide dismutase (SOD).Thus, any increase in NO production can result in $ONOO^-$ production promoting inhibition of hemoproteins such as globins, peroxidases, cytochromes P450, NOS, and

COX-2. (Herold, and Fago, 2005, Floris *et al*., 2003, Mehl *et al*., 1999, Rosen *et al*., 2002 Maréchal *et al*., 2007, Deeb *et al*., 2006).

In each case, the catalytic site of these enzymes was shown to interact with peroxynitrite and to accelerate its decomposition. This potent oxidant and its conjugate acid, peroxynitrous acid (ONOOH), are capable of promoting both protein nitration and initiation of lipid peroxidation, processes known to occur during tissue injury associated with inflammation in vivo. (Radi, 1991). Our studies demonstrated that MPO exposure to $ONOO^-$ is associated with heme depletion and protein nitration resulting with a loss of enzyme activity (Galijasevic *et al.,* 2007). Collectively, these studies showed that interplay between MPO and NO plays a major role at the sites of inflammation. By influencing MPO compound II formation, duration and decay, NO affects overall rate of peroxidation of substrates and the ability of MPO to execute one versus two electron oxidation reactions. Biologically, NO-MPO interactions as such have a broad implication on initiation and progression of local inflammatory and cardiovascular events in vivo. Molecular mechanism of MPO protective and inflammatory activity in relationship with NO is presented in Figure 4.

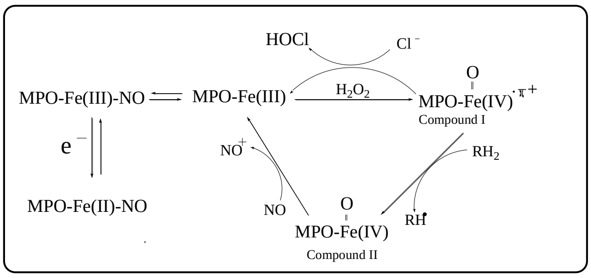


Figure 4. Catalytic cycle of MPO in the presence of nitric oxide.

MOLECULAR MECHANISM OF MPO AND NO AT THE SITES OF INFLAMMATION

Role of leukocyte derived MPO system in a destruction of microorganisms during phagocytosis is well known. Number of studies has shown that MPO system, besides its essential role in immune surveillance and host defense mechanism, has ability to promote tissue injury through a number of oxidative reactions resulting in an initiation and development of different diseases. Increased levels of MPO in walls of coronary arteries were directly related to the risk of heart disease or heart attack. (Shishehbor and Hazen 2004).MPO derived species can oxidize LDL which is considered a major event in the progression and eventual development of arthrosclerosis (Nicholls and Hazen, 2004). Another important consequence of MPO activity is a consumption of NO and induction of endothelial dysfunction. Endothelial dysfunction is defined as a loss of ability to promote vasodilatation by a shift in production and release of several vasoactive molecules. It has been linked directly to a number of diseases such as cardiovascular disease, hypertension, coronary artery disease, chronic heart failure, diabetes (Cohen, 1993, Busee, and Fleming, 1996, Harrison, 1997, Rees, Palmer and Moncada, 1989). On molecular level, a major culprit in a development of ED is decreased NO bioavailability, that is a result of impaired NO production and/or increased inactivation of NO by reactive oxygen species (Gryglewski, Palmer and Moncada, 1986). Additionally, our previous research showed involvement of MPO system by functioning as a catalytic sink for NO at the sites of inflammation thereby affecting the NO availability. Increased concentrations of MPO and reduced bioavailability of NO have been detected in models of type 2 diabetes, what is expectable considering that some of hallmarks of this disease are endothelial dysfunction, increased risk of cardiovascular diseases and elevated oxidative stress (elevated production of ROS and RNS),(Lanngenstroer and Piper, 1992, Graier, Pusch, and Wascher, 1999, Huie and Padmaja, 1993). Elevated activity and biomarkers of MPO mediated molecular damage such as 3-chlorotyrosine, protein carbonyls, 3-nitrotyrosine, lipid oxidation products and oxidized DNA have been detected in Alzheimer's disease (Green *et al*., 2004), while elevated concentrations of MPO and NO detected of synovial fluid of rheumatoid arthritis patients (Sampaio *et al*., 2012). In a **Table 1** MPO derived species, mechanisms of action and target molecules related to a different disease are presented.

Apparently, activity of MPO derived HOCl and modulation of NO bioavailability is major factor of detrimental MPO activity in a number of pathologies. Thus, the interplay between MPO products, nitric oxide , superoxide and target molecules results in a damaged molecules that can be considered as a biomarkers of atherosclerosis, diabetes, ED and continuous inflammation.

Inducible NOS is considered to be nonconstitutive, activated only after the exposure to cytokines at the sites of inflammation, while endothelial NOS is mainly associated with endothelium cell membranes. However, both type of enzyme generates NO from the amino acid L-arginine in the presence of O_2 and NADPH using cofactor tetrahydrobiopterin, H_4B. MPO and inducible NOS are both co-localized and secreted from the primary granules of activated leukocytes (Galijasevic *et al.,* 2003) hence, MPO typically performs its functions in environments where NO is formed. One mechanistic pathway of NO modulation involves inactivation of eNOS by MPO generated HOCl, inducing uncoupling of the eNOS dimere. Generally, uncoupling of NOS occurs when cofactor or substrate are lacking, resulting of production of superoxide by NOS. Dissociation of homomeric NOS can be induced by HOCl oxidation of the Zn-thiolate active center of the enzyme. Another pathway is reaction of HOCl with L-arginine giving chlorinated products that can act as NOS inhibitors, again inhibiting NO synthesis that leads to vasoconstriction manifested as an elevated blood pressure. The link between hyperglycemia and ED has been supported by a number of studies showing reduced bioavailability of NO in ED induced by MPO system (Cohen, 1993, Langenstroer and Piper, 1992). Considering all the possible reaction pathways of MPO system with NO it is clear that contribution of these interactions to ED is significant (Table 2).

Table 2. Modulation of nitric oxide bioavailabiliy.

Pathways for modulation of nitric oxide bioavailability by MPO

1. Formation of MPO-Fe(III) complex
2. Substrate for Compound I and II
3. HOCl induced L-Arginine chlrination
4. Uncoupling of iNOS by HOCl- shutdown of NO production

In inflammatory condition, superoxide radical is produced in neutrophiles during respiratory burst by NDPH oxidase. Superoxide dismutase converts it to hydrogen peroxide that is used by MPO in a production of HOCl. In addition, nonphagocyte NDPH oxidase produces superoxide radical but only a fraction of amount produced by phagocyte NDPH oxidase, and functions through intracellular signaling (Figure 5).

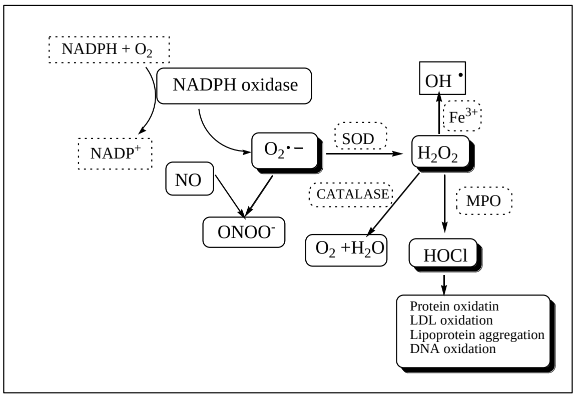


Figure 5. Fate of superoxide radical in inflammatory milieu.

However, high glucose stimulates production of ROS whose main sources is vascular NAD(P)H oxidase that can be either consume as a substrate by MPO or converted to H_2O_2 and again used by MPO leading to inflammation and diabetic vascular disease (Zhang, Yang, and Jennings, 2004). Apparently, after oxidative burst that is initial event in any inflammatory condition is stopped, stimulated MPO stays bound for vascular wall and can consume high glucose stimulated H_2O_2. Additional sources of superoxide is uncoupled NOS. The role of SOD inhibition under pathological conditions is not known yet and data are somewhat conflicting. If superoxide is not consumed directly or indirectly by MPO system, can react with available NO forming $ONOO^-$ highly toxic and reactive molecule causing tissue damage by oxidizing a number of biological molecules. Any of these pathways for superoxide consumption that are highly dependable on MPO, NOS and SOD will lead to development of pathophysiological condition either as HOCl induced tissue damage or ONOO- action in addition to ED that depends on NO bioavailability.

Considering all the available data and our previous research, we proposed the general mechanistic pathway coupling activity of MPO and NOS and their production of free radicals that can contribute directly to the initiation and development of several diseases (Figure 6).

Additional studies, but with the focus on specific concentration depended reactions done *in vivo* are necessary to further deduct the controlling conditions. As a result, the development of specific inhibitory compounds could lead to inactivation of enzymes or reaction pathways causing disturbance of normal physiological processes.

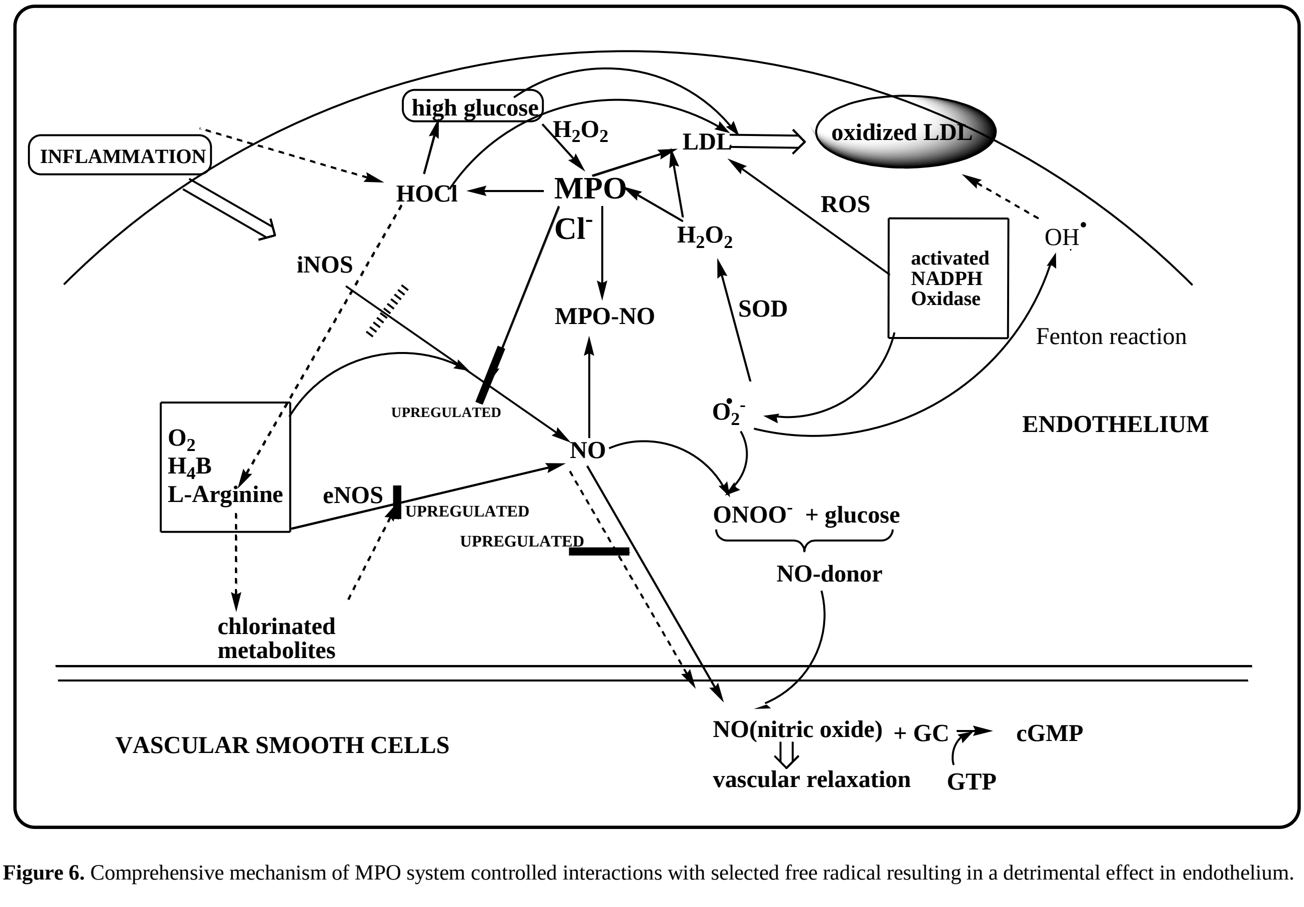


Figure 6. Comprehensive mechanism of MPO system controlled interactions with selected free radical resulting in a detrimental effect in endothelium.

REFERENCES

Abu-Soud, H.M., Hazen S.L. (2000). Nitric oxide modulates the catalytic activity of myeloperoxidase. *Journal of Biological Chemistry*, **275**, 5425-30.

Abu-Soud, H.M., Khassawneh, M.Y., Sohn, J.T., Murray, P., Haxhiu, M.A., Hazen, S.L. (2001). Peroxidases inhibit nitric oxide (NO) dependent bronchodilation: development of a model describing NO-peroxidase interactions. *Biochemistry*, **40**, 11866-75.

Abu-Sound, H. M., Ichimori, K, Presta, A, Stuehr, D.J. (2000), Electron transfer, Oxygen Binding, and nitric oxide feedback inhibition in endothenial nitric-oxide synthase. *Journal of Biological Chemistry 275***,** 17349-17357.

Abu-Sound, H.M., Roussseau, D.L., Stuehr, D.J (1996). Nitric Oxide binding to the heme of neuronal nitric-oxide synthase links its activity to changes in oxygen tension. *Journal of Biological Chemistry*, **271**, 32515-32158.

Albrich, J. M., McCarthy C. A., Hurst, J. K. (1981). Biological reactivity of hypochlorous acid: implications for microbicidal mechanisms of leukocyte myeloperoxidase. *Proceedings of National Academia of Sciences USA*, **78**, 210-214.

Allegra, M., Furtmuller, P.G., Regelsberger, G., Turco-Liveri, M.L., Tesoriere, L., Perretti, M., Livrea, M.A., Obinger, C. (2001). Mechanism of reaction of melatonin with human myeloperoxidase. *Biochemistry and Biophysics Research Communication*, **282**, 380-386.

Beckman, J.S, Beckman, T.W., Chen, J, Marshall P.A., Freeman, B.A. (1990). Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. *Proceedings of National Academia of Sciences USA*, **87**, 1620-1624.

Bolscher, B. G., Wever, R. A. (1984). The nitrosyl compounds of ferrous animal haloperoxidases. *Biochim. Biophys. Acta*, **788**, 1-10.

Busse, R, Fleming, I. (1996). Endothelial dysfunction in atherosclerosis. *Journal Vascular Research*, **33**, 181-194.

Cohen, R.A. (1993).Dysfunction of vascular endothelium in diabetes mellitus. *Circulation* **87**, V67-v76.

Daugherty, A., Dunn, J. L., Rateri, D., L., and Heinecke, J. W. (1994). Myeloperoxidase, a catalyst for lipoprotein oxidation, is expressed in human atherosclerotic lesions. *Journal of Clinical Investigation*, **94**, 437-444.

Deeb, R. S., Hao, G., Gross, S. S., Lainé, M., Qiu, J. H., Resnick, B., Barbar, E. J., Hajjar, D. P., Upmacis, R. K. (2006). Heme catalyzes tyrosine 385 nitration and inactivation of prostaglandin H2 synthase-1 by peroxynitrite. *Journal of Lipid Research*, 47, 898–911.

Fang, F.C. (1997). Perspective series: host/pathogen interactions. Mechanism of nitric oxide-related antimicrobial activity. *Journal of Clinical Investigation*, **99,** 2818-2825.

Floris, R., Piersma, S. R,; Yang, G., Jones, P., Wever, R. (2003). Interaction of myeloperoxidase with peroxynitrite: a comparison with lactoperoxidase, horseradish peroxidase and catalase. *European Journal of Biochemistry*, **215**, 767-775.

Galijasevic**,** S., Saed, G. M, Diamond, M.P., Abu-Soud. H.M. (2003). Myeloperoxidase up-regulates the catalytic activity of inducible nitric oxide synthase by preventing the nitric oxide feedback inhibition. *Proceedings of National Academia of Sciences of U S A.* **100,** 14766-14771.

Graham, A, Hogg, N., Kalyanaraman, B., O'Leary, V, Darley-Usmar, V., Moncada, S. (1993). Peroxynitrite modification of low-density lipoprotein leads to recognition by the macrophage scavenger receptor. *FEBS Letters* **330**,181-185.

Graier, W.F., Pusch, K., Waschert, T. (1999). Increased superoxide formation in endothelial cells during hyperglycemia. *Diabetes Research and Clinical Practice*, **45,** 153-160.

Green, P.S., Mendez, A.J., Jacob, J.S., Crowley, J.R., Growdon, W., Hyman, BT., Heinecke, J.W., (2004). Neuronal expression of myeloperoxidase is increased in Alzheimer’s disease. *Journal of Neurochemistry*, **90,** 724-733.

Griffith, O. W. & Stuehr, D. J. (1995). Nitric oxide synthase properties and catalytic mechanism. *Annual Review of* Physiology, **57**, 707–736.

Gryglewski, R.J., Palmer, R.M., Moncada, S.(1986). Superoxide anion is involved in the breakdown of endothelium – derived vascular relaxing factor. *Nature*, **320**, 453-456.

Hazen S.L., Zhang, R., Shen, Z., Wu, W., Podrez E.A., MacPherson, J.C., Schmitt, D., Mitra, S.N, Mukhopadhyay, C., Chen. Y., Cohen, P.A., Hoff, H.F., Abu-Soud, H.M. (1999). Formation of nitric oxide-derived oxidants by myeloperoxidase in monocytes: pathways for monocyte-mediated protein nitration and lipid peroxidation. *In vivo*. *Circculation Research*, **85**, 950-958.

Hazell, L. J., Arnold, L., Flowers, D., Waeg, G., Malle, E., Stocker, R. (1996). Presence of hypochlorite-modified proteins in human atherosclerotic lesions. *Journal of Clinical Investigation*, **97**, 1535-1544.

Hazen, S. L., Gaut, J. P., Hus, F. F., Crowley, J. R., d’Avignon, A. Heinecke, J. W. (1997). p-Hydroxyphenylacetaldehyde, the major product of L-tyrosine oxidation by the myeloperoxidase-H_2O_2-chloride system of phagocytes, covalently modifies epsilon-amino groups of protein lysine residues. *Journal of Biological Chemistry*, **272**, 16990-16998.

Harrison, D.G. (1997).Cellular and molecular mechanisms of endothelial cell dysfunction. *Journal of Clinical Investigation*, **100**, 2153-2157.

Harrison, J. E., Schultz J. (1976). Studies on the chlorinating activity of myeloperoxidase. *Journal of Biological Chemistry*, **251**, 1371-1374.

Hurst, J. K. (1991). Myleperoxidase-active site structure and catalytic mechanisms. In: Everse J.; Everse K., Grisham, M. B., eds. Peroxidases in Chemistry and Biology. 1st edn. Boca Raton: *CRC Press*, 37-62.

Huie, R.E. Padmaja, S. (1993). The reaction of NO with superoxide. *Free Radicals Research Communication*, **18**, 195-199.

Herold, S., Fago, A.(2005). Reactions of peroxynitritewith globin proteins and their possible physiological role. Comp. Biochem. Physiol. Mol. Integr. Physiol. **142**,124–129.

Ignarro, L. J. (1990). Biosynthesis and metabolism of endothelium-derived nitric oxide. *Annual Review of Pharmacology and* Toxicology, **30**, 535-560.

Ignarro, L.J., Wood, K.S., Wolin, M.S. (1984). Regulation of purified soluble guanylate cyclase by porphyrins and metalloporphyrins: a unifying concept. *Advanced Cyclic Nucleotide Protein Phosphorylation Research*, **17**, 267-7.

Ignarro, L.J. (1990). Haem-dependent activation of guanylate cyclase and cyclic GMP formation by endogenous nitric oxide: a unique transduction mechanism for transcellular signaling. *Pharmacol Toxicol.* **67**:1-7.

Kettle, A.J., Candaeis, L.P. (2000). Oxidation of tryptophan by redox intermediates of myeloperoxidase and inhibition of hypochlorous acid production. *Redox Report,* **5**,179-84.

Kettle, A. J., Winterbourn, C. C. (1988). Superoxide modulates the activity of myeloperoxidase and optimizes the production of hypochlorous acid. *Biochemistry Journal*, **252**, 529-536.

Kettle, A. J., Winterbourn, C. C. (1997). Myeloperoxidase: a key regulator of neutrophil oxidant production. *Redox Report*, **3**, 3-15.

Langenstroer, P., Piper, G.M. (1992). Regulation of spontaneous EDRF release in diabetic rat aorta by oxygen free radicals. *American Journal of Physiology*, **263**, H257-262.

Liu, X., Miller, M.J., Joshu, M.S., Sadowska-Krowica, H., Clark, D., A., Lmacaster, J.R., (1998). Diffucuion limited reactions of free nitric oxide witrh erytrocites. *Journal of Biological Chemistry*, **273,** 18709-16713.

Leeuwenburgh, C., Hardy, M. M., Hazen, S. L., Wagner, P., Oh-ishi, S., Steinbrecher, U. P., Heinecke, J.,W. (1997). Reaction nitrogen intermediates promote low density lipoprotein oxidation in human atherosclerotic intima. *Journal of Biological Chemistry*, **272**, 1433-1436.

Leeuwenburgh, C., Rasmussen, J. E., Hsu, F. F., Mueller, D.M., Pennathur, S., Heinecke, J.,W. (1997). Mass spectrometric quantification of markers for protein oxidation by tyrosyl radical, copper, and hydroxyl radical in low density lipoprotein isolated from human atherosclerotic plaques. *Journal of Biological Chemistry*, **272, 3520**-3526.

Lymar, S.V., Jiang, Q, Hurst J.K. (1996). Mechanism of carbon dioxide-catalyzed oxidation of tyrosine by peroxynitrite. *Biochemistry*. **35**, 7855-7861.

Malle, E., Buch, T., Grone, HJ., (2003). MPO in kidney disease. *Kidney International*, **64**, 1956 -1967.

MacMicking, J., Xie, Q.W., Nathan, C. (1997). Nitric oxide and macrophage function. *Annual Reviews in Immunology*, **15**, 323-350.

Maréchal, A., Mattioli, T. A., Stuehr, D. J., Santolini, J. (2007). Activation of peroxynitrite by inducible nitric-oxide synthase: a direct source of nitrative stress. *Journal of Biological Chemistry,* **282**, 14101–14112.

Mc Veigh, G.E, Brennan, G.M, Johnston, G.D, (1992). Impaired andothelium- dependent and independent vasodilatation in patients with type 2 diabetes mellitus. *Diabetologia* **35**:771-776.

Mehl, M., Daiber, A., Herold, S., Shoun, H., Ullrich, V. (1999). Peroxynitrite reaction with heme proteins. *Nitric Oxide* **3**,142–152.

Moncada, S., Palmer, R. M. J. & Higgs, E. A. (1991) Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacology Review*, **43,**109–142.

Nicholls, S.J, Hazen, S.L. (2004). The role of myeloperoxidase in the pathogenesis of coronary artery disease. *Japanese Journal of Infectious Diseases*, **57**, S21-2.

Radi, R., Beckman, J. S., Bush, K. M., and Freeman, B. A. (1991). Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Archives of Biochemistry and Biophysics*, **288**, 481-487.

Rees, D.D., Palmer, R.M, Moncada, S. (1989).Role of endothelium-derived NO in the regulation of blood pressure. *Proceedings of National Academia of Sciences USA*, **86**, 3375-3378.

Rosen, G.M., Tsai, P., Weave,r J., Porasuphatana, S., Roman, LJ., Starkov, A.A., Fiskum, G, Pou, S. (2002). The role of tetrahydrobiopterin in the regulation of neuronal nitric-oxide synthase-generated superoxide. *Journal of Biological Chemistry*, **277**,40275-80.

Sampaio, R.M., Fernandes, N., da Silva, N.P. Sato, E.I. (2012). Increased myeloperoxidase plasma levels in rheumatoid arthritis. *Rheumatology International*, **32**, 1605-1609.

Schmidt, H. H. H. W. & Walter, U. (1994). NO at work. *Cell*, **79,** 919–925.

Shishehbor M.H., Hazen S.L. (2004). Inflammatory and oxidative markers in atherosclerosis: relationship to outcome. *Current Atherosclerosis Report,* **6**, 243-50.

Stone, J.R., Marletta M.A. (1996). Spectral and kinetic studies on the activation of soluble guanylate cyclase by nitric oxide. *Biochemistry* **35,**1093-9.

Stuehr, D.J (1997) Structure-function aspects in the nitric oxide synthasis. *Annual Reviews Pharmacoal. Toxical* **37**, 339-359.

Stuehr, D.J., Ikeda-Saito, M. (1992) Spectral characterization of brain and macrophage nitric oxide synthases. Cytochrome P-450-like hemeproteins that contain a flavin semiquinone radical. *Journal of Biological Chemistry,* **267,** 20547-20550.

Tang, W. H., Brennan, M. L., Philip, K., Tong, W., Mann, S., Van Lente, F., Hazen, S. L. (2006). Plasma myeloperoxidase levels in patients with chronic heart failure. *American Journal of Cardiologists,* **98**, 706-799.

Vincent, S. R. (1994) Nitric oxide: A radical neurotransmitter in the central nervous system. *Progress in Neurobiology.* **42,** 129–160.

van der Vliet A, Eiserich, J.P., Halliwell, B, Cross, C.E. (1997). Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. *Journal of Biological Chemistry,* **272**,7617-7625.

Wang, J., Rousseau, D.L, Abu- Sound, H.M, Stuehr, D.J (1994). Heme coordination of **NO** in **NO** synthase. *Proceedings of National Academia of Sciences USA,* USA **91,** 10512-10516.

Weinberger, B.,Heck, D.L., Laskin, D.L., Laskin, J. D. (1999) Nitric oxide in the lung : therapeutic and cellular mechanism of action. *Pharmacology Therapies*, **84**, 401-411.

White, K. A, Marletta, M. A. (1992). Nitric Oxide synthase is a cytochrome P-450 type hemoprotein. *Biochemistry, 81,* 6627-6631.

Zgang, C., Yang, J, Jennings, L.K., (2004). Leukocyte-derived myeloperoxidase amplifies high-glucose-induced endothelial dysfunction through interactions with high-glucose stimulated vascular non-leukocyte derived oxygen species. *Diabetes,* **53**, 2950-9.

Summary/Sažetak

Fagocitni enzim mijeloperoksidaze (MPO) igra bitnu ulogu u inflamatornom procesu pri čemu katalizira stvaranje reaktivnih vrsta uključenih u ubijanju mikroba generiranjem hipoklorične kiseline (HOCl) s H_2O_2 pri fiziološkim ($\geq$ 100 mm) Cl^- koncentracijama.

Međutim, povećana MPO aktivnost je povezana s nizom patoloških stanja bilo u inicijaciji i/ili progresiji upalnih procesa. Na primjer, nivoi leukocita i serumske MPO su povišeni kod pacijenata sa koronarnom bolesti srca, te se MPO može marker za kardiovaskularne događaje. MPO proizvedeni oksidansi su povezani sa neurodegenerativnim poremećajima, kancerogenezom, bolestima pluća i disajnih puteva reumatoidnim artritisom, oštećenjima bubrega i aterosklerozom. Nedavni podaci pokazuju vezu između povećanja razine MPO i razvoja dijabetesa, implicirajući enzim kao katalizator oksidativnih reakcije u vaskularnom zidu. Jedan od važnih molekula izravno modulirana sa MPO je nitrogen oksid, čija bioraspoloživost igra središnju ulogu u razvoju različitih bolesti. Nakon analize dosadasnjih istraživanja predloženi su sveobuhvatni reakcijski mehanizmi koji povezuju upalno djelovanje MPO i bioraspoloživosti NO što rezultira poremećajima normalnih fizioloških funkcija.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: ________________________

Review

2013
40
9-14

Structure of Matter – Diagnosis of Misconceptions and Challenge

Barke H.-D.

University of Muenster, Institute of Didactics of Chemistry, Fliednerstr. 21, 48149 Muenster, Germany

Article info
Received: 18/02/2013
Accepted:13/06/2013

Keywords:
Structure of matter,
misconceptions,
atoms and ions as basic particles of matter,
ionic bonding,
ionic lattice

***Corresponding author:**
E-mail: barke@uni-muenster.de
Phone: 0049 251 8339383
Fax: 0049 251 8338313

Abstract: Students at schools and universities may know formulae and chemical equations, but are weak in mental models according the structure of metal- and salt crystals. Especially concerning ions as particles in solid salts or in salt solutions they show a lot of misconceptions, i.e. NaCl molecules in rock salt or Na-O-H molecules in sodium hydroxide solution. One way to challenge those misconceptions can be a periodic table containing symbols of atoms and ions on the base of Daltons atomic model, showing atoms and ions by spheres of different sizes. Combining metal atoms “left and left in PSE” to giant structures will show structures of pure metal crystals and alloys, combining nonmetal atoms “right and right in PSE” to molecules will show molecular structures of volatile substances, combining ions “left and right in PSE” will show ionic lattices of salt crystals. With those steps in early chemistry education the understanding of salts and electrolyte solutions will be scientifically correct – misconceptions should be minimized.

INTRODUCTION

Teaching chemistry for chemists at universities seems easy: you have to deal with well-known textbooks of General chemistry, with Inorganic, Organic and Physical chemistry - mostly in this order. Teaching chemistry education for chemistry teachers is a difficult subject - there are not many textbooks, there is no special order of all topics. One suitable idea to teach chemistry education or chemistry didactics at universities is the "Pie chart" of Figure 1. Within chemistry education, many researches are talking about misconceptions of pupils at schools. A chemist has not to consider them, but the chemistry teacher should know the misconceptions to avoid them, and to find lessons to prevent from those well-known misconceptions.

Therefore the topic "Learners ideas and misconceptions" is one of the biggest sectors (see Fig.1) – it may be taught as a first chapter in a lecture and is reflected in the following paper.

“At last I found a lecture worth to come up early in the morning; excellent examples and experiments of teaching chemistry; now I know what chemistry education means and why it is so important for my studies; good to have the clear concept of the ‘pie chart’ from the beginning of all lectures” (Barke *et. al.*, 2012). These comments of would-be-chemistry teachers show that the lectures of chemistry education in our Institute at University of Muenster are helping them very much for their first steps to think about teaching chemistry at school.

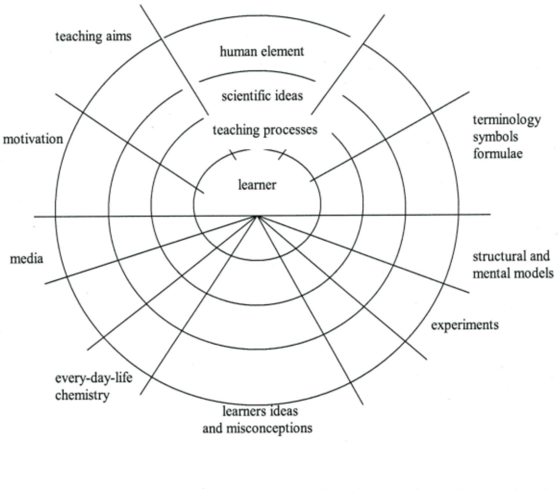


Figure 1. Main subjects of a lecture in chemistry education, “pie chart” metaphor (Barke *et. al.*, 2012).

The most important subjects of the lectures in one semester can be presented in a kind of “pie chart” (see Fig. 1): “Learners ideas and misconceptions; experiments; structural and mental models; terminology, symbols and formulae; every-day-life chemistry; media; motivation; teaching aims” (Barke *et. al.*, 2012). Because we want to give much emphasis to the “learner”, she or he is therefore placed in the centre of the diagram. Secondly, “scientific ideas" should be reflected in association with appropriate "teaching processes" for the learner. Finally there should be reflections on the "human element" or the "chemistry contexts" (Mahaffy *et. al.*, 2006).

In our experience, students like the subject "learners ideas and misconceptions" very much. At the beginning of courses in chemistry education, would-be-chemistry teachers are really not clear with "preconcepts" and "misconceptions", with the existence of these concepts in the mind of young people – sometimes also in their own mind. They don't have the idea how important it is to know more about these concepts and how to integrate them into chemistry education at school. Therefore we published the book "Misconceptions in Chemistry" (Barke *et. al.*, 2009) for all who are studying chemistry for chemistry teaching or are already teaching chemistry at school. We also want it, in the sense of Professor Jung, a physics educator in Germany: "One should really write a book on diagnosing misconceptions and give it to all teachers". The psychologist Langthaler made similar comments: "If you, as a teacher, would have more diagnostic abilities and tools, many problems with your students would never even arise".

In planning coursework in the past few decades, teachers were under the impression that young pupils are bringing hardly any knowledge of science. Therefore, teachers had only to decide how to plan a lecture in order to transmit scientific ideas to their pupils, perhaps incorporating laboratory experiments or new technology-based methods.

However, latest studies in science education show that children and adolescents have many images and ideas about nature and their own surroundings: in the area of combustion they have mental models according to a destruction concept instead of the conservation of mass, in the area of gases children are thinking that gases have no mass or density, in the area of substances they think that substances can change the color: "Copper roofs on churches are changing from red to green, silver spoons are changing to black color".

Research of students' conceptions in chemistry is based on the constructivist approach to learning, in which students are supposed to construct their own cognitive structure. According to this approach, learners before, during and after instruction generate their own meaning based on their background, attitudes, abilities, experiences etc. As long as students construct or build their own concepts, their constructions differ mostly from the scientific ones. These different concepts are variously described by different researchers as: misconceptions, alternative conceptions, naïve beliefs, erroneous ideas, private versions of science, personal models of reality, spontaneous reasoning, developing conceptions, misunderstanding, mistakes, misinterpretation of facts, personal constructs and persistent pitfalls - to name just a few (Blosser, 1987; Elizabeth, 1990; Eylon and Linn, 1988; Fensham *et. al.*, 1994; McGuigan and Schilling, 1997; Nakhleh and Mary, 1992; Wandersee *et. al.*, 1994). The term "misconceptions" is used for the simple reason that researchers refer to it more often.

In order to promote successful learning or at least to simplify it, science educators should diagnose which preconceived images and explanations students hold. In this regard, Treagust (1988) suggests using specific questionnaires to diagnose misconceptions of content and basic ideas: "By using a diagnostic test at the beginning or upon completion of a specific science topic, a science teacher can obtain clearer ideas about the nature of students' knowledge and misconceptions in the topic" (Treagust, 1988).

With this knowledge, teachers are better able to plan their own questionnaires and interviews in order to find out specific preconceptions and misconceptions of their students. Teachers become more aware of such misconceptions and are able to discuss them in their classrooms. Once the alternative conceptions of the students have been identified, the teacher has to decide how to deal with them: giving the scientific idea first and then discussing misconceptions, or go over students' misconceptions first, make them uncomfortable with their own ideas and instruct the scientific concept afterwards (Gilbert *et. al.*, 2002).

Gabel (1999) found out that many teachers are not familiar with or do not acknowledge the science education research regarding misconceptions. Therefore, they do not intend to incorporate them into their lecture plan: "Probably nine out of ten instructors are not aware of the research on student misconceptions, or do not utilize ways to counteract these misconceptions in their instruction". Gilbert *et. al.* (2004) call upon all teachers, not only to increase their awareness of the diagnostic methods available for finding misconceptions, but also to implement them in their lessons. They also suggested that teachers should be aware of these diagnostic tools during their teacher-training curriculum: "The pre-service and in-service education of prospective and experi-enced chemistry teachers can play a crucial role in bridging the gap between chemical education research and classroom practice". In this regard, they point out "increasing chemistry teachers' awareness of chemical education research, improving the use of chemical education research findings and involving chemistry teachers in chemical education research" (Gilbert *et. al.*, 2004).

STUDENTS' MISCONCEPTIONS AND HOW TO OVERCOME THEM

Misconceptions are not only to be observed of today's children or students – even scientists and philosophers developed and lived with many misconceptions in the past: see the Phlogiston Theory of the German scientist Stahl in the 17th century (Barke *et. al.* (2009). Historical concepts and their changes are very interesting: as the early scientists the young students today develop their own ideas by similar observations e.g., the destruction concept with regard to combustion. Ideas that are developed without having any prior knowledge of the subject are not necessarily wrong - they can be described as **preconcepts** instead of misconceptions.

Increasingly however, researchers are finding alternative conceptions in advanced courses also. Because they cannot be only attributed to the students but mainly caused by inappropriate teaching methods and materials, they can be called **school-made misconceptions**. They are clearly different from preconcepts that tend to be unavoidable. Inappropriate teaching methods can be stopped by keeping teachers up-to-date in their subject through advanced education.

One should attempt to find important preconcepts and school-made misconceptions and discuss them with pre-service and in-service teachers. Another important task is to make suggestions of instructional **strategies to improve**

lessons, which will lead to challenge preconcepts and school-made misconceptions: recommending alternative strategies to the traditional approaches, setting up convincing laboratory experiments, using more structural models and mental models, or new technology-based methods etc.

Students' preconcepts. Self-developed concepts made by students do not often match up with today's scientific concepts. One does not take into account that these young folks, through observation, come up with their own mostly intelligent ideas of the world. In this sense, they are in good company with ancient scientists and natural philosophers: they also used their observation and logic in order to develop their ideas. Often, these scientists and philosophers did not use additional experiments to back up their theories.When students talk about combustion, saying that "something" disappears and observe that the remaining ash is lighter than the original portion of coal or wood, then they have done their observation well and have come up with logical conclusions - this is why we cannot describe their conclusions as incorrect. Therefore the teacher has to demonstrate convincing experiments according to the fact that colorless gases are formed by the combustion of a candle, of wood or paper: carbon dioxide and water steam.

In the same sense the following examples of pupil's preconcepts will show that teachers experiments, models and explanations are highly needed to come up with the scientific concept:

- the sun revolves around the earth,
- a puddle is sucked up by the sun's rays,
- the wood of a tree comes from the soil.

Sun and earth. Most children's first experiences regarding the sun are accompanied by comments made by their families and neighbors: "Look, the sun will rise in the morning, at midday it will be at its highest point and in the evening it will set". Observations regarding sunrise, sunset, its own cycle and the common manner of speech regarding the sun must lead the child to the idea: "The sun cycles around the earth". In some of her interviews, Sommer (2002) even comes across the idea of the earth as being a disc: "Children imagine the earth to be a disc over which the sky stretches parallel. The sun, the moon and the stars are to be found in the sky; there is no universe" (Sommer, 2002).Greek natural philosophers developed their ideas 2000 years ago. Ptolemaeus especially imagined the earth to be at the center of everything and pondered: "The sun moving around the earth". It was at the end of the 16th century that Copernicus, after exact observation of the movement of the planets, came up with the heliocentric image of the earth: "The earth is one of the sun's many planets, like these planets, the earth is revolving in a particular pathway around the sun and it also revolves on its own axis". Considering the uproar of the church at that time and the ensuing inquisitions, one can imagine how stable Ptolemaeus' theory was present in the minds of people of the time. It was the real wish of the church to keep people in this ignorance: The earth was supposed to be the center of the universe.

Children and adolescents often, through their own observations, come up with similar concepts like Ptolemäus, of course – there is no way to make discoveries like Copernicus and to develop the heliocentric view of the earth. Teachers have to use the best methods and technology, e.g. a planetarium, in order to convince the kids to free themselves from their original ideas and to accept that the earth is revolving around the sun.

In order to have convincing lessons, it is important that pupils have enough opportunities to first express and compare their ideas of the universe. Only after children are feeling uncomfortable with their ideas the new and current worldview should be introduced. The children should realize that their view of the world is also quite common and even scientists in the past believed that "the sun moves around the earth". Good teaching with models like moving spheres in a planetarium should finally convince children of the revolving earth.

Puddles and sun rays. Through conversations with elementary school children regarding the disappearance of puddles on a sunny day, it is obvious that they believe that the sunrays "soak up the water", that "water disappears to nothing". When asked, many teachers admit that they find this explanation "cute" and often do not bother to correct or discuss it: they let the children be with their "sunray theory" and their view of the "elimination of water".

If, on the other hand, the teachers would carry out experiments showing the vaporization of water and the resulting condensation of the steam to liquid water, the scientific view could be started. If one also introduces the idea of particles and the mental model of increasing movement of the water particles through heat, a child would much better understand that the water particles mix with air particles and therefore remain in the air.

They, furthermore, would understand that particle movement and diffusion of energy-rich particles are responsible for the evaporation of water. This would lead the children to a logical understanding of the conservation of mass for later science lessons and understanding chemical reactions, especially regarding combustion. It is necessary however, that children can express their own view about the "disappearance of water" before they learn the scientific concept. To be convinced by the scientific concept they should look to demonstrated or self-done experiments and compare with their own view. Following these discussions after more experiences with evaporation and condensation of water children may realize their conceptual change.

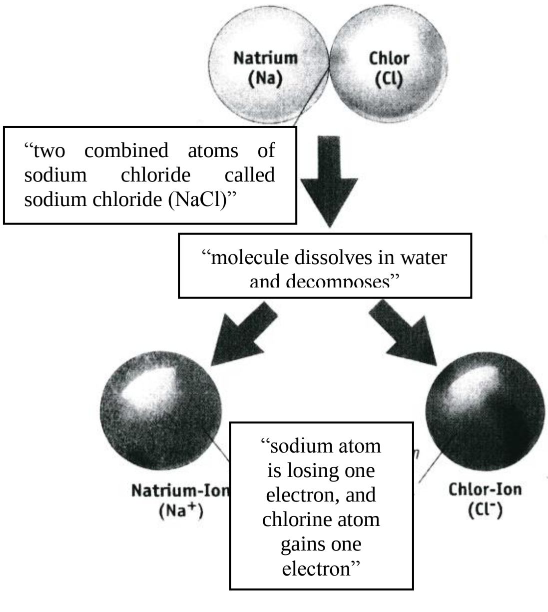
Wood and earth. "When people are given a piece of wood and asked how the material got into the tree, they commonly reply that most of it came from the soil" (Bark *et. al.*, 2009). Even though, in biology, the subject of photosynthesis is taught with the use of carbon dioxide, water, light and heat for the synthesis of sugar and starch, still many students when asked where wood comes from, reply: "from the soil". Most students seem to have their knowledge in special "compartments" of their brain. They do not link them to their every-day life understanding: "Presumably most of the graduates would have been able to explain the basics of photosynthesis (had that been the question), but perhaps they had stored their learning about the scientific process (where carbon in the tree originates from gaseous carbon dioxide in the air) in a different compartment from their 'everyday knowledge' that plants get their nutrition from the soil" (Taber, 2002).

This example should indicate that preconcepts can even still be used for a subject when the related lectures have dealt with the appropriate scientific idea. When one forgets or deliberately avoids making connections between this newly attained knowledge and well-established observations, the new scientific knowledge will not stay stable – the learner is going back to his or her previous preconcepts:

both, preconcept and scientific thinking are stored in “compartments”, in separated areas of the cognitive structure.

Teachers cannot automatically assume that in a particular lesson any preconcepts regarding this lesson will appear. It is necessary to diagnose such preconcepts and to plan a lesson which integrates new information with these concepts. If the lesson is about photosynthesis it would be advisable to bring in everyday aspects, that wood is made up of carbon dioxide and water steam from the air, that starch or sugar molecules are made up of carbon dioxide and water molecules. One could emphasize that plants need the earth in order to transport minerals from the roots to the branches but that, as hard as it is to believe, the solid and massive wood develop due to chemical reactions of colorless gases. Again, one could point out that even ancient scientists believed of the historical humus theory and could not understand when the German Justus von Liebig experimentally verified the photosynthesis in the middle of the 19th century.

School-made misconceptions. When students get involved in a subject matter of advanced courses that is more difficult, a different type of problem arises: school-made misconceptions. Due to their complexity and difficulties in teaching these subjects, it is not often possible to address certain themes in a way to understand them completely. Despite competent and qualified teachers, occasionally questions remain open and problems are not really solved for a full understanding according the actual scientific concept: school-made misconceptions are developing. A few examples should illustrate this.



“two combined atoms of sodium chloride called sodium chloride (NaCl)”

“molecule dissolves in water and decomposes”

“sodium atom is losing one electron, and chlorine atom gains one electron”

Figure 2. Today’s misconceptions about common salt and salt solution (Gerlach, 2004).

Composition of salts. A famous example of school-made misconceptions of our students arises from the Dissociation Theory of Arrhenius. In 1884, he postulated that "salt molecules are found in solid salts as the smallest particles and decompose into ions by dissolving in water”. Later with the concept of electrons, the misconception that “atoms of salt molecules form ions through electron exchange” was born. Today, experts recognize that there are no salt molecules, that ions exist all the time – even in the solid salt. By dissolving the solid salt, water molecules surround the ions, hydrated Na^+(aq) ions and Cl^-(aq) ions are moving free in the salt solution.

Amazingly one can observe that even today the historic misconceptions are quite common: “Sodium chloride consists of sodium and chlorine atoms. Each chlorine atom takes an electron from the sodium atom so the chlorine atom will have a negative electrical charge, the sodium atom a positive one” (Gerlach, 2004). Also a magazine for young students - published in the year 2004 (Welt der Wissenschaft) - contains the same misconceptions (see Fig. 2).

In the related subject of chemical bonding, one elaborates mostly on electron-pair bonding and only briefly on ionic bonding. The result is that students will not have any lasting concept of ions in an ion lattice or in salt solutions. Regarding the question which particles are found in mineral water which contains calcium chloride, many students are answering “Cl-Ca-Cl molecules” (Barke *et. al.*, 2003).

In this case, misconceptions have been developed during lessons - these misconceptions are school-made! Such misconceptions even occur if ions in the recommanded issue of electrolysis of salt solutions have been taught (Hilbing and Barke, 2004).

Chemical reactions. It is traditional in chemistry lessons to separate chemical reactions from physical processes. The formation of metal sulfides from its elements by releasing energy is described in every case as a chemical reaction. In contrast, the dissolving of substances in water is often regarded as a “physical process” because matter “does not actually change”, the dissolved substance can be regained through “physical” separation. If one takes sodium hydroxide and dissolves it in a little water, a colorless solution appears and releases heat; the solution conducts electricity and produces a high pH value. This solution is of course a new material and the production of heat shows an exothermic reaction. From this example one can see that it does not make any sense to separate “chemical” and “physical” processes (Barke and Schmidt, 2004). If we routinely continue to do this in the sense of “we’ve always done it this way”, automatic school-made misconceptions would arise based on teaching traditions in school.

Composition of water. “Water is composed of hydrogen and oxygen” (Barke *et. al.*, 2009) - one often hears these or similar statements in classrooms about compounds, which supposedly “contain” certain elements. These expressions arise from the 19th century when it was common to analyze and find out which elements make up certain compounds. Insiders know the background of these statements – for novices however, they will lead to school-made misconceptions: students would associate the substances copper and sulfur in the black copper sulfide, particularly as experiments show that one can remove these elements out of copper sulfide. It would be better, in introductory classes, to point out that the metal sulfides could be produced from metals and sulfur or to show that one can obtain the elements from the compound. Later on, if one is aware of “atoms” and “ions” as the smallest particles of matter, one can expand on these statements, that the compound “contains” special atoms or ions, that one water molecule contains two H atoms and one O atom connected and arranged in a particular spatial structure. But the pure sentence “water contains hydrogen and oxygen” will develop school-made misconceptions!

Students’ concepts and scientific language. One should be aware that newly acquired concepts are not sustainable forever and can be easily affected when lessons are over. Concepts regarding life in general, which have been sustained over several years, are more deeply rooted than new concepts, which have more recently been picked up in lessons. It is therefore necessary to repeat and intensify these newly “acquired” concepts in order to reach their deep-rooted integration in the minds of students.

Teachers should also be aware that students will have certain insecurity when discussing these new scientific concepts with friends or relatives – they will resort to slang or every-day language. Although they know about conservation of mass they will have to deal with terms like “the fuel is gone” or “spots are removed” (Barke *et. al.*, 2009). One should try to help students begin to reflect on the use of such every-day language and to describe the reaction of fuel with oxygen to form carbon dioxide and water, or to point out that the fat of spots is dissoved in gasoline or ethanol. Then, they could discuss these thoughts with friends or relatives – in this sense, they would become competent and improve the much wished ability to be critical.

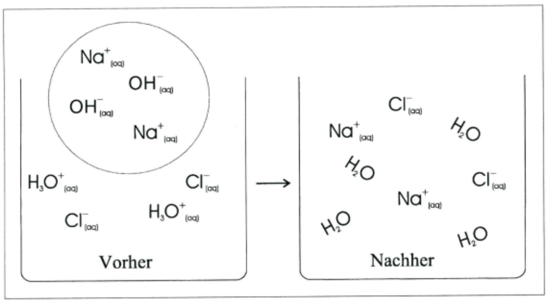


Figure 3. Mental model of the hydrochloric acid and sodium hydroxide reaction (Barke *et. al.*, 2009).

Many school-made misconceptions occur because the specific terminology and the scientific language are not clearly differentiated. Especially for involved substances, particles and chemical symbols, it is not easy to apply the specific terminology. If the neutralization reaction is purely described only through the usual equation $HCl + NaOH \rightarrow NaCl + H_2O$, then students have to memorize it with nearly no understanding. To give them the chance to develop an acceptable mental model one has to use the ions as smallest particles and to offer a mental model of the neutralization (see Fig. 3). After pointing out that Na^+(aq) ions and Cl^-(aq) ions are “spectator ions” and have nothing to do with the reaction, students will better accept the real neutralization reaction with the equation: H_3O^+(aq) + OH^- (aq) $\rightarrow$ 2 H_2O(aq).

Without these considerations on the “submicro level” (see Fig. 4) students mostly come up with mental models of H-Cl molecules and of Na-O-H molecules, or come up with the “formation of salt” or with the formation of “Na-Cl molecules”. If they discuss ions in hydrochloric acid and sodium hydroxide solution, and if one would sketch them in the form of model drawings (see Fig. 3), it would probably be possible for the students to develop the right mental model and scientific language at this level.

Johnstone (2000) elucidated this connection (see Fig. 4): “We have three levels of thought: the macro and tangible, the submicro atomic and molecular, and the representational use of symbols and mathematics. It is psychological folly to introduce learners to ideas at all three levels simultaneously. Herein, lay the origins of many misconceptions. The trained chemist can keep these three’s in balance, but not the learner” (Johnstone, 2000). Gabel (1999) points out, that teachers like to go from the macro level directly to the representational level and that students have no chance to follow in this way: “The primary barrier to understanding chemistry is not the existence of the three levels of representing matter. It is that chemistry introduction occurs predominantly on the most abstract level, the representational level” (Gabel, 1999).

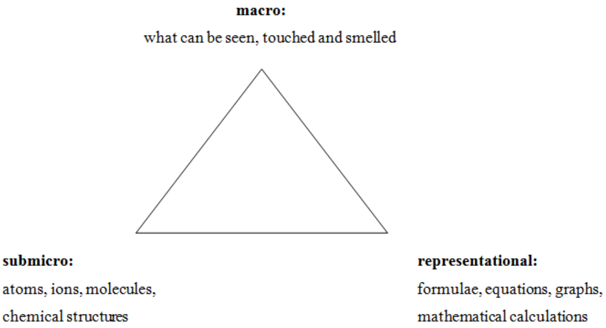


Figure 4. Three levels of representing matter in a “Chemical Triangle” (Johnstone, 2000; Gabel, 1999).

The misconceptions concerning the neutralization example above could be avoided if, after carrying out the experiment, one would describe the observations at the **macro level**. By interpreting these observations, one could ask questions regarding the particles related to the reaction. These could be answered using ions and ionic symbols at the **submicro level**. It would be even better if one uses model drawings related to the hydrated ions in hydrochloric acid and in sodium hydroxide solution (Fig. 3). Only when the reaction of H^+ (aq) ions with OH^- (aq) ions to form H_2O molecules has been made clear on the submicro level, the **representational level** and the chemical symbols will be successfully attained. On this level other reaction equations may be written or related calculations could be done.

EFFECTIVE STRATEGIES FOR TEACHING AND LEARNING

“All teaching should begin with childrens’ experiences - each new experience made by child-ren in a classroom is organized with the aid of existing concepts” (Ausubel, 1974). “Without explicitly abo-lishing misconceptions, it is not possible to come up with scientific sustainable concepts” (Piaget and Inhelder, 1971). “Lessons should not merely proceed from ignorance to knowledge but should rather have one set of knowledge replace another. Chemical education should be a bridge between students’ preconcepts and today’s scientific concepts” (Pfundt, 1975).

These statements make it quite obvious that teachers should not assume their students enter their classroom with no knowledge or ideas. A lesson, which does not take into account that students have existing concepts, usually leads them to barely following the lecture until the next quiz or exam. After that, newly acquired informations will gradually be forgotten: students tend to return to their old and trusted concepts.

Nowadays, teachers and pedagogy experts agree that one should be aware of student’s ideas before the “bridge can be successfully made between the preconcepts and the scientific ones" (Pfundt, 1975). Therefore, an important goal is to allow students to express their own preconcepts during a lesson or, in the attempt to introduce new subject matter in a lesson, to let them be aware of inconsistencies regarding their ideas and the up-to-date scientific explanation. In this way, they can be motivated to overcome these discrepancies. Only when students feel uncomfortable with their ideas, and realize that they are not making any progress with their own knowledge they will accept the teacher's information and thereby build up new cognitive structures (Duit, 1996).

If a student does not believe that "sunrays absorb a puddle", he or she can then, using the particle model of matter with the idea of moving particles, successfully develop a scientific concept about the evaporation of water. There is an extension of the already established particle concept taught in lessons before – a **conceptual growth** appears.

Should yet another student believe that "sunrays soak up the puddle", perhaps through having learned it at the elementary school, then he or she is unlikely to want to let go of this concept. Even if lessons about the particle model of matter are plausible and logical, he or she is unlikely to integrate it or to swap it against the "sun's absorption ability". If the teacher helps to understand the scientific concept through the introduction of self-moving particles, then this student has to take a huge step in releasing his old ideas: a **conceptual change** has to develop in his cognitive structure. To push this development to a new mental model it would be advantageous to do his or her own active experiments and model drawings according the particle model of matter and self-moving particles.

Taber came up with the picture of a "**Learning Doctor"** as a means of discovering individual misconceptions and a suitably-related science class regarding conceptual growth or conceptual change (Taber, 2002): "A useful metaphor here might be to see part of the role of a teacher as being a learning doctor: a) diagnose the particular cause of the failure-to-learn; and b) use this information to prescribe appropriate action, designed to bring about the desired learning…… Two aspects of the teacher-as-learning-doctor comparison may be useful. First, just like a medical doctor, the learning doctor should use diagnostic tests as tools to guide action. Secondly, just like medical doctors, teachers are 'professionals' in the genuine sense of the term. Like medical doctors, learning doctors are in practice (the 'clinic' is the classroom or teaching laboratory). Just as medical doctors find that many patients are not textbook cases, and do not respond to treatment in the way the books suggest, so many learners have idiosyncrasies that require individual treatment" (Taber, 2002).

In a project in progress, Barke and Oetken agree to diagnose preconcepts and school-made misconceptions, but in addition they will integrate them into lectures to develop sustainable understanding of chemistry (Barke and Oetken, 2008). Hence being convinced that preconcepts and school-made misconceptions have to be discussed in chemistry lectures, there are two hypotheses to influence instruction:

1. One discusses first the misconceptions and come up with the scientific explanation afterwards, 2. One instructs first the scientific concept and afterwards students compare it with their own or other misconceptions from literature.

Oetken and Petermann (2008) are taking the first hypothesis for their empirical research concerning the famous preconcept of combustion: "Something is going into the air,…..some things are going away". In their lectures they showed the burning of charcoal and discussed alternative conceptions like: "charcoal is destroyed, nothing remains". Afterwards they used the idea of a cognitive conflict: little pieces of charcoal are deposited in a big round flask, the air is substituted by oxygen, the flask is closed by an air balloon and the whole thing is weighed using analytical balance. The flask is heated at the area of the charcoal; the pieces ignite and burn until no charcoal remains. The whole flask is weighed again: the scales afterwards present the same mass as before.

Working with this cognitive conflict the students find out that there must be a reaction of carbon with oxygen to form another invisible gas. After testing this gas by the well-known lime water test, one can derive: the gas is carbon dioxide. Presenting misconceptions first and instructing afterwards the scientific concept can enable students to compare and investigate by themselves what is wrong with statements like "some things are going away" or "combustion destroys matter, mass is going to be less than before". Integrating preconcepts in lectures by this way will improve sustainable understanding of chemistry.

Barke, Doerfler and Knoop (2007) planned lectures according to the second hypothesis in middle school classes: 14 - 16 years old students were supposed to understand acids, bases and neutralization. Instead of taking the usual equation "$HCl + NaOH \rightarrow NaCl + H_2O$" for the reaction, $H^+(aq)$ ions for acidic solutions and $OH^-(aq)$ ions for basic solutions were introduced, the ionic equation of the *formation of water molecules* was explained: "$H^+(aq)$ ions + $OH^-(aq)$ ions $\rightarrow$ H_2O molecules". Later it was told that with regard to the neutralization other students are thinking of a "*formation of salt*" because "NaCl is a product of this neutralization". Students discussed this idea with the result that no solid salt is formed by the neutralization, $Na^+(aq)$ ions and $Cl^-(aq)$ ions are not reacting but only remaining by the neutralization.

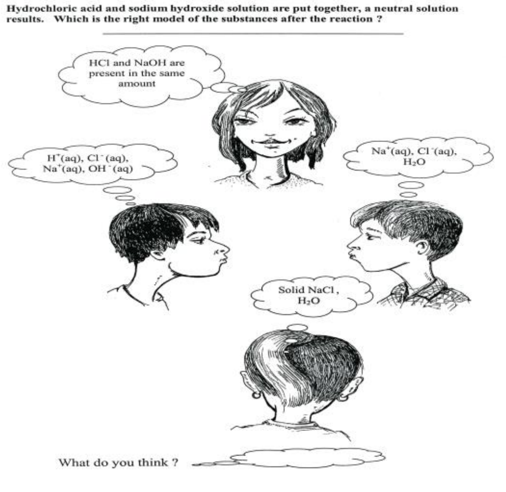


Figure 5. Concept Cartoon according concepts of the neutralization reaction (Temechegn and Sileshi, 2004).

So students were first instructed by the scientific idea of the new topic, and afterwards confronted with well-known misconceptions. By comparing the scientific idea and the presented misconceptions the students could intensify the recently gained scientific concept. Data are showing that this hypothesis is successful in preventing misconceptions.

Temechegn and Sileshi (2004) are proposing another way of discussing misconceptions through concept cartoons. For a special subject or experiment they take three or four persons and let them make proposals for the right explanation. In case of the neutralization reaction they ask: "What is the right model of substances after the reaction?" They offer four different statements according to the most known misconceptions (see Fig. 5), the right answer is of course: "Na^+(aq) ions, Cl^-(aq) ions and H_2O molecules". Students may be tought by the scientific concept first and can study afterwards this concept cartoon. They will find the right answer and should discuss the three wrong ones. They will apply their new scientific concept and will find out what is wrong with the other three statements.

But the concept cartoon may also be discussed first for diagnosis of misconceptions held by the students: they compare all statements of the concept cartoon and decide which one is matching with their own mental model. By this way the teacher knows how his students are thinking and how he should prepare his lessons. With the question of the cartoon "what do you think" the teacher can get even more different thoughts about the subject or experiment he is presenting. The concept cartoon may accompany all the lessons concerning the subject. Taking the new aquired scientific concept and explaining the other alternative answers of the concept cartoon with their new knowledge students will not return to the alternative answers - and will avoid these misconceptions.

Last not least the authors also claim to take concept cartoons for assessment (Temechegn and Sileshi, 2004): finishing the topic of neutralisation the teacher offers the unknown concept cartoon (Fig. 5), asks for the right answer and for explaining the other ones.

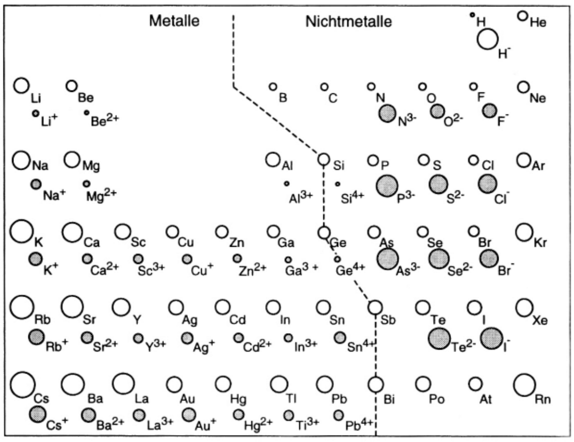


Figure 6. Atoms and ions as basic particles of matter (part of the PSE) (Barke *et. al.*, 2012).

Table 1. Chemical structures by combining atoms and ions (Barke *et. al.*, 2012).

1. Metal atoms "left and left in PSE": (metal structure)	x Ag → Ag_x
2. Nonmetal atoms "right and right in PSE": (molecules)	1 C + 4 H → CH_4
3. Ions "left and right in PSE": (ionic structure)	Na^+ + Cl^- → Na^+Cl^-(s)

With regard to teach ions and ionic bonding **Strehle and Roelleke** (2007) and **Wirbs** (2002) evaluated lectures through the introduction of "atoms and ions as basic particles of matter" on base of Dalton's atomic model (Fig. 6). For introducing chemical structures of important substances needed in chemistry lectures giant structures of metal crystals and salt crystals are reflected, also the structure of some molecules. If the Periodic Table of "atoms and ions" (Fig 6) is devided in metal atoms and ions "left in PSE", and in nonmetal atoms and ions "right in PSE" (H atom and hydride ion belongs to this right side!) some rules are helpful in combining the basic particles of matter and visualizing the most important chemical structures (see Table 1). Following this way students are able to develop mental models according important chemical structures - through this strategy of combining ions and using ion symbols most of the related world-wide found misconceptions can be prevented!

The Structure-oriented approach (Barke *et. al.*, 2012) offers not only a way to understand chemistry through chemical structures, but also a way to improve spatial ability of students and specially the ability of girls. **Temechegn** (2001) could show that in Germany and Ethiopia boys are better than girls in interpreting chemical structures, that chemistry lectures with spatial models of metal and ionic structures and molecules are helpful to develop spatial ability.

In his empirical research **Sopandi** (2004) has shown that there are good correlations between spatial ability of students and their understanding of chemistry. The conclusion seems to be: take structural models of matter and you will not even improve spatial ability as an important ability for many subjects in school and for many professions after school you will also develop a good understanding of chemistry and avoid school-made misconceptions!

REFERENCES

Ausubel, D.P. (1974). Psychologie des Unterrichts. Weinheim (Beltz).

Barke, H.-D. (2002). Das Chemische Dreieck. Unterricht Chemie 13, 45.

Barke, H.-D., Doerfler, T., Knoop, C. (2007). Neutralisation von Saeuren und Basen: Entwicklung und Evaluation eines Unterrichtskonzepts unter Beruecksichtigung empirisch erhobener Fehlvorstellungen. Staatsexamensarbeit. Muenster.

Barke, H.-D., Harsch, G., Schmid, S. (2012). *Essentials of Chemical Education*. Berlin, Heidelberg (Springer).

Barke, H.-D., Hazari, A., Sileshi, Y. (2009). Misconceptions in Chemistry. Addressing Perceptions in Chemical Education. Heidelberg (Springer).

Barke, H.-D., Oetken, M. (2008). Das an Schuelervorstellungen orientierte Unterrichtsverfahren. CHEMKON 15.

Barke, H.-D., Schmidt, F.K. (2004). Chemische Reaktionen und physikalische Vorgaenge: mit untauglichen Schubladen zur Nulleffizienz. MNU 57, 115.

Barke, H.-D., Selenski, T., Sopandi, W. (2003). Mineralwasser und Modellvorstellungen. PdN-ChiS 52, H.2, 15.

Barke, H.-D., Strehle, N., Roelleke, R. (2007). Das Ion im Chemieunterricht – noch Vorstellungen von gestern? MNU 60, 366.

Barke, H.-D., Temechegn, E. (2001). Structural chemistry and spatial ability in different cultures. CERP 2, 227.

Barke, H.-D., Wirbs, H. (2002). Structural units and chemical formulae in chemical education. CERP 3, 185.

Blosser, P. (1987). Science Misconceptions Research and Some Implications for the Teaching to Elementary School Students. ERIC/SMEAC Science Education, 1.

Duit, R. (1996). Lernen als Konzeptwechsel im naturwissenschaftlichen Unterricht. Kiel (IPN).

Gabel, D. (1999). Improving Teaching and Learning through Chemistry Education Research: A Look to the Future. *J.Chem.Educ.* 76, 548.

Gerlach, H. (2004). Salz auf unserem Steak. Sueddeutsche Zeitung, Magazin. Stuttgart, 34.

Gilbert, J.K., Justi, R., De Jong, O., Justi, R., Treagust, D.J., van Driel, J.H. (2002). Chemical Education: Towards Research-based Practice. Dordrecht. (Kluwer Academic Publishers).

Gilbert, J.K., Justi, R., Driel, J.H., De Jong, O., Treagust, D.J. (2004). Securing a Future for Chemical Education. CERP 5, 5.

Elizabeth, H. (1990). *The students Laboratory and the Science Curriculum*. Chatham.

Eylon, B. and Linn, M. (1988). Learning and Instruction: An Examination of Four Research Perspectives in Science Education. *Review of Educational Research.* 58, 251.

Fensham, P. et al. (Ed). (1994). The Content of Science: A constructive approach to its learning and teaching. The Falmer Press.

Hilbing, C., Barke, H.-D. (2004). Ionen und Ionenbindung: Fehlvorstellungen hausgemacht! Ergebnisse empirischer Erhebungen und unterrichtliche Konsequenzen. CHEMKON 11, 115.

Johnstone, A.H. (2000), Teaching of Chemistry – logical or psychological? CERAPIE 1, 9.

Mahaffy, P. (2006). Moving Chemistry Education in to 3D: A tetrahedral metaphor for understanding chemistry. *J.Chem.Educ.* 83, 1.

McGuigan, L. and Schilling, M. (1997). Children Learning in Science. In: Ed Alan Cross and Gill Peet: Teaching Science in the Primary School, Book One: A Practical Source Book of Teaching Strategies. Northcote House Publishers Ltd, 24.

Nakhleh, B., Mary. M. (1992). Why Some Students Don't Learn Chemistry. *J.Chem. Educ.* 69, 191.

Petermann, K., Oetken, M.(2008). Das an Schuelervorstellungen norientierte Unterrichtsverfahren – exemplarische Vorstellung des Unterrichtsverfahrens an Hand einer Unterrichtseinheit zum Kohlenstoffkreislauf. CHEMKON 15.

Piaget, J., Inhelder, B. (1971). Die Entwicklung des raeumlichen Denkens beim Kinde. Stuttgart (Klett).

Pfundt, H. (1975). Urspruengliche Erklaerungen der Schueler für chemische Vorgaenge. MNU 28, 157.

Sommer, C. (2002). Wie Grundschueler sich die Erde im Weltraum vorstellen – eine Untersuchung von Schuelervorstellungen. ZfDN 8, 69.

Sopandi, W. (2004). Raumvorstellungsvermögen und Chemieverstaendnis im Chemieunterricht. Muenster (Schueling).

Taber, K. (2002). Chemical misconceptions – prevention, diagnosis and cure. Volume I. London (Royal Society of Chemistry).

Temechegn, E., Sileshi, Y. (2004). Concept Cartoons – as a Strategy in Learning, Teaching and Assessment in Chemistry. Addis Ababa/Ethiopia (Addis Ababa University).

Treagust, D.F. (1988). Development and use of diagnostic tests to evaluate students' misconceptions in science. *Int.J.Science Educ.* 10, 159.

Wandersee, J.H. et al. (1994). Research on alternative conceptions in science. In: Gabel, D.: Handbook of Research on Science Teaching and Learning. New York.

Welt der Wissenschaft (deutsche Übersetzung). Bath, England. (2004). (Parragon), 34.

Summary/Sažetak

Učenici, đaci i studenti u školama i na univerzitetima mogu znati formule i hemijske jednadžbe, ali mogu imati problema kod mentalnih modela koji se tiču kristalne strukture metala i soli. Osobito kada se radi o ionima kao česticama u čvrstom stanju ili u otopini soli, pokazuju mnoge pogrešne predodžbe (miskoncepcije), kao što su, na primjer molekule NaCl u čvrstoj kuhinjskoj soli, ili Na-O-H molekule u otopini natrijevog hidroksida. Jedan od načina da se prevaziđu ove miskoncepcije može biti Periodni sistem elemenata koji sadrži simbole atoma i iona na bazi Daltonovg atomskog modela, koji prikazuje atome i ione kao loptice različitih veličina. Kombiniranje atoma metala "lijevo i lijevo" u PSE u velike strukture pokazat će kristalne strukture čistih metala i legura, kombiniranje atoma nemetala "desno i desno u PSE" u molekule pokazat će molekularne strukture isparljivih tvari, kombiniranje iona "lijevo i desno u PSE" pokazat će ionske kristalne strukture soli. Ovim koracima u ranom poučavanju hemije, razumijevanje otopina soli i elektrolita bit će znanstveno ispravno - miskoncepcije bi se trebale umanjiti.

Glasnik hemičara i
tehnologa
Bosne i Hercegovine

Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: ________________________
Original Scientific Article

2013
40
17-20

Cyclic Conjugation in Benzo-annelated Ovalenes

Gojak-Salimović, S.[a,*], Gutman, I.[b], Đurđević, J.[b], Janošević, M.[b]

[a]*University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina*

[b]*Faculty of Science, University of Kragujevac, P. O. Box 60, 34000 Kragujevac, Serbia*

Article info
Received: 28/02/2013
Accepted: 14/03/2013

Keywords:
cyclic conjugation
benzo-annelation
ovalene
benzenoid hydrocarbon

***Corresponding author:**
E-mail: sgojak@pmf.unsa.ba
Phone: 00-387-33-279 907
Fax: 00-387-33-649 359

Abstract: The effect of small structural changes on the electronic properties of large benzenoid molecules is studied in the case of ovalene and its benzo-annelated derivatives. Two quantitative indicators of the intensity of cyclic conjugation in individual rings were used: the π-electron content and the energy effect. Most regularities observed can be rationalized by means of the classical Clar aromatic sextet theory, although a few second-order effects point towards the need to go beyond the Clar model.

INTRODUCTION

Klaus Müllen and his coworkers have recently succeeded to synthesize benzenoid molecules of unprecedently large size (Watson et al. 2001). These π-electron systems proved to possess remarkable electronic, optical, and optoelectronic properties, suitable for a variety of technical applications and thus of great commercial value (Berresheim et al., 1999; Li et al., 2010; Figueira-Duarte and Müllen, 2011). The design of large benzenoids with technically desired properties requires the knowledge of the fine details of their electron configuration, in particular of the distribution and energetics of their π-electrons. The present work is the continuation of our earlier efforts to contribute to the better understanding of the electronic properties of large benzenoid molecules, and to help designing such molecules with desired properties (Balaban et al., 2010; Marković et al., 2012; Gutman et al., 2012a, 2012b; Radenković et al., 2012).

It is well known (Gutman and Cyvin, 1989) that the main physical and chemical properties of benzenoid molecules are determined by their π-electrons. These electrons are in a complicated manner cyclically delocalized, and the pattern of their cyclic conjugation can be followed by a variety of theoretical methods. In this work, as well as in our previous studies, we focus our attention to two quantitative indicators of cyclic conjugation, namely the π-electron content (*EC*) and the π-electron energy effect (*ef*) of individual rings. Details of the *EC*-method can be found in the papers (Randić and Balaban, 2004; Balaban and Randić, 2004; Gutman et al., 2004), whereas the details of the *ef*-method are described in the reviews (Gutman, 2005, 2009).

Ovalene is one of the largest benzenoid hydrocarbons known before Müllen's works. Its structure as well as Clar formula are depicted in the Figure 1. Also shown in Figure 1 are the *EC*- and *ef*-values of all rings of ovalene.

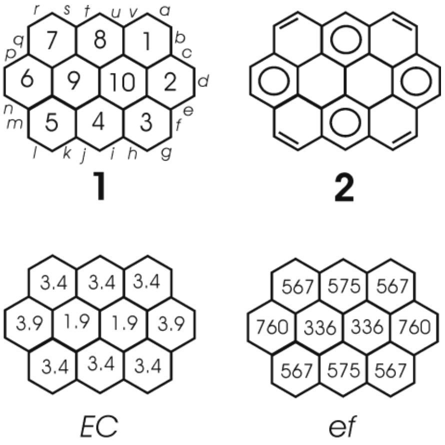


Figure 1: Ovalene (**1**) and its unique Clar formula (**2**). According to formula **2** it is expected that there is a strong cyclic conjugation in the rings 2, 4, 6, and 8, and that this conjugation is particularly weak in the rings 9 and 10. This is well reproduced both by the *EC*-values (= the effective number of π-electrons in the underlying ring) and by the *ef*-values (here and later given in β-units, multiplied by 10000; thus the *ef*-value of the ring 1 is 0.0567β, where β≈-137 kJ mol^{-1}).

NUMERICAL WORK

Ovalene has a total of 23 benzo-annelated derivatives (two mono-, six di-, six tri-, six tetra, two penta-, and one hexabenzo congener). For all six-membered rings of ovalene and all its derivatives (a total of 312 rings), the *EC*- and *ef*-values were calculated using our in-house software. These numerical results can be obtained from the authors (S.G-S. and M.J.) upon request. In what follows, we discuss in detail only a few selected examples.

In Figure 2 we present the plot of the calculated *EC*-values versus the respective *ef*-values. As expected, there is a reasonably good monotonically increasing correlation between these two measures of cyclic conjugation, reflecting the fact that the (stabilizing) energy-effect of cyclic conjugation is proportional to the π-electron content in the respective ring.

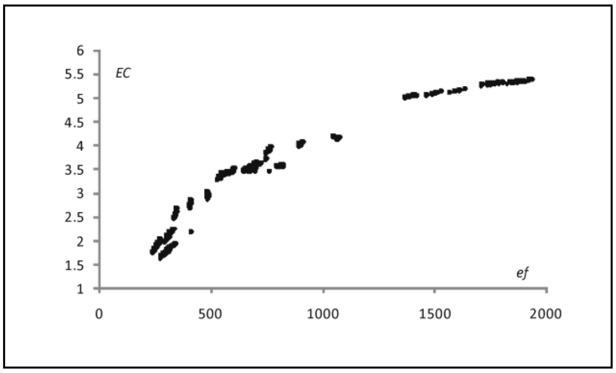


Figure 2: The π-electron contents *EC* of all six-membered rings of ovalene and all its benzo-annelated congeners, plotted versus the respective energy effects *ef*, 312 data points.

DISCUSSION

The simplest and most direct way by which one gets an insight into the modes of cyclic conjugation of a benzenoid molecule is Clar's aromatic sextet theory (Clar, 1972); its details are outlined also in the book (Gutman and Cyvin, 1989). The essence of Clar theory is that so-called "Clar formulas" are constructed by placing "aromatic sextets" into some rings of a benzenoid molecule, obeying certain formal rules (Gutman and Cyvin, 1989; Balaban, 2004). Rings in which "aromatic sextets" are located are predicted to have a high intensity of cyclic conjugation. The original version of Clar's method is qualitative and has no direct foundation in quantum theory. Eventually, much effort has been done to provide a quantitative and theory-based re-formulation of Clar theory (Aihara, 2003; Portella et al., 2005; Gutman et al., 2005, 2006; Salem et al., 2009; Randić and Plavšić, 2011). Anyway, recent works (Gutman et al., 2012a, 2012b, Radenković et al., 2012) showed that in some benzenoid systems, Clar theory yields incorrect results. In what follows we will also point at a property of some benzo-annelated ovalenes, violating the predictions of Clar theory.

In Figure 3 are depicted dibenzo[*a*,*g*]ovalene (**3**) and its unique Clar formula (**4**). This is an example of a benzo-annelated ovalene in which the Clar theory is fully applicable.

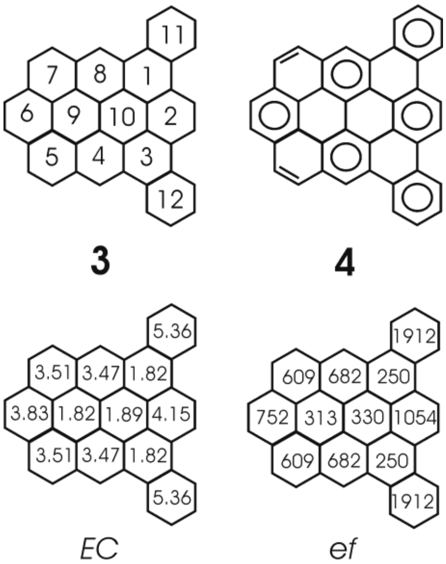


Figure 3: Dibenzo[*a*,*g*]ovalene (**3**) and its unique Clar formula (**4**). The *EC*- and *ef*-values are in good agreement with the predictions of Clar theory: in the rings 2, 4, 6, and 8 cyclic conjugation is strong, and stronger than in ovalene, whereas in the central rings 9 and 10 it is weak and weaker than in ovalene (cf. Figure 1).

From Figure 1 we see that the fixed double bonds in the Clar formula of ovalene are in positions *a*, *g*, *l*, and *r*. Therefore, the benzenoid system obtained by benzo-annelation in any of these positions has a unique Clar formula. Accordingly, cyclic conjugation is increased in the rings 2, 4, 6, and 8, and decreased in the central rings 9 and 10. This regularity in the conjugation pattern of benzo-annelated ovalenes is seen by comparing Figures 1 and 3.

Benzo-annelation in positions *d* and/or *o* causes a significantly different change in the conjugation modes of the ovalene core. As a characteristic example, we consider dibenzo[*d,o*]ovalene. In Figure 4 is depicted its formula (**5**) and three of its 20 Clar formulas (**6,7,8**).

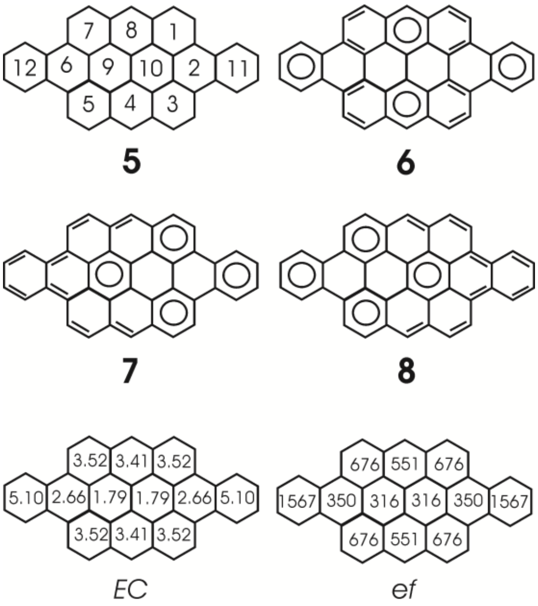


Figure 4: Dibenzo[*d,o*]ovalene (**5**) and three of its 20 Clar formulas (**6,7,8**). Formula **6** seems to be in the best agreement with the calculated *EC*- and *ef*-values. Therefore, our guess is that this is the dominant Clar representation. According to the formulas **7** and **8**, some cyclic conjugation should exist also in the rings 9 and 10, which would require that the *EC*- and *ef*-values of these rings be greater than in unsubstituted ovalene (cf. Figure 1). The fact that this is not the case, points towards a limitation of the Clar model.

Already the fact that dibenzo[*d,o*]ovalene has 20 different Clar formulas, implies that the application of the Clar model to this benzenoid hydrocarbon is neither simple nor unambiguous. The assumption that the all Clar formulas are equally important (Randić and Balaban, 2006, 2008; Randić and Plavšić, 2011) is evidently an oversimplification. From the *EC*- and *ef*-data shown in Figure 4, we conclude that the Clar formula **6** provides the best representation of the true π-electron configuration. In other words, **6** would be the dominant Clar formula of dibenzo[*d,o*]ovalene. Within the Clar model, the remaining 19 aromatic sextet formulas would cause only small perturbations of the initial pattern of cyclic conjugation. If so, then based on formulas **7** and **8**, we would expect that cyclic conjugation in the rings 9 and 10 is stronger than in the unsubstituted ovalene (cf. Figure 1). This, however, is not the case: *d,o*-annelation decreases the intensity of cyclic conjugation in the central rings, a phenomenon violating Clar theory. This second-order effect in the pattern of cyclic conjugation of benzo-annelated ovalenes is one more example for the recently discovered limitations of the Clar model (Gutman et al., 2012a, 2012b). A theoretical explanation of these violations has not yet been achieved.

CONCLUSIONS

Our analysis of cyclic conjugation in benzo-annelated ovalenes can be summarized by the following general regularities. If annelation is done in positions *a*, *g*, *l*, and/or *r* (cf. Figure 1), then the respective benzo-derivative has a unique Clar formula which well reproduces the dominant modes of cyclic conjugation, and to some extent also their intensity. For these benzenoid systems, Clar theory is fully applicable. If annelation is done in positions *d* and/or *o*, then there exist several (sometimes quite numerous) Clar formulas, whose interpretation is difficult and ambiguous. The *EC*- and *ef*-values, calculated in this work, make it possible to single out the Clar formula that provides the best representation of the distribution and energetics of π-electrons. However, some of the fine details of the pattern of cyclic conjugation of such ovalene congeners cannot be correctly predicted by applying the Clar model.

REFERENCES

Aihara, J. (2003). π-Electron currents induced in polycyclic benzenoid hydrocarbons and their relationship to Clar structures. *Journal of Physical Chemistry*, A 107, 11553-11557.

Balaban, A. T. (2004). Clar formulas: How to draw and how not to draw formulas of polycyclic aromatic hydrocarbons. *Polycyclic Aromatic Compounds*, 24, 83-89.

Balaban, A. T., Đurđević, J., Gutman, I., Jeremić, S., Radenković, S. (2010). Correlations between local aromaticity indices of bipartite conjugated hydrocarbons. *Journal of Physical Chemistry*, A 114, 5870-5877.

Balaban, A. T., Randić, M. (2004). Partitioning of π-electrons in rings of polycyclic benzenoid hydrocarbons. 2. Catacondensed coronoids. *Journal of Chemical Infornation and Computer Sciences*, 44, 50-59.

Berresheim, A. J., Müller, M., Müllen, K. (1999). Polyphenylene nanostructures. *Chemical Review*, 99, 1747-1785.

Clar, E. (1972). *The Aromatic Sextet*, Wiley, London.

Figueira-Duarte T. M., Müllen, K. (2011). Pyrene-based materials for organic electronics. *Chemical Review*, 111, 7260-7314.

Gutman, I. (2005). Cyclic conjugation energy effects in polycyclic π-electron systems. *Monatshefte für Chemie*, 136, 1055-1069.

Gutman, I. (2009). Mathematical modeling of chemical phenomena. In Graovac, A., Gutman, I., Vukičević, D. (Eds.), *Mathematical Methods and Modelling for Students of Chemistry and Biology*. Hum, Zagreb, pp. 13-27.

Gutman, I., Cyvin, S. J. (1989). *Introduction to the Theory of Benzenoid Hydrocarbons*. Springer, Berlin.

Gutman, I., Đurđević, J., Matović, Z., Marković, M. (2012a). Verifying the modes of cyclic conjugation in tetrabenzo[*bc,ef,op,rs*]circumanthracene, *Journal of the Serbian Chemical Society*, 77,1401-1408.

Gutman, I., Đurđević, J., Radenković, S., Matović, Z. (2012b), Anomalous cyclic conjugation in the perylene/bisanthrene homologous series. *Monatshefte für Chemie*, 143, 1649-1653.

Gutman, I., Gojak, S., Furtula, B. (2005). Clar theory and resonance energy. *Chemical Physics Letters*, 413, 396-399.

Gutman, I., Gojak, S., Furtula, B., Radenković, S., Vodopivec, A. (2006). Relating total π-electron energy and resonance energy of benzenoid molecules with Kekulé- and Clar-structure-based parameters. *Monatshefte für Chemie*, 137, 1127-1138.

Gutman, I., Morikawa, T., Narita, S. (2004). On π-electron content of bonds and rings in benzenoid hydrocarbons. *Zeitschrift für Naturforschung*, 59a, 295-298.

Li, C., Liu, M., Pschirer, NJ. G., Baumgarten, M., Müllen, K, (2010). Polyphenylene-based materials for organic photovoltaics. *Chemical Review*, 110, 6817-6855.

Marković, M., Đurđević, J., Gutman, I. (2012). Cyclic conjugation in benzo- and benzocyclobutadieno-annelated terrylenes and higher rylenes. *Journal of the Serbian Chemical Society*, 77, 751-759.

Portella, G., Poater, J., Solá, M. (2005). Assessment of Clar's aromatic π-sextet rule by means of PDI, NICS and HOMA indicators of local aromaticity. *Journal of Physical Organic Chemistry*, 18, 785-791.

Radenković, S., Bultinck, P., Gutman, I., Đurđević, J. (2012). On induced current density in the perylene/bisanthrene homologous series. *Chemical Physics Letters*, 552, 151-155.

Randić, M., Balaban, A. T. (2004). Partitioning of π-electrons in rings of polycyclic conjugated hydrocarbons. Part 1: Catacondensed benzenoids. *Polyciclic Aromatic Compounds*, 24, 173-193.

Randić, M., Balaban A. T. (2006). Partitioning of pi-electrons in rings for Clar structures of benzenoid hydrocarbons. *Journal of Chemical Information and Modeling*, 46, 57-64.

Randić, M., Balaban, A. T. (2008). π-Electron partitions, signatures, and Clar structures of selected benzenoid hydrocarbons. *Journal of Physical Chemistry*, A 112, 4148-4157.

Randić, M., Plavšić, D. (2011). Algebraic Clar formulas - Numerical representation of Clar structural formula, *Acta Chimica Slovenica*, 58, 448–457.

Salem, K., Klavžar, S., Vesel, A., Žigert, P. (2009). The Clar formulas of a benzenoid system and the resonance graph. *Discrete Applied Mathematics*, 157, 2565-2569.

Watson, M. D., Fechtenkötter, A., Müllen, K. (2001). Big is beautiful - “Aromaticity” revisited from the viewpoint of macromolecular and supramolecular benzene chemistry. *Chemical Review*, 101, 1267-1300.

Summary/Sažetak

Istraživani su uticaji malih strukturnih promjena na elektronske osobine velikih benzenoidnih molekula, i to na primjeru ovalena i njegovih benzo-aneliranih derivata. Ispitivanja su vršena pomoću dva kvantitativna indikatora intenziteta ciklične konjugacije u pojedinačnim prstenovima, i to: π-elektronski sadržaj i energetski efekat. Većina uočenih pravilnosti može se objasniti pomoću klasične Clarove teorije aromatičnog seksteta, iako neki manje izraženi efekti ukazuju na potrebu da se proširi originalni Clarov model.

Glasnik hemičara i
tehnologa
Bosne i Hercegovine

Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: ___________________

Original Scientific Article

2013
40
21-24

Influence of Monomer Concentration on Capability of Voltammetric Polypyrrole Based Cation Sensor Using Modified Butler-Volmer Equation

Ostojić, J.[a*], Gojak-Salimović, S.[a], Korać, F.[a], Gutić, S.[a], Islamović, S.[a]

[a]*University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina*

Article info
Received: 10/04/2013
Accepted: 06/05/2013

Keywords:
Butler-Volmer equation
voltammetric sensor
conducting polymer
polypyrrole

***Corresponding author:**
E-mail: jelena_ostojic@live.com
Phone: +387 33 279 911
Fax: +387 33 279 896

Abstract: In this work, a modified Butler-Volmer equation is used to determine capability of voltammetric ion sensor based on modified PPy/DBS electrode. This method shows a direct relationship between mid point reversible potential (E_R) and the logarithm of the electrolyte concentration. Slopes from Butler-Volmer equation include electron-transfer coefficient. Modified PPy/DBS electrode was successfully applied as voltammetric cation sensor. It was shown that the capability of the sensor depends on the quality of formed polypyrrole film which is dependent on the initial monomer concentration.

INTRODUCTION

The voltammetric sensors have some advantages when compared to ion-selective electrodes: they dispense with the need to prepare one electrode per ion; the current-time and potential-time curves provide more analytically useful information than single equilibrium potential curves obtained under potentiometric conditions; also, the reversible potential can be calculated from data obtained under dynamic conditions. For voltammetric ion sensors based on polymeric materials, linear relationships between potential and the logarithm of electrolyte concentration can be found. The voltammetric sensors are very attractive for practical applications because they are associated with small-size, portability, low energy consumption, and low cost (Wooster and Bond, 2003; Wooster, Bond and Honeychurch, 2003; Cano, Rodriguez-Amaro and Romero, 2008a, 2008b).

Electrically conducting polymers have also attracted much attention due to their many promising technological applications, as for example microelectronic devices, electroluminescence devices, electrochemomechanical devices, corrosion inhibitors and chemical sensors. Application of conducting polymers in chemical sensors has been extensively studied since 1987. Among conducting polymers, polyaniline (PANI), polypyrrole (PPy) and polythiophene (PTh), are particularly attractive because of their simple synthesis, high conductivity and excellent environmental stability. These polymers offer great possibilities to improve the sensors selectivity, partly by changing the chemical structure of the polymer backbone, and partly because the many possible counterions or neutral molecules can be trapped inside the polymer (Aradilla, Estrany, Azambuja, *et. al.*, 2010; Inzelt, 2008; Jovanović, Stanković, Laninović *et. al.*, 2000; Wallace, Spinks, Kane-Maguire *et. al.*, 2009).

EXPERIMENTAL

Instruments

All electrochemical parameters were determined using Princeton Applied Research model PAR263A potentiostat/galvanostat connected to PowerCV software. A three-electrode cell was used with Pt disc electrode, an Ag/AgCl/KCl$_{(sat)}$ reference electrode (+197 mV vs. SHE), and a Pt foil as counter electrode.

Reagents

Pyrrole (Sigma-Aldrich, 99%) was double distilled under vacuum before use, and kept in the refrigerator, protected from light. Sodium dodecylbenzene sulfonate (DBS) (Sigma-Aldrich), $LiClO_4$, $NaClO_4$, $KClO_4$ and Al_2O_3 (Kemika, Zagreb), were used as received. All solutions were made using doubly distilled water.

Determination of the electrode surface

The "electrochemical surface" of a polished platinum electrode is determined by a chronoamperometry using the electrochemical reduction of complexed Fe(III) ions. The analysis uses the Cottrell equation which establishes the time evolution profile of the current versus the surface, the concentration and the diffusion coefficient of the species for a large potential step at planar electrode. The integration of the Cottrell equation establishes that the slope of *I* versus sqrt (*t*) is directly proportional to the electroactive surface. The electroactive surface is calculated from chronoamperometric response of Pt electrode in 0.01 mol dm^{-3} solution of $K_3[Fe(CN)_6]$ using Cotrell equation (Zoski, 2007):

$$I = nFAc_0(D/\pi t)^{-1/2} \tag{1}$$

where *n* is the number of electrons, *A* is the electrode area (cm^2), *D* is the diffusion coefficient, *F* is the Faraday constant and *c* is the concentration (mol cm^{-3}).

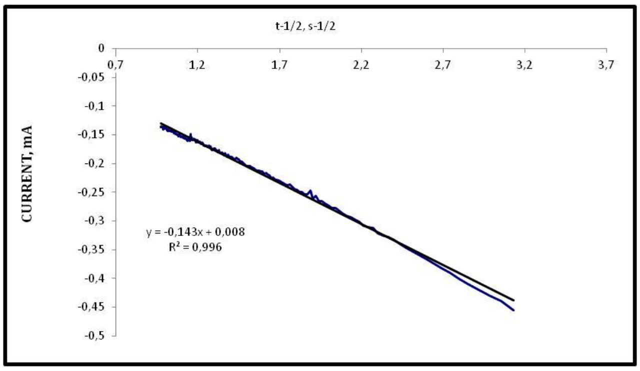


Figure 1: Chronoamperometric determination of electrode area

Using the slope from the chronoamperometric curve, calculated electrode surface of Pt disc electrode was 0.09866 cm^2.

Electrode immobilization

Pyrrole electropolymerization and deposition over the bare Pt electrode (0.09866 cm^2) was preformed potentiostatically at 0.8 V vs. Ag/AgCl from an aqueous solution of three different concentrations of pyrrole monomer (0.05 mol dm^{-3}, 0.1 mol dm^{-3} and 1 mol dm^{-3}) containing also 0.1 mol dm^{-3} DBS, in a three-electrode conventional cell using Pt foil as counter electrode. The amount of polypyrrole deposited can be controlled by choosing proper deposition times, or choosing the charge value. As reported in some previous papers, 0.3 C cm^{-2} value of charge density showed good sensitivity, so the same value of charge density was chosen for the measurements (Goncles, Massafera, Benedetti *et. al.*, 2009).

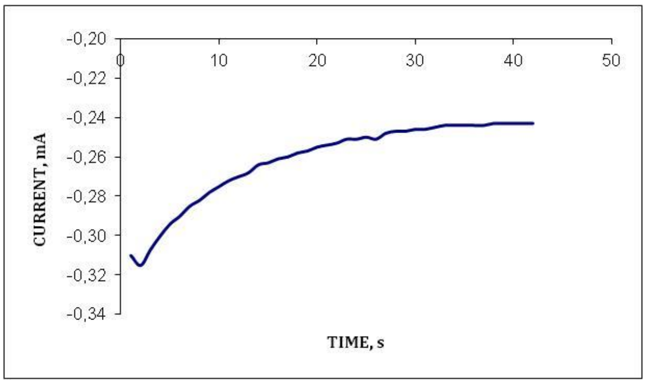


Figure 2: Chronoamperometric curve obtained during formation of modified PPy/DBS electrode

Modified Butler-Volmer equation

Nernst equation was used to characterize ion-selective electrodes. Bond *et. al.* adapted the equation to voltammetric ion sensors (Wooster and Bond, 2003; Wooster, Bond and Honeychurch, 2003). Electromotive force (*EMF*) was changed with mid point reversible potential (E_R) and considering an activity coefficient close to unity.

These authors suggested equations 2 and 3 as followed:

$$E_R = E^0 + S\log\left[M_A^+\right] \tag{2}$$

$$E_R = E^0 - S\log\left[X^-\right] \tag{3}$$

These equations give direct relationship between E_R and the logarithm of the electrolyte concentration. *S* is the slope derived from the curve, which has positive or negative values for cationic or anionic exchanging, respectively. Moreover, ideally *S* will be equal to 2.303*RT*/*nF*. E_R is the mid point reversible potential defined by:

$$E_R = \frac{E_p^{red} + E_p^{ox}}{2} \tag{4}$$

where E_p^{red} is the reduction peak potential and E_p^{ox} is the oxidation peak potential. Equations have also been successfully applied to check 7,7,8,8-tetracyanoquinodimethane (TCNQ) and tetrathiafulvalene (TTF) composite as ion selective electrode.

Romero *et. al.* used equations reported by Bond *et. al.* and modified them to get these equations:

$$E_R = H + 2{,}303\frac{RT}{2\alpha_C n_S}\log\left[M^+\right] \tag{5}$$

$$E_R = H - 2{,}303\frac{RT}{2\alpha_C n_S}\log\left[X^-\right] \tag{6}$$

where *H* is the intercept and α_C is the electron-transfer coefficient.

It can be shown, by comparing equations 2 and 3 with equations 5 and 6, that the only difference is in the slope and the intercept. As Romero *et. al.* demonstrated, the modified Butler-Volmer equation can be successfully applied to modified PPy/DBS electrodes tested as voltammetric cation sensors. Same authors showed that slopes deduced out of semilogarithmic curves were different from Nernstian values, because they included the electron-transfer coefficient (Cano, Rodriguez-Amaro and Romero, 2008a, 2008b).

RESULTS AND DISCUSSION

PPy/DBS modified electrode as a cation sensor

As explained above, modified Butler-Volmer equation gives a direct relationship between E_R and ion concentration.

Three different modified electrodes were immersed in aqueous solution of lithium, sodium and potassium perchlorate to test the capability of polypyrrole modified electrodes as voltammetric sensors.

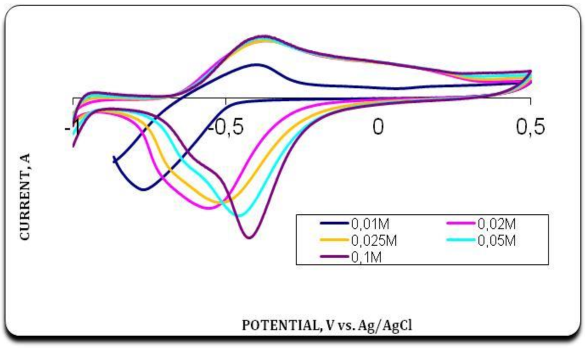


Figure 3: Voltammograms obtained at a scan rate of 10 mV s^{-1} when a PPy(0.1M)/DBS modified electrode was immersed in different concentrations of $LiClO_4$ aqueous solution

Three modified electrodes were PPy(0.05M)/DBS, PPy(0.1M)/DBS and PPy(1M)/DBS electrode. E_R values were calculated from voltammograms as shown in Figure 3 using equation 4, and a linear relationship between E_R and log $[M^+]$ was obtained for all three cations studied. Example of calibration curve is shown in Figure 4.

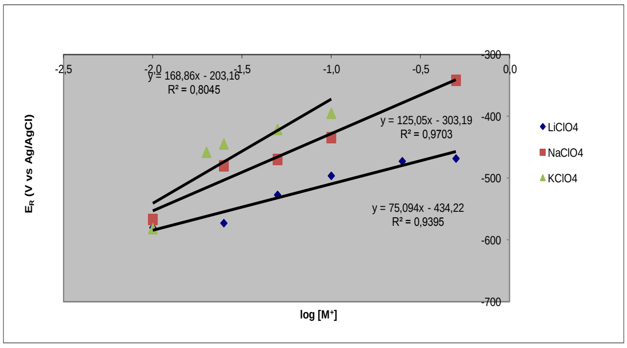


Figure 4: Calibration curves of E_R vs. log $[M^+]$ for a modified PPy(0.1M)/DBS electrode in aqueous solution of $M^+ClO_4^-$

As can been seen from Figure 4 positive slopes are observed, which is in accordance with equations 2 and 5.

Table 1: Voltammetric parameters for the modified **PPy(0.05M)/DBS** electrode in aqueous solution of $KClO_4$, $NaClO_4$ and $LiClO_4$

Cation	*S*, mV dec^{-1}	*α*
Potassium	170.90	0.17
Sodium	122.93	0.24
Lithium	92.69	0.32

Table 2: Voltammetric parameters for the modified **PPy(0.1M)/DBS** electrode in aqueous solution of $KClO_4$, $NaClO_4$ and $LiClO_4$

Cation	*S*, mV dec^{-1}	*α*
Potassium	168.86	0.18
Sodium	125.05	0.24
Lithium	75.09	0.39

Table 3: Voltammetric parameters for the modified **PPy(1M)/DBS** electrode in aqueous solution of $KClO_4$, $NaClO_4$ and $LiClO_4$

Cation	*S*, mV dec^{-1}	*α*
Potassium	98.97	0.30
Sodium	137.42	0.22
Lithium	88.14	0.34

Data in Tables 1, 2 and 3 show that for all three tested electrodes a positive slope from the calibration curves can be derived. In all three cases slopes have the lowest value for lithium ion, and when 0.05 mol dm^{-3} and 0.1 mol dm^{-3} monomer concentration is used the highest value is shown for potassium ion. The only slope value differing from the others is when 1 mol dm^{-3} monomer concentration is used. Regarding the electron transfer coefficients, results show the highest value for lithium ion and the lowest for potassium ion, except again for the electrode made from 1 mol dm^{-3} monomer, when the value of electron transfer coefficient differs from the others.

CONCLUSIONS

Electrochemical synthesis of polypyrrole at a platinum electrode was successfully performed with all three concentrations of monomers. It is proved that the new method based on a modified Butler-Volmer equation can be successfully applied to determine the capabiliity of voltammetric sensors. Modified PPy/DBS electrodes were sensitive to three cations analyzed. It was shown that the capability of the sensor depends on the quality of formed polypyrrole film which is dependent on the initial monomer concentration. In the application of modified PPy/DBS electrode the largest deviations were found when initial monomer concentration was 1 mol dm^{-3} of pyrrole, whereas the results when monomer concentration of 0.05 mol dm^{-3} and 0.1 mol dm^{-3} monomer were used agree with each other.

REFERENCES

Aradilla D., Estrany F., Azambuja D.S., María T. Casas M.T., Puiggali J., Ferreira C.A., Alemán C., (2010). Conducting poly(3,4-ethylenedioxythiophene)-montmorillonite exfoliated nanocomposites, *European Polymer Journal*. 46, 977-983.

Cano M., Rodriguez-Amaro R., Romero A.J.F., (2008). Use of Butler-Volmer treatment to assess the capability of the voltammetric ion sensors: Application to a PPy/DBS film for cations detection, *Electrochemistry Communications*. 10, 190-194.

Cano M., Rodriguez-Amaro R., Romero A.J.F., (2008). A new method based on the Butler-Volmer formalism to evaluate voltammetric cation and anion sensors, *Journal of Physical Chemistry B*. 112, 15596-15603.

Goncles V.R., Massafera M.P., Benedetti T.M., Moore D.G.,Cordoba de Terresi S.I., Torresi R.M., (2009). Nanostructured thin films obtained by electrodeposition over a colloidal crystal template: Applications in electrochemical devices, *Journal of Brazilian Chemical Society*. 20 (4) 663-673.

Inzelt G., (2008). *Conducting Polymers - A new era in electrochemistry*, Springer-Verlag.

Jovanović S.M, Stanković R., Laninović V., Nestorović G., Popović M., Vidic B., Pavlović O., Krstajić N., Grgur B., Vojnović M., Stanković S., (2000). Synthesis and electrochemical properties of polypirole, polyaniline and poly-3-methil thiophene, *Hemijska industrija*. 54 (19), 417-427.

Wallace G.G., Spinks G.M., Kane-Maguire L.A.P., Teasdale P.R., (2009). *Conductive Electroactive Polymers - Intelligent Polymer Systems*, 3rd Edition, Taylor & Francis Group.

Wooster T.J., Bond A.M., (2003). Ion selectivity obtained under voltammetric conditions when a TCNQ chemically modified electrode is presented with aqueous solutions containing tetraalkylammonium cations, *Analyst*. 128 (11), 1386-1390.

Wooster T.J., Bond A.M., Honeychurch M.J., (2003). An analogy of an ion-selective electrode sensor based on the voltammetry of microcrystals of tetracyanoquinodimethane or tetrathiafulvalene adhered to an electrode surface, *Analytical Chemistry*. 75, 586-592.

Zoski C.G., (2007). *Hanbook of Electrochemisty*, Elsevier.

Summary/Sažetak

U ovom radu je primijenjena modifikovana Butler-Volmer jednačina za određivanje kapaciteta voltametrijskog senzora zasnovanog na modifikovanoj elektrodi PPY/DBS. Ova metoda dovodi u direktnu vezu srednju vrijednost reverzibilnog potencijala i logaritam koncentracije elektrolita. Nagib Butler-Volmerove jednačine uključuje i koeficijent prenosa naboja. Modifikovana PPY/DBS elektroda je uspješno primijenjena kao voltametrijski katjon senzor. Dokazano je da polazna koncentracija monomera utiče na formiranje polipirolnog filma, od čije kvalitete zavisi i kapacitet senzora.

Glasnik hemičara i
tehnologa
Bosne i Hercegovine

Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

2013
40
25-30

Print ISSN: 0367-4444
Online ISSN: 2232-7266
UDC: ________________________
Original Scientific Article

Identification and Quantification of Quercetin, Naringenin and Hesperetin by RP LC – DAD in Honey Samples from B&H

Kurtagić H.[a*], **Redžić S.**[b], **Memić M.**[b], **Sulejmanović J.**[b]

[a]*Federal Institute of Agruculture Sarajevo, Butmirska cesta 40, 71 000 Sarajevo, BiH*

[b]*Faculty of Science, University of Sarajevo, Zmaja od Bosne 33 – 35, 71 000 Sarajevo, BiH*

Article info
Received: 24/02/2013
Accepted: 21/05/2013

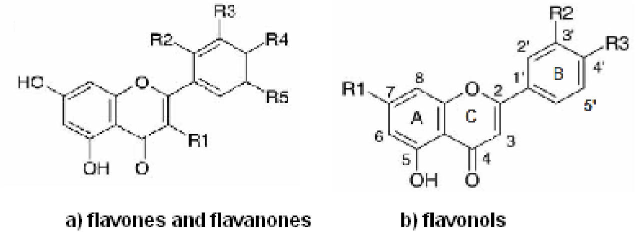
Keywords:
flavonoids
quercetin
naringenin
hesperetin
honey
chromatography

***Corresponding author:**
E-mail: *h.kurtagic@fzzp.com.ba*
Phone: +387-33-637601

Abstract: A large number of different products on the market comes under the name of honey, but many of them are false. Presence of flavonoids in the honey samples may be an indication of its origin. Therefore there is a need for reliable analytical methods for identification and quantification of flavonoids in the honey samples. Flavonoids as constituents of many plants, bees bring to the hive through pollen and honeydew. In this work, identification and quantification of three flavonoids: quercetin, naringenin and hesperetin from twelve honey samples of different botanical origin from Bosnia and Herzegovina were performed. The samples were collected during the period July-September, 2010. Reverse phase liquid chromatography coupled with diode array detector (RP LC-DAD) has been used to separate flavonoids and to quantify them in extractive solutions from honey samples. Results showed that the highest amount of quercentin (43.28 μg/100 g honey) and hesperetin (50.12 μg/100 g honey) was found in honey acacia (K2) and naringenin (41.40 μg/100 g honey) in the linden. The highest total content of all investigated flavonoids was 122.40 μg/100 g honey in sample of honey acacia (K2).

INTRODUCTION

Flavonoids are a group of polyphenolic compounds that are found in many plants, concentrating in seeds, fruit skin or bark, bark, leaves and flowers. As the components of fruits, vegetables, and beverages, such as wine and tea, many of the 4000 - 6400 of known different flavonoids are the part of a regular diet. In recent years, scientists have conducted extensive studies of flavonoids and determined their biological effects, such as antibacterial, antifungal, antiviral, anticancer, and others (Cushnie and Lamb, 2005; Alcerito e*t al.,* 2002; Basle e*t al*., 1999). Flavonols and flavonoids are particularly important because they posses antioxidant and free radical scavenging capacity (Kazazić, 2004). Flavonoids affect the color and flavor of food (Wen *et al*., 2010; Yuan-gang *et al*., 2006; Kazazić, 2004). Flavonoids act as antioxidant, antimicrobial, as photoreceptors and as agents for attracting attention, food rejection and protection from UV radiation (Alcerito *et al.*, 2002). Obviously, these are compounds that play an important role in maintaining and protecting the vital functions of plants. The protective role of flavonoids in biological systems is attributed to their ability to pair ("capture") free radicals electrons, to chelate metal ions (Fe^{2+}, Cu^{2+}, Zn^{2+} i Mg^{2+}), and to activate antioxidant enzymes and inhibit oxidase. Till now, research has shown, that there is a connection between the individual structural components and properties of scavenging, creating chelate complexes and antioxidant activity (Adekunle *et al.*, 2012). The mechanism of action of flavonoids at the molecular level in biological systems is not completely understood, due to large differences in chemical properties and because of their large structural heterogenity. In every organism there is a balance between oxidative stress and antioxidant reparations. Absence of antioxidant protection can cause oxidative stress in several ways (Khazai *et al.,* 2011). Free radicals are involved in the development processes of many diseases, such as asthma, cancer, cardiovascular disease, cataracts, diabetes, gastrointestinal inflammatory disease, liver disease, and other inflammatory processes (Hiran *et al.,* 2004). Free radicals can damage the lipid membrane by creating a carbon radical which reacts with oxygen producing peroxide radical. The resulting peroxide radicals react with fatty acids creating new carbon radicals. Initiation of lipid peroxidation chain reactions, can damage many molecules by one radical. It is important that there are different mechanisms of antioxidant defense including enzymes, proteins, and antioxidants soluble in water and fats and flavonoids which react as scavengers of free radicals, because of the potentially damaging effects of free radicals in the body. (Olszewska, 2007; Kazazić, 2004). Flavonoids are the subject of research of many scientists, because of the all positive qualities (characteristics), (Stanojević *et al*., 2009; Robbinsa, 2009). Phenolic compounds, as very important secondary metabolites of plant life, have different chemical structures and functions and generally possess an aromatic ring whit one or more hydroxyl substituents. Phenolic flavonoids, such as monomeric compounds of flavanols, flavanones, anthocianidines, flavones and flavanones have diphenylpronane ($C_6C_3C_6$) skeleton (Djilas *et al*., 2002). So far, several thousand of phenolic compounds, which occur in free form or more often in the form of glycosides, are isolated and identified from plants. Given the great diversity of plant phenols, their classification is very complex. In the literature there are different classifications mostly by chemical structure and biosynthetic origin (Grbović, 2001). Honey is the product resulting from the processing of bee nectar and / or honeydew. Honeydew is a sweet product of aphid and whiteflies ears, and bees collect it from leaves and other parts of trees. Nectar honey is sweeter than honey of honeydew. Flavor of nectar honey, its color, viscosity and chemical structure are characteristics of the honeydew from which the nectar is collected (Dujmović and Hulina, 2007; Chang *et al*., 2001). There is a growing interest in establishing the authenticity of food products, particularly for natural products, like honey (Nozal *et al*., 2005). Many authors that studied phenolic compounds and flavonoids have found there is a relationship between the antimicrobial activity of honey and its geographical location, as well as botanical origin. The presence of certain flavonoids in honey primarily depends on its botanical origin, thus flavonoids are markers of botanical origin of honeys (Bertoncelj, 2008; Bogdanov and Martin, 2002). Thus, Assma and others (2009) used the flavanones hesperetin as a marker for the socalled citrus honeys. Flavonol kampferol is the marker of rosemary honey, quercetin for sunflower honey, some phenolic acids as markers of hazelnuts honey and hidroxicinnams as markers of chestnut honey (Alvarez – Suarez *et al*., 2009; Robbinsa *et al*., 2009; Olszewska, 2007). The main groups of flavonoids in honey are flavones, flavonols and flavanones that differ in structural formulas and in the position of substituents in rings A, B and C (Figure 1 a). Basic structural formulas of phenolic flavonoids (with different positions of the substituents R1 - R5) are shown in Figure 1. Due to poor solubility in water and oils, applications of flavonoids in foods and medicines are limited (Yue *et al*., 2010; Alabedeen *et al*., 2009). Until now, the flavonoids are analyzed by various instrumental techniques. Today, HPLC - DAD is largely being applied in the determination of the active ingredients in the plants as well as of the flavonoids (Yue *et al*., 2010; Wen *et al*., 2010; Yuan-gang et al., 2006). Most of these studies focused on the analysis of flavonoids in honey by HPLC with UV detection. Two UV absorption bands are characteristic for flavonoids, one band with maximum in the range of 240-285 nm, is believed to arise from (A) ring, and the other band with maximum in the range of 300-550 nm, probably come from the (B) ring (Figure 1). A good estimate of the concentrations of flavonoids can be obtained by comparing the data integration of target flavonoids in honey with standard chromatograms of flavonoids (Alabedeen *et al*., 2009; AOAC Officel method, 2002).



a) flavones and flavanones

b) flavonols

Figure 1: Basic structural formulas of flavonoids with different substituents R1 - R5.

EXPERIMENTAL

Chemicals and Reagents: Quercetin standard was purchased from Sigma - Aldrich/Gemany (Se.No. 020M1566), naringenin from SAFC/Germany (Se.No. MKAA2821), hesperetin from Fluka Analytical/Switzerland (Se.No. 059K1313). Acetonitrile and methanol HPLC grade (purity ≥ 99.6%) were obtained from the firm J & T Baker Ltd. (USA). Ethanol p.a. was obtained from the company Kemika Zagreb (Croatia). Deionized water was produced on the instrument Milli-Q Water Purification System (Millipore Corporation) - Direct Q. SPE - C18 cartridges for extraction were Resprep (6 mL, 500 mg) from Restek Corp. All prepared solutions for HPLC analysis were previously filtered through 0,45 µm pore filter of regenerated cellulose, obtained from Macherey - Nagel (Lot 8301).

Solution of standard substances: Stock solutions of flavonoids quercetin, naringenin and hesperetin (2000 µg/mL) were prepared in methanol. Working solutions of flavonoids at the concentration of 100 µg/mL, 50 µg/mL, 25 µg/mL, 5 µg/mL and 2.5 µg/mL, were prepared in methanol. All these solutions, whose 2 months stability was confirmed by HPLC analysis, were kept in the dark at + 4 ^{0}C. Prior to injection into the HPLC system, all solutions were filtered through 0.45 µm pore membrane filter.

Table 1: Relations between components of mobile phase of acetonitrile (MF) and 5 % aqueous solution of acetic acid and its flow time

Time (min)	15	10	15	30	10	10
MF–A (%)	95-85	85	85-78	78	78-75	75 -95
MF–B (%)	5-15	15	15-22	22	22-25	25-5

Apparatures and instrument conditions: The chromatographic system Agilent Technologies LC 1200 consisted of the following modules; Chemstation software, Degasera model G1322A, Autosampler model G1239A, Quat Pump model G1311A and Photodiode Array Detector model G1315D. Chromatographic separation was performed on the column, Eclipse XDB - C18 reverse phase (4.6 mm x 250 mm, Agilent Technologies, USA) with particles diameter of 5 µm, mobile phases acetonitrile (MF-A) / 5% aqueous solution of acetic acid (MF-B), gradient system, is used. Chromatographic analysis was carried out with a constant flow rate of MF of 1mL/min, with the ratio of mobile phases A and B as shown in table 1.

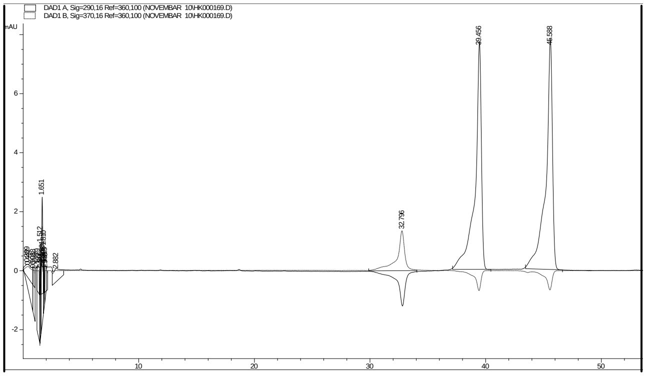


Figure 2: HPLC chromatogram of standard solution quercetin, naringenin and hesperetin in the concentration of 5 µg/mL.

Qualitative - Quantitative analysis of the flavonoids quercetin, naringenin and hesperetin were done with DAD detector at 370 nm for quercetin and 290 nm for naringenin and hesperetin. Flow rate of mobile phase was 1 mL / min, injection volume 20 µL and the temperature of column was 35 ^{0}C. After confirmation the retention time (Rt) and UV spectra of standard substances (Figure 2), calibration curve with 5 points in the concentration range from 2.5 µg/mL to 100 µg/mL was established. Coefficients of correlation for all target analytes were $r^2 \geq 0.999$. In such conditions, quantification of the target flavonoids was performed using an external standard.

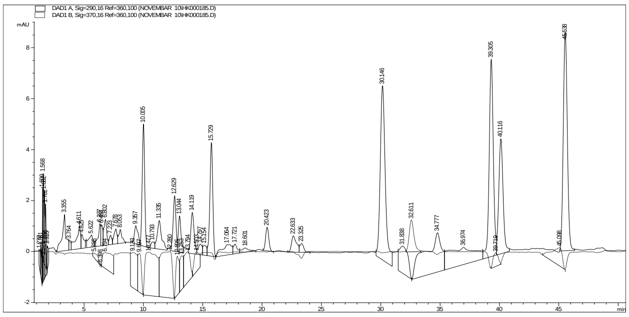


Figure 3: HPLC chromatogram of acacia honey sample from the area of Konjic / BiH (labeled K2).

Sampling and sample preparation for analysis: In the period July - September 2010, twelve samples of honey from BiH region of different botanical and geographical origin and from individual producers were collected (Table 2). Samples for analysis were prepared by dissolving 5 g of sample in 10 mL of deionized water and stirring vigorously. pH values of obtained honey solution were measured at 25 ^{0}C. Before further treatment pH of samples was adjusted to 2 with HCl solution 1 moL/dm^3. The solutions are then passed through the pre-prepared SPE - C18/500 g column with a flow rate of 1mL/min.

Preparation of SPE - C18 column: The column was washed with 9 mL of mixture acetonitrile / methanol / demineralized water (1:1:1), then with 3 mL of acidified demineralized water at pH 2 and finally with 10 mL of demineralized water. Rinsing was conducted with the constant flow rate of 1mL/min, missed fractions were discarded. Samples were extracted with 2 mL of methanol and 1 mL of acetonitrile with the same flow rate of solvent (1mL/min). Collected flavonoids are filtered through 0.45 µm pore filter and immediately analyzed on the chromatograph.

The content of flavonoids was calculated using the formula:

Content of flavonoids (µg/100g) = $c_{\text{read out}}$ (µg/g) x V_{final} (mL)/$m_{\text{weighted sample}}$ (g) x 100

RESULTS AND DISCUSSION

In this study qualitative and quantitative analysis of flavonoids quercetin (QUE), naringenin (NAR) and hesperetin (HES) in the honey samples of different biological and geographical origins from BiH, was performed (Table 2 and Table 3). Presence of flavonoids is confirmed, their content in analyzed samples of honey were significant. Obtained pH values of aqueous solutions are ranged from minimum value 3.78 to maximum value 5.29 (Table 2).

Table 2: Overview of pH values, geographical and botanical origin of 12 honey samples from BiH collected during the period July-September 2010

Label of samples	Geografic orignin	Botanical origin	pH values
O1	Olovo	Mountain honey	3.90
C1	Cazin	Linden (*Tilia* sp.)	3.82
Z1	Zenica	Meadow	3.78
Lj1	Ljubuški	Sages (*Salvia officinalis* L.)	5.29
K1	Konjic	Meadow	3.90
C2	Cazin	Chestnut (*Castanea sativa*)	3.90
K2	Konjic	Acacia (*Robinia pseudacacia*)	5.28
K3	Konjic	Acacia (*Robinia pseudacacia*)	4.45
C3	Cazin	Chestnut (*Castanea sativa*)	4.14
K4	Konjic	Meadow	4.01
G1	Gradačac	Linden (*Tilia* sp.)	4.32
C4	Cazin	Chestnut (*Castanea sativa*)	5.14
		Median value	**4.07**
		Avarage value	**4.33**
		Min value	**3.78**
		Max value	**5.29**

Average pH value for all tested samples was 4.33. Calibration curves were established through three injections of standard solutions, whereby the retention time (Rt), and the coefficient of correlation (r^2) were specified (Table 4). Extraction reproducibility R (%) was established by spiking of sample Z1 which has the lowest flavonoid content. According to the established conditions, the chromatographic analysis of the content of QUE, NAR and HES in the final extracts of honey samples were done in three parallel determinations. Different varieties of honey from 6 geographic regions; Olovo, Cazin, Zenica, Ljubuški, Konjic and Gradačac (Table 2) were analyzed. Six varieties of honey; 1 sample of mountain honey (mark O1), 2 samples of linden honey (C1 and G1), 3 samples of meadow honey (Z1, K1 and K4), 1 sample of sage honey (Lj), 3 chestnut honey samples (C2, C3 and C4) and 2 samples of acacia honey (K2 and K3) were analyzed. Contents of QUE, NAR and HES in relation to the origin of honeys is presented in Table 3.

Table 3: Contents of flavonoid QUE, NAR and HES in BiH honeys of different botanical and geographical origin

Label of samples	**Individual amounts of tested flavonoids (µg / 100 g honeys)**		
	Quercetin	**Naringenin**	**Hesperetin**
O1	15.98	3.98	2.88
C1	22.84	9.34	5.84
Z1	11.20	5.00	4.40
Lj1	17.20	6.44	10.08
K1	18.40	4.64	6.40
C2	27.34	5.80	13.80
K2	43.28	29.00	50.12
K3	14.74	5.40	5.80
C3	17.52	5.32	1.02
K4	16.40	7.60	0.88
G1	12.40	41.40	5.90
C4	16.80	34.00	4.40
Median value	17.00	6.12	5.82
Average value	19.51	13.16	9.29
Min value	11.20	3.98	0.88
Max value	43.28	41.40	50.12

Chromatograms of samples in Figure 3 show that beside the targeted flavonoids of QUE, NAR and HES in honey samples significant amounts of other flavonoid compounds were detected whose identity has not been established in this study. Content of investigated flavonoids QUE, NAR and HES varies depending on the origin of honey samples.

Table 4: Overviews the results of developing a method for the qualitative and quantitative targeted flavonoids analysis

	Quercetin	Naringenin	Hesperetin
Rt (min)	32.81± 6%	39.46± 6%	45.66± 6%
Coefficient of correlation (r^2)	0.99926	0.99996	0.99993
Recovery – R (%)	101.02	99.98	99.76

Among the three tested flavonoids the lowest amount of HES (0.88 µg /100 g) was found in the meadow honey sample (K4). The highest contents of QUE and HAS (Table 3) as well as the highest total content of all three investigated flavonoids was found in honey of acacia (K2) (Table 5).

The obtained results confirm the presence of significant amounts of flavonoids quercetin, naringenin and hesperetin in BiH honeys of different geographical and biological origins.

Contents of quercetin, naringenin and hesperetin in BiH honeys

Contents of quercetin, naringenin and hesperetin are given in Table 3.

Quercetin: Regardless of the variety of honey, a certain amount of QUE was detected in all samples. Average content of QUE was 19.51 µg/100 g. Content of QUE ranged from 11.20 µg/100 g of honey to 43.28 µg/100 g of honey (acacia honey). Based on the obtained results, it could be observed that there are no statistically significant differences in the content of QUE in the samples tested, regardless of their variety and geographical origin. However, higher contents were found in acacia honey of 43.28 µg/100 g (label K2), chestnut honey of 27.34 µg/100 g (label C2) and linden honey of 22.84 µg/100 g (label C1).

Naringenin: Significant amount of naringenin was found in all varieties of honeys and the highest content was in linden honey 41.40 µg/100 g of honey (label G1). The average content of NAR of 13.16 µg/100g honey differs substantially from the median value of 6.12 µg/100 g of honey, which shows that there is a statistically significant difference in the content of NAR in relation to the origin of honey. The minimum content was found in the sample of mountain honey of 3.98 µg/100 g of honey (label O1). High amounts of NAR were found in linden honey (label G1), chestnut honey (label K4) and acacia honey (label K2).

Hesperetin: Hesperetin was detected in all varieties of the honey. Its average content was 9.29 µg/100 g honey and the median value was 5.82 µg/100 g honey. The obtained results for HES content ranged from 0.88 µg/100 g of meadow honey (label K4) to 50.12 µg/100 g of honey acacia (label K2), it represents high variability of the content of this flavonoid. Considering all obtained results, it can be concluded that the amounts of HES are relatively small. However, the high content of HES of 50.12 µg/100 g of honey was found in acacia honey, then in chestnut honey (label C2) of 13.80 µg/100 g of honey and in sage honey (label Lj1 samples) of 8.10 µg/100 g of honey.

The total content of quercetin, naringenin and hesperetin in BiH honeys

The total content of quercetin, naringenin and hesperetin are given in Table 5.

The average total content of all investigated flavonoids (QUE, NAR and HES) is amounted to 41.96 µg /100 g of honey whereas the median value is 31.55 µg /100 g of honey. The minimum content of all investigated flavonoids was recorded in the sample of meadow honey of 20.60 µg /100 g (Z1) and the highest content in the sample of honey acacia of 122.40 µg/100 g (K2). According to the total content of QUE, NAR and HES, tested honey samples can be ranked by accessing the total content of all three flavonoids, in the following order,: Z1 (meadow honey) <O1 (mountain honey) <C3 (chestnut honey) <K4 (meadow honey) <K3 (acacia honey) <K1 (meadow honey) <LJ1 (sage honey) <C1 (linden honey) <C2 (chestnut honey) <C4 (chestnut honey) <G1 (linden honey) <K2 (acacia honey). The obtained results show that greater amounts of tested flavonoids were found in the examined samples of the botanical origin of honey acacia (K2), linden and chestnut, whereas smaller amounts were found in mountain and meadow honeys, while their content in sage honey (Lj1) is near the median value of all tested samples.

Differences of QUE, NAR and HES contents in the same kind of honeys, probably occur due to variation in the vegetation period of honey collecting, elevation, scale pastures, climatic factors, the number of sunny days, types of bees, etc. (Marghitas, et . al., 2009).), which may explain the differences between the content of investigated flavonoid in the same kind and geographical origin of honey. From the foregoing, it can be concluded that the honey of acacia has the highest content of investigated flavonoids, while the honeys of meadows and mountain honey regardless of the geographical origin have almost the same content of these flavonoids.

Table 5: Total contents of flavonoid QUE, NAR and HES in BiH honeys of different botanical and geographical origin

Label of samples	Map location		Total amount of QUE, NAR i HES (µg / 100 g honey)
	Latitude	Longitude	
O1	$44^{0}13'$	$18^{0}60'$	22.84
C1	$44^{0}97'$	$15^{0}95'$	38.02
Z1	$44^{0}20'$	$17^{0}93'$	20.60
Lj1	$43^{0}19'$	$17^{0}55'$	33.66
K1	$43^{0}65'$	$17^{0}97'$	29.44
C2	$44^{0}97'$	$15^{0}95'$	46.94
K2	$43^{0}65'$	$17^{0}97'$	122.40
K3	$43^{0}65'$	$17^{0}97'$	25.94
C3	$44^{0}97'$	$15^{0}95'$	23.86
K4	$43^{0}65'$	$17^{0}97'$	24.88
G1	$44^{0}87'$	$18^{0}43'$	59.70
C4	$44^{0}97'$	$15^{0}95'$	55.20
Median			31.55
Avarage value			41.96
Min value			20.60
Max value			122.40

CONCLUSION

Twelve samples pH values of six different varieties of BiH honey, which were collected during the period July - September 2010 from 6 geographical locations (Olovo, Cazin, Zenica, Ljubuski, Konjic and Gradacac) range from 3.78 to 5.29 and it can be concluded that they are in a good correlation with last scientific knowledge (National Honey Board, 2007). This study shows that BH honeys, regardless of geographical or botanical origin contain significant amounts of quercetin, naringenin and hesperetin. Minimum amounts of tested flavonoids were found in the following samples: Quercetin in an amount of 11.20 µg / 100 in the meadow honey sample originated from the region of Zenica (Z1), naringenin of 3.98 µg / 100g sample in mountain honey (O1) from the site of Olovo and hesperetin of 0.88 µg / 100 g sample in meadow honey originating from Konjic (K4). The maximum amounts of these investigated flavonoids are found in the following honey samples: quercetin in amount of 43.28 µg / 100 g was found in a sample of acacia honey from the site of Konjic (K2), naringenin of 41.40 µg / 100g sample in linden honey originated from the site Gradačac (G1) and hesperetin of 50.12 µg / 100 g sample of acacia honey from the site of Konjic (K2). The total content of three investigated flavonoids depends on the botanical and geographical origin as well as from the other factors (vegetation period of honey collecting, elevation, scale of bee pastures, climatic factors, the number of sunny days, types of bees, etc.), which confirms the different flavonoid content in the samples from the same locality and the same botanical origin.

The honeys of linden, acacia and chestnut have higher amounts of quercetin, naringenin and hesperetin than the honeys of meadow and mountain. The maximum total amount of the three flavonoids per 100 g sample was found in honey acacia originated from Konjic (K2) of 122.40 µg / 100 while the minimum amount of 20.60 µg / 100 g honey was in meadow sample from area of Zenica (Z1). The total amount of the tested flavonoids found in 100 g of sage honey was between the values of the two mentioned varieties of honey. Considering the results, it can be concluded that BiH honeys have significant amounts of quercetin, naringenin and hesperetin which makes them powerful antioxidants.

REFERENCES

Adekunle AS., Aline AB., Afolabi AK., Rocha JBT. (2012). Determination of free phenolic acids, flavonoid contents and antioxidant capacity of ethanolic extracts obtained from leaves of mistletoe (*tapinanthus globiferus*). Asian Journal of Pharmaceutical and Clinical Research 5(3):36 - 41.

Alabedeen Makawi SZ., Gadkariem EA and Hussein Ayoub SM. (2009). Determination of Antioxidant Flavonoid in Sudanese Honey Samples by Solid Phase Extraction and High Performance Liquide Chromatography. E-Journal of Chemistry 6(S1): 429-437.

Alcerito T., Barbo F.E., Negri G., Santos D. Y.A.C., Meda C.I., Young M.C.M., Chávez D., Blatt C.T.T. (2002). Foliar epicuticular wax of *Arrabidaea brachypoda*: flavonoids and antifungal activity. Biochemical Systematics and Ecology 30(7): 677 – 683.

Alvarez – Suarez JM., Tulipani S., Romandini S., Vidal A and Batino M. (2009). Methodological Aspects about Determination of Phenolic Compounds and In Vitro Evaluation of Antioxidant Capacity in the Honey. Curent Analytical Chemistry 5: 293 – 302.

AOAC Officel Method 999.05. (2002). Naringenin and Hesperetin in Orange Juice, LC Method Firtst Action 1999. J. AOAC Int. 83: 1155.

Asmaa A.H., Hanna M.I., Abd-Al Moneimn A.A.A., Naglaa F.G. (2009). Determination of Flavonoid and Phenolic Acid Contents of Clover, Cotton

Basile A., Giordano S., López-Sáez, J. A., Cobianchi R.C. (1999). Antibacterial activity of pure flavonoids isolated from mosses. Phytochemistry 52(8): 1479 – 1482.

Bertoncelj J. (2008). *Identifikacija in vsebnost nekaterih antioksidantov v slovenskem medu,* Dokt. Disertacija, Ljubljana, Univ. V Ljubljana, Biotehniška fakulteta, Ljubljana, Slovenia.

Bogdanov S. and Martin P. (2002). Honey Authenticity. Mitteilungen aus dem Gebiete der Lebensmitteluntersuchung und Hygiene 93: 232 – 254.

Chang PR., Anderson DP., and Thompson K. (2001). Flavonoids of Buckwheat Honeys, Saskatoon Research Centre, Agriculture and Agri-Food Canada 107 Science Place, Saskatoon, SK, S7N OX2, Canada, The proceeding of the 8Ib ISB:633-636.

Dujmović Purgar D., Hulina N. (2007). Medonosne biljne vrste plešivičkog prigorja (SZ Hrvatska). Agronomski Glasnik 1/2007.

Djilas SM., Čanadanović – Brunet JM., Ćetković GS. (2002). Antioxidants in food. Chem. Ind. 58 (3): 105 – 112.

Grbović S. (2010). Hemijska karakterizacija i biološke aktivnosti vrsta eucalyptus camaldulensis dehnh. i eucalyptus gunniihook. Iz Cgrne Gore, Univerzitet u Novom Sadu, Departman za hemiju, Novi Sad, Serbia.

Hiran C.C., Tavan J., Hendrik W. van V., Stephen B.H., Margery A.B. (2004). Interaction of the breast cancer resistance protein with plant polyphenols. Biochemical and Biophysical Research Communications 317: 269–275

Kazazić SP. (2004). Antioksidacijska i antiradikalska aktivnost flavonoida. Arh Hig Rada Toksikol 55:279-290.

Khazai V., Piri1 KH., Nazeri1 S., Karamian R., Zamani N. (2011). Free Radical Scavenging Activity and Phenolic and Flavonoid Contents of Echinophora Platyloba DC. Asian J. Med. Pharm. Res. 1(1): 09-11.

Marghitas L.A., Daniel D., Moise A., Bobis O., Laslo L. and Bogdanov S., 2009. Physico-chemical and bioactive properties of different floral origin honeys from Romania. Food Chem. 112, 863–867.

National Honey Board, 2007. Honey and therapeutic qualities. Longmont, The National Honey Board: *http://www.biologiq.nl/UserFiles/Compendium%20Honey%202002.pdf.*

Nozal Nalda MJ., Bernal Yague JL., Diego Calva JC., Martin Gomez MT. (2005). Classifying honeys from the Soria Province of Spain via multivariate analysis. Anal Bioanal Chem 382: 311 – 319.

Olszewska M. (2007). Quantitative HPLC analysis of flavonoids and chlorogenic acid in the leaves and inflorescences of prunus serotina ehrh. Acta Chromatographica, 19: 253 – 269.

Robbinsa RJ., Leonczaka J., Christopher Johnsona J., Lia J., Kwik-Uribea C., Priorb RL., Guc L. (2009). Method performance and multi-laboratory assessment of a normal phase high pressure liquid chromatography–fluorescence detection method for the quantitation of flavanols and procyanidins in cocoa and chocolate containing samples. Journal of Chromatography A 1216: 4831 – 4840.

Stanojević Lj., Stanković M., Nikolić V., Nikolić Lj., Ristić D., Čanadanovic-Brunet J & Tumbas V. (2009). Antioxidant Activity and Total Phenolic and Flavonoid Contents. Sensors 9: 5702-5714.

Wen J., Liu B., Yuan E., Ma Y and Zhu Y. (2010). Preparation and Physicochemical Properties of the Complex of Naringenin with Hydroxypropyl-β-Cyclodextrin. Molecules 15: 4401-4407.

Yuan-gang Zu, Yu-jie Fu& Wei Liu, Chun-lian Hou, Yu Kong Key. (2006). Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, P. R. Chin.

Yue P., Sun J., Zhang C., Ye R. (2010). HPLC-DAD separation and determination of major active constituents in an important Tibetan medicine Meconopsis quintuplinervia from different regions of Qinghai-Tibet Plateau. Journal of Medicinal Plants Research Vol. 4(11): 1053-1058.

Summary/Sažetak

Veliki broj različitih proizvoda na tržištu dolazi pod imenom med, ali često značajan broj tih proizvoda predstavlja falsifikat. Prisustvo flavonoida u uzorcima meda, može biti znak porijekla meda. Stoga, postoji potreba za pouzdanim analitičkim metodama za identifikaciju i određivanje sadržaja flavonoida u uzorcima meda. Flavonoide kao sastojke mnogih ljekovitih biljaka pčele putem polena i medljike donose u košnice. U ovom radu provedena je identifikacija i kvantifikacija tri flavonoida: Kvercetina, naringenina i hesperetina iz 12 uzoraka meda različitog botaničkog porijekla sa područja Bosne i Hercegovine. Uzorci su sakupljeni tokom perioda juli – septembar 2010. Za separaciju flavonoida i njihovo određivanje iz ekstrakata uzoraka meda korištena je metoda tečne hromatografija sa reverznom fazom uz diodni detektor (RP LC – DAD). Rezulati pokazuju da su najveći sadržaji kvercetina (43,28 µg/ 100 g meda.) i hesperetina (50,12 µg/100 g meda) utvrđeni u medu bagrema (K2) (*Robinia pseudacacia L.*), a naringenina (41,40 µg/100 g meda) u lipovom medu (*Tilia sp.*). Najveći ukupni sadržaj sva tri ispitivana flavonoida (122,40 µg /100 g) nađen je u uzorku meda bagrema (K2).

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2013

40

31-34

Print ISSN: 0367-4444

Online ISSN: 2232-7266

UDC: ________________________

Original Scientific Article

Thermal and Crystallographic Characteristics of Synthesized Xanthen-3-one Derivatives

Završnik D.[a], Veljović E.[a*], Bajrović A.[b], Vrankić M.[c], Gržeta B.[c], Osmanović A.[a], Špirtović-Halilović S.[a], Muratović S.[a]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Zmaja od Bosne 8, Sarajevo, Bosnia and Herzegovina*

[b] *Bosnalijek d.d., Jukićeva 53, Sarajevo, Bosnia and Herzegovina*

[c] *Division of Materials Physics, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia*

Article info
Received: 26/04/2013
Accepted: 28/05/2013

Keywords:
xanthen-3-one derivatives,
differential scanning calorimetry,
powder diffraction analysis,
purity,
crystallinity

***Corresponding author:**
E-mail: *elmacrncevic@gmail.com*
Phone: +387 62616899

Abstract: Series of synthesized xanthen-3-one derivatives were analyzed for thermal characteristics by differential scanning calorimetry (DSC), as well as for crystallinity using X-ray powder diffraction. Xanthen-3-one derivatives are prepared according to the well known procedure, which includes two-fold Friedel-Crafts alkylation. The aim of this research was to determine purity and crystallinity of synthesized xanthen-3-one derivatives.

Thermograms of synthesized compounds showed that compounds have purity above 98%, while crystallographic analysis of powder showed that the compounds have a 10-37% monocrystalline form.

INTRODUCTION

Studies of natural and synthetic xanthones and its derivatives have been present for a number of years. Derivatives of xanthone are characterized by excellent chemical reactivity and different bioactivity. Their remarkable biological potential is the reason for synthesis of many new products, suitable for application in modern therapy.[1]

Over recent years, there was increased interest in these compounds because of their important biological activities, such as inhibition of monoamine oxidase (MAO) enzymes, antiprotosoal[2], antioxidant[3,4], antiulcer, bronchodilatation action and it is also used *in vivo* and *in vitro* as antitumoral agents.[5-7]

Natural xanthones are highly biologically active, possess anti-inflammatory properties such as COX inhibition and have cardiovascular protective effects.[8]

Also, recent studies have shown that xanthene derivatives have cardioprotective effect acting as antiarrhythmics.[9]

In addition, we discuss the synthesis of novel xanthene-3-on derivatives and their thermal behavior and crystallinity.

EXPERIMENTAL

General procedure for the preparation of xanthen-3-one derivatives

A round-bottomed flask equipped with a condenser and mechanical stirrer was filled with 1,2,4-triacetoxybenzene (5 g) in 50% EtOH (75 mL). Conc. sulfuric acid (3 mL) was added and the white suspension was heated to reflux, resulting in a clear, honey colored solution. To this mixture various benzaldehydes (10 mmol) were added dropwise within 2 min. The stirred mixture was kept at reflux for another 60 min. Subsequently, potassium peroxodisulphate (2.70 g) was added at 80°C within a period of 50 min in small portions. The contents were brought to reflux for another 20 min and then poured onto ice water. Obtained red fluorescent crystals were washed with cold water and after drying in vacuum at 60°C crystallized from glacial acetic acid or from ethanol.

General procedures for analytical and experimental section

Microanalyses for C, H and N were preformed on Perkin Elmer 2400 elementary analyzer (Germany). IR spectra were recorded on Perkin Elmer FT-IR 1000 (Germany) in KBr discs. The ^{1}H NMR and ^{13}C NMR spectra were recorded at 300,075 MHz, in DMSO-*d*6, on NMR Spectrometer, Varian Unity Plus 500 MHz and Bruker Advance DPX 300 MHz (Varian, UK). Thermal characteristics of synthesized compounds were determined by differential scanning calorimetry (DSC) using a Perkin Elmer Pyris Diamond DSC calorimeter, at heating rate of 10˚C/min with a sample volume of 2 mg. Scanning was performed by heating the sample for 1 min at 50°C, and then in the temperature range 50-300°C.

Differential scanning calorimetry (DSC) may be used as simple and rapid method of estimating the purity of compounds. The method is based on the van't Hoff law of melting point depression expressed as:

$$T_0 - T_m = (RT_0^2 X_2/\Delta H_f) \cdot (1/F) \qquad (1)$$

Crystallinity of compounds was examined by X-ray powder diffraction (XRPD) using a Philips PW 1730/10 diffractometer with a graphite-monochromatized Cu $K\alpha_1\alpha_2$ radiation [$\lambda(K\alpha_1)$ = 1.54056 Å, $\lambda(K\alpha_2)$ = 1.54439 Å]. X-ray diffraction patterns were recorded in steps of 0.02° (2θ) in the 2θ range from 5° to 100° with a fix counting time of 1 s per step. Determination of the crystallinity level for the samples was performed with the program X'Pert HighScore Plus, version 2.1.[11] according to equation:

$$Crystallinity[\%] = 100 \cdot \frac{\sum I_{net}}{\left(\sum I_{tot} - Bgr._{const.}\right)} \qquad (2)$$

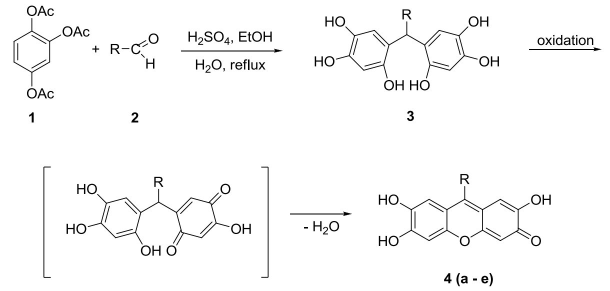
where I_{net} is diffracted intensity from the crystalline part of sample, I_{tot} is complete measured intensity from the sample (both, intensity of X-ray diffraction from the crystalline part of sample and intensity of X-ray scattering from the amorphous part of sample). $Bgr._{const.}$ is a constant background intensity determined for the crystalline standard. The summation is taken over measured 2θ steps in the whole measured range of Bragg angle.

RESULTS AND DISCUSSION

Chemistry

Novel derivatives of xanthene-3-ones have been prepared according the procedure described by Liebermann and Linndenbau[10,11], which includes two-fold Friedel-Crafts alkylation of 1,2,4-triacetoxybenzene (**1**), reflux in ethanol and sulphuric acid with addition of potassium peroxodisulphate as an oxidizing agent. The oxidation of **3** occurs under aerobic conditions over an extended period of time and by dehydratation afford xanthene-3-one derivatives (**4a** - **4e**), (Scheme 1).

The composition of the synthesized compounds was confirmed by elemental analysis, and structure of synthesized compounds was confirmed by spectroscopic methods: infrared spectroscopy (IR spectroscopy), proton nuclear magnetic resonance (^{1}H-NMR) and carbon nuclear magnetic resonance (^{13}C-NMR).



	R
4a	C_6H_5-
4b	o-OCH_3-C_6H_4-
4c	p-OCH_3-C_6H_4-
4d	o-Cl-C_6H_4-
4e	p-Cl-C_6H_4-

Scheme 1. Synthetic pathway for the preparation of xanthen-3-one derivatives.

9-Phenyl-2,6,7-trihydroxyxanthen-3-one (4a). Yield= 60.28%. Anal. calcd for $C_{19}H_{12}O_5$: C 71.40, H 3.45. Found: C 71.25, H 3.50. All values are given in percentages. IR (KBr) n 3600 2400 1590 1300 1200 690 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*6) d 7.8 (m, 2H, H-12 and H-16), 7.5 (m, 2H, H-13 and H-15), 7.2 (m, 1H, H-14), 6.8 (s, 2H, H-1 and H-8), 6.5 (s, 2H, H-4 and H-5).^{13}C NMR (150MHz, DMSO-*d*6) d 177.5 (C-3, C-6), 172 (C-3), 152.17 (C-2, C-7), 151.77 (C-4a, C-10a), 149.2 (C-11), 147.56 (C-9), 133.58 (C-14), 129.21 (C-13, C-15), 128.81 (C-12, C-16), 115.31 (C-9a, C-8a), 108.78 (C-4, C-5), 106.96 (C-1, C-2).

9-(2-Methoxyphenyl)-2,6,7-trihydroxyxanthen-3-one (4b). Yield= 71.76%. Anal. calcd for $C_{20}H_{14}O_6$: C 68.50, H 3.71. Found: C 68.54, H 3.78. All values are given in percentages. IR (KBr) n 3300 1715 1500 1370 1200 770 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*6) d 7.4 (m, 1H, H-14), 7.35 (d, *J* 7.9 Hz, 1H, H-16), 7.125 (m, 1H, H-15), 6.62 (d, *J* 6.81 Hz, 1H, H-13), 3.704 (s, 2H, H-1 and H-8), 3.57 (s, 2H, H-4 and H-5). ^{13}C NMR (150MHz, DMSO-*d*6) d 173.1 (C-3, C-6), 162.698 (C-2, C-7), 156.28 (C-10a, C-4a), 152.86 (C-9), 141.8 (C-11), 131.46 (C-13), 130.46 (C-14), 121.30 (C-16), 121.19 (C-15), 120.97 (C-12), 116.55 (C-8a, C-9a), 108.12 (C-4, C-5), 102.29 (C-1, C-8).

9-(4-Methoxyphenyl)-2,6,7-trihydroxyxanthen-3-one (4c). Yield= 91.00%. Anal. calcd for $C_{20}H_{14}O_6$: C 68.50, H 3.71. Found: C 68.43, H 3.75. All values are given in percentages. IR (KBr) n 3200 1715 1450 1390 1200 860 780 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*6) d 7.47 (d, *J* 8.4 Hz, 2H, H-12 and H-16), 7.3 (d, *J* 8.4 Hz, 2H, H-13 and H-15), 6.98 (s, 2H, H-1 and H-8), 3.97 (s, 2H, H-4 and H-5). ^{13}C NMR (150MHz, DMSO-*d*6) d 176.8 (C-3, C-6), 161.6 (C-14), 160.80 (C-2, C-7), 153.20 (C-4a, C-10a), 147.6 (C-12, C-16), 131 (C-9), 124.50 (C-11), 117.00 (C-13, C-15), 115.00 (C-9a, C-8a), 109.8 (C-4, C-5), 102.00 (C-1, C-8).

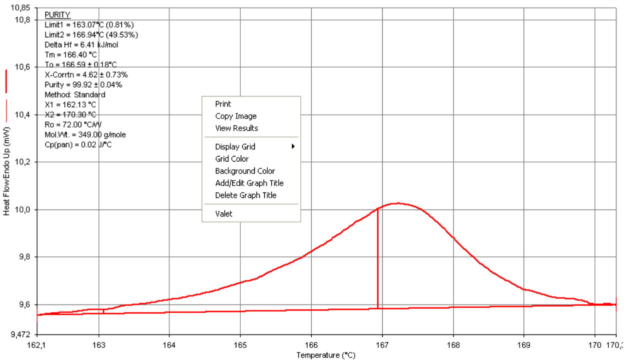
9-(2-Chlorophenyl)-2,6,7-trihydroxyxanthen-3-one (4d). Yield= 81.70% Anal. calcd for $C_{19}H_{11}ClO_5$: C 64.28, H 2.82. Found: C 64.11, H 2.76. All values are given in percentages. IR (KBr) n 3200 2700 1600 1550 1500 1056 770 735 540 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*6) d 7.78 (d, *J* 7.76 Hz, 1H, H-13), 7.682 (m, 1H, H-14), 7.74 (m, 1H, H-15), 7.54 (d, *J* 6.85 Hz, 1H, H-16), 7.18 (s, 2H, H-1 and

H-8), 6.52 (s, 2H, H-4 and H-5). ^{13}C NMR (150MHz, DMSO-*d*6) d 176.8 (C-3, C-6), 163.00 (C-2, C-7), 153.00 (C-4a, C-10a), 148.00 (C-9), 132.00 (C-11), 131.00 (C-13), 130.62 (C-14), 129.76 (C-16), 129.12 (C-15), 127.9 (C-12), 116.00 (C-8a, C-9a), 107.80 (C-4, C-5), 102.70 (C-1, C-8).

9-(4-Chlorophenyl)-2,6,7-trihydroxyxanthen-3-one (4e). Yield= 96.70%. Anal. calcd for $C_{19}H_{11}ClO_5$: C 64.28, H 2.82. Found: C 64.12, H 2.65. All values are given in percentages. IR (KBr) n 3200 1500 1400 1270 1070 780 512 cm^{-1}. ^{1}H NMR (300 MHz, DMSO-*d*6) d 7.76 (d, *J* 4.4 Hz, 2H, H-12 and H-16), 7.535 (d, *J* 4.4 Hz, 2H, H-13 and H-15), 7.10 (s, 2H, H-1 and H-8), 6.66 (s, 2H, H-4 and H-5). ^{13}C NMR (150MHz, DMSO-*d*6) d 172.76 (C-3, C-6), 163 (C-2, C-7), 152.8 (C-4a, C-10a), 148 (C-9), 135 (C-14, C-11), 131.80 (C-13, C-15), 131 (C-12, C-16), 116 (C-9a, C-8a), 107.80 (C-4, C-5), 102.70 (C-1, C-8).

DSC and XRPD results

In the study, 1-2 mg accurately weighed samples were run at a scanning speed of 10˚C/min. Figure 1 shows the endotherm of compound **4c**.



PURITY
Limit1 = 163.07°C (0.81%)
Limit2 = 166.94°C (49.53%)
Delta Hf = 6.41 kJ/mol
Tm = 166.40 °C
To = 166.59 ± 0.18°C
X-Corrtn = 4.62 ± 0.73%
Purity = 99.92 ± 0.04%
Method: Standard
X1 = 162.13 °C
X2 = 170.30 °C
Ro = 72.00 °C/W
Mol.Wt. = 349.00 g/mole
Cp(pan) = 0.02 J/°C

Figure 1. Endotherm of compound **4c.**

Endothermic peaks of all synthesized compounds were wide. Explanation for this is a small percentage of monocrystalline form which is thermostable. If compound has amorphic composition, endotherm will be wide because amorphic parts will soften at first and then melt when heated.

For all synthesized compounds we determined crystallinity using X-ray powder diffraction. Figure 2 shows diffractogram of compound **4e**.

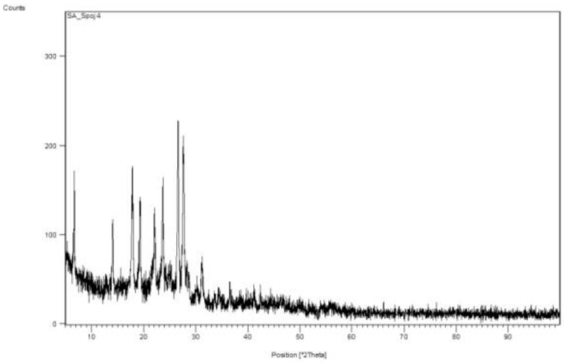


Figure 2. Diffractogram of compound **4e**.

Melting points, heat of melting, purity and crystallinity of synthesized compound are listed in Table 1.

Table 1. DSC and XRPD data for synthesized compounds.

		DSC			XRPD
Entry	**Mw**	**m.p. (°C)**	**Purity (%)**	**$\Delta H_{melt.}$ KJ/mol**	**Crystall. (%)**
4a	319.00	357.61	100.06 ± 0.03	-2.70	25
4b	349.00	166.00	99.85 ± 0.06	9.35	30
4c	349.00	166.04	99.92 ± 0.04	6.41	10
4d	353.50	235.97	98.82 ± 0.42	29.72	20
4e	353.50	235.42	98.54 ± 0.55	36.69	38

CONCLUSION

All synthesized compounds showed wide peaks on thermograms, as a result of low percentage of monocrystalline form. This was also confirmed by X-ray diffraction of the powder (XRPD). As a result of higher percentage of amorphic composition, which is thermolabile and starts to soften at first and then to melt when heated, endotherms do not have sharp peaks.

REFERENCES

Kappe, C. (2004). Controlled microwave heating in modern organic synthesis. *Angew. Chem.*, 43, 6250–6284.

Eunice, M. T. (2006). Antiprotozoal Activity Against Plasmodium falciparum and Trypanosoma cruzi of Xanthones Isolated from Chrysochlamys tenuis. *Pharm. Biol.*, 7, 550-553.

Hyun-Ah, J., William, K., Douglas, K. et al. (2006). Antioxidant Xanthones from the pericarp of Garcinia mangostana (Mangosteen). *J. Agric. Food Chem.*, 54, 2077-2082.

Fotie, J. and Bohle, S. (2006). Pharmacological and biological activities of xanthones. *Curr. Med. Chem. - Anti-Infect. Agents*, 5, 15-31.

Madalena, P. et al. (2002). Xanthones as Inhibitors of growth of human cancer Cell lines and Their Effects on the Proliferation of Human Lymphocytes In Vitro. *Bioorg. And med. Chem.*, 10, 3725-3730.

Nicole Pouli and Panagiotis M. (2009). Fused Xanthone Derivatives as Antiproliferative Agents. *Anti-Cancer Agents in Med. Chem.*, 9, 77-98.

Yukihiro, A., Yoshihito, N., Munekazu, I. and Yoshinori, N. (2008) Anti-cancer Effects of Xanthones from Pericarps of Mangosteen. *Int. J. Mol. Sci.*, 9, 355-370.

Lih-Geeng, C., Ling-Ling, Y. and Ching-Chiung, W. (2007) Anti-inflammatory activity of mangostins from Garcinia mangostana. *Food Chem. Toxicol.*, 15, 5548-5552

Jiang, D. J. et al. (2003). Protective effects of xanthones against myocardial ischemia-reperfusion injury in rats. *Acta Pharmacol. Sin.*, 24 (2), 175-180.

Schrick P. et al. (2008). Reliable Synthesis of 9-Aryl-Substituted-2,6,7-Trihydroxyxanthen-3-ones. *Synthesis*, *14*, 2211-2216.

Scott H. and Ralf W. (2007). One-Pot synthesis of New Symmetric and Asymmetric Xanthene Dyes. *Tetrahedron Tet*ters, 48(25), 4383-4385.

Summary/Sažetak

Seriji sintetiziranih ksanten-3-on derivata ispitane su termalne karakteristike diferencijalnom skenirajućom kalorimetrijom (DSC), te kristalografskom analizom praha (XRPD) utvrđen je stupanj kristaličnosti. Ksanten-3-on derivati sintetizirani su po već poznatoj proceduri, u osnovi koje su dvije Friedel-Craftsove alkilacije. Cilj ovog rada bio je utvrditi stupanj čistoće i kristaličnost sintetiziranih ksanten-3-on derivata.

Termogrami sintetiziranih spojeva pokazali su da spojevi imaju stupanj čistoće iznad 98 %, dok je kristalografska analiza praha pokazala da spojevi imaju 10-37% monokristalnih formi.

Glasnik hemičara i tehnologa Bosne i Hercegovine
Bulletin of the Chemists and Technologists of Bosnia and Herzegovina
2013
40
35-38
Print ISSN: 0367-4444
Online ISSN: 2232-7266
UDC: ________________________
Original Scientific Article

Change in Acidity of L-Ascorbic Acid in the Mixed Solvent DMSO - Water Followed by Conductometric Determination of Dissociation Constants

Rimpapa, Z. [a]**, Pleho-Kapić, A.** [a]**, Galijašević, S.** [b]**, Šapčanin, A.** [c]**, Korać, F.** [b]

[a]*University of Sarajevo, Medical Faculty Sarajevo, Čekaluša 90, 71 000 Sarajevo, Bosnia and Herzegovina*

[b]*University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71 000 Sarajevo, Bosnia and Herzegovina*

[c]*University of Sarajevo, Faculty of Pharmacy Sarajevo, Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina*

Article info
Received: 06/06/2013
Accepted: 13/06/2013

Keywords:
L-ascorbic acid
Dissociation constant
Conductometric
Mixed solvent

***Corresponding author:**
E-mail: rimpapaz@gmail.com
Phone: 00387 33 210725

Abstract: Using conductometric method, the changes in the acidity of L-ascorbic acid in the mixed solvent dimethylsulfoxide (DMSO) - water were monitored. The mixed solvent used was two-component system consisting of nonpolar and polar solvents. Dissociation constants of L-ascorbic acid was determined in pure water and in the mixed solvent DMSO - water with different molar ratios of two solvents: x_{DMSO} = 0.500, 0.333, 0.250, 0.200, 0.166, corresponding respectively to DMSO - H_2O, DMSO - $2H_2O$; DMSO - $3H_2O$, DMSO - $4H_2O$; DMSO - $5H_2O$. Concentrations of freshly prepared solution of ascorbic acid were in the range from 0.00002 to 0.05 $moldm^{-3}$. Molar conductivity resistance values were measured for a given concentration of freshly prepared solutions of L-ascorbic acid and 24 hours old solutions. Using Ostwald's dilution law values for the molar conductivity at infinite dilution (λ_0) and dissociation constants (K), i.g. pK (– log K) were calculated. The results obtained for different concentrations of L-ascorbic acid in the mixed solvent DMSO - water indicate that during a time, pK values declined and acidity increased. Following pK values in the dependence on x_{DMSO} for a freshly prepared solution of L-ascorbic acid. The curve with two inflection points was obtained corresponding to the system of mixed solvent DMSO and DMSO - $2H_2O$ and DMSO - $3H_2O$. In 24 hours old solution of L-ascorbic acid, inflection points were not observed. The results indicate the possibility of different adducts in the mixed solvent or in old solutions as well as accumulation of degradation products over time.

INTRODUCTION

Since L-ascorbic acid (AA) called Vitamin C was isolated from the adrenal glands by Szent-Györgia in 1928 it generated great interest as physiologically and pharmacologically active substance (Davey, 2000). Most animal species are able to synthesize this vitamin, while the man and other primates, due to enzymatic deficiency, are unable to synthesise it. The lack of this essential microsupstance is manifested through the symptoms of avitaminosis.

After a number of research studies, the structure and synthesis of Vitamin C has been established. L-ascorbic acid has four isomers that show different physiological effect while some of the isomers, in general, show no physiological effect (Animon, 1974; Enler, 1957). L-ascorbic acid can be represented by a structure shown in Figure 1. which shows the formation keto-γ-lactone structure.

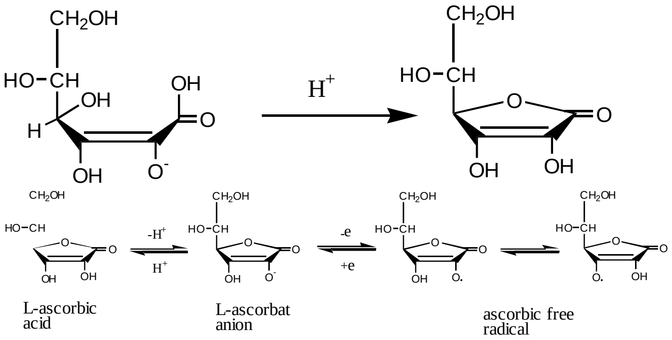


Figure 1. L-Ascorbic acid in all its forms.

According to the Figure 1, it is concluded that L-ascorbic acid has enediol configuration adjacent to the carbonyl group, and can be clasified as an acidic reductone. By increasing pH, increases the speed of oxidation of L-ascorbic acid, in addition, temperature, oxygen in the water, oxygen from the air, light, as well as a presence of polyvalent metal salts contributes to autooxidation process. Oxidation of L-ascorbic acid gives dehydroascorbic acid, and the subsequent hydrolytic cleavage of the ring produced 2,3-diketo-L-gulonic acid that can degrade further giving treonic acid, L-lyxosic acid and L-xylonic acid. L-ascorbate anion with a loss of electrons can give ascorbic free radical (AFR), Figure 1.

L-Ascorbic acid dissociations in two steps depending on pH of water solution and can be changed by addition of hydroxide (Figure 2). On 37 °C pK$_1$ is 4.18, and pK$_2$ = 11.6 (Karrer, 1933; Borskok, 1937). Some authors cited different values that are dependable on temperature. This dissociation reaction occurs on C$_2$ and C$_3$ hydroxil groups giving ions of L-ascorbic acid.

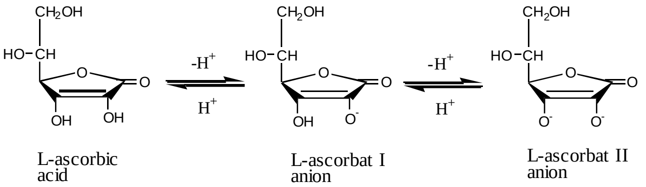


Figure 2. L-Ascorbic acid ions.

The acidity of the solution is determined by the concentration of H$^+$ ions in solution. The term proposed by King (King, 1965) gives the logic to the name "acidity constant", avoiding separate descriptions of constants, one for acids, and other for bases. Brönstad-Lowry theory (Albert, 1971) is widely accepted for description of acids and bases. According to this theory, the acid dissociation constant is described by the chemical equation:

$$K_a = \frac{[H^+][A^-]}{[HA]} \quad \text{or} \quad pK_a = pH + \log\frac{[HA]}{[A^-]}$$

and includes equilibrium concentrations of all spices present in solution.

This equation can be used in a simplified form if constants are determined in solutions with a concentration of less than 0.01 moldm^{-3} or monovalent ions are used (Albert, 1971).

For the evaluation of conductivity data by using recently developed conductance equations, the most essential parameters are the viscosity and the dielectric constants of the solvent media. To evaluate the conductivity measurements the Kraus and Bray conductance equation can be used:

$$\lambda_c c = \frac{1}{\lambda_c}\lambda_0^2 K - \lambda_0 K$$

where λ_c is molar conductance of the acid at concentration c, λ_0 is molar conductance of the acid at infinite dilution and K is dissociation constant. Plots of 1/λ_c versus cλ_c for the acid in the various media gave straight lines. The limiting molar conductance and dissociations constants were determined from slopes and intercepts (Jaiswal, 2005).

EXPERIMENTAL

Materials

All chemicals used for the preparation of the solution were of the highest purity grade and prepared in redistilled water. Prepared mixtures of solvent and solute were kept at 20 °C. Mixture DMSO - water was prepared by mixing calculated volume of solvents, and the molar proportion of DMSO: water were the following: x_{DMSO} = 0.500, 0.333, 0.250, 0.200, 0.166. The composition of mixed solvent can be presented as DMSO - H_2O, DMSO - $2H_2O$; DMSO - $3H_2O$, DMSO - $4H_2O$, DMSO - $5H_2O$. Preferred concentrations of L-ascorbic acid were prepared by accurate weighing and dissolving it in these mixed solvent systems. Fresh solutions were used immediately after preparation for experiment. Conductometric measurements were performed using conductometer ″Iskra″ MA 5962. Conductometric cell was from the same manufacturer type MA 5963. Initially, the conductometric cell was plated, and the value of the cell constants determined. For all measurements, conductivity temperature was maintained at 25 ± 0.2 °C.

To confirm the method that can be used for conductometric determination of dissociation constants for L-ascorbic acid, solution of acetic acid was utilized as a control. By measuring the molar resistance of solutions with different concentrations of acetic acid, the molar conductivity λc was calculated. Here, using the method of linear regression, molar conductivity at infinite dilution, λ_0 and dissociation constants K i.e. pK were calculated. The obtained results for acetic acid showed that conductometric method was suitable for determining the ionization constants of L-ascorbic acid in water and mixed solvent. Table 1 shows the measured and calculated parameters for acetic acid and linear dependence is shown in Figure 1.

Table 1. The measured resistance values and calculated physical values used for calculations of λ_0 and K$_c$ for different acetic acid concentrations

c×10^{-5} (moldm^{-3})	R (Ω)	κ×10^{-6} (Ω^{-1}cm^{-1})	λ_c (Ω^{-1}cm^{2}mol^{-1})	X = 1/λ_c	Y = λ_c c
5000	805	382	7.635602	0.130965	0.38178
1000	1820	169	16.88643	0.059219	0.168864
100	6050	50.8	50.79884	0.019685	0.050799
50	8700	35.3	70.65126	0.014154	0.035326
10	21600	14.2	142.2838	0.007028	0.014228
5	34100	9.01	180.254	0.005548	0.009013

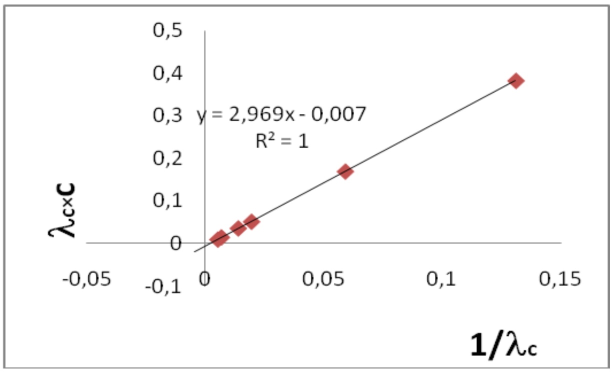


Figure 1. Plot of $\lambda_c C$ = f(1/λ_c) for a water solution of acetic acid.

After extrapolation, the value for λ_0 = 418.74 Ω^{-1}cm^{2}mol^{-1} was obtained while dissociation constant was K$_a$ =1.693×10^{-5} moldm^{-3} or pK$_a$ = 4.77 which is in agreement with literature values (Albert, 1971).

This process of solving algebraic computation that limits molar conductivity at infinite dilution and pK was conducted in order to determine the dissociation constants that are related to L-ascorbic acid in pure water and mixed solvent immediately after preparation and in 24 hours old solution. The values obtained are represented in Table 2 and Figure 2.

Table 2. The measured resistance values and calculated physical values used for algebraic and graphic computed values for λ_0 and K_c for a different concentrations L-ascorbic acid (freshly prepared solutions)

c×10^{-5} (moldm^{-3})	**R** (Ω)	**κ×10^{-6}** (Ω^{-1}cm^{-1})	**λ$_c$** (Ω^{-1}cm^{2}mol^{-1})	**X = 1/ λ$_c$**	**Y = λ$_c$ c**
2	17900	4.19441	209.7207	0.004768	0.004194
12	8150	9.21227	76.76892	0.013026	0.009212
25	5120	14.6641	58.65625	0.017048	0.014664
50	3230	23.2446	46.48916	0.02151	0.023245
250	1310	57.313	22.92519	0.04362	0.057313
500	910	82.5055	16.5011	0.060602	0.082505
1000	625	120.128	12.0128	0.083245	0.120128
5000	270	278.074	5.561481	0.179808	0.278074

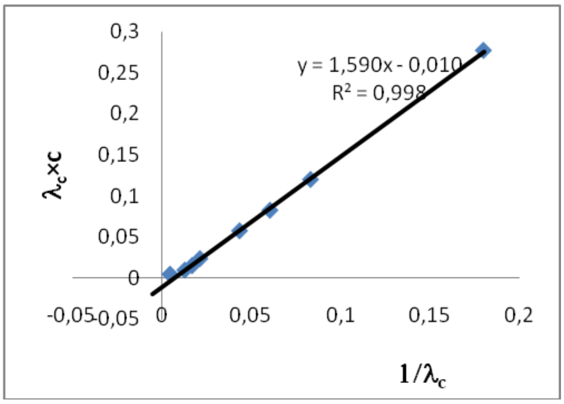


Figure 2. Representation of equation: $\lambda_c C = f(1/\lambda_c)$ for water solution of L-ascorbic acid.

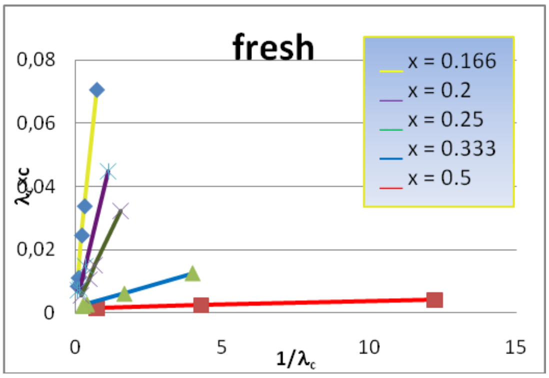


Figure 3a. Linear dependance where $\lambda_c C = f(1/\lambda_c)$ for 24 hours old L-ascorbic acid solutions in mixed solvent with different mole ratios of DMSO.

After extrapolation, the obatined values were as follows: λ_0 = 149.48 Ω^{-1}cm^{2}mol^{-1} and K_a = 7.15 × 10^{-5} moldm^{-3} or pK_a = 4.147 that are in an agreement with literature values.

Using the same procedure, conductometric measurements were performed for different concentrations (0.0005, 0.001, 0.005, 0.01, and 0.05 moldm^{-3}) of L-ascorbic acid in the mixed solvent DMSO - water (x_{DMSO} = 0.166, 0.200, 0.250, 0.333 and 0.500), and the obtained experimental data were used to calculate the acidity constants of freshly prepared and 24 hours old solution of L-ascorbic acid. Results are shown in graph 3a and 3b graph.

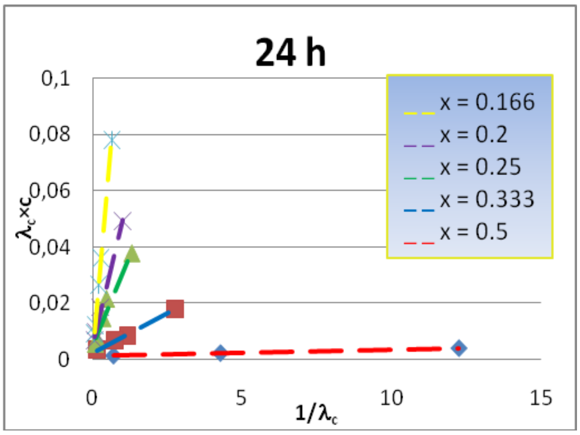


Figure 3b. Linear dependance where $\lambda_c C = f(1/\lambda_c)$ for 24 hours old L-ascorbic acid solutions in mixed solvent with different mole ratios of DMSO.

By using linear extrapolation method, the values for concentration dissociation constants i.e. acidity constant K or pK for L-ascorbic acid in the mixed solvent of varying moles of constituent components are obtained.

RESULTS AND DISCUSSION

Based on the conductometric measurements, the dissociation constant of acetic acid in water were determined by solving Ostwald's law of dilution equations using the method of the least squares. The calculated pK value of acetic acid in water at 25 °C was 4.771 which is in good agreement with literature data (4.75) (Albert, 1971). The results indicate the possibility of monitoring pK with deviation of 0.02 pK units.

Since our goal was monitoring changes in acidity mixed solvent, rather than determining absolute values of pK, selected method was suitable for the study. The same method and the same procedure were used to calculate pK of L-ascorbic acid at 25 °C and the resulting value of pK_a = 4.147 coincided with literature data with value of 4.12 (Kumler, 1935). Measurements were performed with a freshly prepared solution to avoid ″autoxidation″ of L-ascorbic acid in aqueous solution leading to reduced pK values. pK values were determined for L-ascorbic acid in the system of mixed solvents: DMSO - water with different molar components of the mixed solvent and calculated corresponding pK values are given in Table 3.

Table 3. pK of freshly prepared and 24 hours old solutions of L-ascorbic acid in two-component mixed solvent DMSO - water with different molar ratios of solvents

$x_{(DMSO)}$	pK_{fresh}	pK_{24}	ΔpK
0.166	3.795	4.008	-0.213
0.200	3.805	3.258	0.547
0.250	4.333	2.987	1.346
0.333	3.072	2.953	0.119
0.500	3.836	2.951	0.885

Concentrations of dissolved L-ascorbic acid in each two-component solvent were 0.0005, 0.001, 0.005, 0.01 and 0.05 moldm^{-3}. Calculated values of pK listed in Table 3 show decline in pK values over a time and an increase in the acidity of the solution, which is caused by decomposition products of L-ascorbic acid.

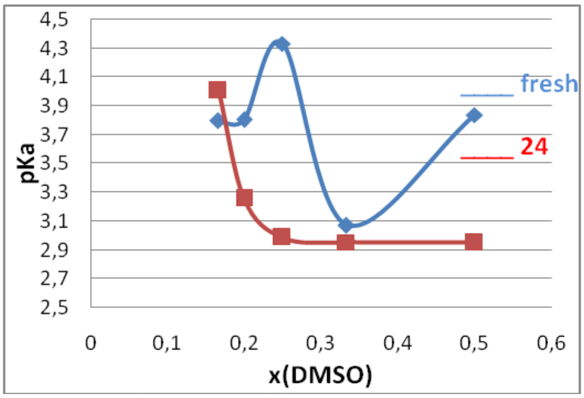


Figure 4. Plot of pK values of freshly prepared and 24 hours old solutions of L-ascorbic acid in two-component mixed solvent DMSO - water in dependence on DMSO molar ratio in a solvent mixture.

It is evident from the diagram pK = f (x_{DMSO}) that, for a freshly prepared solution of L-ascorbic acid, the data curve is obtained with the inflection points that correspond to the composition of a mixed solvent system of DMSO and DMSO - $2H_2O$ and DMSO - $3H_2O$. The data curve for pK = f (x_{DMSO}) for solutions of 24 hours old L-ascorbic acid in Figure 4, shows no inflection points. It is most likely that the solution contains degradation products of L-ascorbic acid and various products of solvolysis. Measurement results show that one can, on the basis of conductivity measurements and determination of pK values, define the specific interaction between the components and mixed solvent.

This study can contribute to the understanding of relationship that exists between the physical-chemical properties (Milićević, 1983) of the solution and interactions between the components and mixed solvent.

CONCLUSION

Selected physico-chemical method for the conductometric monitoring of acidity of L-ascorbic acid proved to be relaibale. Because of the tendency toward autooxidation, measurement of L-ascorbic acid needs to be conducted in freshly prepared solution in order to avoid adverse effects that could disturb the measured values.

Monitoring changes in acidity of L-ascorbic acid in freshly prepared solution showed that at a given molar ratio of components in a mixed solvent solution, there is an interaction between the components of solution that contribute to the effect of unexpected or anomalous values of acidity.

Our experiment showed that changes in acidity is recorded in the mixed solvent systems that can be best described by quantitative composition: DMSO - $2H_2O$ and DMSO - $3H_2O$. Twenty four hours old solutions of L-ascorbic acid in the mixed solvent showed a progressive increase in acidity with increasing moles of DMSO in a two-component system with the assumption of a possible increase in the acidic degradation products of L-ascorbic acid, but, without additional measurements and parameters, this hypothesis cold not be confirmed.

REFERENCES

Albert, A., Serjeant, E.P. (1971). The Determination of Ionization constants, 2nd ed; *Chapman Hall*, London

Animon, R., Dirscherl, W. (1974). Fermonte, Hormone, Vitamine (Band III/1 Vitamine), *Georg Thieme Verlag*, Stuttgart

Borskok, H. at all. (1937). The oxidation of ascorbic acid and its reduction in vitro and in vivo, *J. Biol. Chem.* 117, 237-279

Davey, M.W., Van Montagu, M., Inze, D., Sanmartin, M., Kanellis, A., Smirnoff, N., Benzi, I.J.J., Strain, J.J., Favell, D., Fletcher, J. (2000). Plant L-ascorbic acid: chemistry, function, metabolism, biovailability and effects of processing. *J. Sci. Food Agric*. 80, 825-860

Enler, H., Eistert (1957). Chemie und Biochemie der Reduktonate, *Enke Verlag*, Stuttgart

Jaiswal, P.V., Ijeri, V.S., Srivastava, A.K. (2005). Effects of surfactains on the dissociation constants of ascorbic and maleic acids, *Colloids and surfaces B*: *Biointerfaces* 46, 45-51

Karrer, P., Schwarzenbach, K. and Schopp, G. (1933). Uber Vitamin C, *Helvetica Chimica Acta* 16, 305-305

King, E.J. (1965). Acid-base Eqilibria, *Pergamon Pres*, Oxford.

Kumler, W.D. and Daniels, T.C. (1935). Titration curves and dissociation constants of L-ascorbic acid (vitamin C) and diethyl dihydroxymalate, *J.Amer.Chem.Soc.* 57, 1929-1930

Milićević, V., Škerlak, T., Babić, N. (1983). Srebro – srebro halogene elektrode u mješovitom rastvaraču DMSO – voda, *Savjetovanje tehnologa i hemičara BiH*, Banja Luka, Izvodi radova sa programom.

Summary/Sažetak

Praćena je promjena kiselosti L-askorbinske kiseline u mješovitom vodenom rastvaraču dimetilsulfoksid (DMSO) - voda konduktometrijskom metodom. Odabir mješovitog rastvarača uzet je kao dvokomponentni sistem nepolarnog i polarnog rastvarača. Određivana je konstanta disocijacije L-askorbinske kiseline u čistoj vodi i u mješovitom rastvaraču DMSO - voda u različitim molskim udjelima mješovitog rastvarača: x_{DMSO} = 0.500; 0.333; 0.250; 0.200; 0.166 što odgovara respektivno kombinaciji DMSO - H_2O; DMSO - $2H_2O$; DMSO - $3H_2O$; DMSO - $4H_2O$; DMSO - $5H_2O$. Koncentracije svježe pripremljenih rastvora askorbinske kiseline kretale su se u intervalu od 0.05 do 0.00002 moldm^{-3}. Konduktometrijski je određivana molarna provodljivost mjereći vrijednosti otpora za date koncentracije svježe pripremljenih rastvora različitih koncentracija L-askorbinske kiseline i odstajalih 24 sata. Koristeći Ostwaldov zakon razblaženja izračunate su vrijednosti za graničnu molarnu provodljivost (λ_0) i konstantu disocijacije (K) tj. – log K (pK). Rezultati dobiveni za različite koncentracije L-askorbinske kiseline u mješovitom rastvaraču DMSO - voda pokazuju da sa vremenom dolazi do opadanja pK vrijednosti, odnosno do povećanja kiselosti. Prateći pK u funkciji x_{DMSO} za svježe pripremljen rastvor L-askorbinske kiseline dobiva se kriva sa dvije prevojne tačke (infleksije) koje odgovaraju sistemu mješovitog rastvarača DMSO - $2H_2O$ i DMSO - $3H_2O$. Kod rastvora L-askorbinske kiseline koji su odstajali 24 sata nije primijećena prevojna tačka. Dobiveni rezultati ukazuju na mogućnost asocijata u mješovitom rastvaraču ili pak kod odstajalih rastvora na degradacione produkte koji nastaju tokom vremena.

Glasnik hemičara i
tehnologa
Bosne i Hercegovine

Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

2013

40

39-46

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: ______________________

Original Scientific Article

Energy Consumption and Environmental Impact of the Foundry Industry

Šehić-Mušić N.[1,*], Goletić Š.[2], Pihura D.[3], Mušić L.[4], Hasanović K.[5], Kalajdžisalihović A.[1]

[1] *Chamber of Economy of the Federation of B&H, Branislava Đurđeva 10, Sarajevo, B&H*

[2] *Faculty of Mechanical Engineering, University of Zenica, Fakultetska 1, Zenica, B&H*

[3] *B&H Association of Foundry Workers, Travnička 1, Zenica, B&H*

[4] *Faculty of Political Science, University of Sarajevo, Skenderija 72, Sarajevo, B&H*

[5] *European University Brcko District, Bulevar mira 4/III, Brčko District, BiH*

Article info
Received: 31/05/2013
Accepted: 14/06/2013

Keywords:
climate change,
castings,
iron,
steel industry,
energy

***Corresponding author:**
E-mail: n.sehic@kfbih.com
Phone: 00-387-33-566-306
Fax: 00-387-33-217-783

Abstract: Climate change has a negative effect on water and food production, which poses a serious problem, as both resources are necessary for human survival. Sustainable development is the basic approach to the environment in modern times. Energy consumption and environmental issues such as climate change are global problems and industries such as the foundry industry have an influence over them. Therefore, environmental awareness, eco ethics, bioethics, and social reflexivity can be the agents of change in the future.

Integrating the environment into development issues is of particular importance in the industry, so the aim of the research in this paper is to define a model for implementation and monitoring of key indicators of energy efficiency, as an instrument of effective environmental and energy management in the metal sector of Bosnia and Herzegovina. Using these environmental tools contributes to more efficient use of energy as well as reducing emissions and negative impact on the environment. The study includes

INTRODUCTION

The relatively permanent components of the Earth's atmosphere: O_2, N_2, CO_2, H_2O and other gases, are an indestructible mixture of gases. However, emissions of pollutants of anthropogenic origin may drastically change the atmosphere's properties, both locally and globally. In present-day urban and industrial areas, significant changes in the composition of the atmosphere have been registered and they have had an unfavorable impact on all aspects of the environment. The emitted anthropogenic gases cause damage to the ozone layer and we are already experiencing the adverse consequences. Damage to the ozone layer above the Earth's poles, extending to the Equator, has undesirable implications and consequences which will become more visible in the future. Large numbers of compounds, gases and different particles can be released into the atmosphere as pollutants in urban surroundings in the form of: SO_2, soot (smoke), CO_2, CO, C_xH_y, NO_x, NH_3 and hard particles.

The destruction of the ozone layer is caused by higher rates of CO_2 emission. The Kyoto and Montreal protocols push to analyze the problem of climate change because of its influence on food production, water quality, and, ultimately, poverty. [1] Therefore it is necessary to develop adequate strategies for protection of food and water supplies. Industrial corporations in B&H spend significantly more energy to make their products than similar corporations from other countries. It is particularly important to note that more than 50% of energy is consumed in the metal industry. Within the metallurgy and foundry industrial branch, cast iron and steel production in the foundry industry is a large energy consumer. Specific energy consumption for certain castings is high as well, which at the same time shows the effect of greater or lesser efficiency in energy use. When observed through the prism of energy efficiency in the economy and the metal sector, those in charge of energy consumption must bear in mind the following: (1) the economy of use, (2) safety of equipment and (3) environmental management. Integration of the environment into development issues has special importance in this work, therefore the goal of this paper is to investigate, define implementation models, and observe key indicators of efficient energy use as instruments of efficient environmental and energy management. This study included companies at the intermediate (mezzo) level (foundries). [2] [3]

FOUNDRY INDUSTRY

In addition to the reasons already mention, the interest in this subject matter also arises from the following:

- Profitability of companies can be increased by minimizing energy costs.
- Every sustainable development strategy has the aim of ensuring the availability of energy and its sustainable consumption.
- With energy indicators, it is possible to connect human activities, changes in energy, and the effects of undertaken measures.
- The metal and electrical industry sector in B&H is traditionally a leading sector.
- The foundry industry is a propulsive branch.
- The foundry industry is the key factor in ferrous and non-ferrous metal waste recycling, as the materials can be re-melted into new products and used 100 %. [4] [5]
- The foundry industry is an intensive energy consumer. [6]
- The foundry industry is also an intensive polluter: soil, air, and water. [7][8]

The foundry industry can negatively impact the environment through its use of thermal processes and mineral additives. Managing its environmental footprint is therefore related mainly to acid gases and the recycling of mineral waste. [5]

METAL AND FOUNDRY INDUSTRY IN BOSNIA AND HERZEGOVINA (B&H)

Data on B&H foundry industry production for the years 2009 and 2010 are shown in Figures 1 and 2. Production is divided into categories according to the NACE Rev. 2 classification. Each production category is denoted by its NACE code.

In 2009, foundry output consisted mainly of light metal castings for land vehicles, excluding for locomotives or rolling stock and construction industry vehicles. This group makes up 45% of total production. Two categories (1) ductile iron castings for locomotives/rolling stock/parts, for uses other than in land vehicles, bearing housings, plain shaft bearings, piston engines, gearing, pulleys, clutches, machinery and (2) non-ferrous metal parts for other uses have the smallest production share (in both cases 2%).

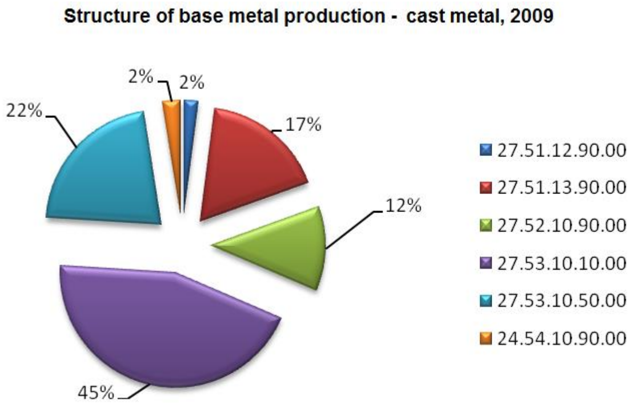


Figure 1: Foundry industry production, 2009
Source: BHAS, 2010

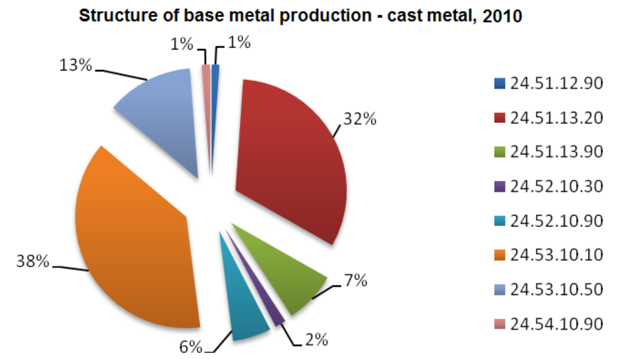


Figure 2: Foundry industry production, 2010
Source: BHAS, 2011

In 2010, the production structure was somewhat wider than the previous year, with two new product groups. While the parts for land vehicles still dominate (38% of production), second by percentage (32% of production) are grey iron castings for transmission shafts, crankshafts, camshafts, cranks, bearing housings and plain shaft bearings (excluding bearing housings incorporating ball or roller bearings). Steel castings for bearing housings and plain shaft bearings (excluding for bearing housings incorporating ball or roller bearings) were also a new category in relation to 2009.

PROSPECTS FOR THE FOUNDRY INDUSTRY IN B&H AND THE WORLD

Analyses of casting production in the world have shown that EU countries are first in terms of non-ferrous metal castings and second in cast steel. The EU foundry industry produces 50 % of its castings for the automobile industry, while another large portion is for machine production. In spite of the dramatic changes in 1990 and those caused by the recent recession, prospects for the EU countries in the mid-term, i.e. until 2020, are good and they are related to castings for automobiles and trucks, but especially for electrical units' castings. According to the EU development programme, casting production for the automobile industry is expected to grow in both relative and absolute terms, even with the present crisis, as a result of current expansion in global vehicle production. In that sense, we have to bear in mind that regional production and casting production structure will change continuously. The rest of the transportation sector, such as air and ship transportation, expects significant investments.

Therefore, the EU casting industry will stay stable until 2020, with very small deviations from the rule in certain regions or countries. It is estimated that the casting market in the four BRIC countries will grow by up to 60 % in 2020 (Figure 3). Availability of materials and prices that the foundry industry must pay will be of vital importance.

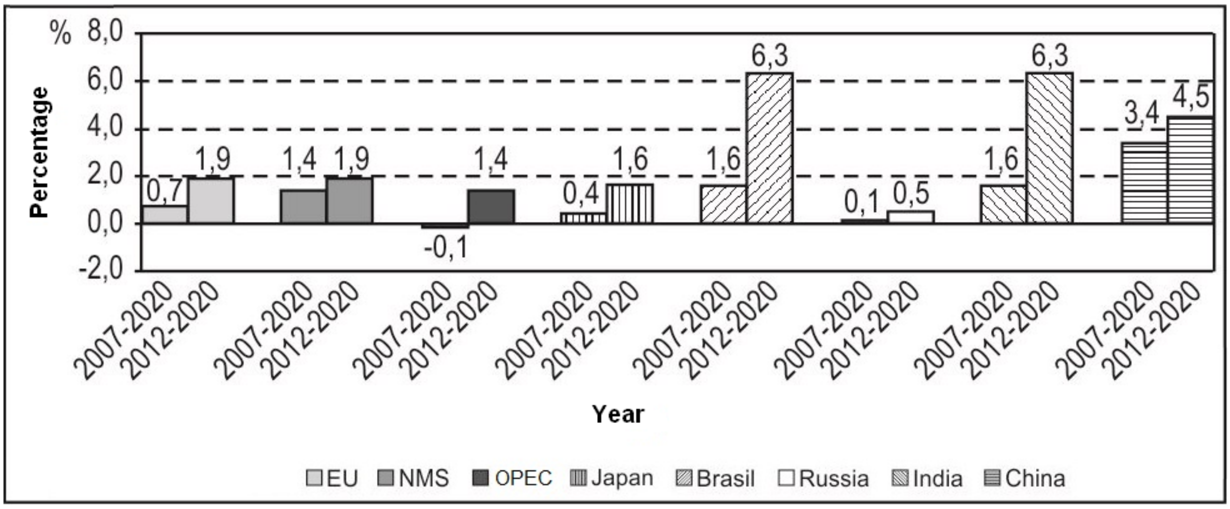


Figure 3: World casting production between 2007– 2012 and estimates until 2020

Table 1 shows an estimate of casting production by industry and metal type until the year 2020.

Table 1: Casting production estimates CAEF until 2020

	Castings by industry (%)			
Metals	**Vehicles**	**Machine construction**	**Construction**	**Other**
Steel-based	56	27	3	14
Non-ferrous	63	9	4	24

Source: The Casting Industry and Cast Production Perspectives in Bosnia and Herzegovina

All data shows the foundry industry in Bosnia and Herzegovina has great possibilities, if it is supported financially and with the proper regulation. The projected structure of foundries in B&H is based on present foundry capacities: 3 iron foundries, 5 foundries producing grey, nodular or vermicular cast iron, and 4 non-ferrous metal foundries, for which we do not have data at our disposal. The B&H foundry industry, in the period until 2015, should focus on the problems facing this branch, including: analyzing production to determine market goals for the European, EU, and other markets, entering the projected markets and finding suitable market "niches", which would be the best possible solution for certain B&H foundries. On the other hand, producing quality castings in a cost-efficient manner requires large investments in equipment as well as increased investments in R&D. [8] [9] [5]

In regulatory terms, the B&H foundry industry must be supported by adequate legal acts which should include not only environmental protection, but also the regulation of growing costs of materials and uncontrolled export or import of materials.

In the coming times, the foundry industry must undergo a reconstruction, thus showing its readiness to implement European and international norms related to technology and environmental protection.

WASTE RESOURCES AND EMISSIONS OF ACID GASES

Developing and implementing an EI indicator on the selected business systems

In order to develop and implement an EI indicator while bearing in mind the main hypothesis of this paper and the reasons mentioned above, we have selected foundries as a business system that represents a propulsive branch of the B&H metal sector and also a challenge from the point of managing the environment and energy. The foundry industry is an intensive energy consumer and polluter. Foundries and their production represent an important part of the metal sector, particularly in terms of the casting-based mechanical industry including the automobile industry, where almost 50% of each automobile is made from castings.

Gathering and categorization of collected data

The general questionnaire used in this study was based on the ESCO Company questionnaire. In some companies, a limited preliminary audit, interviews and meetings with management were carried out. In order to gain insight on energy intensity, data on output (expressed both in physical and monetary units) and use of energy were collected. Out of 10 examined foundries, 9 filled out the questionnaire. Additional data was obtained through interviews in certain companies, while BAT and BREF were used as sources of comparative data.

Table 2 shows electricity consumption in kWh in the surveyed foundries, categorized by its different uses.

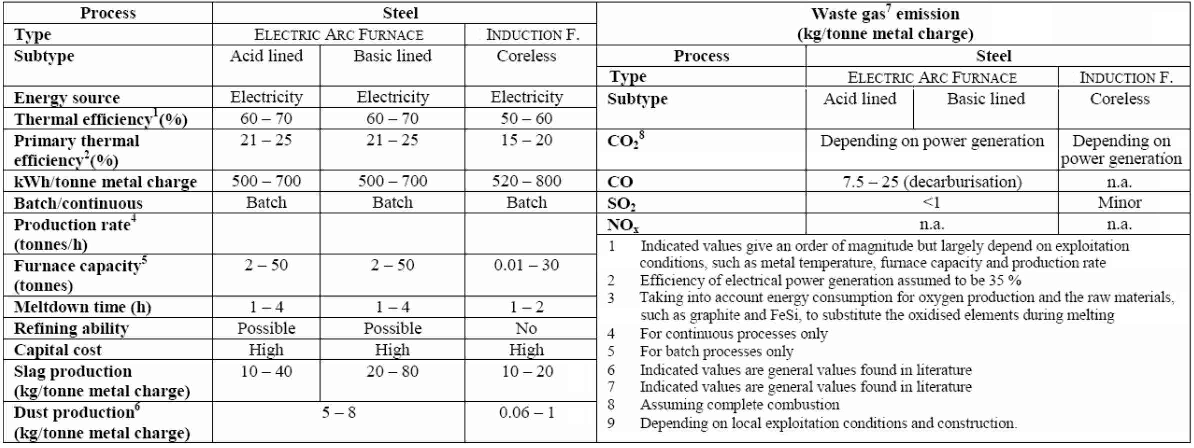
Table 2: Consumption of electricity in surveyed foundries during the year (kWh)

Uses	Foundries						
	CIMOS TMD Casting doo	Zenica P.J. Novi zivot	Bosnia Vales Tuzla	Foundry Visoko d.d.	Jelšingrad d.d.	Foundry Turbe d.d.	Iron and Steel Works Ilijaš d.d.
	F1	F2	F3	F4	F5	F6	F7
Shop	18.524.147	4.000.000	2.400.000	86.000	13.440.000	93.676	
Technology		100.000	100.000				3.500.000
Heating		600.000	800.000		960.000		
Lighting	4.705.853	300.000	300.000		1.600.000		36.000
Total	23.230.000	5.000.000	3.600.000	86.000	16.000.000	93.676	3.536.000
Shop + Technology	18.524.147	4.100.000	2.500.000	86.000	13.440.000	93.676	3.500.000
Percentage (%)	79,74	82,00	69,44	100,00	84,00	100,00	98,98

Environmental, production and energy characteristics of the selected business systems

In the contemporary market, casting is a well-known, very competitive and efficient production process. In addition, metal casting is a very complex process and can, at times, lead to unsatisfactory outputs, as the process involves the compounding of a great number of variables which must be controlled, including above all: quality of molds, melting process, metallurgic state of melting, temperature and speed of casting, etc. The technological process of casting must also be placed within the context of protecting the environment as the process is a significant polluter. Therefore, the demands placed on foundries are very complex. Table 3 shows the basic characteristics and emission levels of steel melting by furnace type.

Table 3: Basic characteristics and emission levels of steel melting by furnace type



Process	Steel		
Type	ELECTRIC ARC FURNACE		INDUCTION F.
Subtype	Acid lined	Basic lined	Coreless
Energy source	Electricity	Electricity	Electricity
Thermal efficiency[1](%)	60 – 70	60 – 70	50 – 60
Primary thermal efficiency[2](%)	21 – 25	21 – 25	15 – 20
kWh/tonne metal charge	500 – 700	500 – 700	520 – 800
Batch/continuous	Batch	Batch	Batch
Production rate[4] (tonnes/h)			
Furnace capacity[5] (tonnes)	2 – 50	2 – 50	0.01 – 30
Meltdown time (h)	1 – 4	1 – 4	1 – 2
Refining ability	Possible	Possible	No
Capital cost	High	High	High
Slag production (kg/tonne metal charge)	10 – 40	20 – 80	10 – 20
Dust production[6] (kg/tonne metal charge)	5 – 8		0.06 – 1

Waste gas[7] emission (kg/tonne metal charge)

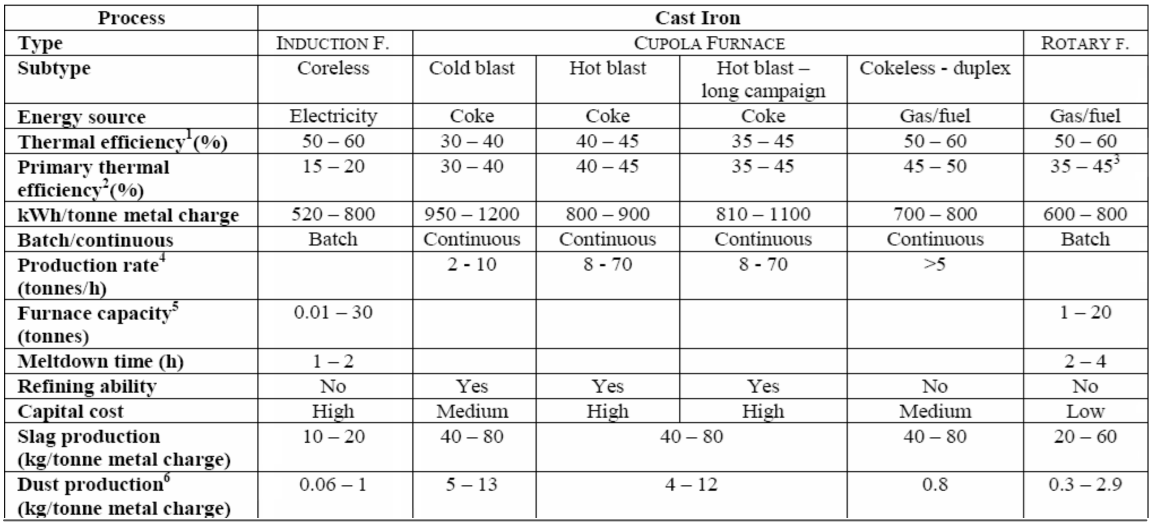
Process	Steel		
Type	ELECTRIC ARC FURNACE		INDUCTION F.
Subtype	Acid lined	Basic lined	Coreless
CO_2[8]	Depending on power generation		Depending on power generation
CO	7.5 – 25 (decarburisation)		n.a.
SO_2	<1		Minor
NO_x	n.a.		n.a.

1 Indicated values give an order of magnitude but largely depend on exploitation conditions, such as metal temperature, furnace capacity and production rate
2 Efficiency of electrical power generation assumed to be 35 %
3 Taking into account energy consumption for oxygen production and the raw materials, such as graphite and FeSi, to substitute the oxidised elements during melting
4 For continuous processes only
5 For batch processes only
6 Indicated values are general values found in literature
7 Indicated values are general values found in literature
8 Assuming complete combustion
9 Depending on local exploitation conditions and construction.

Source: Metalcasting Industry Energy Best Practices Guidebook, 2011 [10] [4]

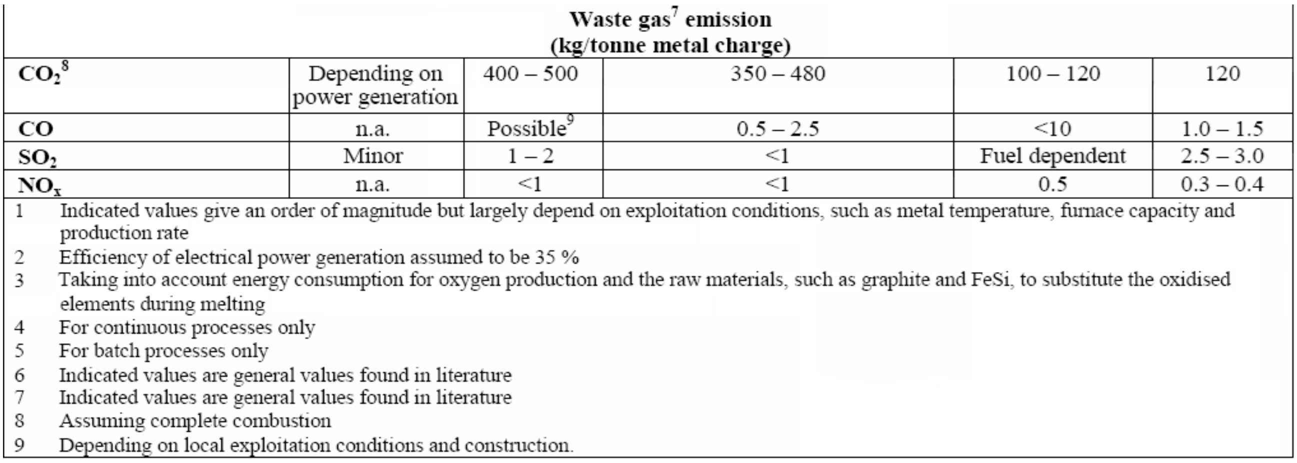
Table 4 contains data on characteristics and emission levels for the production of cast iron.

Table 4: Basic characteristics and emission levels of iron melting by furnace type



Process	Cast Iron					
Type	INDUCTION F.	CUPOLA FURNACE				ROTARY F.
Subtype	Coreless	Cold blast	Hot blast	Hot blast – long campaign	Cokeless - duplex	
Energy source	Electricity	Coke	Coke	Coke	Gas/fuel	Gas/fuel
Thermal efficiency[1](%)	50 – 60	30 – 40	40 – 45	35 – 45	50 – 60	50 – 60
Primary thermal efficiency[2](%)	15 – 20	30 – 40	40 – 45	35 – 45	45 – 50	35 – 45[3]
kWh/tonne metal charge	520 – 800	950 – 1200	800 – 900	810 – 1100	700 – 800	600 – 800
Batch/continuous	Batch	Continuous	Continuous	Continuous	Continuous	Batch
Production rate[4] (tonnes/h)		2 - 10	8 - 70	8 - 70	>5	
Furnace capacity[5] (tonnes)	0.01 – 30					1 – 20
Meltdown time (h)	1 – 2					2 – 4
Refining ability	No	Yes	Yes	Yes	No	No
Capital cost	High	Medium	High	High	Medium	Low
Slag production (kg/tonne metal charge)	10 – 20	40 – 80	40 – 80		40 – 80	20 – 60
Dust production[6] (kg/tonne metal charge)	0.06 – 1	5 – 13	4 – 12		0.8	0.3 – 2.9

Table 4: Continuous.



Waste gas[7] emission (kg/tonne metal charge)					
CO_2[8]	Depending on power generation	400 – 500	350 – 480	100 – 120	120
CO	n.a.	Possible[9]	0.5 – 2.5	<10	1.0 – 1.5
SO_2	Minor	1 – 2	<1	Fuel dependent	2.5 – 3.0
NO_x	n.a.	<1	<1	0.5	0.3 – 0.4

1 Indicated values give an order of magnitude but largely depend on exploitation conditions, such as metal temperature, furnace capacity and production rate
2 Efficiency of electrical power generation assumed to be 35 %
3 Taking into account energy consumption for oxygen production and the raw materials, such as graphite and FeSi, to substitute the oxidised elements during melting
4 For continuous processes only
5 For batch processes only
6 Indicated values are general values found in literature
7 Indicated values are general values found in literature
8 Assuming complete combustion
9 Depending on local exploitation conditions and construction.

Source: Metalcasting Industry Energy Best Practices Guidebook, 2011 [10] [4]

Data on average emissions from induction furnaces in the process of melting steel and iron is provided in Table 5.

Table 5: Average emissions from melting iron and steel in an induction furnace

	Capacity No. oven x tona/module	**Off- gas collection**	**Flux** m^3/sat	**Equipment for cleaning the gases**	**Dust** mg/m^3	**SO_2** mg/m^3	**CO** mg/m^3	**NO_x** mg/m^3	**HF** mg/m^3	**O_2** vol %
IP	(2 x 10) + (3 x 3)	Side-draught	54000	Bag-like filter	5	No data available	No data available	No data available	No data available	21

Source: [9] [4]

Measurements of CO and SO_2 emissions in foundries F1 and F2 taken by the ”Kemal Kapetanović” Institute University of Zenica are presented in Tables 6 and 7.

The average values for all gases measured in foundry F1 were obtained by taking three measurements of waste gases in one hour. The results are shown in Table 7. In this case, data for CO_2 (%), CO_2-IR (%), NO (ppm), NO_2 (ppm), EffN (%), EffG (%), ΔP (mbar), T1 and T2 (^{o}C), speed (m/s) and volume flow (m^3/s), dewpoint (^{o}C), as well as mass flow of CO, SO_2 and NO_x (kg/h) have been measured, but have not been recorded.

Table 6: Average values for all gases measured in foundry F1

Measured characteristics	Units	Values
O_2	%	20,95 ± 0,02
CO	Ppm	3,67 ± 1,15
Temp. fluida	^{o}C	29,03 ± 0,47
NO_x	Ppm	0,00 ± 0,00
SO_2	Ppm	0,00 ± 0,00
H_2	Ppm	0,00 ± 0,00
Amb, temp.	^{o}C	18,73 ± 0,21
Device temp.	^{o}C	21,67 ± 0,06
Oild		0,00 ± 0,00
Pump flow	l/m	0,82 ± 0,25
O_2 ref	%	5,10 ± 1,82
CO_2 max	%	11,9 ± 0,00

The average values for all gases measured in foundry F2 were obtained by taking four measurements from the foundry’s boiler room in one hour. These averages are presented in Table 6. Data for ΔP (mbar), T1 and T2 (^{o}C), speed (m/s) and volume flow (m^3/s), dewpoint (^{o}C), as well as mass flow of CO, SO_2 and NO_x (kg/h) have been measured, but have not been recorded.

Table 7: Average values for all gases measured in foundry F2

Measured characteristics	Units	Values
O_2	%	5,77 ± 0,03
CO	Ppm	3,25 ± 0,50
CO_2	%	11,24 ± 0,03
CO_2 – IR	%	10,43 ± 0,01
NO	Ppm	89,74 ± 1,15
NO_2	Ppm	-0,30 ± 0,35
Temp. fluida	^{o}C	242,65 ± 5,45
NO_x	Ppm	89,00 ± 1,15
SO_2	Ppm	41,25 ± 33,63
H_2	Ppm	3,25 ± 2,22
EffN	%	88,23 ± 0,17
EffG	%	83,08 ± 0,22
Rati		0,00 ± 0,00
Amb, temp.	^{o}C	21,98 ± 1,49
Device temp.	^{o}C	21,18 ± 0,96
Oild		0,00 ± 0,00
Dewpoint	^{o}C	47,83 ± 0,05
Pump flow	l/m	0,77 ± 0,02
O_2 ref	%	3,0 ± 0,00
CO_2 max	%	15,5 ± 0,00

The experiments and observations presented here show that emissions can cause serious damage to the air, soil, and environment.

EXPLANATION

- Harmful emissions caused by the production of castings are related mainly to the use of additives and fuels, as well as to pollutants. The use of coke or other natural fuels can cause emissions related to combustion. The use of additives can generate reaction products. The presence of pollutants in waste materials which are melted can cause the formation of incomplete combustion products and dust. Dust from the process may contain metals and oxides. During melting, elements evaporate and dust particles are liberated. Metal particles also appear during finishing [10].

- Emission of pollutants from cast iron foundries [11]
- A study involving 20 foundries showed that the total annual emission of dust-like pollutants into the atmosphere was between 0.1 and 94 kg or approximately 4.7 kg/t. Annual emissions of waste gases consisted mainly of SO_2, NO_x and CO. Quantities of castings from 0.1 to 108 kg/t amount to approximately 5.4 kg gases/t. In addition to the above-mentioned compounds, the following chemical components were also present: xylene, butanols, terpenes, benzo(a)pyrene, and esters.
- Emission of pollutants from cast steel foundries [11]
- Experiments have shown that the total amount of dust produced in the casting of steel is between 6.65 and 35.55 kg/t, while Si dust varies from 3.46 to 21.09 kg/t. The annual emissions of SO_2, NO_x, and CO from production vary from 0.01 to 20 kg. The biggest source of NO_x emissions are electric furnaces, with up to 90% of emissions, while the remainder comes from induction furnaces, etc. Emissions of CO from production amount to up to 18 kg/t. This gas is emitted primarily from electric and induction furnaces, while emissions of SO_2 originate from different sources and furnaces, etc. [11]

STATISTICAL DATA PROCESSING

Statistical methods were used to turn the data collected through surveys into information on trends in energy intensity, which can serve as a basis for decision-making and undertaking necessary measures to improve the production process. Statistical methods are important in that they make it possible to define mass processes, establish their tendencies and the laws governing them.

For the surveyed foundries, the following method was used to develop an energy intensity indicator and estimate its value.

- The energy intensity [EI] of the surveyed foundries was calculated with the following formula:

$$EI = \frac{K_e}{Y} \qquad (1)$$

where:
EI – energy intensity of foundry
K_e – electricity use in MWh
Y – Total output of foundry in physical units

- For processing of numerical data, values were summed up to calculate average values and the percent share of main elements in certain values were estimated. In estimating these values, special consideration was given to compounding values in total sums, while determining the participation of different categories of compounding elements depended of accessible data.
- Different types of diagrams were used to graphically illustrate the data: spherical, linear, etc. The diagram type is chosen based on what was the best way to present the given data and compare complementary values, etc.
- In certain cases, trend lines are marked for changes in data, as lines of aberration, etc.
- Foundry CIMOS Casting Zenica submitted complete data, followed by the best analyses, a regression model, and practical experiences for the measurement of certain performances.
- Comparison of data for the region.

Table 8 shows the energy intensity of each surveyed foundry, as well as their average intensity.

Of the observed foundries, F2 has the highest energy intensity, while F5 uses the least electricity in its production. On average, the foundries need around 2 MWh of electricity to produce one ton of castings.

Table 8: Energy intensity of surveyed foundries

Foundry	**Electricity use (K_e)**		**Output (Y)**		**Energy intensity (EI)**	
F1	21.485	MWh	7.873	t	2,728947	MWh/t
F2	5.000	MWh	1.000	t	5,000000	MWh/t
F3	3.600	MWh	970	t	3,711340	MWh/t
F4	86	MWh	1.333	t	0,064516	MWh/t
F5	16	MWh	2.420	t	0,006612	MWh/t
F6	93,676	MWh	60	t	1,561267	MWh/t
F7	3.500	MWh	2.000	t	1,750000	MWh/t
Total	**33.781**	**MWh**	**15.656**	**t**	**2,157703**	**MWh/t**

CLIMATE CHANGE

The negative consequences of climate change affect human life as a whole. The greenhouse effect and higher emissions of gases and pollutants cause global warming and thinning of the ozone layer. The reason for writing a paper on this topic is that combining different disciplines can offer the best possible solution for energy efficiency and environmental management.

GIDDENS PARADOX REGARDING CLIMATE CHANGE

Cars are a main source of benzene emissions to the environment. Apart from food, benzenes enter the human organism through the air. The environment has high concentrations of benzenes which vary from 3 to 160 $\mu g/m^3$ (urban or rural). Benzene is a hematological poison [7]. The behavior of NO_x in the atmosphere has been the subject of several experiments, which have shown that its high chemical reactivity has an important role. NO_x and C(OH) (aliphatic and aromatic) accumulate in the atmosphere during the night and, during the day, NO_x transforms into NO_2 through photolytic cycles (Figure 3).

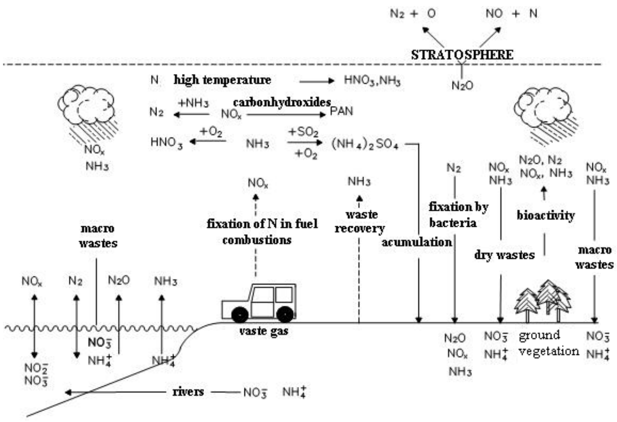


Figure 4: Circulation of nitrogen in nature
Source: Perina, I., Mihanović, B.: Ispitivanje onečišćenja zraka, SKHT/Kemija u industriji, Zagreb, 1988.

There is wide-spread knowledge about a possible apocalyptic scenario and the uncertain future of global climate change [12] [13] [14].

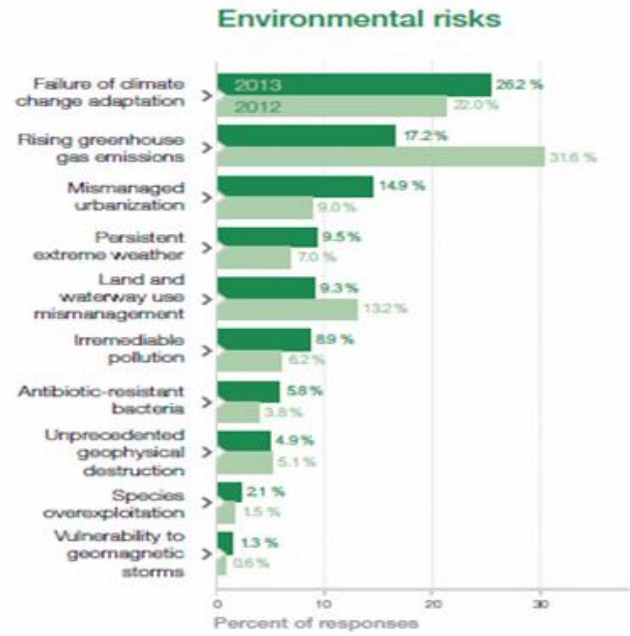


Figure 5: Environmental risks, 2012 and 2013
Source: Global risks report 2013:54-56

The eco-pathological sociology of this is the fact that it is dealing with "abstract and elusive dangers, however potentially devastating they might be". This is called Giddens paradox.

ECO ACTIVISM

When pollutants (CO_2, SO_2, NO_x) are in the atmosphere, their existence very much depends on physical processes such as dispersion, transport, deposition as well as the very complex chemical processes that are developing all the time, from the moment of emission to precipitation onto the Earth's surface. It is not simple to find the correlation between emissions and the acidity of the layers of the atmosphere. The numerous variables related to the origin of pollutants, their types and conversions, their transmission through the atmosphere and their precipitation are a complex system to which the response is not simple. They also require a complex system of modeling. The implementation of legislation on air pollution would bring many changes in the regulation of climate change and protect the environment from its negative consequences. [15] [14]

CONCLUSIONS

Through its present technology, the electrical, metal, and foundry industries pollute the air, water, and soil to some degree, which in turn affects the climate. Although moderate polluters, foundries in B&H also contribute to the process of climate change through the use of outdated equipment and the resulting emissions of gases such as CO_2 and SO_2 and air pollutants. Minimal production of castings in B&H is associated with a minimal consumption of energy in melting furnaces and production processes. The existing casting production amounts to several tens of thousands of tons and is at least six times lower than it was before the war. By increasing the total production and fuel consumption, no drastic contamination is anticipated, but only an increase in environmental pollution. It is expected that increased production and fuel consumption will result in increased environmental pollution. From the above considerations and available literature, it is possible to calculate energy intensity. At the same time, it is also possible to mitigate higher pollution resulting from higher production by modernizing foundries in order to create the so-called "ideal foundry".

Recent studies of energy efficiency in the B&H metal sector offered the following recommendations: special methodology must be involved in the legislative and pre-legislative act, formation of the Energy Efficiency Agency (which is foreseen in the draft Law on Energy Efficiency in the Federation of B&H), formation of the Industrial Energy Efficiency Network of B&H (MEEIBH), etc.

REFERENCES

Adam, Barbara, Zeitvielfalt in der Evolution aus gesellschaftstheoretischer Sicht, February, Akademievorlesung an der Berlin-Brandenburgischen Akademie der Wissenschaften, Berlin, 2010.

Eisler, Riane, *Sacred Pleasure, Sex, Myth and Politics of Society*, Harper, San Francisco, 1996.

Emisije CO_2 od sagorijevanja energenata CO_2 (emissions from fuel combustion), IEA 2011.

European Commission Integrated Pollution Prevention and Control Reference Document on Best Available Techniques in Forging and Foundries Industry, May 2005.

Fair Globalization's Eating Opportunity for All, The World Commission on the Social Dimension of Globalization, ILO Publication, April 2004.

Goletić, Š., Pihura, D., Upravljanje otpadnim materijalima u ljevaonicama. Savjetovanje "Ekologija u ljevarstvu", Zenica, 1998.

Hasanović, K.: Identificiranje i disperzija benzena i produkata benzena i policikličkih ugljikovodika metodom GC–MS u zraku tuzlanskog bazena, doktorska disertacija.

Marković S., Najbolje dostupne tehnike za livnice (ograničenja i mogućnosti), decembar 2011.

Metalcasting Industry Energy Best Practice Guidebook, 2011.

Oprašić. S., Uticaj karakteristika opreme na ekološku efikasnost u proizvodnji metala sa posebnim osvrtom na ljevarstvo, dokt. Disertacija, Mostar, 2002.

Perina, I., Mihanović, B.: Ispitivanje onečišćenja zraka, SKHT/Kemija u industriji, Zagreb, 1988.

Pihura D., Perspektive ljevarstva u BiH, Prilog Strategiji razvoja FBIH i BIH, Sarajevo, 2009.

Šehić-Mušić N., Mušić L., Interakcija menadžmenta i okoline sa akcentom na okolinsko i energijsko upravljanje, IX Naučno/stručni simpozij sa međunarodnim učešćem „Metalni i nemetalni materijali“, Zenica, 2012.

The Energy Sector Study in BH, 3rd project, EES Module 12- BHP3-EES-TEPRP-Q-04/05 WB.

Wild S.R., Jones K.C., Polinuclear Aromatic Hidrocarbons in the United Kingdom Environment - A Preliminary Source Inventory and Budget, Environ Pollut, 1995.

Summary/Sažetak

Analiza potrošnje u industrijskom sektoru BiH pokazuje da više od 50% energijske potrošnje otpada na metaluršku industriju. Industrija lijevanja željeznih proizvoda veliki je potrošač energije unutar široke grane metalurgije.

Interes za obradom ovakve teme, koja iziskuje pluriperspektivni pristup**,** proistekao je, i iz slijedećeg:

U centru svake Strategije održivog razvoja je vizija poboljšanja obezbjeđenja i potrošnje energije na održiv način. Dakle, potrebno je da se upravlja energijom i sa stanovišta ekonomije i ekologije, a da bi se nečim upravljalo moraju se poznavati i odrediti mjerila, odnosno, indikatori. Stoga je bez energijskih indikatora, nezamislivo energijsko i okolinsko upravljanje iz razloga što oni upravo daju vezu između ljudskih aktivnosti, energijskih promjena i efekata mjera.

Sektor metalske i elektro industrije BiH, odabran je i zbog tog što je to tradicionalno vodeći sektor , u ukupnom izvozu BiH, u kojem pojedine kompanije izvoze i do 100% proizvodnje (npr. automobilske dijelove za „prvu ugradnju”). Livnice, odnosno livarski proizvodi, su značajan dio ovog sektora, naročito u mašinskoj industriji koja se bazira na ljevarstvu, uključujući automobilsku industriju, uz spoznaju da je u svaki automobil ugrađeno do 50 % odljevaka.

Livnice su, međutim, intenzivni potrošači energije i intenzivni zagađivači okoline, a što je i predstavljeno radom.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

2013

40

47-49

Print ISSN: 0367-4444

Online ISSN: 2232-7266

UDC: ________________________

Original Scientific Article

Treatment of Antimony-Rich Waste Streams

Avdić N.[a], **Müller-Czygan G.**[b]

[a] *University of Sarajevo, Faculty of Science, Department of Chemistry, Zmaja od Bosne 33-35, 71000 Sarajevo, Bosnia and Herzegovina*

[b] *HST Systemtechnik GmbH & Co. KG, Sophienweg 3, 59872 Meschede/Germany*

Article info
Received: 06/06/2013
Accepted: 14/06/2013

Keywords:
Antimony
Wastewater
Lead-acid batteries
Extraction

***Corresponding author:**
E-mail: technoprocur@yahoo.com
Phone: +387 33 279 862

Abstract: The use of antimony in various chemical industries represents a potential source of environmental pollution, that occurs by discharg of untreated wastewater generated in the watercourses. Another sources of this pollutants can be individual sources from improper recycling of products containing antimony as, for example, of old batteries containing antimony in the bars and in the electrolyte.

Here, it is suggested to extract antimony from waste water, with the possibility of application to different flows and concentrations of metals.

INTRODUCTION

Lead-acid batteries production and recycling plants are common sources of antimony in waste streams, because of its common usage as alloying element in lead grids. Antimony can also be found as an additive in car brake linings (in form of antimony(III) sulfide), in heads of safety matches, as a catalyst in some industrial processes (PET production) (Erosa 2008) and as a colorant for ceramics. In waste streams antimony can be found in many forms, including very toxic and corrosive fluorides and chlorides, in concentrations as high as 44 μg/l (PET production), which is well above maximum allowed concentration of 5 μg/l for drinking water. Extraction of antimony from waste streams (with 4 – 12 mass % of antimony) is cost effective, considering its price (10$ per kilogram). However, removal of antimony requires special conditions that can only be achieved in separate facility.

EXPERIMENTAL

Removal of antimony from waste water

Research and scientific approach to the problem of antimony in waste streams is rarely found in publications, partly because of its very low concentrations in most of industrial waste streams. Apart from sources mentioned above, antimony can be found in waste waters from glass production facilites (up to the 450 mg/l, depending on the technology), in streams from incineration plants (up to the 4 mg/l) and in waste waters from anode sludge treatment (up to the 500 mg/l) (Fujita 2006). In Germany, as well as in Bosnia and Herzegovina, upper limit of antimony that is alowed in waste waters infusing natural streams is 0.3 mg/l.

Due to the amphoteric nature of its ions, reactions of antimony in waste streams are pH dependent and are quite diverse. In general, in alkaline envirnoments antimony is mainly in the form of antimonite anions, while antimone cations are main form in acidic environments.

Behavior of antimony(III) and (V) in different pH is shown on Figures 1 and 2. Analysis of the influence of pH is important because its precise adjustment allows precipitation and extraction of all present forms of antimony. If pH value is below or above provided limits precipitation of antimony is hindered, with oxidation and reduction becoming major processes.

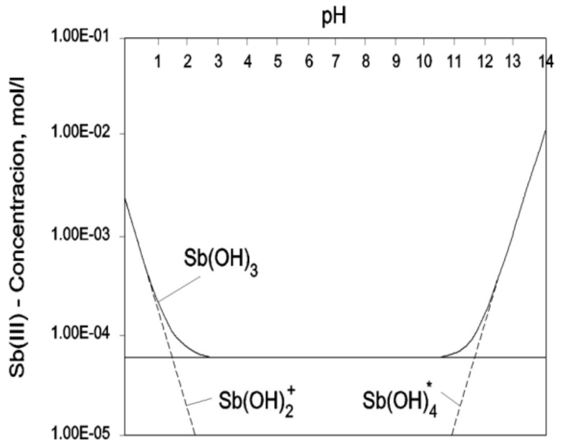


Figure 1: Solubility and concentration of antimony (III).

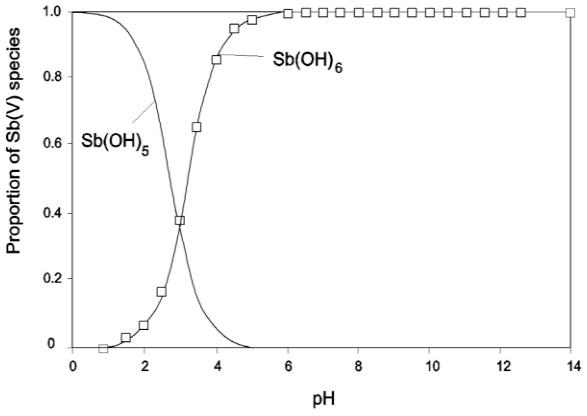


Figure 2: Solubility and concentration of antimony (V)

Main difficulites in the process of antimony removal arise from inability to accurately controll the pH value, by adding acid or base. Each sample that is to be treated must be tested separately and, on the basis of obtained results, sequence and quantities of required reagents has to be determined.

It has been found, in some experiments conducted in Japan, that the addition of adsorbent (eg. iron(III) chloride) can have significant influence on removal efficiency (Gannon 1986). In some research dealing with removal of antimony(III) oxide, published by Ganon and Wilson, significant improvement was attributed to the presence of calcium hydroxide rather than the chosen sequence of pH alteration. They also found that addition of aluminium hydroxide had no effect on the removal of antimony, while iron(III) salts significantly improved the process. Detailed insight into different aspects of the process can be found in works of Erosa (Erosa 2008). He showed that some methods that were succesfully used to remove antimony in one case can be absolutely inefficient in the other (eg. active charcoal is good adsorbent for antimony(V), but in some cases, as in pH range from 4 – 10, shows no effect).

Numerous researches showed that the addition of iron chloride leads to a very satisfying results, which is, for neutral and alkaline environment, shown on Figure 3.

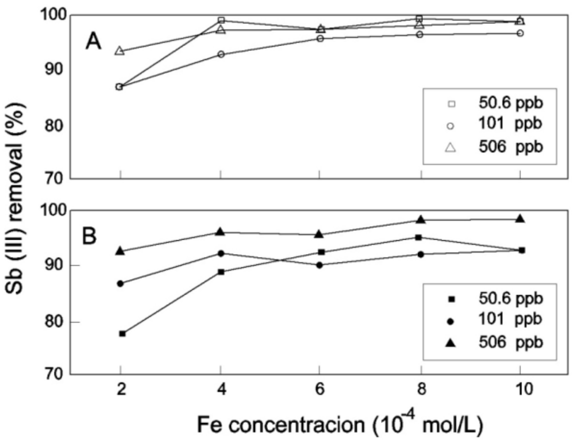


Figure 3: Extraction of antimony(III) compounds by adding iron(III) chloride: A. acidic environment (pH = 6 ± 0.2); B. weak alkaline (pH = 7.8 ± 0.2); temperature 25 ± 1 °C.

In the pH range between 6 – 8, efficiency of extraction is as high as 98%, regardless of initial concentrations.

Treatment of waste streams from battery-recycling plants

Recycling of used lead-acid batteries includes extraction of metallic components and their recycling, during which antimony(III) and (V) oxides can be found in waste gas. Waste gas is purged through waste water, which is than treated with iron(III) chloride and sodium hydroxide. Alkaline environment causes hydrolysis and preciptation of red-brown iron(III) hydroxide, antimony compounds adsorbs on the obtained precipitate, and are easily removed from the waste water.

Proposed technological process is based on classical flocculation-precipitation approach, the precise control of pH being the most important issue.

A typical scheme for the treatment of wastewater containing antimony is shown in Figure 4.

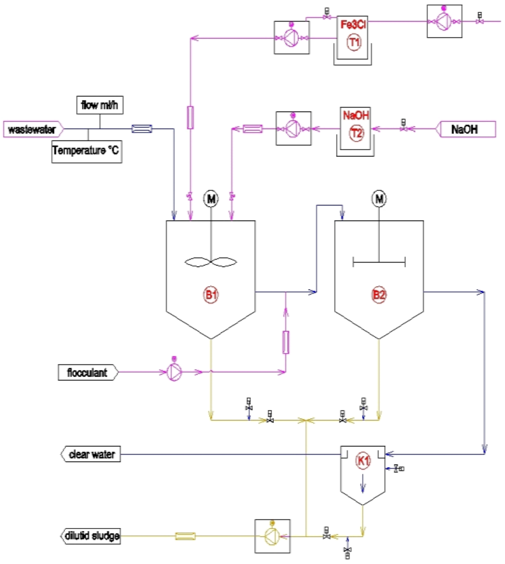


Figure 4: Schematic diagram of a typical antimony-containing wastewater treatment plant (Anlagenkonzept der HST 2012)

CONCLUSION

Antimony compounds are considered as potential polutants of drinking water and natural water streams. Some researchers compare its damaging effect to that of arsenic. Some research studies showed that iron(III) chloride can be used to reduce antimony levels to allowed limits. Described methods were succesfully used in large scale production plants. Considering that every waste water has distinctive properties, separate testings should be done, including on-site testings. For optimal process operation it is necessary to use precise measuring and automated process-controling technique, with well defined process conditions, which includes optimal pH values and time intervals for adding desired reactants. This represents the only way to enhance succesful and effective operation of industrial plant.

REFERENCES

MSc. Mercy Sugey Dzul Erosa „Entfernung von Selen- und AntimonSpezies aus wässrigen Lösungen mit Hilfe“, Dissertation an der Fakultät für Chemieingenieurwesen und Verfahrenstechnik der Universität Fridericiana Karlsruhe (TH), 2008

Toyohisa Fujita, Gjergj Dodbiba , Jun Sadaki, Atsushi Shibayama „Removal of Anionic Metal Ions from Wastewater by Hydroxide-type Adsorbents“, The Chinese Journal of Process Engineering, Vol. 6, No. 3, June 2006

Gannon, K. U. Wilson, D.J. , Removal of antimony from aqueous systems. , Sep. Sci. Technol. 21, 475-493 (1986).

Anlagenkonzept der HST Systemtechnik GmbH & Co. KG, copyright 2012

Summary/Sažetak

Upotreba antimona u različitim hemijskim industrijama predstavlja i potencijalni izvor zagađenja životne sredine, ispuštanjem nastalih neprečišćenih otpadnih voda u vodotoke. Slijedeći izvor ovog zagadživača predstavljaju i pojedinačni izvori nastali nepropisnim recikliranjem proizvoda koji sadrže antimon kao naprimjer starih akumulatora koji sadrže antimon u rešetkama a i u elektrolitu. Ovdje je dat predlog za izdvajanje antimona iz otpadnih voda, sa mogućnošću primjene na različite protoke i koncentracije metala.

Glasnik hemičara i
tehnologa
Bosne i Hercegovine

Bulletin of the Chemists and Technologists
of Bosnia and Herzegovina

2013

40

51-56

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: ________________________

Original Scientific Article

100 Years Anniversary of the Bohr Model of the Atom: How Chemistry Freshmen Understand Atomic Structure of Matter

Hadžibegović Z.*, Galijašević S.

Faculty of Science, Zmaja od Bosne 33-35, Sarajevo, Bosnia and Herzegovina

Article info
Received: 15/05/2013
Accepted: 14/06/2013

Keywords:
Chemistry student
Model of the atom
History of science
Student picture of atom
The Bohr model of the atom
Worksheet rubric

***Corresponding author:**
E-mail: zalkidah@yahoo.com
Phone: 00-387-33-279-996

Abstract: Hundred years ago the Danish physicist Niels Bohr presented his model of the atom for what he received Nobel Prize. His theory led to the revolutionary development of science in the beginning of 20th century. Later one, quantum mechanics developed a new theory explaining all the shortcomings of Bohr’s model. However Bohr’s atom theory is presented and studied in most introductory General Chemistry textbooks. This anniversary was an opportunity to see how science students understand and visualize the structure of the atom. The data reported in this paper derived from a pilot research with 58 chemistry freshmen, were collected to explore their ideas of an atom, their knowledge about the Bohr model of the atom, and history and philosophy of science approach to Bohr atomic theory. Students' knowledge about Bohr's postulates is in agreement with chemistry textbook content except the part about Bohr's idea of angular momentum quantization, which they discuss within the General Physics 2 course. Using historical point of view when studying Bohr’s theory and his model of the hydrogen atom can help students to understand contemporary theory of the atom better.

INTRODUCTION

The 100th anniversary of the Bohr model of the atom is an opportunity to check whether science students are aware of Bohr’s contribution to the revolutionary development of science at the beginning of 20th century. In 1922, Danish physicist Niels Bohr received Nobel Prize in the field of theoretical nuclear physics "for his services in the investigation of the structure of atoms and of the radiation emanating from them" (The Nobel Prize in Physics 1922). Victor Weisskopf (1985) placed Niels Bohr at the level of great scientists saying that “his name ranks beside Galileo, Newton, Maxwell and Einstein”. Weisskopf highlighted his colossal role in the human problems related to the science, society and politics. Bohr’s contribution to science has been important for the understanding of atomic structure and for the development of quantum mechanics ideas (Bentzen, 2000). The Bohr model of the atom has been used for decades as a useful atomic model in physics and chemistry education. Today, this model of the atom has its historical value. It was the first atomic model offering explanation for paradoxical stability of the Rutherford model of the atom (Bohr, 1913; Niaz & Costu, 2009) and explanation of hydrogen line spectrum (Niaz & Costu, 2009). In the Bohr’s Nobel Award lecture (December 11, 1922), his two central topics were the periodic table and an atom of an element. According to our present understanding, an atom of an element is built up of a nucleus that has a positive electrical charge and carries the greatest part of the atomic mass. Negatively charged electrons move around the nucleus at the distances that are very great compared to the dimensions of the nucleus or of the electrons themselves. In this picture, we at once see a striking resemblance to a planetary system, such as we have in our own solar system (Bohr, 1922).

In the *Philosophical Magazine*, Niels Bohr published the three papers under common title „On the Constitution of Atoms and Molecules’’. The first paper appeared in the July 1913 journal issue where Bohr was focused on the hydrogen atom. His second paper was published in the September journal issue covering the atomic theory of the complex atoms, and Bohr published the last paper in November of 1913 where he considered the structure of molecules. Bohr proposed his atomic theory based on the postulate of stationary electron orbits and frequency postulate. Bohr’s theory was successful when applied to the simplest element, the hydrogen atom and ionized helium, but failed for systems such as the hydrogen molecule and helium atom (Kragh, 2011). Bohr’s paper on atomic structure was “a highly original and truly revolutionary contribution to physics that more than anything reflects Bohr’s own genius” (Bentzen, 2000).

Niels Bohr used an idea, introduced by Planck in 1900, of the atomic quantization for finding a solution for the atomic stability, but he did not try to find a theoretical solution for the Balmer and Pashen line spectra. In that time Bohr was influenced by Planck’s and Einstein’s quantum theory ideas and by work of many other scientists as he said:

“In the following years many efforts were made to apply the concepts of the quantum theory to the question of atomic structure, and the principal emphasis was sometimes placed on one and sometimes on the other of the consequences deduced by Einstein from Planck’s result. As the best known of the attempts in this direction, from which, however, no definite results were obtained, I may mention the work of Stark, Sommerfeld, Hasenöhrl, Haas, and Nicholson” (Bohr, 1922, p. 14).

The Bohr model of the atom is a quantum physics-based modification of the Rutherford model of the atom which replaced the Joseph John Thompson model of the atom based on his first paper “installments on the atomic structure” (Heilbron, 1977). Bohr did not have any intention to explain Balmer’s formula for calculating the wavelengths of the spectral lines of the hydrogen atom line spectrum. Victor Weisskopf (1985) highlighted the importance of Bohr's contribution in quantum mechanics and its development that brought „a true revolution in our thinking ...in the new system of concepts and a new way of dealing with atomic structure was introduced; it revolutionized our ideas of material reality“.

Several studies were conducted about how models introduced by Thomson, Rutherford and Bohr have been included in the general chemistry textbooks from History and Philosophy of Science point of view (Niaz, 1998; Rodriguez & Niaz, 2002; Niaz & Costu, 2009). The most emphasized textbook contents were based on the experimental details and heuristic principles of the atomic structure and lacks of context on the history and philosophy of science (Niaz & Cosku, 2009). Atomic structure has been found as one of the most difficult concepts for students at high-school and university level of education within both chemistry and physics courses (Niaz, et al., 2002; Taber, 2003; Nakiboglu, 2008). Keith Taber (2003) found that students learn about four atomic theories (theory by Thomson, Rutherford, Bohr, and Quantum Mechanical Theory) using textbooks and shows some difficulties in understanding a basic concept of the atom, very often presented as „ a confused amalgam of historical models“.

Igal Galili (2008), answering to the question „Why to teach physics using history?“, gave a claim based on his research results, that the history of physics (HoP) is a tool for teaching physics. Galili answering to mentioned question gave his view about three distinguished stages how to include HoP in the process of learning physics.

(1) The HoP helps students to better understand physics. Galili has explained that „the historical contents familiarize students with the way of doing physics, the nature of physics as a method of human exploration and learning about the Nature“.
(2) The HoP helps students to use knowledge of physics for practical application in the process of problem solving, using as Galili said „the argumentation employed by scientists in the past in illustrating the contents they teach and persuading their students to consciously reconstruct and build the new for them knowledge“.
(3) The HoP is a basic stage for physics knowledge as main structure elements of discipline-culture paradigm that provide a connection between the past and new knowledge in science.

Science teaching and history of science should be connected at four levels: interest level (includes story based information about scientists, experiments, scientific theory developments methods), socio-cultural level (includes connections between science and society), epistemological level (for introducing scientific inquiry concepts by scientist from the history of science), and conceptual level for including information about „historical development of the concepts to help students understand scientific concepts“ (Seker, 2011).

Our main aim in this study was to investigate the effects of knowledge integration of history of science dealing with Bohr model of the atom and how knowledge transition from chemistry course to physics course is achieved when students’ perception of atoms is used. Learning about atomic models is content of both General chemistry and General physics course syllabi at Bosnia and Herzegovina universities within the first year of science study.

Chemistry freshmen under this study learn about three atomic models (given by Thomson, Rutherford and Bohr) in last year of secondary school and again within both General Chemistry 1 and General Physics 2 as a part of introduction to the quantum mechanical theory (QMT).

The data reported in this paper derived from a pilot research with 58 chemistry freshmen, were collected to explore their accepted idea of an atom, knowledge about the Bohr model of the atom, and history and philosophy of science perspective of Bohr atomic theory.

A BRIEF REVIEW OF THE BOHR MODEL OF THE ATOM

There are several interpretation and versions of the Bohr’s postulates first presented to the scientific community in 1913, in different textbooks used by student included in this study in both courses (general chemistry and general physics). The Bohr’s first and second postulate as a core of his Nobel lecture presented in the Swedish Academy of Sciences in 1922 is as it follows.

Bohr's postulate one (BP1)

„Among the conceivably possible states of motion in an atomic system there exist a number of so-called stationary states which, in spite of the fact that the motion of the particles in these states obeys the laws of classical mechanics to a considerable extent, possess a peculiar, mechanically unexplainable stability, of such a sort that every permanent change in the motion of the system must consist in a complete transition from one stationary state to another“.

Bohr's postulate two (BP2)

„While in contradiction to the classical electromagnetic theory no radiation takes place from the atom in the stationary states themselves, a process of transition between two stationary states can be accompanied by the emission of electromagnetic radiation, which will have the same properties as that which would be sent out according to the classical theory from an electrified particle executing an harmonic vibration with constant frequency. This frequency ν has, however, no simple relation to the motion of the particles of the atom, but is given by the relation

$$h\nu = E' - E''$$

where h is Planck's constant, and E' and E'' are the values of the energy of the atom in the two stationary states that form the initial and final state of the radiation process. Conversely, irradiation of the atom with electromagnetic waves of this frequency can lead to an absorption process, whereby the atom is transformed back from the latter stationary state to the former"(Bohr, 1922, p.15).

Textbooks prepared by local or international textbook authors, contain different Bohr's postulate expressions. Basically, Bohr's postulates are included in many investigated textbooks recommended by syllabi in the manner shown here (as Bohr's original postulates marked as BP1 and BP2). But in some textbooks, mostly in physics textbooks, there is an addition to the first Bohr's postulate expression. A difference is related to the size of the allowed electron orbit which is determined by a condition imposed on the electron's orbital angular momentum. The allowed, electron orbits are those for which the electron's orbital angular momentum is given by relation

$$m_e v_n r_n = \frac{h}{2\pi} n$$

where m_e is the electron's mass, v_n and r_n is the electron velocity and radius respectively in a certain stationary state, h is Planck's constant, and n is the principal quantum number (Serway & Jewett, 2006, p. 353).

The Bohr's theory had several limitations, but Bohr's model of the atom was "a first picture of what an atom is like" (Giancoli, 1998, 859). The Bohr theory did not explain why intensity of some spectral lines is different, and how atoms are connected in molecules. The Bohr theory was a mixture of classical and quantum theory without a wave-particle duality ideas. In the same time, the Bohr model of the atom and the Bohr theory was an impetus for finding a comprehensive theory to solve theoretical problems opened in the early 1920s, thus giving him a great role in the history of science. The Bohr model of the atom was replaced by the quantum mechanics model based upon the Schrödinger equation in the 1920's.

METHOD

Sample

The sample of this study consists of N = 58 chemistry freshmen (c. 19-22 years of age). Of these students, 80% finished *Gymnasium* (secondary school) education, and 20% of students came at university from the technical secondary school environments. All participating students did not differ in the number of years of studying chemistry at secondary school level (four years).

Students were given a worksheet containing three rubrics and were advised to give answers to the each of three questions. The data collection took place from April to May, in the spring semester of the first year of chemistry study at university.

Worksheet rubrics

The worksheet rubrics created to explore students' knowledge and attitudes toward the Bohr model of the atom contains questions and tasks as follows:

Rubric 1: Draw a sketch of an atom as you imagine it and give the explanation of your idea about atoms.

Rubric 2: In the provided space, you are asked to express your knowledge about the Bohr's postulates that have appeared at scientific scene 100 years ago, according to the Bohr model of the atom as you know it.

Rubric 3: Do you think that Bohr atomic theory was revolutionary idea at that time? If yes, explain why?

Students' concept of atomic structure was included as a target variable for which knowledge integration effect was assumed. It is important to note that a need for knowledge integration and transfer effect related to the students' scientific view of atoms has not been highlighted in both syllabi content (General Chemistry and General Physics course). There are not any highlighted learning strategies for achieving students' common general physics and chemistry courses outcomes. As a numerous research showed, students usually have a difficulties to understand atom structure as is explained by modern quantum theory (Harrison and Treagust, 2000; Taber, 2002).

RESULTS AND DISCUSSION

Worksheet rubric 1 results

In this study, focus was directed to the historical models of atom proposed by: Dalton (the atom as a sphere), Thomson (the atom as a positive sphere filled by electrons), Rutherford (planetary model of atom), Bohr (the atom as a nucleus that electron orbit it) and quantum-mechanical model of the atom or Schrödinger theory (the atom as a nucleus surrounded by an electron cloud).

The students were divided into four groups depending on visual-textual representation of atoms (in students' minds) and giving their personal views of atoms.

Model of the atom 1/MA1: students who built-in the Thomson model of the atom;

Model of the atom 2/MA2: students who presented the Rutherford model of the atom;

Model of the atom 3/MA3: students who presented the Bohr model of the atom;

Model of the atom 4/MA4: students who included in their representations one or more elements of the quantum-mechanical model of the atom (the Schrödinger theory).

Students' answers distribution among such defined groups is shown in Figure 1.

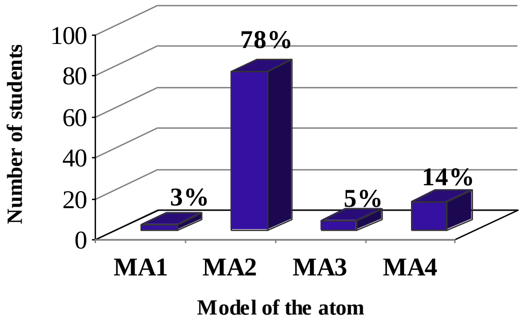


Figure 1. Distribution of student visual-textual representations of model of the atom.

MA1: Students gave a model of the atom that presented their visual-textual representation of atom as a sphere uniformly filled with positive electricity, and with electrons inside the sphere. This model is a characteristic Thomson's classical picture of the atom as whole a neutral particle.

MA2: Students' model of the atom is a planetary model as a system of positive nucleus which is a massive center of atom and with negative electrons distributed that travel about the nucleus along circled trajectories.

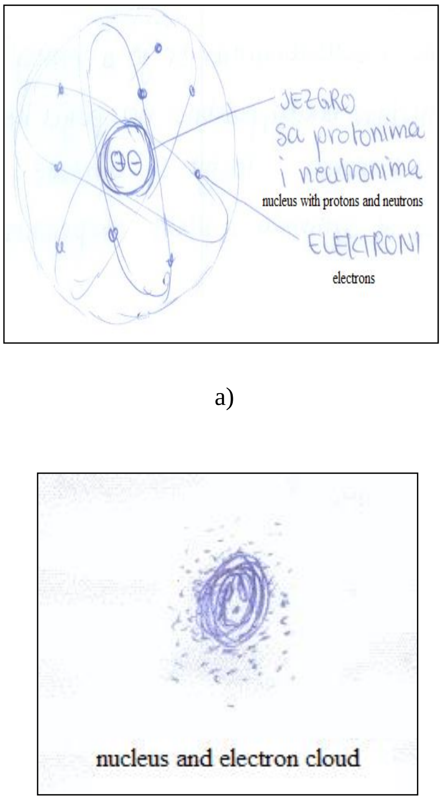
MA3: Students presented their model of the atom as Bohr model of the atom with additional information. They mentioned infinite multitude of electron orbits that are existing as a discrete orbits in the proper quantum conditions. In this stationary state atom does not emit or absorb electromagnetic energy. The emission and absorption of the light occur only if an electron jumps from one energy level to another.

MA4: An atom with its particles is described by wave function where the square of the magnitude of the wave function gives the density of the probability of finding an electron in certain place in area around nucleus. The electrons are in electronic cloud (complex shapes of orbital) without a definite locations and trajectories. In this model of students' views of atoms, characterization of an electron named as s-electron, p-electron, d-electron, f-electron are given.

Four students gave textual explanations showing the atom as a sphere (Dalton's approach), but three students wrote down their statements about atoms being the smallest particles of matter.

Students included in this study mostly presented their ideas about atoms using Rutherford's model as an expression of their prior knowledge of the particle model of matter introduced to them in ages 13-15 during their primary school education. A possible reason for the fact that only few of students gave their pictures of atoms according to the modern scientific model of the atom is that, students have usually difficulties to understand the particles of matter which they can not see. In another words, any abstract concept about a matter is too difficult for them to understand. In the literature, one can find that learners showing their view of atoms demonstrate it as a difficult and confusing epistemological obstacle (Taber, 2003).

There are two selected, but typical, student drawings shown in Figure 2. On the (a) drawing is presented student's idea of an atom which has a nucleus contains protons and neutrons and around them orbiting electrons. On the (b) drawing, an atom is presented, which consists of a nucleus and an electron cloud. In summary, 80% of students have presented their drawings depicting an atom as system of nucleus containing positive protons and neutrons and negative electrons around its nucleus.



a)

b)

Figure 2. Two selected drawings of the atom presented by students under this study.

Worksheet rubric 2 results

In this rubric, students had the opportunity to demonstrate their knowledge about the Bohr's postulates after they completed their required assignments and final exam in General Chemistry 1 course.

The analyses of student responses to the second rubric describe students' knowledge about Bohr's postulates. Students have written evidences that they know different number of Bohr's postulates: two postulates was an answer of the 42 students (72% of students), three Bohr's postulates were an answer of five students (9% of students) and a fact that there are four Bohr's postulates was mentioned only by one student. It is important to note that students generally wrote down a textual expression for (1) only one postulate in correct form (8 or 14% of students), (2) two Bohr's postulates (34 or 59% of students), in very similar or the same form that can be found in their textbooks. Among the 16 students who did not give answers about Bohr's postulates, half of them repeated their description of atoms according to the MA3, and other eight students left a blank space on the worksheet.

It is important to note that three students had written a formula ($h\nu = E_m - E_n$) attached to the second Bohr's postulate expression. None of students wrote down Bohr's ideas of discrete values (quantized) of angular momentum of electrons for the allowed orbits in an atom.

Worksheet rubric 3 results

Most of the students understand the concept about a revolutionary role of the Bohr model of the atom. These concepts were not formed through their acquisition of knowledge of the history of science using some arguments. Therefore, most of them did not give any explanation for what Bohr did for establishing the contemporary scientific model of the atom. Only three students left their blank rubric in the answer space. Analysis of the students' answers to the third worksheet rubric resulted by grouping students answers into a four groups as follows.

A Answer: Bohr was the first scientist who introduced the concept of stationary states of electrons in an atom and had explained why the electromagnetic radiation comes from the atom.

B Answer: Bohr's model marked the beginning of spectral analysis as a research method of composition of matter.

C Answer: The Bohr model of the atom had influenced a fast development of atomic physics.

D Answer: The Bohr model of the atom was a revolutionary one because it is still a valid model to explain the structure of matter.

Quantitative data analysis outcome, as answer distribution of 55 students according to the answer category is shown in Figure 3.

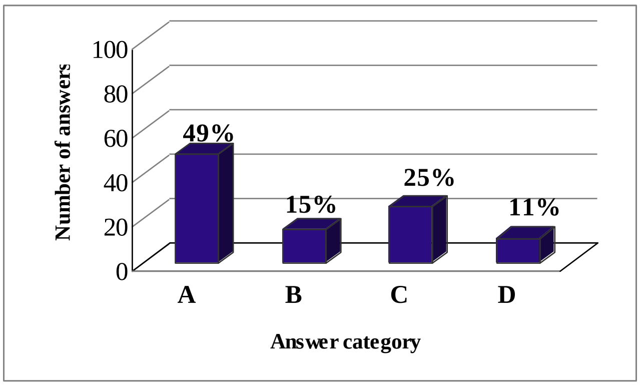


Figure 3. Distribution of students' results by answer category to the third worksheet rubric.

Looking at the students' D answer (Figure 3), one could note that 11% of students in this study did not show their understanding of an importance of Bohr model of the atom looking from the point of view of the history of science. There are still students who think about this model as a model, which is taught in the secondary schools or at the university, as one the entire time valid scientific model. It can be assumed that this group of students does not understand the historical context of the Bohr model of the hydrogen atom. Still, it is the topic in current curricula of both general chemistry and general physics courses.

A total of ten chemistry students gave unacceptable answers and wrong thinking about an atom as structural particulate matter. Several selected answers are as follows.

Significance of Bohr model of the atom is a great because it confirmed that the atom is the smallest particle of matter.

The significance of the Bohr model of the atom because it was the first experimentally confirmed that there is an atom as particulate matter.

When Bohr established his model of the atom all previous studies have lost importance.

According to the obtained students' answers it may be noted that 80% of students who did not know how to express Bohr's postulates have given nevertheless their comments about revolutionary and historic role of this model. Several students' statements are as follows.

Bohr introduced new ideas in physics and chemistry.

Bohr gave the best picture of the atom at the time.

Bohr made a flourishing of science, and his ideas still apply.

Bohr's model of the atom initiated at the time of new experiments in atomic physics.

Comments from both, students who had acceptable or unacceptable answers to the third worksheet rubric are very poor according to the number of sentences used in their writings. It is a proof that they did not show well developed ability to express complex ideas in writing. The students' justifications and comments were in accordance with authors' experiences that students can not easily express their thoughts and knowledge using more sentences and other ways for presenting their knowledge contained in their written test.

Number of 15% of students who gave the answer B is significant evidence that there are chemistry students that were aware of the importance of spectral analysis for research and investigation using optical methods in chemistry and physics.

CONCLUSION

The first and second stage of using history of science in science learning showed by worksheet rubric results that 86% of students failed to give their pictures of atoms according to contemporary scientific model of atom based on quantum theory. Since this is a large percentage of the students, future knowledge integration of students' learning outcomes according to the content of both general chemistry and general physics syllabus has to be a main strategy of curriculum implementation in chemistry study.

Students need to understand the historical context of previous models of the atom, and that, these historically significant models of atoms are used to understand the process of scientific theories creation and scientific knowledge gaining.

Presented students' evaluation results about showed knowledge of Bohr's postulates introduced by Bohr in 1913 are not quite satisfactory. Around 60% of students attached their answers about Bohr model of the hydrogen atom with quantized energy levels and electron moving in circular orbits corresponding to the various allowed energy levels. Students showed knowledge that Bohr suggested that the electron could jump to a different orbit by absorbing or emitting a photon of light with exactly the correct energy content. Each of 60% students knew that Bohr's postulate has explanation of the energy levels in the hydrogen atom represented certain allowed circular orbits. Students' knowledge about Bohr's postulates is in agreement with chemistry textbook content except for a part about Bohr's idea of angular momentum quantization which they will discuss within the General Physics 2 syllabus.

Using historical point of view the Bohr theory and his model of the hydrogen atom can help that students better understand development of contemporary theory of the atom. Students should learn that Bohr's main objective in the process of creating his model was to give an explanation to the stability of the Rutherford model of the atom, instead to learn an incorrect interpretation that Bohr had intention to explain the Balmer spectral formula and the hydrogen line spectrum. Students need to learn "that progress in science evolves through competition between rival and conflicting frameworks, and the work of Thomson, Rutherford, and Bohr is particularly illustrative of this tentative nature of science"(Niaz, 1998, p. 548).

REFERENCES

Bentzen, S.M. (2000). Lise Meitner and Niels Bohr-A Historical Note. *Acta Oncologica*, 39(8), 1002-1003.

Bohr, N. (1922). The structure of the Atom-Nobel Lecture, December 11, 1922. Nobelprize.org, http://www.nobelprize.org/nobel_prizes/physics/laureates/1922/bohr-lecture.pdf (13/05/2013)

Galili, I. (2008). History of Science as a Tool for Teaching. In M. Vicentini & E. Sassi (eds.) Connecting research in Physics Education with Teachers Education.ICPE, http://web.phys.ksu.edu/icpe/Publications/teach2/Galili.pdf, (12/04/2013=

Giancoli, D.C. (1998). *Physics: Principles with Applications*, Sixth Edition. New Jersey Prentice Hall International.

Harrison, A.G., Treagust, D.F. (2000). Learning about Atoms, Molecules, and Chemical Bonds: A Case Study of Multiple-Model Use in Grade 11 Chemistry. *Science Education,* 84, 352-381.

Heilborn, J. L. (1977). Far from being merely „scientific curiosities, " J.J. Thomson's seemingly naive models actually contained some of the fundamental ideas of Niels Bohr's revolutionary quantum theory of the atom. *Physics Today*, April 1977, 23-30.

Kragh, H. (2011). Resisting the Bohr Atom: The Early British Opposition. *Physics in Perspectiv*e, 13, 4-35.

Niaz, M. (1998). From cathode rays to alpha particles to quantum of action: a rational reconstruction of structure of the atom and its implications for chemistry textbooks, *Science Education,* 82, 527-552.

Nakiboglu, C. (2008). Using word associations for assessing non major science students' knowledge structure before and after general chemistry instruction: the case of atomic structure. *Chemistry Education and Research Practice*, 9(4), 309-322.

Niaz, M. and Costu, B. (2009). Presentation of atomic structure in Turkish general chemistry textbooks. *Chemistry Education and Research Practice,* 10(3), 233-240.

Niaz, M., Aguilera, D., Maza A. and Liendo, G., (2002), Arguments, contradictions, resistances, and conceptual change in students' understanding of atomic structure, *Science Education*, 86, 505-525.

Niaz, M. (1998). From cathode rays to alpha particles to quantum of action: A rational reconstruction of structure of the atom and its implications for chemistry textbooks. *Science Education*, 82(5), 527-552.

Rodríguez, M. A. , Niaz, M. (2002). How in spite of the rhetoric, history of chemistry has been ignored in presenting atomic structure in textbooks, *Science & Education,* 11(5), 423-441.

Seker, H (2011). A Facilitator Model for the Use of History of Science in Science Teaching. *Journal of Turkish Science Education*, 8(3), 59-68.

Serway, R.A., Jewett, J. W. (2006). *Principles of Physics - A calculus based text fourth edition*. London: Thomson Learning.

Taber, K.S. (2002). Conceptualizing Quanta: Illuminating the Ground State of Student Understanding of Atomic Orbitals. *Chemistry Education Research and Practice*, 3(2), 145-158.

Taber, K.S. (2003). The atom in the chemistry curriculum: fundamental concept, teaching model or epistemological obstacle? *Foundations of Chemistry*, 5(1), 43-84.

The Nobel Foundation (1922). The Nobel Prize in Physics 1922.Nobelprize.org, www.nobelprize.org/nobel_prizes (12/04/2013)

Weisskopf, V. F. (1985). Niels Bohr, the quantum, and the world. *Physics Today,* October 1985, 191-192.

Summary/Sažetak

Obilježavanje 100 godina Bohrovog modela atoma je prilika da se skrene pažnja studentima o doprinosu Bohra i revolucionarnom razvoju znanosti početkom 20. stoljeća. Podaci prezentirani u ovom radu su rezultati jednog početnog istraživanja u kojem je sudjelovalo 58 studenata prve godine studija hemije, a prikupljeni su da se istraži kako studenti zamišljaju atom, kakvo je njihovo poznavanje Bohrovog modela atoma i historijsko-filozofskog značaja Bohrove teorije. Znanje studenata o Bohrovom modelu atoma se slaže sa sadržajima u udžbenicima koje studenti koriste, osim u dijelu o Bohrovoj ideji kvantizacije momenta količine kretanja elektrona, koji se izučava u okviru kursa Opće fizike 2. Koristeći historijski aspekt Bohrove atomske teorije i njegovog modela atoma vodika, može se pomoći studentima da bolje razumiju razvoj savremene teorije o atomu.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

UDC: ________________________

Technical Paper

2013
40
57-60

Influence of Hydroxypropyl Guar on Water Retention of Cement Based Renders

Medić V.

BFS Franchise Systeme, PJ Kreševo-Laboratory for quality control, Resnik bb, 71260 Kreševo, Bosnia and Herzegovina

Article info
Received: 15/03/2013
Accepted: 06/05/2013

Keywords:
Mortar
Cement based render
Water retention
Cellulose ethers
Guar

***Corresponding author:**
E-mail: vesna.medic.bfs@gmail.com
Phone: +387 30 807 370
Fax: +387 30 807 077

Abstract: Cellulose ethers are polymers introduced into mortar formulations to improve thickening and water retention capacity. Cellulose industry require technology with chemicals treatment which causes the production of industrial wastewater and made this industry for one of the largest pollutants of water and air. This work present the analysis of possibile application of the guar beans powder in cement based renders for partical replacement of the cellulose ether. Technology of basic guar bean powder production is very simple process and present only mechanical operations which don't include a chemical treatments that causes pollutant effects to ecology. Experimental part involves a meassurement of water retention capacity in freshly mixed cement based render independency of quantity of aplicient hydroxypropyl guar. For measuring are Filter plates and conical plastic rings according to EN 459-2 were used for measurements. The results are presented as mass percente of hydroxypropyl guar (HPG) which can be replaced with hydroxyethyl methyl cellulose (HEMC) including high water retention capacity in optimal conditions of used water for preparing fresh mixed mortars. Thereby, the possibility for decraseing consuption of celluloseused in t building material products and constructions has been established which can improve a positive effect by decreasing wood exploatation and decreasing enviromental pollutants.

INTRODUCTION

Factory-made mortars are mainly composed of mineral binders (cement or gypsum and hydrated lime), aggregates and fillers. They also contain different kinds of additives, mostly organics (polysaccharides), in order to impart some specific properties to the mortar, from the fresh paste to the hardened material. Among all polysaccharides, cellulose ethers seem to be the most suitable molecules to produce mortars with very high water retention ability (higher than 97 %). When mortar is applied to substrate, water may be absorbed by the substrate which can induce insufficient hydratation of cement and thus decrease a mechanical properties of the mortar.

Among all polysaccharides, cellulose ethers are able to improve excellent thickening, water retention capacity and workability of fresh mixed mortar. Water retention is a fundamental property, which affects workability and bonds between mortar and masonry (Patural et al, 2011).

The most widespread cellulose ethers used in practice for mortar's formulations are hydroxypropyl methyl cellulose (HPMC) and hydroxyethyl methyl cellulose (HEMC). The cellulose industry is one of the most serious produces of environmental pollutants because of the nature of technological process. The production of basic technical cellulose involves a chemical treatments which results in a production of a different chemical waste.

The guar plant (*Cyamopsis tetragonoloba* L.) is source of natural polysaccharide found in the seeds. Technological process of basic guar gum production is simple and involves only mechanical steps. The plants have the capability to produce basic guar, modified ethers, cationic guar as well as other single and mixed derivates. Guar beans grow in pods which are three to five centimeters long and consists of three main components, the seed coat, the endosperm and the cotyledon. The endosperm accounts for about one third of the bean weight and contains the majority of the polysaccharide (galactomannan).

Basic guar is produced by a process in which the bean splits are hydrated and then milled under high pressure and shear conditions that provide efficient rupturing of the cell walls and release the galactomannan content. After the initial milling stage, the product is dried and reground to produce the basic guar gum powder.

Hydroxypropyl guar (HPG) is one of the guar ethers which is produced by etherification of highly pure guar with base and propylene oxid. HPG is nonionic product which is soluble in cold water and forms thick solutions at low concentrations The molecular structures of basic guar gum and HPG are shown on Figure 1.

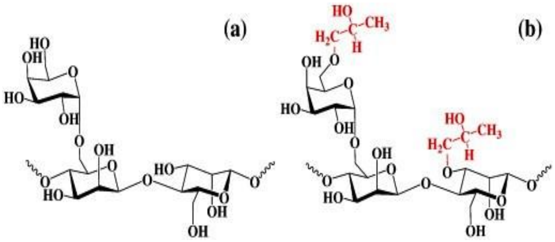


Figure 1: Molecular structures of basic guar gum (a) and HPG (b)

HPG have good thickening property and water retention capacity and can be partially replaced with quantity of cellulose which is used in mortar formulations as a thickener and water retention agent. In this work we used well known recipe and additives in order to produce a mortars with known quality and very high water retention capacity.

EXPERIMENTAL

The renderer has been used in experiment in order to provide adequately consistence and workability of freshly mixed mortar as well as granulometry of aggregates and filler according to Fuller granulometry curve to produce mortar without empty spaces between aggregates.

Amount of 1kg mortar were prepared for each measurement of cement based render. For preparing freshly mixed mortar, water requirement should be optimum, between 20-25%, to provide adequate consistency for workability.

Preparation of dry cement based render

For preparing dry cement based render, portland cement CEM I 52,5 N (properties according to EN 197-1 : 2005) and hydrated lime CL 80 S (properties according to EN 459-1:2010) have been used.

Table 1: Properties of CEM I 52,5 N

Parameters	Properties
SO_3	3,0 %
Cl^-	0,0005 %
Loss on ignition	1,3 %
Insoluble residue	1,0 %
Initial setting time	150 min
Soundness (Le Chatelier)	0,8 mm
Compressive strength/28 days	58 MPa

Table 2: Properties of hydrated lime CL 80 S.

Parameters	Properties
CaO+MgO	3,0%
MgO	0,81%
CO_2	1,3%
SO_3	1,0%
Free water	150 min
Soundness (Le Chatelier)	0,8 mm
Penetration	26 mm
Air content	3,3%

Limestone sand 0,8 – 1,4 mm and 0,0 – 0,125 mm, have been used as aggregates and filler whose properties are given in Table 3.

Table 3: Chemical composition of limestone sand and filler.

Parameters	%
CaO	53,61
MgO	1,66
Cl^-	0,0006
SiO_2	0,45
R_2O_3	0,51
Loss on ignition	43,57
Free water	0,04

HEMC and HPG have been used for thickening and water retention capacity.

Table 4: Properties of HEMC.

Parameters	Properties
Appearance	white powder
Viscosity	20,000-27,000 mPa*s
Particle size	<125µm: min 90%
Moisture	< 7 %
Water retention	98,8 %

Table 5: Properties of HPG.

Parameters	Properties
Appearance	beige powder
Viscosity	3 000- 6 000mPa*s
Particle size	<125µm: min 85%
Moisture	< 3 %
Water retention	92,7 %
pH (2 % solution)	7,1-8,9

Starch ether for slip resistance and air entraining agent have been used for preparation of dry mortar whose properties are given in Table 6 and 7.

Dry cement based render is prepared in order to produce freshly-mixed mortar which can provide very good quality of rheological requirements and workability.

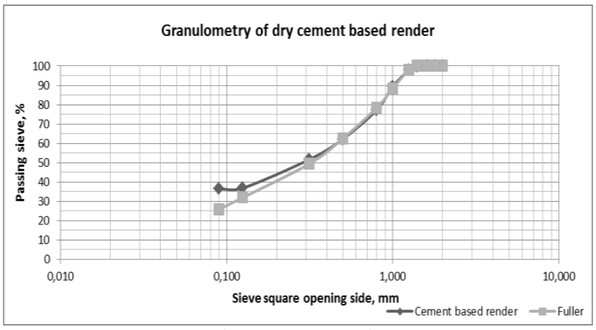


Figure 2: Composition of aggregates and filler.

Granulometry is determined according to Fuller's curve of aggregates for cement concretes in order to provide optimal composition of aggregates and filler.

Table 6: Properties of starch ether.

Parameters	Properties
Appearance	powder
Oleinic sulphonat	90-98 %
Potassium carbonate	< 5 %
pH	10-11
Bulk density	300g/L

Table 7: Properties of starch ether.

Parameters	Properties
Appearance	powder
Viscosity	20 mPa*s
Particle size	<125µm: min 60%
Moisture	4 %
Bulk density	600g/L

The recipe which is used for preparing cement based render is given in Table 8.

Table 8: The recipe for preparing dry cement based render

Composition	%
Portland cement	12
Hydrated lime	6
Filler	7
Limestone sand	65
Thickener and WR agent	0.12
Starch ether	0.025
Air entraining agent	0.025

Preparation of freshly mixed mortar and measuring of water retention

Freshly mixed mortars have been prepared with enought amount of water in order to produce consistency which flow diameter's value is 170 ± 3 mm. In this way good workabillity and apllication of material at surface have been provided , as well as sufficient hydration of the cement. For determination, a sufficiente quantity of water as a thickener in the recipe is used together with HEMC with zero composition of HPG to provide determination the variety of consistency and water retention capacity dependency of quantity of aplicient HPG.

The amount of water has been placed into laboratory mixer and mixed, then the timer was started and dry mortar placed into mixer during first 10 sec. Mixture was continuously mixied in period of 60 seconds. After mixing was finished, mortar was placed into mould in order to determined the flow diameter according to EN 459-2. When the required flow diameter has not been achived with the selected amount of water, the correct amount of water was determined by using other mixtures with different quantities of water. Freshly mixed mortar with correct consistency has been used for measuring of water retention.

For determining the water retention filter paper plate (190 mm x 190 mm x 2 mm), conical plastic ring (140 mm smaller and 150 mm larger inside diameter, 12 mm in height) , two plastic plates (200 mm x 200 mm x 5 mm) and nonwoven tissue according to standard have been used.

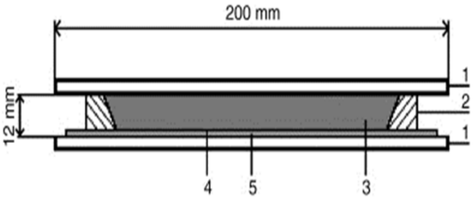


Figure 3: Apparatus for water retention measuring according to standard EN 459-2 (1- plactic plate, 2- conical plastic ring, 3- freshly-mixed render, 4- nonwoven tissue, 5- filter plate).

Water retention of the freshly mixed render is expressed as that percentage of water which remains in the mortar after short suction time on filter paper.

Water retention (WR) was calculated following formulations according to the EN 459-2:

$$WR = 100 - W_4$$

$$W_4 = \frac{m_{20} - m_{17}}{(m_{19} - m_{18})\ x \left(\frac{m_{21}}{m_{21} + m_{22}}\right)}\ x\ 100\%$$

where W_4 is the relative loss of water after suction time, m_{21} is the total mass of water in fresh mortar, m_{22} is the mass of dry mortar, m_{23} is the mass of mortar in the plastic ring, m_{20} is the mass of the soaked filter plate and the plastic plate, m_{19} is the mass of the plastic plate, filter plate, nonwoven tissue and plastic ring with mortar filling, m_{18} is the mass of the plastic plate, filter plate, nonwoven tissue and plastic ring.

RESULTS AND DISCUSSION

Freshly mixed render with HEMC prepared with correct consistency for workability and parameters was measured in order to provide observation of parameters variabillity dependency of quantity apllied HPG. Water retention has been mesured twice at each sample of freshly mixed mortar and avarege is used as the result. Following the increase of applied HPG which is replaced with HEMC correlation between consistency and water retention capacity has been detected. The results demonstrated that the increase of HPG has effect on dimeter's value of consistency and the water retention capacity of freshly mixed render decreasing both values. This also have influence on workabillity and application of material at the surface, which is shown in Table 9.

Table 9: Results of water retention measuring

HPG %	Water %	Consistency mm	WR %
0	22.5	170	98.8
5	22.5	170	98.6
10	22.5	170	98.3
15	22.5	169	98.1
20	22.5	168	98.0
25	22.5	168	97.8
30	22.5	168	97.5
35	22.5	167	97.2
40	22.5	166	97.0
45	22.5	165	96.6
50	22.5	165	96.2
55	22.5	164	96.0
60	22.5	163	95.8
65	22.5	161	95.4

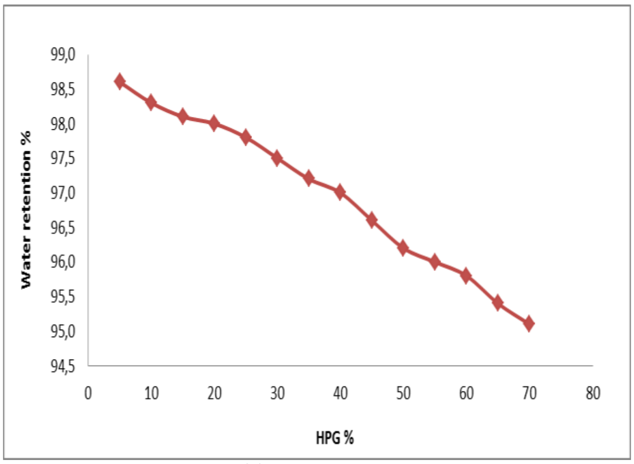


Figure 3: Water retention of freshly mixed cement based render-

CONCLUSION

The conclusion of this study is that HPG as a product of guar gum plant can be used as an additive in building materials. About 25-30 % of cellulose ether in cement based render can be replaced with HPG at conditions of optimal demand of water for preparing freshly mixed mortars. This quantity of HPG is acceptable for consistency and workability of freshly mixed mortar as well as water retention capacity wich is higher than 97 %.

REFERENCES

Bayer, R., Lutz, H. (2003), *Dry mortars, Documents and resources for small bussineses and professionals* www.docstoc.com/docs/97096514/dry-mortar, (29/09/2011).

Jaganjac, A., Tahirović, I. (2005). *Osnove hemijske tehnologije za studente hemije*.

Murray, P., *Fracturing fluid properties, Documents and resources for small bussineses and professionals*, www.docstoc.com/docs/74092945/Fracturing-Fluid-Properties,
(18/03/2011).

Nandhini Venugopa, K., Abhilash, M. (2010), *Study of hidratation kinetics and rheological behaviour of guar gum*, International Journal of Pharma Sciences and Research (IJPSR),1(1),28-39.

Patural, L., Marchal, P., Govin, A., Rout, B., Deves, O., Grosseau, P. (2011), *Cellulose ethers influence on water retention and consistency in cement based mortars*, Cement and concrete research, 41(1), 46-55.

Sultan, M., Ashiq Rabbani, M., Shinwari, Z.K., Shahid Masood, M. (2012), *Phenotypic divergence in guar (Cyamopsis tetragonoloba) landrace genotypes in Pakistan*, Pakistan Journal of Botany, 44(SI1), 203-210.

Standard EN 459-2:2010, (2011). *Building lime-Part 2: test methods, Physical tests: Water retention (Procedure and Evaluation).*

Summary/Sažetak

Celulozni eteri primjenjuju se u proizvodnji mortova kao učvršćivač i sredstvo za retenciju vode svježe pripremljenog morta, što olakšava obradu materijala. Sama proizvodnja tehničke celuloze zahtijeva tehnološki postupak obrade kemikalijama, što industriju celuloze čini jednom od najvećih zagađivača vode i zraka. Cilj ovog rada je analizirati mogućnosti primjene sjemena guara u cementnim mortovima kao djelomičnu zamjenu za celulozu. Obzirom da proces proizvodnje primarnog oblika guarovog praha uključuje samo mehaničke postupke, bez tretiranja kemijskim supstancama, sam proces nema negativan utjecaj na okoliš. U eksperimentalnom dijelu primijenjen je eterificirani oblik guara, hidroksipropil guar (HPG), kao zamjena za hidroksietil-metil celulozu (HEMC). Rezultati obuhvataju mjerenje sposobnosti retencije vode u cementnom malteru u odnosu na količinu primijenjenog HPG u optimalnim granicama zahtijeva za vodom suhog morta. Za mjerenje retencije vode korištene su filter ploče i kalupi prema propisanoj normi. Rezultatima analiza ustanovljena je granična količina HPG koja se može zamijeniti sa HEMC , a da se pri tome zadrži visoka retencija vode (> 97 %) i zahtjev za konzistencijom ostane u optimalnim granicama u odnosu na količinu dodane vode. Na ovaj način dat je uvid u mogućnosti primjene biljke guara kao aditiva u proizvodnji građevinskih mortova, što ima pozitivan utjecaj na smanjenje eksploatacija šumskih površina i zagađenja okoliša uzrokovanog otpadnim tvarima iz industrije celuloze.

Udruženje Društvo kemičara i tehnologa Kantona Sarajevo

Društvo kemičara i tehnologa Kantona Sarajevo je osnovano 03.02.2000. godine s ciljem promoviranja kemije u različitim granama naše društvene zajednice. U martu mjesecu 2013.godine ponovo je održana Izborna skupština na kojoj se okupio veći broj kemičara i tehnologa na kojoj je izabrano novo rukovodstvo Društva. Društvo kemičara i tehnologa Kantona Sarajevo je jedinstveno, samostalno, dobrovoljno, stručno i naučno udruženje kemičara, kemijskih inženjera i tehnologa, fizičkih i pravnih osoba.

Zadatak Društva je razvijati i unaprijeđivati naučnu, stručnu i nastavnu djelatnost na svim područjima čiste i primjenjene kemije. Svoj zadatak Društvo ostvaruje tako što okuplja kemičare, kemijske inženjere i tehnologe radi unaprjeđenja kemijske i kemijsko inženjerske struke te srodnih struka; održava predavanja i rasprave; štampa naučne i stručne periodične publikacije te monografije; priređuje naučne i stručne sastanke, simpozije, kongrese i izložbe, kao i mnoge druge aktivnosti.

Sjedište Društva je u Sarajevu, ulica Zmaja od Bosne 35. Prvi korak Društvo je napravilo aktiviranjem web stranice: http://www.dktks.ba/. Na web stranici su postavljeni razni sadržaji koji mogu biti korisni za sve kemičare i tehnologe. Svi oni koji žele postati članovi Društva kemičara i tehnologa Kantona Sarajevo, mogu se učlaniti i putem ove web stranice.

Izborna skupština, mart 2013. godine

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA

Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 11.03.2013. godine, donijela je

ODLUKU O IMENOVANJU ČLANOVA

Skupštine Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Skupštinu udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Hajrudin Hajdar, predsjednik
2. Dalibor Karačić, podpredsjednik
3. Fehim Korać, glavni urednik društvenih glasila

Član 2.

Izabrani članovi Skupštine Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Skupštine traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-03/13

11. mart 2013. Predsjednik Skupštine

Sarajevo

Hajrudin Hajdar

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA

Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 11.03.2013. godine, donijela je

ODLUKU O IMENOVANJU PREDSJEDNIKA I ČLANOVA

Upravnog Odbora
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Upravni odbor udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se :

1. Harun Kurtagić, predsjednik
2. Aida Šapčanin, podpredsjednik
3. Jelena Ostojić, sekretar
4. Sabina Žero, blagajnik
5. Sanja Ćavar, glavni urednik
6. Tarik Fetahagić, član
7. Reuf Bajrović, član

Član 2.

Izabrani članovi Upravnog odbora Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Upravnog odbora traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-04/13

11. mart 2013.

Sarajevo

Predsjednik Skupštine

Hajrudin Hajdar

DKT KS
DRUŠTVO KEMIČARA I TEHNOLOGA
KANTONA SARAJEVO
BOSNA I HERCEGOVINA

Zmaja od Bosne 35, 71000 Sarajevo, Bosna i Hercegovina
Tel. +387 33 279 911
Web: www.dktks.ba, E-mail: dktks.sekretar@gmail.com

Na osnovu člana 18. Zakona o udruženjima i fondacijama ("Službene novine Federacije BiH", broj 45/02), i člana 19. Statuta Udruženja "Društvo kemičara i tehnologa Kantona Sarajevo" Skupština Društva na sjednici održanoj dana 11.03.2013. godine, donijela je

ODLUKU O IMENOVANJU ČLANOVA

Nadzornog Odbora
Udruženja – Društvo kemičara i tehnologa Kantona Sarajevo

Član 1.

U Nadzorni odbor udruženja „Društvo kemičara i tehnologa Kantona Sarajevo“ imenuju se:

1. Faiza Muštović Bišćević, predsjednik
2. Sead Hrustanović, član
3. Ismet Tahirović, član

Član 2.

Izabrani članovi Nadzornog odbora Udruženja su postali kandidati sa najvećim brojem glasova. Mandat članova Nadzornog odbora traje 2 godine.

Član 3.

Ova odluka stupa na snagu danom donošenja, a objavit će se u glasilu Društva.

Broj: P-05/13

11. mart 2013.

Sarajevo

Predsjednik Skupštine

Hajrudin Hajdar

IN MEMORIAM

Mira Glavaš

(1932-2011)

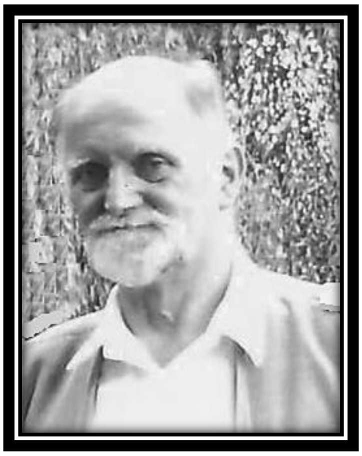
Mira (Jakova) Glavaš, rođena Banjalučanka, diplomirala je na Filozofskom fakultetu Univerziteta u Sarajevu 1956.g. i dobila zvanje diplomirani hemičar, a doktorsku disertaciju odbranila je 1964 g. na Prirodno-matematičkom fakultetu Sarajevu. Svoj radni vijek, profesorica Glavaš je provela na Prirodno-matematičkom fakultetu, najprije kao asistent, a onda kao dugogodišnji nastavnik u oblasti anorganske hemije. Penzionisana je 1994 g. u zvanju redovnog profesora.

Sa sigurnošću se može reći da profesorica Mira Glavaš ima ogromne zasluge u razvoju savremene anorganske hemije u Bosni i Hercegovini. Njen naučni opus skoncentriran je na hemiju kompleksnih jedinjenja, mehanizame anorganskih reakcija kao i termičke metode analize. Objavila je 31 naučni rad od kojih su mnogi u, još uvijek, najprestižnijim časopisima svijeta iz oblasti anorganske hemije kao što su Polyhedron, Journal American Chemical Society, Inorganic Chemistry i sl. Uz brojne naučne projekte, profesorica Glavaš je učestvovala na 14 naučnih skupova u zemlji i inostranstvu. Njen naučni rad, rezultirao je i međunarodnom saradnjom sa kolegama sa Univerziteta u Minesoti (USA) sa kojima je objavila radove u koautorstvu.

Uz sve to, profesorica Glavaš je bila dugogodišnji rukovodilac Katedre za opštu i anorgansku hemiju Prirodno-matematičkog fakulteta u Sarajevu *koja predstavlja jednu od najstarijih Katedri današnjeg Prirodno-matematičkog fakulteta. Za doprinos razvoju Univerziteta u Sarajevu dodjeljena joj je Plaketa Univerziteta. Njen uporan i predan rad na razvoju moderne anorganske hemije ostavio je neizbrisiv trag među nama koji dalje idemo njenim stopama.*

Prof.dr. Emira Kahrović
Šef katedre za opštu i anorgansku hemiju

IN MEMORIAM



Vladimir Milićević

(1933-2006)

Profesor Vladimir (Nika) Milićević rođen je u Sarajevu gdje se i školovao. 1958 g. diplomirao je hemiju na Filozofskom fakultetu Univerziteta u Sarajevu od kada je započela njegova univerzitetska karijera. Radio je na Medicinskom, Filozofskom i Prirodno-matematičkom fakultetu kao asistent i nastavnik, najviše na Opštoj hemiji i Metodici nastave hemije. Njegova nastavnička aktivnost i entuzijazam u širenju ljubavi prema hemiji rezultirala je i brojnim udžbenicima, naročito za srednje škole.

Obavljao je brojne dužnosti u privrednim organizacijama i na Univerzitetu. Njegov doprinos razvoju Odsjeka za hemiju i Prirodno-matematičkog fakulteta ne može se mjeriti samo činjenicama da je obavljao funkcije počevši od Šefa Katedre za opštu i anorgansku hemiju, šefa Odsjeka do Dekana Fakulteta, nego ukupnošću njegovog djelovanja na polju nastave, nauke i pomoći u stručnom usavršavanju u oblasti hemije. Za ukupan rad dobio je čak 13 priznanja od društvenog i naučno-stručnog značaja među kojima treba istaći Plaketu Univerziteta u Sarajevu za višegodišnji uspješan rad i značajan doprinos u razvoju Univerziteta i Orden zasluga za narod-srebrna zvijezda.

U mirovinu je otišao kao redovni profesor 2003 g. a dvije godine kasnije izabran je u zvanje profesor emeritus. Ostavio je neizbrisiv trag među svojim saradnicima i studentima.

Prof.dr. Emira Kahrović
Šef katedre za opštu i anorgansku hemiju

INSTRUCTIONS FOR AUTHORS

GENERAL INFORMATION

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

Categories of Contributions

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

Reviewing the Manuscript

All contributors are evaluated according to the criteria of originality and quality of their scientific content, and only those deemed worthy will be accepted for publication. To facilitate the reviewing process, authors are encouraged to suggest three persons competent to review their manuscript. Such suggestions will be taken into consideration but not always accepted.

The Editor-In-Chief and Editors have the right to decline formal review of a manuscript when it is deemed that the manuscript is:

1. on a topic outside the scope of the Journal;
2. lacking technical merit;
3. of insufficient novelty for a wide international readership;
4. fragmentary and providing marginally incremental results; or
5. is poorly written.

Proofs

When a manuscript is ready for printing, the corresponding author will receive a PDF-formatted manuscript for proof reading, which should be returned to the journal within one week. Failure to do so will be taken as the authors are in agreement with any alteration which may have occurred during the preparation of the manuscript.

Copyright

Subscribers may reproduce tables of contents or prepare lists of articles including abstracts for internal circulation within their institutions. Permission of the Publisher is required for resale or distribution outside the institution and for all other derivative works, including compilations and translations.

Professional Ethics and Publication Policy

The journal expects the Editors, Referees and authors to adhere to the well-known standards of professional ethics. Authors are responsible for the factual accuracy of their contributions. Submission of the paper commits the author not to submit the same material elsewhere. Referees should act promptly. If certain circumstances preclude prompt attention to the manuscript at the time it is received, the non-received manuscript should be returned immediately to the Editor or the Referee should contact the Editor for possible delay of the report submission date. The Editor accepts full responsibility for his decisions on the manuscripts.

PREPARATION AND SUBMISSION OF MANUSCRIPT

Cover Letter

Manuscripts must be accompanied by a cover letter in which the type of the submitted manuscript. It should contain:

1. full name(s) of the author(s),
2. mailing address (address, phone and fax numbers, e-mail) of the author to whom correspondence should be addressed,
3. title of the paper (concise, without any abbreviations),
4. type of contribution,
5. a statement that the article is original and is currently not under consideration by any other journal or any other medium, including preprints, electronic journals and computer databases in the public domain, and
6. the names, full affiliation (department, institution, city and country), and
7. e-mail addresses of three potential Referees.

Contributors from Bosnia and Herzegovina should provide the name and full affiliation of at least one Referee from abroad.

Authors are fully encouraged to use ***Cover Letter Template*.**

Manuscript preparation

The submitted articles must be prepared with Word for Windows. Manuscripts should be typed in English (either standard British or American English, but consistent throughout) with 1.5 spacing (12 points Times New Roman; Greek letters in the character font Symbol) in A4 format leaving 2.5 cm for margins. Authors are fully encouraged to use ***Manuscript Template***.

All contributions should be written in a style that addresses a wider audience than papers in more specialized journals. Manuscripts with grammar or vocabulary deficiencies are disadvantaged during the scientific review process and, even if accepted, may be returned to the author to be rewritten in idiomatic English. The authors are requested to seek the assistance of competent English language expert, if necessary, to ensure their English is of a reasonable standard. The journal maintains its policy and takes the liberty of correcting the English of manuscripts scientifically accepted for publication.

Tables and figures and/or schemes should not be embedded in the manuscript but their position in the text indicated. In electronic version (Word.doc document) tables and figures and/or schemes should follow the text, each on a separate page. Please number all pages of the manuscript including separate lists of references, tables and figures with their captions.

IUPAC and International Union of Biochemistry and Molecular Biology recommendations for the naming of compounds should be followed.

SI units, or other permissible units, should be employed. The designation of physical quantities should be in Times New Roman font. In text, graphs, and tables, brackets should be used to separate the designation of a physical quantity from the unit. Please do not use the axes of graphs for additional explanations; these should be mentioned in the figure captions and/or the manuscript (example: “pressure at the inlet of the system, kPa” should be avoided).

Percents and *per mills*, although not being units in the same sense as the units of dimensioned quantities, can be treated as such. Unit symbols should never be modified (for instance: w/w %, vol.%, mol.%) but the quantity measured has to be named, *e.g.* mass fraction, w=95 %; amount (mole) fraction, x=20 %.

Latin words, as well as the names of species, should be in *italic*, as for example: *i.e., e.g., in vivo, ibid, Artemisia annua* L., *etc*. The branching of organic compound should also be indicated in *italic*, for example, *n*-butanol, *tert*-butanol, *etc*.

Decimal numbers must have decimal points and not commas in the text (except in the Bosnian/Croatian/Serbian abstract), tables and axis labels in graphical presentations of results. Thousands are separated, if at all, by a comma and not a point.

Structure of the Manuscript

The manuscript must contain, each on a separate page, the title page, abstract in English, (abstract in Bosnian/Croatian/Serbian), graphical abstract (optional), main text, list of references, tables (each table separately), illustrations (each separately), and legends to illustrations (all on the same page).

1. ***Title page*** must contain: the title of the paper (bold letters), full name(s) of the author(s), full mailing addresses of all authors (italic), keywords (up to 6), the phone and fax numbers and the e-mail address of the corresponding author.
2. A one-paragraph ***abstract*** written of 150–200 words in an impersonal form indicating the aims of the work, the main results and conclusions should be given and clearly set off from the text. Domestic authors should also submit, on a separate page, a Summary/Sažetak. For authors outside Bosnia and Herzegovina, the Editorial Board will provide a Bosnian/Croatian/Serbian translation of their English abstract.
3. Authors are encouraged to submit a ***graphical abstract*** that describes the subject matter of the paper. It should contain the title of the paper, full name(s) of the author(s), and graphic that should be no larger than 11 cm wide by 5 cm tall. Authors are fully encouraged to use ***Graphical Abstract Template.***
4. ***Main text*** should have the following form:
    - ***Introduction*** should include the aim of the research and a concise description of background information and related studies directly connected to the paper.
    - ***Experimental*** section should give the purity and source of all employed materials, as well as details of the instruments used. The employed methods should be described in sufficient detail to enable experienced persons to repeat them. Standard procedures should be referenced and only modifications described in detail.
    - ***Results and Discussion*** should include concisely presented results and their significance discussed and compared to relevant literature data. The results and discussion may be combined or kept separate.
    - The inclusion of a ***Conclusion*** section, which briefly summarizes the principal conclusions, is highly recommended.
    - ***Acknowledgement*** (optional).
    - Please ensure that every ***reference*** cited in the text is also present in the reference list (and *vice versa*). Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with either "Unpublished results" or "Personal communication" Citation of a reference as "in press" implies that the item has been accepted for publication. As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. No more than 30 references should be cited in your manuscript.

      In the text refer to the author's name (without initials) and year of publication (e.g. "Steventon, Donald and Gladden (1994) studied the effects..." or "...similar to values reported by others (Anderson, Douglas, Morrison, *et al.*, 1990)..."). Type the names of the first three authors at first citation. At subsequent citations use first author *et al.* The list of references should be arranged alphabetically by authors' names and should be as full as possible, listing all authors, the full title of articles and journals, publisher and year.

Examples of ***reference style***:

a) Reference to a journal publication:
   Warren, J. J., Tronic, T. A., Mayer, J. M. (2010). Termochemistry of proton-coupled electron transfer reagents and its implications. *Chemical Reviews*, *110 (12)*, 6961-7001.
b) Reference to a book:
   Corey, E. J., Kurti, L. (2010). *Enantioselective chemical synthesis*. (1st Ed.) Direct Book Publishing, LLC.
c) Reference to a chapter in an edited book:
   Moody, J. R., Beck II, C. M. (1997). Sample preparation in analytical chemistry. In Setlle, F. A. (Ed.), *Handbook of instrumental techniques for analytical chemistry*. (p.p. 55-72). Prentice Hall.
d) Reference to a proceeding:
   Seliskar, C. J., Heineman, W.R., Shi, Y., Slaterbeck, A.F., Aryal, S., Ridgway, T.H., Nevin, J.H. (1997). *New spectroelectrochemical sensor*, in Proceedings of 37th Conference of Analytical Chemistry in Energy and Technology, Gatlinburg, Tenesee, USA, p.p. 8-11.
e) Patents:
   Healey, P.J., Wright, S.M., Viltro, L.J., (2004). *Method and apparatus for the selection of oral care chemistry*, The Procter & Gamble Company Intellectual Property Division, (No.US 2004/0018475 A1).
f) Chemical Abstracts:
   Habeger, C. F., Linhart, R. V., Adair, J. H. (1995). Adhesion to model surfaces in a flow through system. *Chemical Abstracts,* CA 124:25135.
g) Standards:
   ISO 4790:1992. (2008). *Glass-to-glass sealings - Determination of stresses*.
h) Websites:
   Chemical Abstract Service, www.cas.org, (18/12/2010).

- ***Tables*** are part of the text but must be given on separate pages, together with their captions. The tables should be numbered consequently in Latin numbers. Quantities should be separated from units by brackets. Footnotes to tables, in size 10 font, are to be indicated consequently (line-by-line) in superscript letters. Tables should be prepared with the aid of the Word table function, without vertical lines. Table columns must not be formatted using multiple spaces. Table rows must not be formatted using Carriage returns (enter key; ↵ key). Tables should not be incorporated as graphical objects.
- ***Figures and/or Schemes*** (in high resolution) should follow the captions, each on a separate page of the manuscript. High resolution illustrations in TIF or EPS format (JPG format is acceptable for colour and greyscale photos, only) must be uploaded as a separate archived (.zip or .rar) file.

Figures and/or Schemes should be prepared according to the artwork instructions.

- ***Mathematical and chemical equations*** must be numbered, Arabic numbers, consecutively in parenthesis at the end of the line. All equations should be embedded in the text except when they contain graphical elements (tables, figures, schemes and formulae). Complex equations (fractions, inegrals, matrix…) should be prepared with the aid of the Word Equation editor.

Artwork Instructions

Journal accepts only TIF or EPS formats, as well as JPEG format (only for colour and greyscale photographs) for electronic artwork and graphic files. MS files (Word, PowerPoint, Excel, Visio) are NOT acceptable. Generally, scanned instrument data sheets should be avoided. Authors are responsible for the quality of their submitted artwork.

Image quality: keep figures as simple as possible for clarity - avoid unnecessary complexity, colouring and excessive detail. Images should be of sufficient quality for the printed version, i.e. 300 dpi minimum.

Image size: illustrations should be submitted at its *final size* (8 cm for single column width or 17 cm for double column width) so that neither reduction nor enlargement is required.

Photographs: please provide either high quality digital images (250 dpi resolution) or original prints. Computer print-outs or photocopies will not reproduce well enough for publication. Colour photographs rarely reproduce satisfactorily in black and white.

The facility exist for color reproduction, however the inclusion of color photographs in a paper must be agreed with Editor in advance.

Reporting analytical and spectral data

The following is the recommended style for analytical and spectral data presentation:

1. ***Melting and boiling points***:
   mp 163–165°C (lit. 166°C)
   mp 180°C dec.
   bp 98°C
   Abbreviations: mp, melting point; bp, boiling point; lit., literature value; dec, decomposition.
2. ***Specific Rotation***:
   $[a]^{23}_{D}$ –222 (c 0.35, MeOH).
   Abbreviations: a, specific rotation; D, the sodium D line or wavelength of light used for determination; the superscript number, temperature (°C) at which the determination was made; In parentheses: *c* stands for concentration; the number following *c* is the concentration in grams per 100 mL; followed by the solvent name or formula.

3. ***NMR Spectroscopy***:
   ^1H NMR (500 MHz, DMSO-*d*$_6$) d 0.85 (s, 3H, CH_3), 1.28–1.65 (m, 8H, 4´CH_2), 4.36–4.55 (m, 2H, H-1 and H-2), 7.41 (d, *J* 8.2 Hz, 1H, ArH), 7.76 (dd, *J* 6.0, 8.2 Hz, 1H, H-1'), 8.09 (br s, 1H, NH).
   ^{13}C NMR (125 MHz, $CDCl_3$) d 12.0, 14.4, 23.7, 26.0, 30.2, 32.5, 40.6 (C-3), 47.4 (C-2'), 79.9, 82.1, 120.0 (C-7), 123.7 (C-5), 126.2 (C-4).
   Abbreviations: d, chemical shift in parts per million (ppm) downfield from the standard; *J*, coupling constant in hertz; multiplicities s, singlet; d, doublet; t, triplet; q, quartet; and br, broadened. Detailed peak assignments should not be made unless these are supported by definitive experiments such as isotopic labelling, DEPT, or two-dimensional NMR experiments.
4. ***IR Spectroscopy***:
   IR (KBr) n 3236, 2957, 2924, 1666, 1528, 1348, 1097, 743 cm^{-1}.
   Abbreviation: n, wavenumber of maximum absorption peaks in reciprocal centimetres.
5. ***Mass Spectrometry***:
   MS *m/z* (relative intensity): 305 (M^+H, 100), 128 (25).
   HRMS–FAB (*m/z*): $[M+H]^+$ calcd for $C_{21}H_{38}N_4O_6$, 442.2791; found, 442.2782.
   Abbreviations: *m/z*, mass-to-charge ratio; M, molecular weight of the molecule itself; M^+, molecular ion; HRMS, high-resolution mass spectrometry; FAB, fast atom bombardment.
6. ***UV–Visible Spectroscopy***:
   UV (CH_3OH) l_{max} (log e) 220 (3.10), 425 nm (3.26).
   Abbreviations: l_{max}, wavelength of maximum absorption in nanometres; e, extinction coefficient.
7. ***Quantitative analysis***:
   Anal. calcd for $C_{17}H_{24}N_2O_3$: C 67.08, H 7.95, N 9.20. Found: C 66.82, H 7.83, N 9.16. All values are given in percentages.
8. ***Enzymes and catalytic proteins relevant data:***
   Papers reporting enzymes and catalytic proteins relevant data should include the identity of the enzymes/proteins, preparation and criteria of purity, assay conditions, methodology, activity, and any other information relevant to judging the reproducibility of the results[1]. For more details check Beilstein Institut/STRENDA (standards for reporting enzymology data) commission Web site (http://www.strenda.org/documents.html).

---

[1] For all other data presentation not mentioned above please contact Editor for instructions.

Submission Checklist

The following list will be useful during the final checking of an article prior to sending it to the journal
for review:

- E-mail address for corresponding author,
- Full postal address,
- Telephone and fax numbers,
- All figure captions,
- All tables (including title, description, footnotes),
- Manuscript has been "spellchecked" and "grammar-checked",
- References are in the correct format for the journal,
- All references mentioned in the Reference list are cited in the text, and *vice versa*.

Submissions

Submissions should be directed to the Editor by e-mail: ***glasnik@pmf.unsa.ba***, or ***glasnikhtbh@gmail.com***. All manuscripts will be acknowledged on receipt (by e-mail) and given a reference number, which should be quoted in all subsequent correspondence.

Glasnik hemičara i tehnologa Bosne i Hercegovine

Bulletin of the Chemists and Technologists of Bosnia and Herzegovina

Print ISSN: 0367-4444
Online ISSN: 2232-7266

Zmaja od Bosne 33-35, BA-Sarajevo
Bosnia and Herzegovina
Phone: +387-33-279-918
Fax: +387-33-649-359
E-mail: glasnik@pmf.unsa.ba
glasnikhtbh@gmail.com

Sponsors

www.prevent.ba

HIGRACON

HIGRACON d.o.o. Sarajevo
Dzemala Bijedica br.2
71000 Sarajevo
Bosnia and Herzegovina

Tel. +387 33 718 286
Fax. +387 33 718 285
GSM: +387 62 994 254
E-mail: higracon@bih.net.ba

www.higracon.ba