

UNIVERSITY OF BELGRADE

FACULTY OF CHEMISTRY

Najat M. Aburas

**ELECTROCHEMICAL BEHAVIOR AND  
ANTIOXIDANT ACTIVITY OF  
TETRADENTATE SCHIFF BASES AND  
THEIR COPPER(II) COMPLEXES**

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Najat M. Aburas

**ELEKTROHEMIJSKO PONAŠANJE I  
ANTIOKSIDATIVNA AKTIVNOST  
TETRADENTATNIH ŠIFOVIH BAZA I  
NJIHOVIH BAKAR(II) KOMPLEKSA**

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**MENTOR:**

Dr Rada Baošić, docent Hemijskog fakulteta

Univerziteta u Beogradu

**ČLANOVI KOMISIJE:**

Dr Živoslav Tešić, redovni profesor Hemijskog fakulteta

Univerziteta u Beogradu

Dr Snežana Mandić, redovni profesor Hemijskog fakulteta

Univerziteta u Beogradu

Dr Dragan Manojlović, vanredni profesor Hemijskog fakulteta

Univerziteta u Beogradu

Dr Ljiljana Jakšić, redovni profesor Rudarsko-geološkog fakulteta

Univerziteta u Beogradu

**DATUM ODBRANE:**

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## Elektrohemijsko ponašanje i antioksidativna aktivnost tetradentatnih Šifovih baza i njihovih bakar(II) kompleksa

### Abstrakt

U doktorskoj disertaciji je ispitano elektrohemijsko ponašanje tetradentatnih Šifovih baza i njihovih bakar(II) kompleksa primenom ciklične voltametrije. Izvršena je optimizacija uslova pri kojima je urađeno ispitivanje. Ispitan je uticaj strukture i uticaj supstuenata na elektrohemijsko ponašanje. Utvrđeno je da supstituenti imaju merljiv efekat na elektrohemijsko ponašanje Šifovih baza i njihovih bakar (II) kompleksa. Induktivni i sterni efekti, različitih supstituenata, utiču na promenu elektronske gustine jona metala, kao i na baznost atoma azota, koji je prisutan u diaminskom mostu. Utvrđena je razlika i pravilnost u elektrohemijskom ponašanju posmatranog seta jedinjenja, što se detektuje pomeranjem anodnih i katodnih potencijala zbog prirode Šifovih baza, kada su slobodni (kao ligandi) ili koordinativno vezani u kompleksima bakra(II).

Pored toga određena je antioksidativna aktivnosti navedenih jedinjenja primenom TLC-DPPH eseja. Primenom ove metode omogućen je kontakt, a samim tim i reakcija između jedinjenja i slobodnih radikala nezavisno od primenjenog rastvarača što je prednost u poređenju sa spektrofotometrijskom metodom. Ispitana je veza između strukture (definisane molekulskim deskriptorima) i antioksidativne aktivnosti, elektrohemijskog ponašanja i antioksidativne aktivnosti i pretpostavljen je mehanizam ispoljavanja antioksidativne aktivnosti posmatrane serije jedinjenja.

Uspostavljene su korelacije između redoks potencijala i antioksidativne aktivnosti. Jedinjenja sa jakim antioksidativnim aktivnostima pokazuju sposobnost oksidacije na niskim potencijalima, što omogućava da se oksidacioni potencijal primeni kao pokazatelj sposobnosti eliminacije slobodnih radikala.

U cilju boljeg razumevanja reakcionog puta i mehanizma ispoljavanja antioksidativne aktivnosti urađena su DFT izračunavanja kvantno-hemijskih deskriptora. Dipolni moment, energija disocijacije veze, naelektrisanje na atomima kiseonika i azota (N7 i N10) pokazuju jako visoku korelaciju sa eksperimentalno određenom antioksidativnom aktivnošću. Antioksidativna aktivnost bakar (II) kompleksa je u direktnoj zavisnosti od

dela strukture koju definiše helatni ugao ravni i ukupna spinska gustina. Na osnovu odabranih deskriptora, koji pokazuju visoku korelaciju sa antioksidativnom aktivnošću, pretpostavljen je mehanizam reakcije ispoljavanja ove aktivnosti. Slobodni radikal (DPPH) može da bude neutralisan ili direktnom redukcijom putem premeštanja elektrona (kompleksi Šifovih baza) ili proton (Šifove baze).

Rezultati ukazuju na činjenicu da poznavanje elektronskih i prostornih efekata koji kontrolišu redoks procese posmatranog seta jedinjenja omogućava ciljnu sintezu novih, strukturno sličnih, jedinjenja sa izraženijom antioksidativnom aktivnošću.

Ključne reči: Šifove baze, bakar(II) kompleksi Šifovih baza, elektrohemijsko ponašanje, antioksidativna aktivnost, mehanizam antioksidativne aktivnosti, DFT, ciklična voltometrija

Naučna oblast: Hemija

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## Electrochemical behavior and antioxidant activity of tetradentate Schiff bases and their copper(II) complexes

### Abstract

In this doctoral dissertation was examined the electrochemical behavior tetradentate Schiff bases and their copper (II) complexes using cyclic voltammetry. The optimization of conditions and investigation of effects of the structure and impact of substituents the electrochemical behavior was done. It has been found that the substituents have a detectable effect on the electrochemical behavior of the Schiff bases and their copper(II) complexes. Their inductive and steric effects influence the change in electron density on metal ion and basicity of nitrogen. Therefore, there is the difference in electrochemical behavior, which is detected by shifting of anodic and cathodic potentials values strongly influenced by the nature of the Schiff base when they are free or bound as ligands in the complex compounds.

In addition it was determined the antioxidant activity of investigated compounds with TLC-DPPH assay. This method provides an opportunity to contact and reaction of the compounds and free radicals independent of the solvent used to prepare the solution, which is an advantage compared to the spectrophotometric method. Based on results was investigated the relationship between structure (defined molecular descriptors) and antioxidant activity, electrochemical behavior and antioxidant activity and assumes a mechanism to manifest antioxidant activity observed series of compounds.

The correlation between the redox potential and antioxidant activity was established. Compounds with a strong antioxidative activities show the ability of the low oxidation potential, which allows the oxidation potential to be applied as indication of the ability of eliminating of free radicals.

For better understanding the reactivity patterns and mechanisms of activity of antioxidants, the DFT-based quantum-chemical descriptors were calculated. Quantum-chemical descriptors are the numerical representation of the molecular structures. Dipole moment, bond dissociation energy, charge on oxygen atoms and charge on hydrogen atoms on N7 and N10 show high correlation with antioxidant activity of the investigated Schiff bases. Antioxidant activity of copper(II) complexes is

highly correlated with chelate plane angle and sum spin density descriptors. Selected descriptors, which have the best correlation with antioxidant activity, shed light on the reaction mechanism of scavenging/antioxidant activity of the studied compounds. Free radical (DPPH) may be neutralized either by direct reduction via electron-transfer (Schiff base complexes) or by radical quenching via hydrogen atom transfer (Schiff bases).

The results point to the fact that knowledge of the physical and electronic effects of the processes which control the redox processes of investigated and structural similar compounds offer very interesting research opportunities and may be critical in the design of new ones.

**Key words:** Schiff bases, Schiff base copper(II) complexes, electrochemical behaviour, antioxidant activity, mechanisms of antioxidant activity, DFT, cyclic voltametry

Scientific field: Chemistry

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## 1. INTRODUCTION

Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antibacterial, antifungal and antitumor activity. Among the organic reagents actually used, Schiff bases possess excellent characteristics, structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural properties. Schiff base complexes play a vital role in designing metal complexes related to synthetic and natural oxygen carriers. Metal complexes make these compounds effective as stereospecific catalysts towards oxidation, reduction, hydrolysis, biological activity and other transformations of organic and inorganic chemistry. Unfortunately, most Schiff bases are chemically unstable and show a tendency to be involved in various equilibria, like tautomeric interconversions, hydrolysis, or formation ionized species. Therefore, successful application of Schiff bases requires a careful study of their characteristics. It is important to determine the potential capability for antioxidant activity of these interesting compounds and find the factors that lead to the manifestation of antioxidant activity and if it is possible to determine the mechanism of antioxidant activity. Also, determination of antioxidant activity using thin-layer and TLC scanner provides an opportunity to contact and reaction of the compounds and free radicals independent of the solvent used to prepare the solution. This allows the determination of antioxidant activity of compounds that are not soluble in the solvent used for preparation of stable free radicals (the same solvents are needed in the spectrophotometric determination).

Investigation and determination of electrochemical behaviour of Schiff bases and their complexes provides an opportunity for control of their behavior under different condition. Cyclic voltammetry has become an important and widely used electroanalytical technique in this area. Substituents have a detectable effect on the electrochemical behavior of the Schiff bases and their complexes. Their inductive and steric effects influence the change in electron density on metal ion and basicity of nitrogen. Knowledge of the electronic and steric effects that control redox processes of these compounds offers very interesting research opportunities and may be critical in the design of new one.

The structural characteristics of molecules are expressed through molecular descriptors and definitions of those molecular parameters that are responsible for the antioxidant activity. It is known that the antioxidant activity is conceivably related to the electrochemical behavior. To facilitate observation of the impact structure and finding the part that is responsible for the redox behavior and therefore the antioxidant activity, the DFT-based quantum-chemical descriptors were calculated. Quantum-chemical descriptors are the numerical representation of the molecular structures.

Aim of this doctoral dissertation is to evaluate the influence of the substituents on electrochemical behavior and the effect of structures on antioxidative activity of these compounds as well as relation between obtained redox potentials and antioxidant activity. Objectives of this thesis were:

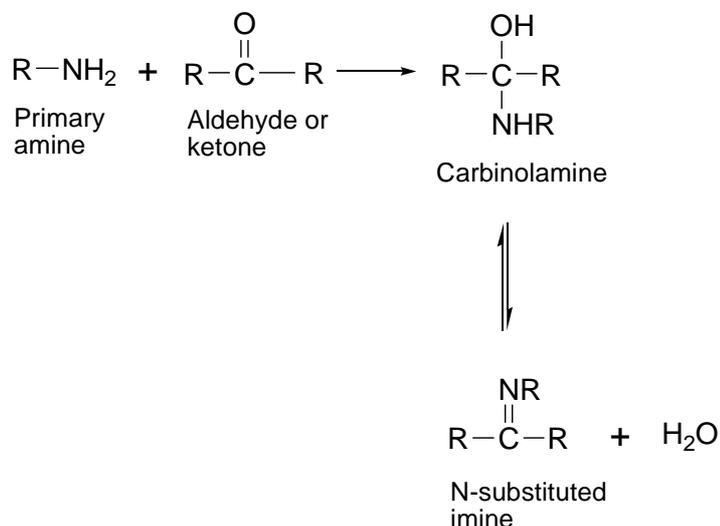
- a) synthesis and purification of the investigated tetradentate Schiff bases, and their copper (II) complexes
- b) cyclic voltammetry conditions optimization
- c) recording the cyclic voltammograms of the investigated compounds at different scanning speeds
- d) discussion of recorded voltammograms
- e) determination of antioxidant activity using the DPPH-TLC assay
- f) correlation of structure and electrochemical behavior
- g) correlation of structure (defined molecular descriptors) and antioxidant activity
- h) determination of mechanism of antioxidant activity of investigated compounds.

## 2. GENERAL PART

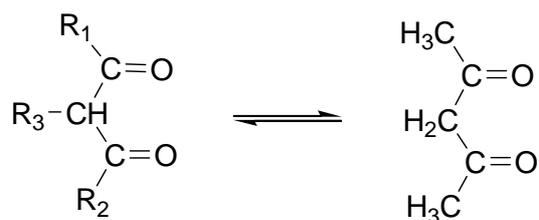
### 2.1. Schiff bases

Schiff bases are compounds containing,  $\text{>C=N-}$  azomethine group. They are nitrogen analogous of an aldehyde or ketone in which the  $\text{>C=O}$  group is replaced by  $\text{>C=N-R}$  group. These compounds are also known as anils, imines or azomethines. It is important to emphasize that Schiff bases are compounds having a formula  $\text{RR}_1\text{C=NR}_2$  where R is an aryl group,  $\text{R}_1$  is a hydrogen atom and  $\text{R}_2$  is either an alkyl or aryl group [1]. The Schiff base class is very versatile as compounds can have a variety of different substituents. They can be unbridged or N,N'-bridged. Most commonly Schiff bases have NO or  $\text{N}_2\text{O}_2$ -donor atoms. The oxygen can be replaced by sulphur, nitrogen, or selenium atoms [2].

The formation of a Schiff base from an aldehydes or ketones is a reversible reaction and generally takes place under acid or base catalysis, or upon heating.

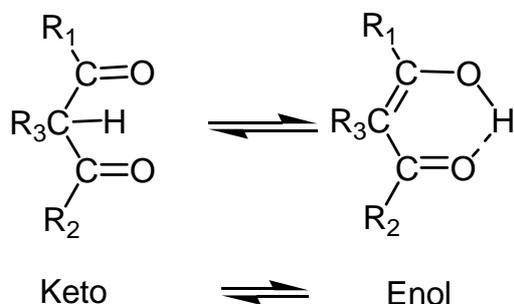


$\beta$ -diketones,  $\text{R}_1\text{COCHR}_3\text{COR}_2$ , from which it is possible to synthesize Schiff base, are very important class of ligands, which have been widely investigated due to their varied coordination modes [3,4]. Extensive work has been done on various substituted  $\beta$ -diketones (Figure 1) in which  $\text{R}_3 = \text{H}$ ,  $\text{R}_1$  and  $\text{R}_2 = \text{aryl}$ , alkyl, cyclo alkyl or heterocyclic groups [5].



**Figure 1.** Structure of  $\beta$ -diketone

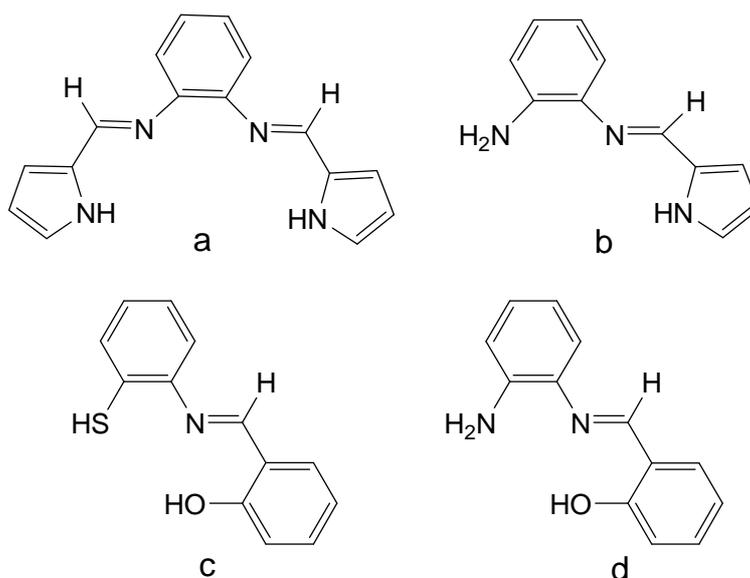
This group of compound is capable of keto-enol tautomerism (Figure 2). The hydrogen atom of the  $CHR_3$  group is activated by the adjacent  $>C=O$  groups, leading to conjugate system via a prototropic shift. These tautomers exist in equilibrium with each other [6].



**Figure 2.** Keto-enol tautomerism in  $\beta$ -diketones

The presence of electron withdrawing groups or phenyl at the  $R_1$  and/or  $R_2$  position(s), increases enolisation, while electron releasing substituents like alkyl and methoxy group increased the keto tautomers [7,8]. Enolisation is not possible if the  $\alpha$ -carbon atoms are disubstituted. Consequently, formation of chelates with metal ions would obviously be impossible. The enol tautomer is stabilized by intramolecular hydrogen bonding, since it is less polar than the keto tautomer. The tautomeric forms of  $\beta$ -diketones are readily distinguished by infrared spectroscopy and NMR studies [9]. Generally, the enol tautomer reacts with appropriate metal salts to form chelate complexes. The high stability of these complexes, have been attributed to presence of „benzenoid resonance“ in addition to the „enolate resonance“ and in fact, these complexes have been treated as aromatic system [10,11]. There are three coordination modes for  $\beta$ -diketones: (i) O,O'-chelation of a keto-tautomer i.e. coordination is via the two carbonyl of a  $\beta$ -diketone, and (ii) O-unidentate coordination of enol.

$\beta$ -ketoamines,  $R_1COR_3C=CR_2NHR_4$  are molecules having a  $\beta$ -diketone structure, in which one  $>C=O$  group has been replaced by an  $NHR_4$  group. They are capable of acting either as weak acids or bases by virtue of the  $>C=O$  or amino groups respectively [12]. They may assume the following structural forms depending on the amine from which they are derived, amines or diamines, (Figure 3).

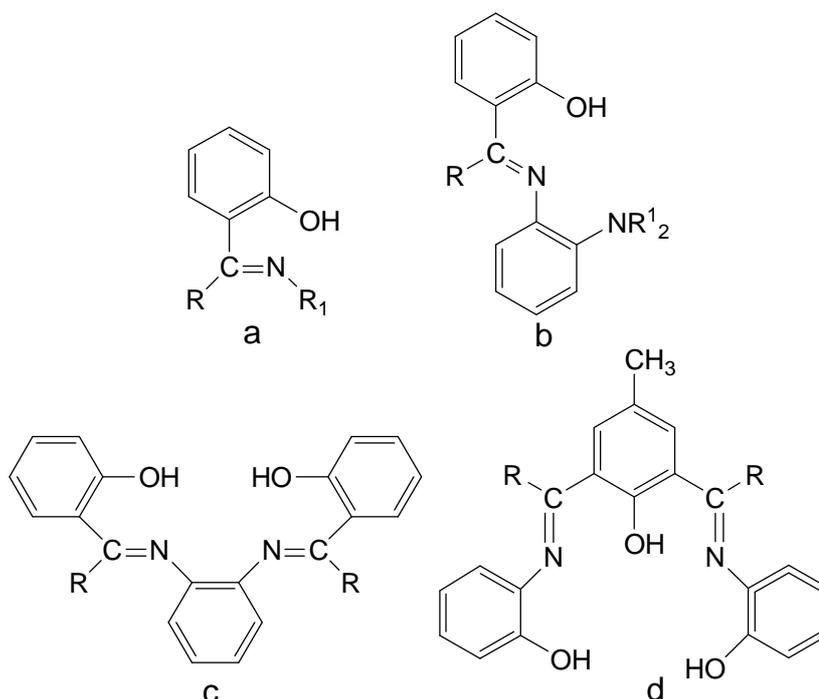


**Figure 3.** Structures of some Schiff bases: a)  $N,N'$ -bis(pyrrole-2-carboxalidene)-1,2-diaminobenzene; b)  $N$ -pyrrole-2-carboxalidene-1,2-diaminobenzene; c)  $N$ -salicylidene-2-aminothiophenol; d)  $N$ -salicylidene-1,2-diaminobenzene

As mentioned above, Schiff bases are functional groups that contain a carbon-nitrogen double bond ( $>C=N-$ ) with the nitrogen atom connected to an aryl or alkyl group, but not hydrogen. They are of the general formula  $R_1R_2C=N-R_3$ , where  $R_3$  is an aryl or alkyl group that makes the Schiff base a stable imine. This chelating ability of the Schiff bases combined with the ease of preparation and flexibility in varying the chemical environment about the  $>C=N-$  group makes it an interesting ligand in coordination chemistry. Presence of a lone pair of electrons in a  $sp^2$  hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical importance and impart excellent chelating ability especially when used in combination with one or more donor atoms close to the azomethine group.

## 2.2. Schiff base complexes

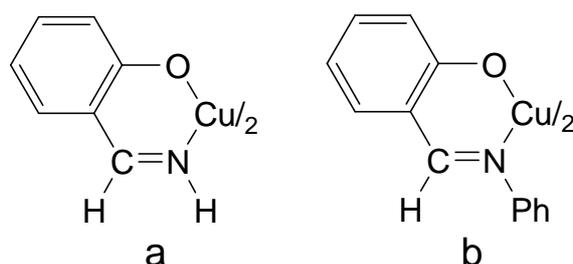
Schiff bases are generally bidentate, tridentate, tetradentate or polydentate ligands capable of forming very stable complexes with transition metals. They can only act as coordinating ligands if they bear a functional group, usually the hydroxyl, sufficiently near the site of condensation in such a way that a five or six membered ring can be formed when reacting with a metal ion (Figure 4). The tetradentate Schiff base class is of the type reported in this thesis.



**Figure 4.** Some classes of Schiff base ligands: a) bidentate; b) tridentate; c) tetradentate; d) pentadentate

Metal complexes of Schiff bases have occupied a central role in the development of coordination chemistry. This is due to the fact that Schiff bases offer opportunities for inducing substrate chirality's, tuning the metal centered electronic factor, and enhancing the solubility and stability of either homogeneous or heterogeneous catalysts [13]. Schiff base complexes are known to offer (i) ease and flexibility of the synthetic procedure; (ii) diverse properties; and (iii) use as biological models or biologically active compounds. Many of them are centered on the catalytic activity of Schiff base complexes in a large number of homogeneous and heterogeneous reactions.

Metal complexes of the Schiff bases are generally prepared by treating metal salts with Schiff base ligands under suitable experimental conditions [14,15]. Transition metal complexes of Schiff bases have been known for 130 years. Dark green crystalline solid, bis(salicylaldimino)Cu(II), had been isolated from reaction of cupric acetate, salicylaldehyde, and aqueous ammonia as well as their phenyl and aryl derivatives [16,17]. Structures of these compounds are shown on Figure 5. Even more Hugo Schiff determined the stoichiometric ratio of ligand and metal (1:2) [18].



**Figure 5.** Structures of a) bis(salicylaldimino)Cu(II) and b) their phenyl derivative

In recent years, there has been a growing interest in the synthesis, characterization and crystal structures of copper(I) and copper(II) Schiff base complexes, not only because they have interesting properties and structural diversity but also because they have found important application in catalysis for the coupling of phenylacetylene with halobenzene, preparation of supramolecular assemblies the design of single and double-stranded architectures and the grid complexes [19-27]. Also, many efforts have been devoted to the design and synthesis of new Schiff base ligands that would be able to control the crystal structure of copper(I) complexes [28-30].

Structure and mechanism of the formation of the Schiff base complexes and stereochemistry of four coordinate chelate complexes formed from Schiff bases and their analogues have been discussed in several reviews [31]. The configuration of the chelate group in the four coordinate complexes may be square-planar, tetrahedral, distorted tetrahedral or distorted trigonal pyramidal with the metal atom at the apex. The configuration depends primarily on the nature of the metal atom and also on the symmetry of the ligand field. A variety of physicochemical investigations on these complexes provide a clear understanding of their stereochemical and electronic properties.

### 2.3. Application of Schiff bases and their metal complexes

Schiff bases and their metal complexes have wide applications in food industry, dye industry, analytical chemistry, catalysis, fungicidal, agrochemical and biological activities [32,33]. They are used, e.g. in optical and electrochemical sensors, as well as, in various chromatographic methods, to enable detection of enhance selectivity and sensitivity [34]. Among the organic reagents actually used, Schiff bases possess excellent characteristics, structural similarities with natural biological substances, relatively simple preparation procedures and the synthetic flexibility that enables design of suitable structural properties. Schiff base complexes of transition metals have been frequently used as catalysts in diverse processes, such as oxygen and atom transfer, enantioselective epoxidation and aziridination, mediating organic redox reactions and as mediators in other oxidation processes. Schiff bases are widely applicable in analytical determination, using reactions of condensation of primary amines and carbonyl compounds in which the azomethine bond is formed (determination of compounds with an amino or carbonyl group); using complex formation reactions (determination of amines, carbonyl compounds and metal ions); or utilizing the variation in their spectroscopic characteristics following changes in pH and solvent (pH of solvent polarity indicators). Unfortunately, most Schiff bases are chemically unstable and show a tendency to be involved in various equilibria, like tautomeric interconversions, hydrolysis, or formation ionized species. Therefore, successful application of Schiff bases requires a careful study of their characteristics.

#### 2.3.1. Antimicrobial and antitumor activities

Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antibacterial antifungal and antitumor activity [35,36]. Schiff base complexes play a vital role in designing metal complexes related to synthetic and natural oxygen carriers. Metal complexes make these compounds effective as stereospecific catalysts towards oxidation, reduction, hydrolysis, biological activity and other transformations of organic and inorganic chemistry [37].

Agrawal et al. [38] were screened a number of substituted Schiff bases for antifungal activity in vitro against pathogenic fungi, namely, *Sclerotium rolfsii* and *Rhizoctonia bataticola*, and for their effect on nitrification inhibition under laboratory conditions. Maximum antifungal activity was exhibited by (2,4-dichlorobenzylidene)-(2,4,5-trichlorophenyl)-amine and (3-nitrobenzylidene)-(2,4,5-trichlorophenyl)-amine against both fungi with range from 3 to 24 µg/mL. Maximum nitrification inhibition was exhibited by (2,4-dichlorobenzylidene)-(2-fluorophenyl)-amine, (4-fluorophenyl)-(3-benzylidene)-amine, (2,6-dichlorobenzylidene)-(4-fluorophenyl)-amine, and (2,6-dichlorobenzylidene)-(3-fluorophenyl)-amine.

Chohan et al [39] synthesized the Schiff bases by the reactions of 2-aminonicotic acid and salicylaldehyde, 5-bromosalicylaldehyde, 5-nitrosalicylaldehyde and 5-methoxysalicylaldehyde, respectively. Similarly, the Schiff bases were prepared by condensing the amino component, 2-amino-1,3,4-thiadiazole with the respective aldehydes i.e. furfuraldehyde, thiophene-2-carboxaldehyde and pyrrole-2-carboxaldehyde. In a similar fashion, the Schiff bases were derived from 5-amino-1,3,4-thiadiazole-2-thiol and the respective aldehydes i.e. furfuraldehyde, thiophene-2-carboxaldehyde, 4-bromothiophene-2-carboxaldehyde, pyrrole-2-carboxaldehyde, salicylaldehyde, and pyridine-2-carboxaldehyde. Some of these Schiff bases, were used as potential ligands for synthesizing their cobalt(II), nickel(II) and zinc(II) complexes. The synthesized Schiff bases and their transition metal complexes were screened for their in vitro antibacterial activity against Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*) and Gram-positive (*Bacillus subtilis*, *Staphylococcus aureus*) bacterial strains by the agar-well diffusion method. The synthesized Schiff bases were found exhibit no or low to moderate activity against one or more bacterial species. On the contrary, all the metal complexes exhibited varied activity against different bacteria. The Schiff bases, which were inactive before complexation became active and less active ones, became more active upon coordination with metal ions. The metal complexes showed comparatively much higher activity.

Schiff bases play an important role in bioinorganic chemistry as they exhibit remarkable biological activity. Schiff bases form an interesting class of chelating ligands that has enjoyed popular use in the coordination chemistry of

thiosemicarbazones. Their complexes have received considerable attention since, because of their pharmacological properties; they have numerous applications (for example as antibacterial and anticancer agents) [40,41]. Besides the structural diversities and bonding interactions, bioisosteric relationship of thiophene to benzene has led to several structures of drug analogs in which benzene rings have been replaced by thiophene rings and the thiophene derivatives have application as important therapeutic agents.

In the field of medicine, a series of aryl azo Schiff bases derived from 4-N,N'-bis(2-cyanoethylaminobenzylidene) with diazotized aromatic amines showed some anticancer activity but no anti-HIV activity and its homologues with different aromatic and heterocyclic amines and its adducts of hypophosphoric acid, showed high activity against human tumor cells *in vitro* and *in vivo* system. The Schiff base, derived from S-benzylthiocarbamate had significant anti-cancer activity. This free Schiff base ligand was very effective against melanoma [42].

Recently, the research relating with metal complexes of heteronuclear Schiff bases has expanded enormously and now comprising their interesting aspects in coordination chemistry with a special emphasis in bioinorganic chemistry. A use of organosilicon and organotin compounds as reagents or intermediates in the inorganic synthesis has further strengthened their applications. Moreover, metal complexes of organosilicon(IV) and organotin(IV) halides with N, O, and S donor atoms have received much more consideration due to their industrial, environmental, and biological applications. These donor atoms have been used to enhance the biological activity of organosilicon and organotin derivatives. Organosilicon(IV) complexes have been subjected of interest for their versatile applications in pharmaceutical and chemical industries. Organosilicon compounds of nitrogen and sulphur containing ligands are well known for their anticarcinogenic, antibacterial, antifungal, tuberculostatic, and insecticidal activities. Similarly, organotin compounds are the active components in a number of biocidal formulations in such diverse areas as fungicides, miticides, molluscicides, antifouling paints and surface disinfectants [43].

Also, Schiff bases complexes exhibited various antimicrobial activities. Divalent nickel and copper complexes of the Schiff bases derived from *o*- and *p*-aminothiophenol and sulphanilic acid, phenylbutazone or oxyphenylbutazone and *o*-aminophenol, iodo-

and bromo-vanillin and different substituted amines; and 1-(3,4-dihydroxybenzylidene)thiosemicarbazone were all effective against *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Cryptococcus neoforms* [44]. Similarly, divalent cobalt, nickel, copper, zinc, cadmium and mercury complexes of Schiff bases: N-(5-phenylazosalicylidene)aniline, 2-(5-phenylazosalicylideneamino)phenol, N-(5-phenylazosalicylidene)-o-toluidine, O-(N-pyrrolideneimino)phenol and O-(N-thiophene-2-carbonalimine) benzoic acid were all effective against *Sarcina lutea*, *Bacillus subtilis*, *Bacillus pumplies* and *Staphylococcus aureus*, while the divalent zinc complex of tetradentate macrocyclic Schiff base derived from 1,10-phenanthroline-2,9-dicarboxyaldehyde-phenylhydrazine and 1,2-dibromoethane showed fungi toxicity against *Aspergillus niger*, *Fusarium solani*, *Shizosaccharomyces arthrosporus* and *Rhizopus spp* [45].

### 2.3.2. Miscellaneous Applications

Schiff bases are used as corrosion inhibitor which is based on their ability to spontaneously form a monolayer on the surface to be protected. Many commercial inhibitors include aldehydes or amines, but presumably due to the C=N bond the Schiff bases function more efficiently in many cases. Fluorinated Schiff base derived from 3,4-difluorobenzaldehyde and 4,4'-benzidine were used as inhibitor in steel [46].

Despite all the above, a large number of different Schiff base ligands have been used as cation carriers in potentiometric sensors. They have shown excellent selectivity, sensitivity, and stability for specific metal ions such as Ag(II), Al(III), Co(II), Cu(II), Gd(III), Hg(II), Ni(II), Pb(II), Y(III), and Zn(II) [47]. Indeed, from the ion sensor functional point of view it is crucial that the electroactive material dispersed in a polyvinylchloride (PVC) membrane exhibits strong affinity for a particular metal ion in order to enable selective permeation of one metal ion through the membrane electrodes [48-50].

Mixed Schiff base complexes are used as pigments in the paint industry e.g.  $[ML_1L_2(H_2O)_2]$  (where M = Ni(II), Cu(II), Zn(II); L<sub>1</sub> and L<sub>2</sub> = Schiff bases derived from 7-formyl-8-hydroxyquinoline and o-hydroxy-4-methoxybenzophenone or 2-amino-5-chlorobenzophenone using o-toluidine and p-toluidine as primary aromatic

amines) were used in making white, blue and green paints [51]. In catalysis, divalent metal complexes of Schiff bases have been extensively used in the oxidation of alcohols, cyclopropanation, and base hydrolysis of amino acid esters. In these reactions, the Fe(II), Ru(II) and Cu(II) compounds of Schiff base were the most effective [52,53].

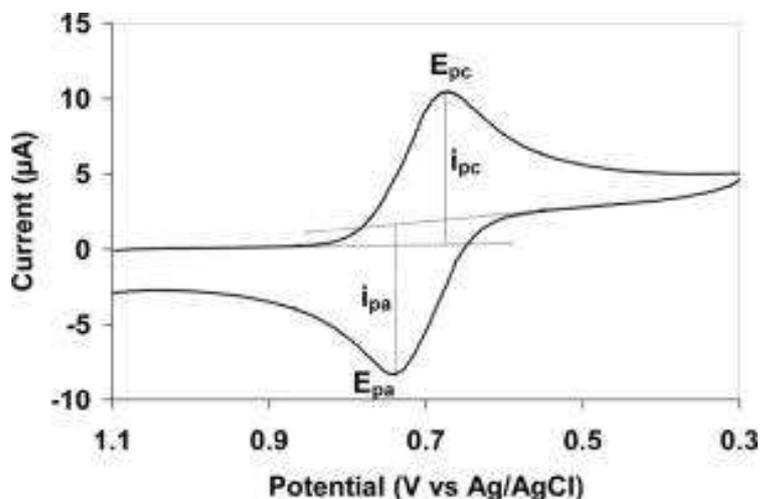
In chromatographic separations/applications, divalent nickel and copper complexes of various  $\beta$ -ketoamines such as bis(acetylaceton)ethylenediimine, bis(acetylaceton)propylenediimine, bis(acetylaceton)-dl-stilbenediimine and bis(acetylaceton)-meso-stilbenediimine were used as stationary phases in the separation of hydrocarbons, heteroaromatic compounds, aldehydes, ketones and alcohols, while the  $\beta$ -ketoamine such as N,N'-propylenebis(acetylacetonimine) was used as the derivatizing ligand in the chromatographic separation of the non-fluorinated tetradentate  $\beta$ -ketoamine chelates of nickel(II), palladium(II) and copper(II) at nanogram level without degradation [54,55].

A ruthenium(III) Schiff base complex was used in the fabrication of chloride PVC-based membrane sensor [56]. It could also be used as an indicator electrode in the potentiometric titration of chloride ions with silver nitrate solution. Gupta et al [57] recently reported a potentiometric aluminium sensor based on the use of N,N'-bis(salicylidene)-1,2-cyclohexanediamine as a neutral carrier in polyvinyl chloride matrix. It was successfully applied for direct determination of aluminium(III) in biological, industrial and environmental samples. In addition to the homogeneous catalytic reaction, supported transition metal Schiff base complexes also find wide application in catalysis [58-60].

#### **2.4. Electrochemical behavior of Schiff bases and their metal complexes**

Cyclic voltammetry (CV) has become an important and widely used electroanalytical technique in many areas of chemistry. It is used for the study of redox processes, for understanding reaction intermediates, and for obtaining stability of reaction products. In a cyclic voltammetry experiment the working electrode potential is ramped linearly versus time like linear sweep voltammetry. Cyclic voltammetry takes the experiment a step further than linear sweep voltammetry which ends when it reaches a set potential. When cyclic voltammetry reaches a set potential, the working electrode's

potential ramp is inverted. This inversion can happen multiple times during a single experiment. The current at the working electrode is plotted versus the applied voltage to give the cyclic voltammogram (Figure 6). Cyclic voltammetry is generally used to study the electrochemical properties of an analytes in solution.



**Figure 6.** Cyclic voltammogram

Cyclic voltammetry is carried out in stable solution to ensure diffusion control. A three-electrode arrangement is used. Mercury film electrodes are often used because of their good negative potential range. Other working electrodes include glassy carbon (GC), platinum (Pt), gold (Au), graphite (C), and carbon paste (CP). In cyclic voltammetry, the electrode potential ramps linearly versus time, as already mentioned. This ramping is known as the experiment's scan rate (V/s). The potential is applied between the reference electrode and the working electrode, and the current is measured between the working electrode and the counter electrode. These data are then plotted as current ( $i$ ) vs. potential ( $E$ ) (Figure 6). As the waveform shows, the forward scan produces a current peak for any analytes that can be reduced or oxidized (depending on the initial scan direction) through the range of the potential scanned. The important parameters in a cyclic voltammogram are the peak potentials ( $E_{pc}$ ,  $E_{pa}$ ) and peak currents ( $i_c$ ,  $i_a$ ) of the cathodic and anodic peaks, respectively. The current will increase as the potential reaches the reduction potential of the analyte, but then falls off as the concentration of the analyte is depleted close to the electrode surface. If the redox couple is reversible then, when the applied potential is reversed, it will reach the

potential that will reoxidize the product formed in the first reduction reaction, and produce a current of reverse polarity from the forward scan. This oxidation peak will usually have a similar shape to the reduction peak. As a result, information about the redox potential and electrochemical reaction rates of the compounds is obtained.

If the electron transfer process is fast compared with other processes (such as diffusion), the reaction is electrochemically reversible, and the peak separation is:

$$\Delta E_p = E_{pa} - E_{pc} = \frac{2.303 \cdot R \cdot T}{n \cdot F}$$

Thus, for a reversible redox reaction at 25 °C with  $n$  electrons  $\Delta E_p$  should be  $\frac{0.059}{n}$  V

or about 60 mV for one electron. In practice this value is difficult to attain because of such factors as cell resistance. Irreversibility due to a slow electron transfer rate results in  $\Delta E_p > \frac{0.059}{n}$  V, greater than 70 mV for a one-electron reaction. The formal

reduction potential ( $E^0$ ) for a reversible couple is given by the following equation:

$$E^0 = \frac{E_{pc} + E_{pa}}{2}$$

For a reversible reaction, the concentration is related to peak current by the Randles–Sevcik expression (at 25 °C):

$$i_p = 2.686 \cdot 10^5 \cdot n^{3/2} \cdot A \cdot c \cdot D^{1/2} \cdot v^{1/2}$$

where  $i_p$  is the peak current (amperes),  $A$  is the electrode area ( $\text{cm}^2$ ),  $D$  is the diffusion coefficient ( $\text{cm}^2 \text{s}^{-1}$ ),  $c^0$  is the concentration in  $\text{mol cm}^{-3}$ , and  $v$  is the scan rate in  $\text{V s}^{-1}$ .

For the reactions that are 'slow' (so called quasi-reversible or irreversible electron transfer reactions) the voltage applied will not result in the generation of the concentrations at the electrode surface predicted by the Nernst equation. This happens because the kinetics of the reaction are 'slow' and thus the equilibria are not established rapidly (in comparison to the voltage scan rate). For quasi-reversible systems the current is controlled by both the charge transfer and mass transport. The voltammograms of a quasireversible system are more drawn out and exhibit a larger separation in peak potentials compared to a reversible system.

For instance if the electron transfer at the surface is fast and the current is limited only by the diffusion of species to the electrode surface, then the current peak

will be proportional to the square root of the scan rate. This relationship is described by the Cottrell equation [61-63].

The electrochemical behavior of three synthesized Schiff base Co(II) complexes with N<sub>2</sub>SO donor group was investigated in different non-aqueous media such as acetonitrile (AcN) and 50% (w/w) dichloromethane-dimethylsulfoxide (DCM-DMSO) mixture as aprotic solvents at the surface of solid electrodes (Pt, Au and GC) using tetrabutylammonium perchlorate as supporting electrolytes by cyclic voltammetry [64]. It has been found that, these compounds exhibit one or two quasi-reversible oxidation peaks. Also, the charge transfer coefficients ( $\alpha$ ) and the diffusion coefficients (D) for these compounds in various solvents were obtained. The effect of scan rate and dielectric constant of solvents on the redox behavior of compounds were evaluated.

The electrochemical behavior of copper(II) complexes with the Schiff base N,N'-ethylenebis(salicylideneimine) in aqueous phosphate buffer (pH 7.0) was studied by polarographic and voltammetric techniques at a mercury electrode by Fariasercio et al [65]. The resulting polarograms and voltammograms consist of a single quasi-reversible one-electron transfer attributable to the coupling of Cu(II)salen/Cu(I) salen via an EC mechanism. In differential pulse adsorptive stripping voltammetry (DPV) the response of the Cu(II) salen complex is linear over the concentration range 0 to  $3.2 \cdot 10^{-7}$  molL<sup>-1</sup>. For an accumulation time of 30 seconds, the detection limit was found to be  $1.0 \cdot 10^{-8}$  molL<sup>-1</sup>. Square-wave (SWV) and cyclic voltammetric (CV) measurements indicated that the Cu(II)-salen complex and its reduction product adsorb at the electrode surface.

Also, the electrochemical behaviors of a series of Schiff base 3-[5-phenylpyrazol-3-ylimino]indol-2-ones synthesized by the reaction of various 5-substituted isatins with 3-amino-5-phenyl-pyrazole has been investigated and compared with corresponding isatin in dimethylformamide in 0.1 M LiCl using cyclic voltammetry at Hanging Mercury Drop Electrode. All synthesized Schiff bases exhibit a single irreversible two-electron reduction wave in contrast with the two discrete one-electron transfer reduction waves observed for isatin in this medium. Observation of a well-developed single reduction wave can be attributed to the higher basicity of the nitrogen species of the imine bond of Schiff bases, making proton abstraction as well as second electron transfer both rapid [66].

Salicylaldehyde, 2-hydroxyacetophenone, and 3,5-dichlorosalicylaldehyde react with 1,2-diaminoethane to give three symmetrical Schiff bases  $H_2L^1$ ,  $H_2L^2$ , and  $H_2L^3$ , respectively. With Ru(III) ions, these ligands lead to three complexes:  $Ru(III)CIL^1$  (1),  $Ru(III)CIL^2$  (2), and  $Ru(III)CIL^3$  (3). The cyclic voltammetry in acetonitrile showed irreversible waves for all three ligands. Under the same experimental conditions, it was proved that the ruthenium is coordinated in the three complexes 1, 2, and 3 showing quasireversible redox systems. The behavior of these complexes and their comparison with cytochrome P450 are investigated using them as catalysts in the presence of molecular oxygen with an apical nitrogen base 1- or 2-methylimidazole [67].

Cyclic voltammetry (CV), the square wave voltammograms (SWV) have been used to investigate the electrochemical behaviour of 2-hydroxy-5-bromobenzaldehyde polyacrylamide, 2-hydroxy-5-chlorobenzaldehyde polyacrylamide and 2-hydroxy-5-methylbenzaldehyde polyacrylamide at the mercury electrode in aqueous media. The bromo- and chloro- derivatives were electroactive while the halide-free compound, the methyl- derivative, was not. The analysis of the experimental data supports the formation of anion radicals as a result of C-X bond cleavage. The large polymeric molecules are adsorbed at the mercury electrode as expected contributing the adsorption current component to the diffusion one [68].

A series of *cis*- and *trans*- tetradentate copper macrocyclic complexes, of ring size fourteen-sixteen, which employ amine and thioether donor groups are reported. Apart from 5,6,15,16-bisbenzo-8,13-diaza-1,4-dithia-cyclohexadecane copper(I) (*cis*-[Cu(H4NbuSen)]<sup>+</sup>). The complexes were subjected to electrochemical analysis in water and acetonitrile. The effect of the solvent, positions of the donor atoms (*cis/trans*) on  $E_{1/2}$  is discussed as the comparison of the electrochemical behaviors of these complexes with their parent Schiff base macrocycles [69].

The electrochemical reduction of 1-[(4-halophenyl)imino]methyl-2-naphthols on graphite electrodes was studied using cyclic voltammetry, chronoamperometry, constant-potential coulometry and preparative constant-potential electrolysis techniques by Uçar et al [70]. The data revealed that the reduction on graphite was irreversible. The diffusion coefficients and the number of electrons transferred were determined using the chronoamperometric Cottrell slope and the ultramicro disc Pt-electrode steady-state current.

Mulazimoglu et al [71] synthesized Schiff base ligand, 4-[(2-hydroxyphenylimino)methyl]benzene-1,3-diol (HIBD) by reaction of 2-aminophenol and 2,4-dihydroxybenzaldehyde. Electrochemical behaviors were investigated on the glassy carbon electrode (GC) surface with cyclic voltammetry. The modification of HIBD on the GC was performed in +0.3 V and +2.8 V potential range using 100 mV s<sup>-1</sup> scanning rate having 5 cycle. For the characterization of the modified surfaces 1 mM ferrocene redox probe in 0.1 M tetrabutylammonium tetrafluoroborate (TBATFB) and 1 mM ferricyanide redox probe in 0.1 M H<sub>2</sub>SO<sub>4</sub> were used. Electrochemical biosensors combine the specificity of the biological species and the analytical power of electrochemical techniques.

Electrochemical measurements exhibit that a novel Schiff base compound based on ferrocene (C<sub>18</sub>H<sub>17</sub>OFeN<sub>3</sub>) undergoes a reversible one-electron redox process. Ferrocenyl Schiff bases have been employed in various fields, such as biosensors, polymer science as active dendrimers, nonlinear optics and liquid crystals. Their redox and electrical properties have resulted in this wide range of applications. A few of hydrazones containing ferrocenyl have been synthesized from the reaction of hydrazides with acetylferrocene because they have very active sites for transition metal to coordinate, such as 1,1'-diacetylferrocene dihydrazone, acetylferrocene thiosemicarbazone, and acetylferrocene semicarbazone. These Schiff bases have shown varied redox and electrical behaviors, depending on the involvement of active coordination sites [72].

## 2.5. Antioxidant activity of Schiff bases and their complexes

Oxidation is one of the most important processes, which produce free radicals in food, chemicals, and even in living systems. Free radicals have an important role in processes of food spoilage, chemical materials degradation, and also contribute to more than one hundred disorders in humans. Antioxidants are defined as substances that even at low concentration significantly delay or prevent oxidation of easy oxidizable substrates. The applications of antioxidants are industrially widespread in order to prevent polymers oxidative degradation, autooxidation of fats, synthetic and natural pigments discoloration, etc. Several methods are used for the estimation of efficiency of

synthetic/natural antioxidants, like the ferric reducing antioxidant power (FRAP) assay,  $\beta$ -carotene/linoleic acid assay, Rancimat method, inhibition of low-density lipoprotein (LDL) oxidation, DPPH assay, etc. This method diversity is due to the complexity of the analyzed substrates, often mixtures of dozens of compounds with different functional groups, polarity, and chemical behavior [73].

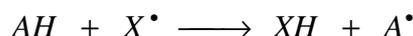
Fats and oils present in many food and cosmetic products may easily deteriorate due to oxidation, in a chain of reactions in which free radicals are formed, propagated and finally converted into stable oxygenated compounds, which are responsible for off-flavors and other undesirable characteristics. Although synthetic antioxidants such as butylhydroxytoluene (BHT) and butylhydroxyanisole (BHA) have been commonly used to prevent such reactions the increasing demand for “natural products” that is free from chemical additives, which is one of the most important consumer trends in the mentioned industries, has motivated the use of a number of natural antioxidants such as tocopherols, flavonoids and terpenoids.

Also, antioxidants play an important role in the human metabolism. Normally, the human body maintains a balance between the amount of antioxidants and oxidation initiators (i.e. free radicals) by simultaneously producing both, as a result of metabolic processes (a phenomenon known as “oxidative stress” is induced). This phenomenon is believed to be an important part of the natural aging process, and plays a significant role on some health problems like arteriosclerosis, and rheumatism. The nutraceuticals industry has thus introduced several natural antioxidants as diet supplements. The development of such natural products, especially from herbs matrices, is an exciting and fast growing field. Several research groups around the world have succeeded in finding and identifying natural antioxidants from herbs and spices. It is well known, for example, those herbs from the *Labiatae* family, such as thymus and rosemary, and other plants such as sage and cloves are a source of antioxidants. Other natural antioxidants are not easily separated from the herbaceous essential oil, which is responsible for flavor and odor. Supercritical fluid (SCF) extraction has been used by several research groups to overcome these limitations. In principle, by manipulating pressure and temperature it is possible to finely control the solubility of the compounds present in the herbal material and thus it may be possible to produce superior products [74].

Antioxidants are a variety of compounds that react differently with different types of free radicals. Determination of antioxidant activity is not easy. Today they are used a number of different tests to determine the antioxidant activity of different compounds. Neutralization of free radicals in the methods for the determination of antioxidant capacity, takes place by one of two mechanisms:

- Hydrogen atom transfer reaction (HAT)

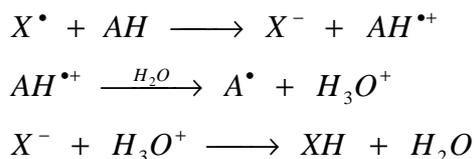
HAT classical method measures the ability to neutralize radicals by donating a hydrogen atom.



These reactions are fast and last a few seconds to minutes, and independent of the pH and the type of solvent,

- Electron transfer reaction (ET)

It is based on the ability to detect the transfer of one electron, from potential antioxidants, in the reaction of reduction of some compounds.



The relative reactivity of antioxidants in the ET method is dependent on their deprotonisation and ionization potential. Therefore, this method is dependent on the solvent pH [75].

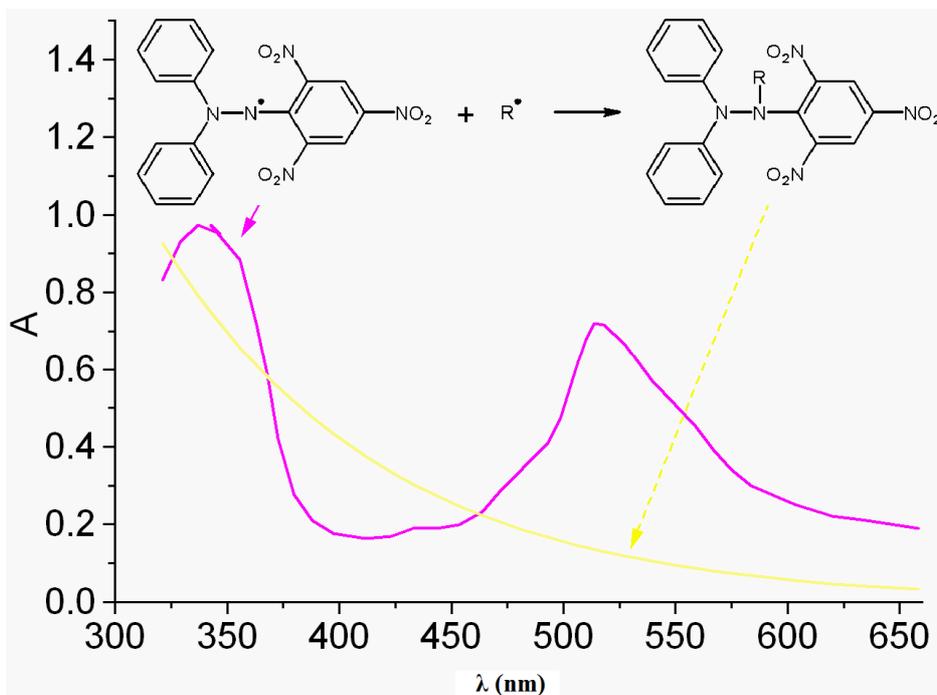
Oxygen Radical Absorbance Assay (ORAC), *Total* Radical-Trapping Antioxidant Parameter (TRAP), Total Oxidant Scavenging Capacity (TOSC), Chemiluminescence (CL), Photochemiluminescence assay (PCL), *Croton* or  $\beta$ -Carotene bleaching by  $LOO^{\bullet}$  assay, and Low-Density Lipoprotein (LDL) oxidation are HAT methods. ET group of methods belong: Ferric Reducing Antioxidant Power (FRAP), Cupric ion Reducing Antioxidant Capacity (CUPRAC), and Folin-Ciocalteu method [76]. Determination of Trolox equivalence antioxidant capacity (TEAC) and DPPH (2,2-difenil-1-pikril-hidrazil) assay usually classified in assays with ET reaction mechanism, but the free radicals in these two essays can be neutralized by both, HAT and ET reactions [77].

Various antioxidant activity methods have been used to monitor and compare the antioxidant activity. In recent years, oxygen radical absorbance capacity assays and enhanced chemiluminescence assays have been used to evaluate antioxidant activity of foods, serum and other biological fluids. These methods require special equipment and technical skills for the analysis. The different types of methods published in the literature for the determinations of antioxidant activity of foods involve electron spin resonance (ESR). Also, analytical methods measure the radical-scavenging activity of antioxidants against free radicals like the DPPH radical, the superoxide anion radical ( $O_2^-$ ), the hydroxyl radical (OH $\cdot$ ), or the peroxy radical (ROO $\cdot$ ) [75]. Antioxidant activity methods using free radical traps are relatively straightforward to perform. The ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation) has been used to screen the relative radical-scavenging abilities of flavonoids and phenolics through their Prior et al. [76,77] have used the Oxygen radical absorbance capacity (ORAC) procedure to determine antioxidant capacities of fruits and vegetables. In the ORAC method, a sample is added to the peroxy radical generator, 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) and inhibition of the free radical action is measured using the fluorescent compound, B-phycoerythrin or R-phycoerythrin [78]. Phenolic and polyphenolic compounds constitute the main class of natural antioxidants present in plants, foods, and beverages and are usually quantified employing Folin's reagent. Vinson et al [79] have measured phenolics in fruits and vegetables colorimetrically using the Folin-Ciocalteu reagent and determined the fruit and vegetable's antioxidant capacity by inhibition of low density lipoprotein oxidation mediated by cupric ions.

A rapid, simple and inexpensive method to measure antioxidant capacity of food involves the use of the free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in recent years.

The DPPH method can be used for solid or liquid samples and is not specific to any particular antioxidant component. A measure of total antioxidant capacity helps understand the functional properties of foods. Oxidized form of DPPH has an absorption maximum at 515-517 nm. After the reaction with antioxidant, i.e. after

reduction of DPPH, the absorbance decreases as a result of discoloration of DPPH (from purple to yellow). DPPH assay is based on monitoring the reduction of DPPH absorption maximum (515-517 nm), which is a consequence of the interaction of radicals with some antioxidant (Figure 7) [73].

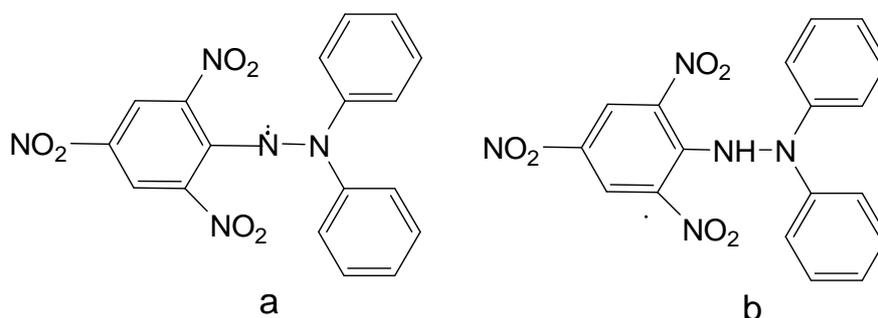


**Figure 7.** Changes in the absorption spectrum of DPPH after reaction with free radical

Antioxidant activity has been expressed in various ways including the percentage of the reagent used, the oxidation inhibition rate and so on. An easier way to present antioxidant activity of foods would be to reference a common reference standard. One common reference standard, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, also known as Trolox, serves this purpose, as well as, use of the parameter “EC50” (equivalent concentration to give 50% effect) [80,81].

The molecule of 2,2-diphenyl-2-picrylhydrazyl (Figure 8, structure **1**) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet color, characterized by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen

atom, then this gives rise to the reduced form (Figure 8, structure 2) with the loss of this violet color.



**Figure 8.** Structures of two form of DPPH: a) free radical, b) nonradical

Although there would be expected to be a residual pale yellow color from the picryl group still present. Representing the DPPH radical the donor molecule by AH, the primary reaction is



where ZH is the reduced form and A<sup>•</sup> is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorised) by one molecule of the reductant [82].

Six complexes, M(HL)<sub>2</sub>·nH<sub>2</sub>O (M=Co(II), Ni(II) and Fe(II); n=4) with two ligands, 2-carboxy-benzaldehydebenzoylhydrazone (H<sub>2</sub>L<sup>1</sup>) and 2-carboxybenzaldehyde-(4'-methoxy)benzoylhydrazone (H<sub>2</sub>L<sup>2</sup>), have been synthesized and characterized on the basis of elemental analyses, molar conductivities, IR spectra and thermal analyses. The 50% inhibitions (IC<sub>50</sub>) of the complexes were studied. This study demonstrated that the complexes have activity in the suppression of O<sub>2</sub><sup>•-</sup> and OH<sup>•</sup>. In general, the antioxidative activities increased as the concentration of these complexes increased up to a selected extent. The complexes exhibit more effective antioxidants than the ligands and the series of the ligand (H<sub>2</sub>L<sup>2</sup>) are better than the series of the ligand (H<sub>2</sub>L<sup>1</sup>) do [83,84].

## 2.6. Quantum-chemical calculations

In the last decade, it was shown that quantum-chemical calculations can be a valuable tool in predicting and explaining biological e.g. antioxidative activity of

various compounds. A good correlation between some quantum-chemical descriptors (i.e., various electronic descriptors, bond dissociation enthalpy and spin density) and antioxidant activity for a series of molecules were reported [85]. Several works were published to describe and explain the antioxidant activity using molecular descriptors. It is well known that the physical properties of chemical substances as well as color, odor, melting point, density, electrical conductivity, etc. are very closely related to the structure of molecules. In addition to the physical properties of the molecular structure vary its chemical and biological activity [86-88]. In other words, if we consider the structure of an independent variable, physical properties, chemical reactivity and biological activity will be the dependent variable.

Based on previous empirical findings can be assumed that the molecular characteristics best defines a particular trait. In order to obtain a satisfactory correlation with the physical sense, it is very important to select parameters of molecules that will be applied. These parameters represent the numeric value of the relevant characteristics of the compounds, called molecular descriptors [89-92]. Molecular descriptors are obtained as the final result of the procedure which with the help of logical and mathematical procedure based on the structure of molecules generated numeric data that symbolically represent particular characteristic of molecules. Today, the modern mathematical software and programs developed to calculate more than 2,000 different molecular descriptors [93-95].

### 3. EXPERIMENTAL PART

#### 3.1. Chemicals and Solutions

All the chemicals used were of reagent-grade quality. Dimethylsulfoxide (DMSO) supplied by J.T.Baker was purified further by the standard procedure used before [96]. The supported electrolyte, sodium perchlorate (Merck) was dried before use. 2,2'-diphenyl-1-picrylhydrazyl (DPPH) and 6-hydroxyl-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) were purchased from Fluka. All of the investigated Schiff bases and their copper(II) complexes (doublet spin state,  $d^9$ ) were prepared according to described procedures [97]. The structures of the compounds investigated in this study are given in Table 1.

**Table 1** Structures of the compounds used in this study.

No	Schiff base	R	R <sub>1</sub>	B	No	Complex
<b>1</b>	H <sub>2</sub> ( <i>acac</i> <sub>2</sub> <i>en</i> ) <sup>a</sup>	CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>2</sub> CH <sub>2</sub>	<b>7</b>	[Cu( <i>acac</i> <sub>2</sub> <i>en</i> )]
<b>2</b>	H <sub>2</sub> ( <i>acac phacac en</i> ) <sup>b</sup>	CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> CH <sub>2</sub>	<b>8</b>	[Cu( <i>acac phacac en</i> )]
<b>3</b>	H <sub>2</sub> ( <i>phacac</i> <sub>2</sub> <i>en</i> )	C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	CH <sub>2</sub> CH <sub>2</sub>	<b>9</b>	[Cu( <i>phacac</i> <sub>2</sub> <i>en</i> )]
<b>4</b>	H <sub>2</sub> ( <i>acac</i> <sub>2</sub> <i>pn</i> ) <sup>c</sup>	CH <sub>3</sub>	CH <sub>3</sub>	CH(CH <sub>3</sub> )CH <sub>2</sub>	<b>10</b>	[Cu( <i>acac</i> <sub>2</sub> <i>pn</i> )]
<b>5</b>	H <sub>2</sub> ( <i>acac phacac pn</i> )	CH <sub>3</sub>	C <sub>6</sub> H <sub>5</sub>	CH(CH <sub>3</sub> )CH <sub>2</sub>	<b>11</b>	[Cu( <i>acac phacac pn</i> )]
<b>6</b>	H <sub>2</sub> ( <i>phacac</i> <sub>2</sub> <i>pn</i> )	C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	CH(CH <sub>3</sub> )CH <sub>2</sub>	<b>12</b>	[Cu( <i>phacac</i> <sub>2</sub> <i>pn</i> )]

<sup>a</sup> *acac* = pentane-2,4-dione ; *en* = ethane-1,2-diamine; <sup>b</sup> *phacac* = 1-phenylbutane-1,3-dione

<sup>c</sup> *pn* = propane-1,2-diamine

#### 3.2. Apparatus and procedures

##### 3.2.1. Cyclic voltammetry

The electrochemical experiment was carried out using a CHI760b Electrochemical Workstation potentiostat (CH Instruments, Austin, TX). Cyclic

voltammetry was performed using a conventional three-electrode cell (5 mL). System consisted of a working electrode, a reference electrode (Ag/AgCl or Ag/Ag<sup>+</sup>) and an auxiliary platinum wire electrode. The working electrodes were pre-polished glassy carbon (GC), gold (Au) or platinum (Pt). Prior to recording the voltammogram all solutions (10<sup>-3</sup> M) were deoxygenated by passing the stream of nitrogen into the solution for at least 10 minutes. Voltammetric measurements were performed in dimethylsulfoxide (DMSO), acetonitrile (AcN) and mixture of dimethylsulfoxide/dichloromethane (DMSO/DCM) (50% v/v) with supporting electrolyte (0.1 M sodium perchlorate or tetrabutylammonium perchlorate) in the potential range of -1.5 to 1.5 V, and at sweep rates between 0,01 and 1 V s<sup>-1</sup>. All measurements were carried out at room temperature (25°C).

### 3.2.2. Antioxidant activity

Antioxidant activities, of observed Schiff bases and their copper(II) complexes (Table 1), were determined *in vitro* by DPPH free radical scavenging assay. Before determination of antioxidant activity by DPPH TLC assay the optimization of chromatographic condition were done using different types of stationary phases: RP 18 silica gel, cellulose, alumina, and silica gel. Trolox, in concentration range of 0-280 ng/spot, were used as a standard (r =0.9993). The spots of 1 µL of compound solution (0.6 mg/mL) in appropriate solvents were applied by autosampler (Linomat 5, Camag) on RP18 silica plate (Merck, Germany) as well as series of standard solutions in methanol followed by applying of 1 µL of methanolic solution of DPPH (0.15·10<sup>-3</sup> M) at the same spots. No development was carried out. This plate was left in the dark. After 30 minutes of incubation plate was scanned. Camag TLC Scanner with CATS evaluation software was used with the following settings: wavelength 515 nm, scanning speed 20 mm/s, multi level calibration via peak area. The values of antioxidant activity of the compounds are expressed as Trolox Equivalent Antioxidant Capacity (TEAC). Also, the radical scavenging activity (%RSA) was calculated as a percentage of DPPH discoloration using the following equation:

$$\%RSA = \frac{A_{DPPH} - A_S}{A_{DPPH}} \cdot 100$$

where  $A_S$  is the absorbance of the spots after 30 minutes of incubation and  $A_{\text{DPPH}}$  is the absorbance of the DPPH.

### 3.2.3. Calculations

Geometries of all molecules were fully optimized using hybrid Density Functional Theory (DFT) method, more specifically Becke three-parameterized exchange functional and Lee-Yang-Parr correlation functional (B3LYP). For the complexes unrestricted B3LYP calculations were used with lanl2dz basis set on the copper atom and 6-31G (d,p) basis set on other atoms. Inner electrons of the copper atom were described with lanl2 effective core potential. Geometries of neutral ligand molecules were optimized with 6-311G (d,p) basis set. All quantum-chemical descriptors were calculated on the optimized geometries with 6-31+G (d,p) basis set on nonmetallic atoms and lanl2dz with EPC on the copper atom. Electronic molecular descriptors obtained after optimization were: HOMO and LUMO energies,  $\Delta E$  HOMO-LUMO calculated as the difference of the energies, dipole moment, total energy, electronic energy, spin density, charge on atoms by Natural Bond Orbital (NBO) analysis, and chelate plane angle.

Relative bond dissociation energies (BDE) for dissociation of N-H bond in ligand molecules in methanol solution were calculated using isodesmic reaction shown in Scheme 1.



**Scheme 1.** Isodesmic reaction for BDE calculations

Geometries of all ligands and radicals were fully optimized using B3LYP method for closed-shell molecules and restricted open B3LYP method (ROB3LYP) for radicals with 6-311G (3df,3pd) basis set. The thermal energy, including zero-point energy, work and translational, rotational and vibrational entropy corrections were evaluated using standard statistical mechanics formulas [98]. Polarizable continuum model (PCM)

calculations in methanol as solvent were done on optimized closed-shell molecules and radicals geometries in order to introduce environment effect in BDE calculations. All DFT calculations were done in Gaussian 03 program package [99]. Statistical calculations were performed by NCCS 2004 software package [100].

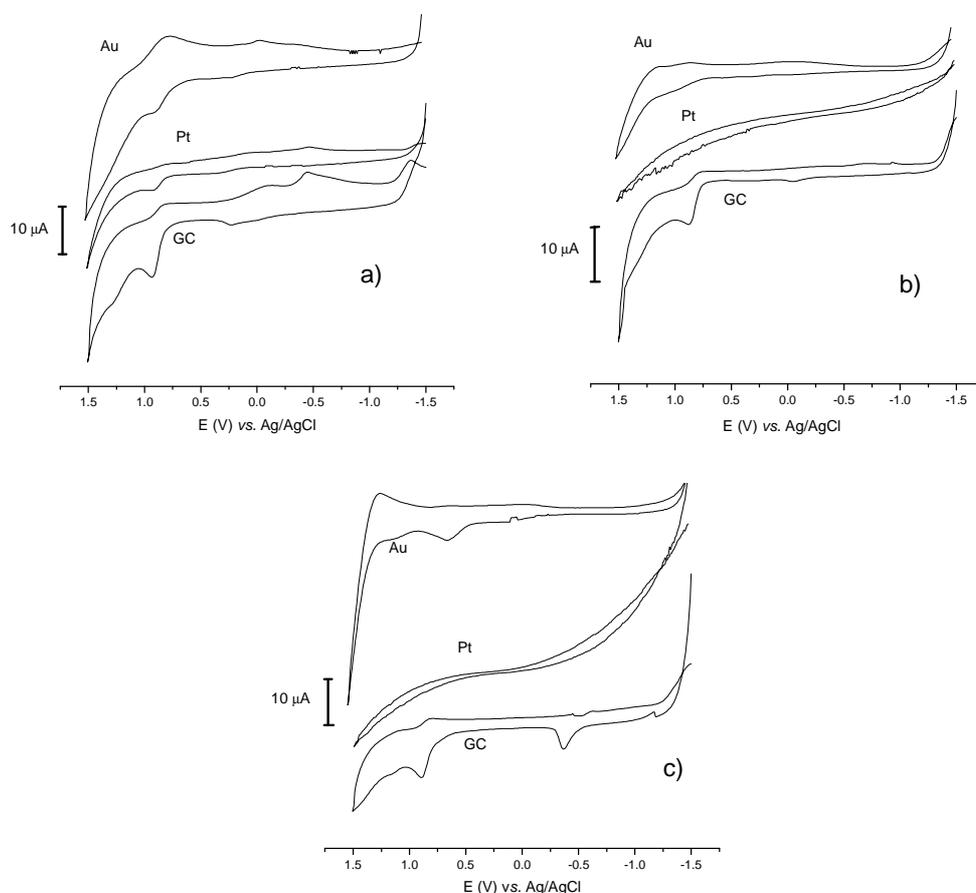
## 4. RESULTS AND DISSCUSION

### 4.1. Optimization of condition for cyclic voltammetry

Optimization of conditions for investigation of electrochemical behavior of Schiff bases and their copper(II) complexes were made in relation to N,N`-bis(acetylaceton)ethylenediimine and corresponding copper(II) complex. Electrochemical behavior was studied by cyclic voltammetry in different non-aqueous media such as dimethylsulfoxide (DMSO), acetonitrile (AcN) and mixture of dimethylsulfoxide/dichloromethane (DMSO/DCM) as aprotic solvents at the surface of different solid electrodes (platinum, gold and glassy carbon) using sodium perchlorate as supporting electrolyte. The effect of concentration of the investigated compounds, solvents, type of electrodes and scan rates on the redox behavior of these compounds were discussed.

#### 4.1.1. The solvent effect

The cyclic voltammograms of copper(II) complex, with N,N`-bis(acetylaceton)ethylenediimine as ligand, is solvent dependent (Figure 9). Solvents dependence can be accounted for by differences in dielectric constant and donor number of the solvent. The dielectric constant for DMSO is 46.7 while the value for AcN is 37.5. Although both are aprotic solvents, meaning that they do not have a hydrogen atom joined to an electronegative atom, acetonitrile has a weaker basic character than dimethylsulfoxide, based on the value of their donor number. Obtained potentials in different solvents are listed in Table 2.



**Figure 9.** Cyclic voltammograms of 1 mM  $\text{Cu}(\text{acac}_2 \text{en})$  recorded in different solvents containing 0.1 M sodium perchlorate as supporting electrolyte at the surface of different solid electrodes, scan rate  $0.1 \text{ V s}^{-1}$  a) DMSO b) DMSO/DCM (50% v/v) c) AcN

**Table 2.** Cyclic voltammetric data for  $\text{Cu}(\text{acac}_2 \text{en})^{\text{a}}$

Solvent	Potentials (V vs. Ag/AgCl)							
	$E_{\text{pc}}^{\text{b}}$				$E_{\text{pa}}^{\text{c}}$			
DMSO	-0.104	-0.448	-1.367	/	+0.239	+0.933	+1.281	+0.900
DMSO/DCM	/	/	/	-0.048	/	+0.870	+0.780	/
AcN	-0.450	-0.620	/	-0.375	/	+0.829	+0.890	+1.180

<sup>a</sup>1 mM; 0.1 M sodium perchlorate; scan rate  $0.1 \text{ V s}^{-1}$

<sup>b</sup>cathodic potential; <sup>c</sup>anodic potential.

As can be seen from Table 1 the voltammogram recorded in the DMSO, with high dielectric constant, shows a higher number of peaks. In addition, dimethyl sulfoxide is a highly polar aprotic solvent with higher value of donor number (125 kJ/mol) in comparison with AcN (59 kJ/mol) [101]. This results in the ability to solvate cations i.e. ability to be coordinated to the complex and to stabilize the Cu(II). This is observed on anodic potential peak in DMSO at + 0.239 V which is shifted in AcN onto more negative values (Figure 9).

Linear region of the Randles-Sevcik equation was used for calculation of diffusion coefficient (D) of the compound in applied solvent [102]. The Randles-Sevcik equation gives a straightforward relation between the voltammetric peak current and D. The diffusion coefficient is a measure of how fast the analyte moves through the solution as a result of random collisions with other molecules. From cyclic voltammograms D can be calculated from Randles-Sevcik equation: peak current =  $0.4463 n F A C (n F \nu D / R T)^{1/2}$  where:

n = number of electrons transferred

F = Faraday constant

A = the electrode surface area in  $\text{cm}^2$

$\nu$  = scan rate in Volt / s

R = gas constant

T = temperature.

Obtained diffusion coefficient values (D) are summarized in Table 3.

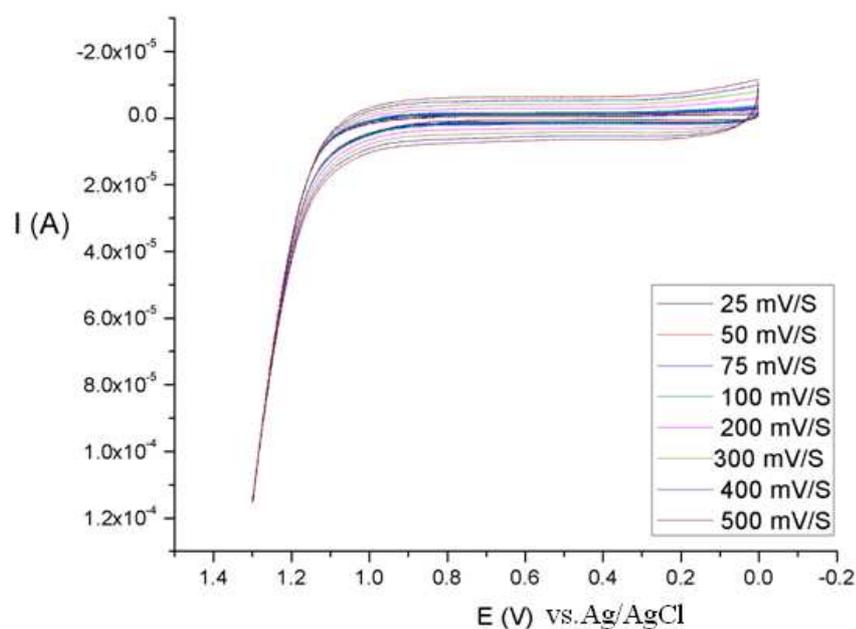
**Table 3.** Diffusion coefficient values data for Cu(*acaca*<sub>2</sub>*en*) in different solvents

Solvent	D ( $\text{cm}^2/\text{s}$ ) $\cdot 10^{-6}$
DMSO	0.28
DMSO/CH <sub>2</sub> Cl <sub>2</sub>	0.48
AcN	0.94

Analysis of the values of diffusion coefficient, shows that the values in AcN are higher than those obtained in DMSO and DMSO/DCM. Solvent dependence can be ascribed to

differences in solvents dielectric constants [103]. The values of  $D$  increase with decreasing of dielectric constant. Thus, we used dimethylsulfoxide as electrolyte for further investigations. With its high polarity combined with a high dielectric constant, dimethylsulfoxide (DMSO) is an excellent solvent for polar or polarizable organic compounds, but also for many acids, alkalis and mineral salts.

On Figure 10 shown the cyclic voltammograms of dimethylsulfoxide with sodium perchlorate as supporting electrolyte,  $c = 0,1$  mol/L scanned on different scan rates.



**Figure 10.** Cyclic voltammograms of dimethylsulfoxide with sodium perchlorate as supporting electrolyte,  $c = 0.1$  mol/L

#### 4.1.2. The effect of different types of working electrodes

The material should exhibit favourable redox behavior with the analyte, ideally fast, reproducible electron transfer without electrode fouling. Secondly, the potential window over which the electrode performs in a given electrolyte solution should be as wide as possible to allow for the greatest degree of analyte characterization. Additional considerations include the cost of the material, its ability to be machined or formed into useful geometries, the ease of surface renewal following a measurement, and toxicity.

In order to examine the impact of different types of electrodes on the electrochemical behavior of investigated compounds their cyclic voltammograms were recorded on glassy carbon, platinum, and gold as working electrodes.

Platinum is likely the favorite, demonstrating good electrochemical inertness and ease of fabrication into many forms. The biggest disadvantage to the use of platinum, other than its high cost, is that the presence of even small amounts of water or acid in the electrolyte leads to the reduction of hydrogen ion to form hydrogen gas (hydrogen evolution) at fairly modest negative potentials ( $E = -0.059 \cdot \text{pH}$ ). This reduction obscures any useful analytical signal. Gold electrodes behave similarly to platinum, but have limited usefulness in the positive potential range due to the oxidation of its surface. It has been very useful, however, for the preparation of modified electrodes containing surface structures known as self-assembled monolayers. Carbon electrodes allow scans to more negative potentials than platinum or gold, as well as good anodic potential windows. The most common form of carbon electrode is glassy carbon, which is relatively expensive and difficult to machine.

The obtained cyclovoltammograms of  $\text{Cu}(\text{acac})_2 \cdot \text{en}$  in DMSO, DMSO/DCM (50% v/v), and AcN are shown in Figure 9. Potentials are shown in V versus Ag/AgCl at the scan rate of  $0.1 \text{ V s}^{-1}$ . As can be seen from Figure 9 the platinum electrode is inert in these voltammetric systems thus there is no detectable peaks in recorded voltammograms. On some voltammograms obtained at gold electrode low intensity peaks were detected (Figures 9a and 9c). The surface of glassy carbon electrode is much more suitable as active area for redox behavior of investigated compounds compared to platinum or gold electrodes. Thus, we used glassy carbon electrode as working electrode for further investigations.

#### 4.1.3. Effect of scan rates

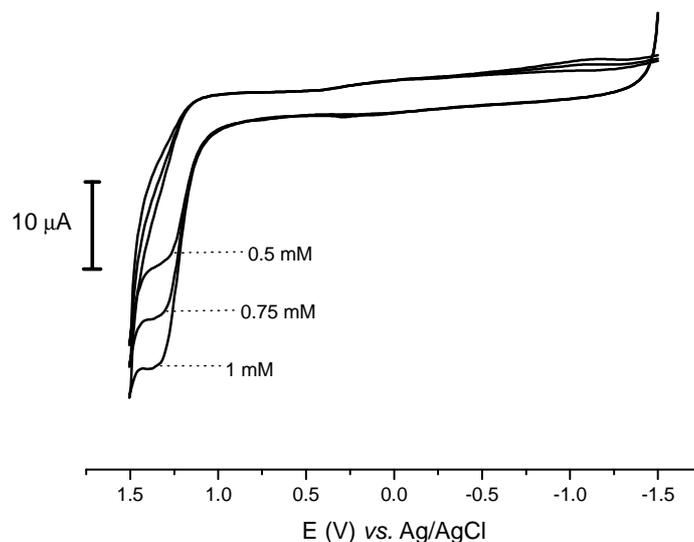
Scan rate studies were carried out to assess whether the processes on glassy carbon electrode were controlled by diffusion or adsorption. The scan rate has a great influence on the peak current as expected according to Randles-Sevcik equation [102]. It is directly proportional to peak current. Studied  $\text{N,N}'$ -bis(acetylacetonate) ethylenediimine as well as its  $\text{Cu}(\text{II})$  complex show increase in the peak current with

increase in scan rate 0.01 to 0.1 V s<sup>-1</sup>. The difference between anodic and cathodic peak potential increased with the increase of the scan rate.

Cyclic voltammetry is also very useful in determining the mode of transport for the system. The system is designed so that there are two possible modes of transport, adsorption and diffusion. The system might exhibit either of these or a combination of both. In an adsorption-controlled system, the analyte does not have to travel to the electrode. Thus, when the potential required for reaction is reached, the current increases and decreases much more rapidly. The result is a sharp (narrow) peak with a high current (since all the analyte can react at once). Peak currents were correlated with the scan rates and square root of scan rates. A straight line was observed with the former correlation and curved line was obtained with the later correlation suggesting adsorption controlled oxidation. Also, because of this quicker reaction, the anodic and cathodic peaks are closer than in a diffusion-controlled system. In our case the peak current linearly increases with the increase of the square root of the scan rates. This confirms that the electrode processes are diffusion controlled. However, another evidence of the negative shift of the peak potential with increasing scan rate indicates that there is also adsorption of the Cu(*acac*<sub>2</sub> *en*) at the surface of electrode [104]. Therefore, we can conclude that redox processes of Cu(II) complex is controlled by both diffusion and adsorption.

#### 4.1.4. The effect of concentration of investigated compounds

According to electrochemical response, the redox peak current increased with increase of analyte concentration. Figure 11 presents cyclic voltammograms of H<sub>2</sub>(*acac*<sub>2</sub> *en*) at different concentration.



**Figure 11.** Cyclic voltammograms of  $H_2(acac_2en)$  at different concentration.

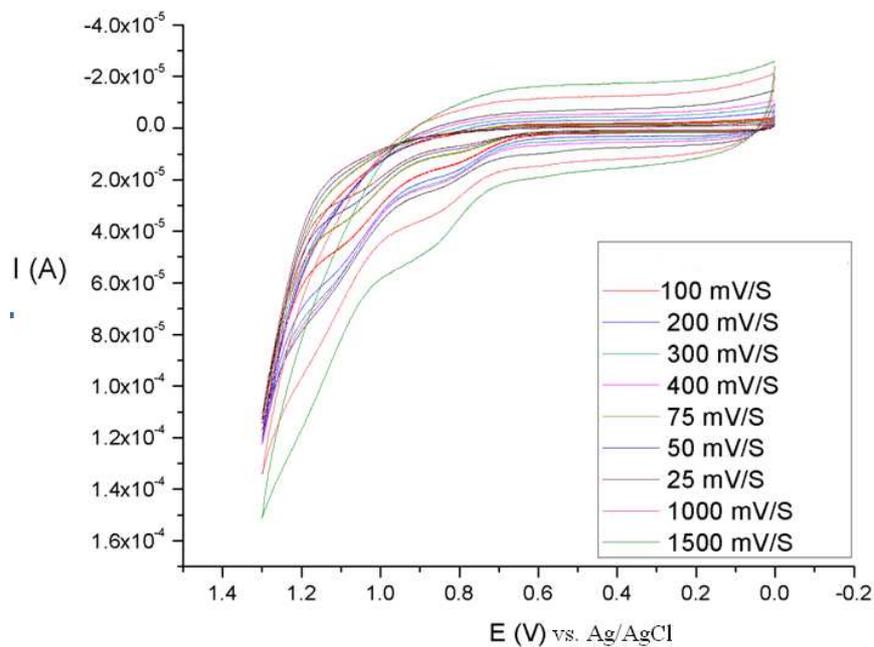
The peak corresponds to a particular electroactive analyte in the investigated solution, and the height of a peak is proportional to the concentration of that analyte. Due to the fact that the the magnitude of this current is proportional to the concentration of the analyte in solution, cyclic voltammetry can be used in an analytical determination of concentration. The plot of peak current against concentration of  $H_2(acac_2en)$  shows linearity with very high values of correlation coefficient ( $r = 0.999$ )

## 4.2. Cyclic voltammetry of series of Schiff bases and their copper(II) complexes

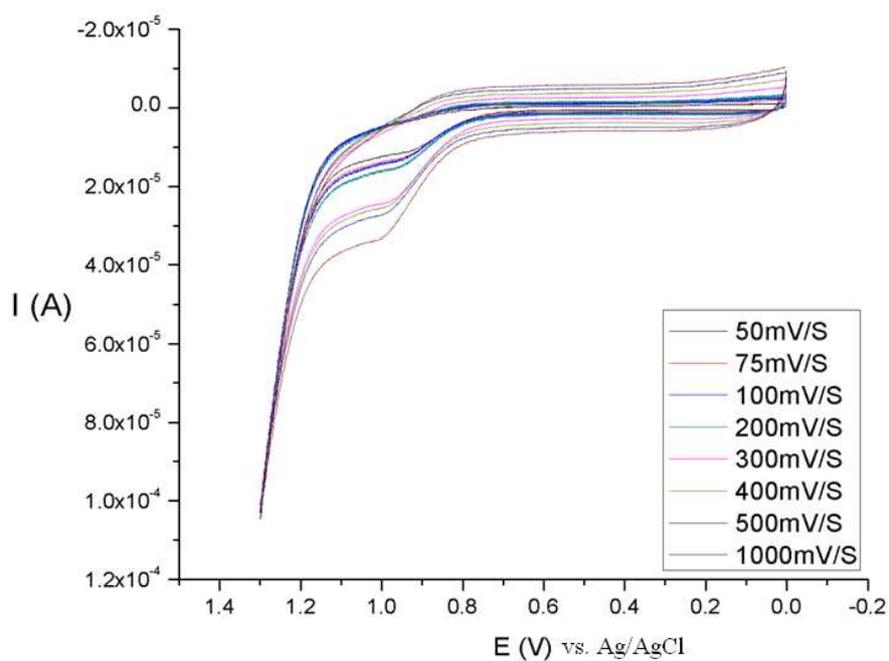
### 4.2.1. Electrochemical behavior of Schiff bases

Analisis of recorded cyclic voltammograms clearly shown effect of substituents in structure of Schiff bases on electrochemical behavior. Inductive and steric effect are responsible for different electrochemical compounds which are contained different substuents such as methyl-, phenyl-, ethyl- as well as isopropyl- present in diammine bridge. Inductive and steric effect affected distribution of electrones on donor atoms (O, N).

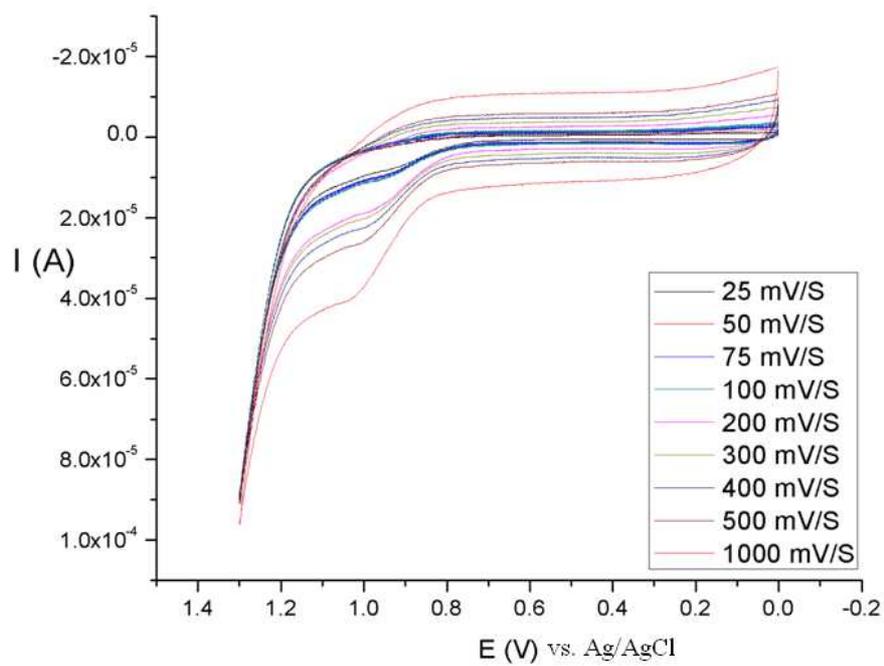
Recorded cyclic voltammograms of investigated Schiff bases under menteniod conditions are shown on Figures 12-16.



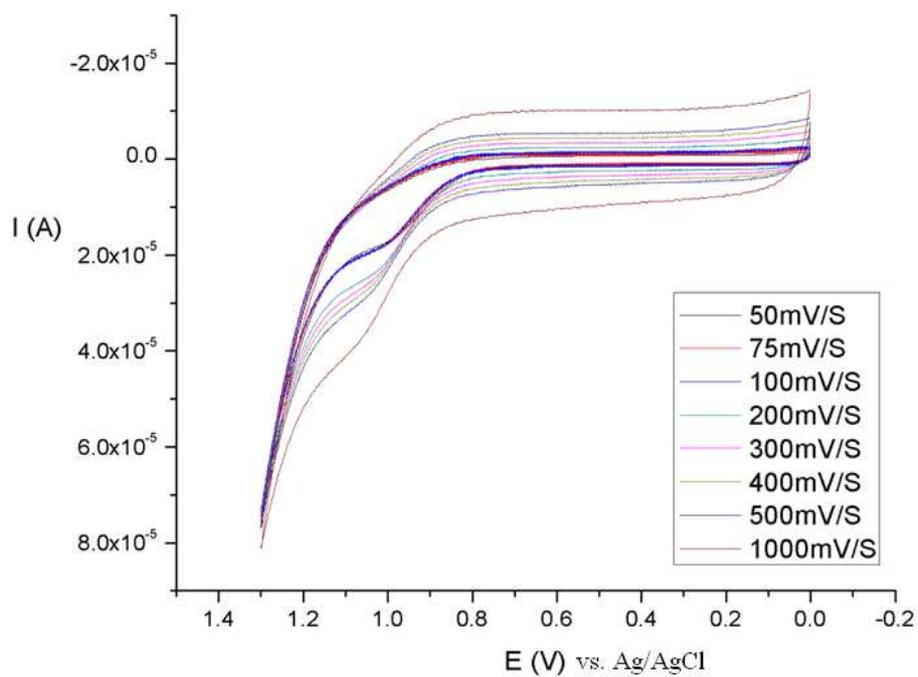
**Figure 12.** Cyclic voltammograms of  $H_2(acac_2 en)$



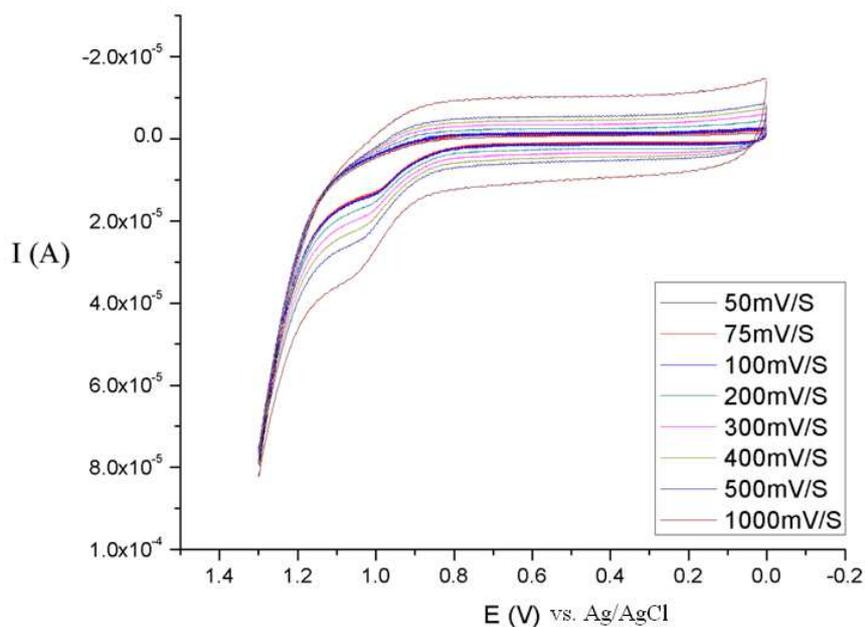
**Figure 13.** Cyclic voltammograms of  $H_2(phacac_2 en)$



**Figure 14.** Cyclic voltammograms of  $H_2(acac\ phacac\ en)$



**Figure 15.** Cyclic voltammograms of  $H_2(acac_2\ pn)$



**Figure 16.** Cyclic voltammograms of  $H_2(\text{phacac}_2 \text{pn})$

From obtained figures we concluded that Schiff bases characterised totally irreversible redox process. For all investigated Schiff bases is characteristic only one irreversible peak corresponding to oxidation potential of imino group which is responsible for redox reaction of observe tetradendate Schiff bases. On Figures 12-16 are clearly defined anodic peak as consequence of irreversible reduction of imino-azomethin group to amino group. This is in accordance with results of A. Fry at al [105]. This intensive characteristic peak appear at potential about +1.0 V (Table 4).

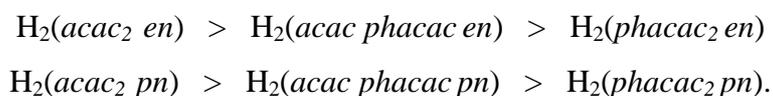
**Table 4.** Values of potential for observed Schiff bases

Schiff base	Potential E (V)
$H_2(\text{acac}_2 \text{pn})$	+ 0.999
$H_2(\text{phacac}_2 \text{pn})$	+ 0.984
$H_2(\text{acac}_2 \text{en})$	+ 0.992
$H_2(\text{phacac}_2 \text{en})$	+ 0.950
$H_2(\text{acac phacac en})$	+ 0.929

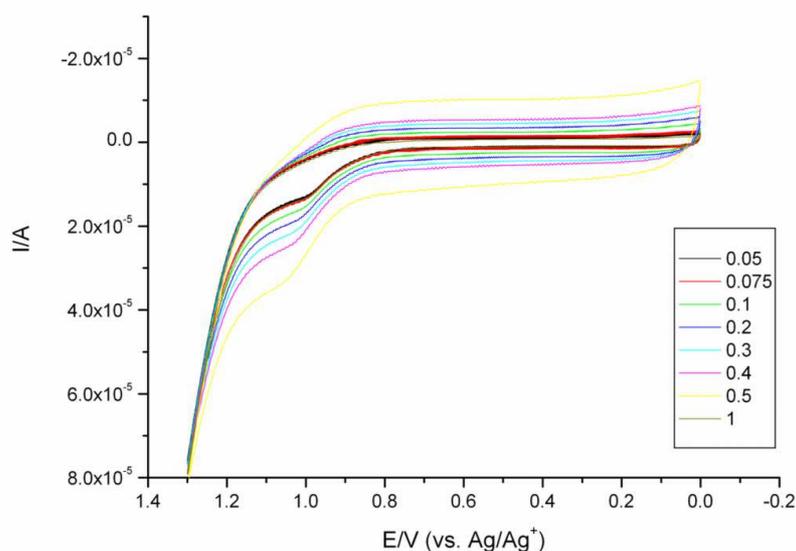
#### 4.2.1.1. Effect of Schiff base substituents

The obtained cyclic voltammograms of Schiff bases clearly indicate that the redox processes of the ligands are highly irreversible. They show the presence of only one anodic peak at potential value in the range from +1.0 to +1.1 V; no cathodic wave occurs in the reverse scan. This behavior was observed for a wide range of scan rates from 0.05 to 1 V/s. Hence, such an oxidation process should correspond to a totally irreversible electron transfer. For all Schiff bases under study, this irreversible oxidation peak would be ascribed to the oxidation of the imine group through which these compounds participate in redox processes [106].

The value of anodic potential varies depending on the substituents present in the compound in accordance with the intramolecular interactions of the imine group. These interactions are changing, as expected, depending on the substituents electronic effect that is present (inductive effect and  $\pi$ -electron interaction) [107]. The effect of anodic potential shift is less pronounced in the substitution of first methyl group in comparison to substitution of both methyls by phenyl-groups. The introduction of two phenyl groups in the structure of Schiff bases due to the symmetry of the observed compounds has a more pronounced effect on the basicity of the nitrogen atom, which participates in redox processes. Differences in electrochemical behaviour are not only affected by the substituents of Schiff bases but also by structure of imine bridge. All of investigated Schiff bases containing propane-1,2-diamine have lower values of potentials comparing to ethane-1,2-diamine. The potentials decrease in following order

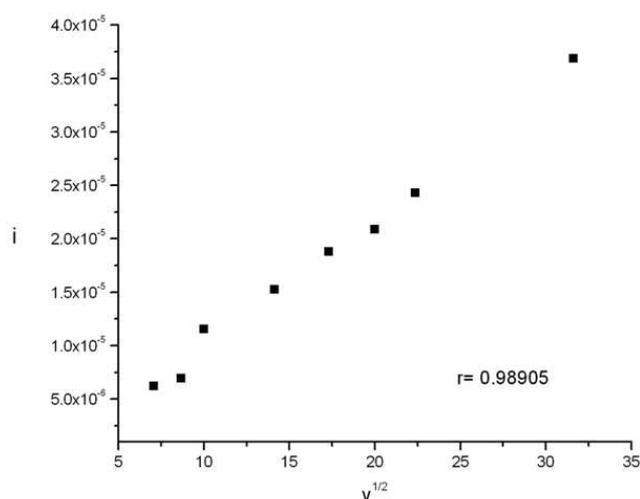


Also, by analyzing the position of peaks obtained at different scan rates may be concluded that the electrochemical processes of ligand are mainly controlled by diffusion. With increasing the scan rates all Schiff bases show a positive peak potential shift, as well as an increase in current intensity. Voltammograms of  $\text{H}_2(\text{phacac}_2 \text{ en})$  on different scan rates are shown on Figure 17 as example.

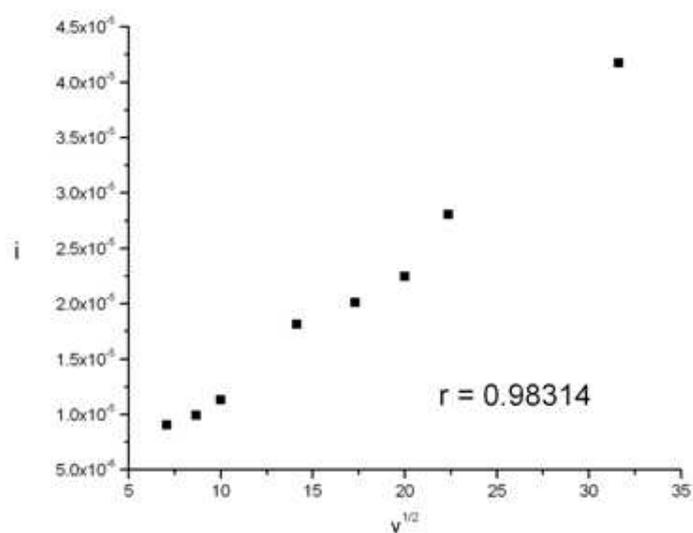


**Figure 17.** Voltammograms of  $H_2(phacac_2 en)$  at different scan rates  $0.05 - 1 \text{ V s}^{-1}$

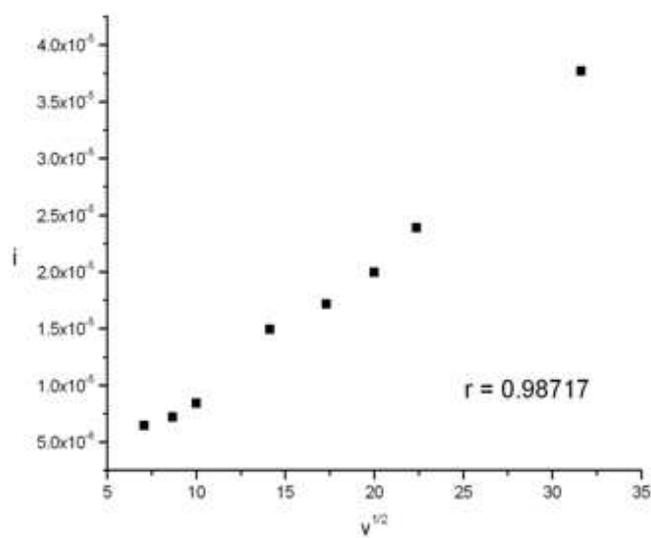
Observation of dependance of current intensity *vs.* the square root of scan rate provides an opportunity for information which of processes affect on redox behavior: diffusion and/or adsorption i.e. the mode of transport and retention of the compounds under conditions of cyclic voltammetry. These dependences are shown on Figures 18-22.



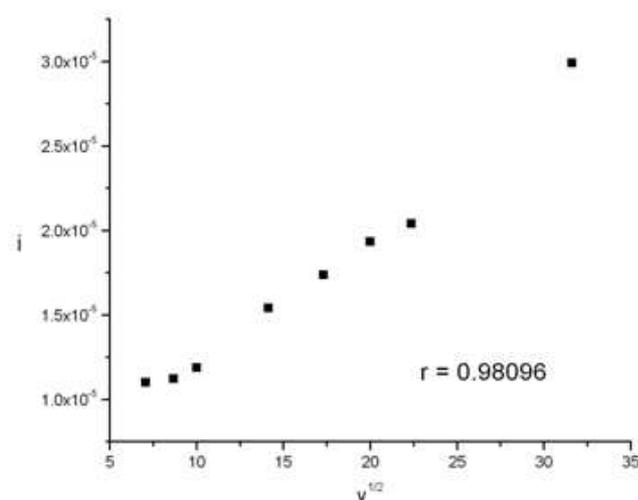
**Figure 18.** Dependance of current intensity *vs.* square root of scan rate for  $H_2(acac_2 en)$



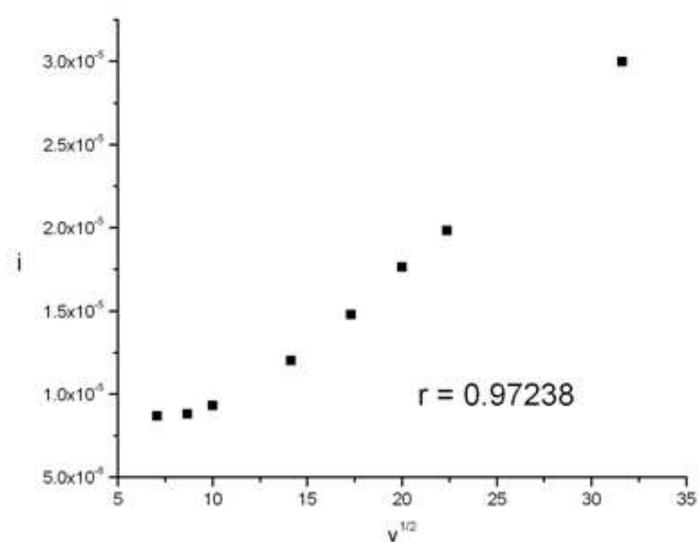
**Figure 19.** Dependence of current intensity vs. square root of scan rate for  $H_2(\text{phacac}_2 \text{ en})$



**Figure 20.** Dependence of current intensity vs. square root of scan rate for  $H_2(\text{acac phacac en})$



**Figure 21.** Dependence of current intensity vs. square root of scan rate for  $H_2(acac_2 pn)$



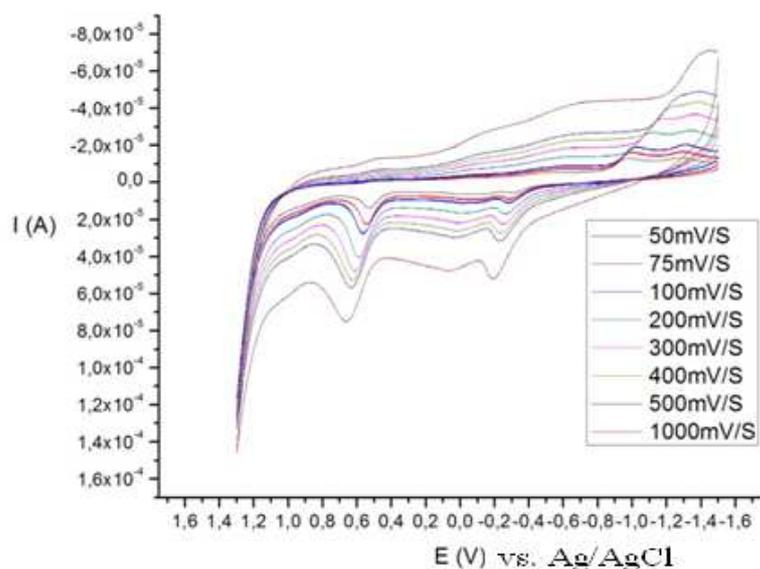
**Figure 22.** Dependence of current intensity vs. square root of scan rate for  $H_2(phacac_2 pn)$

From this figures it is obviously that the electrochemical processes are mainly diffusion controled. Therefore, on some scan rates we established different effect. Adsorption also happened in electrochemical cell. This irreversible oxidation peak would be assigned to the oxidation of the imine group through which these compounds participate in redox processes. Also, by analyzing the position of peaks obtained at different speeds it can be seen that the electrochemical processes of ligand are mainly

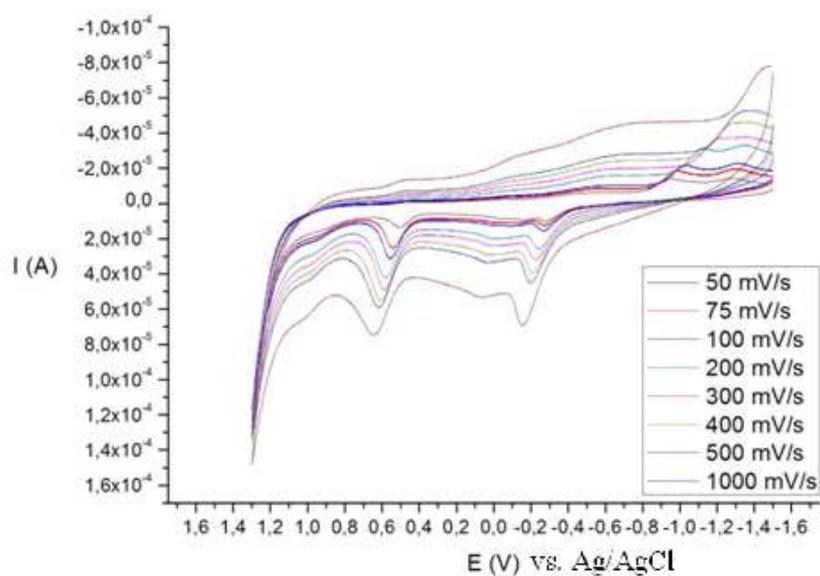
controlled by diffusion in comparison to these processes of complex which are controlled by both diffusion and adsorption.

#### 4.2.2. Electrochemical behavior of copper(II) complexes

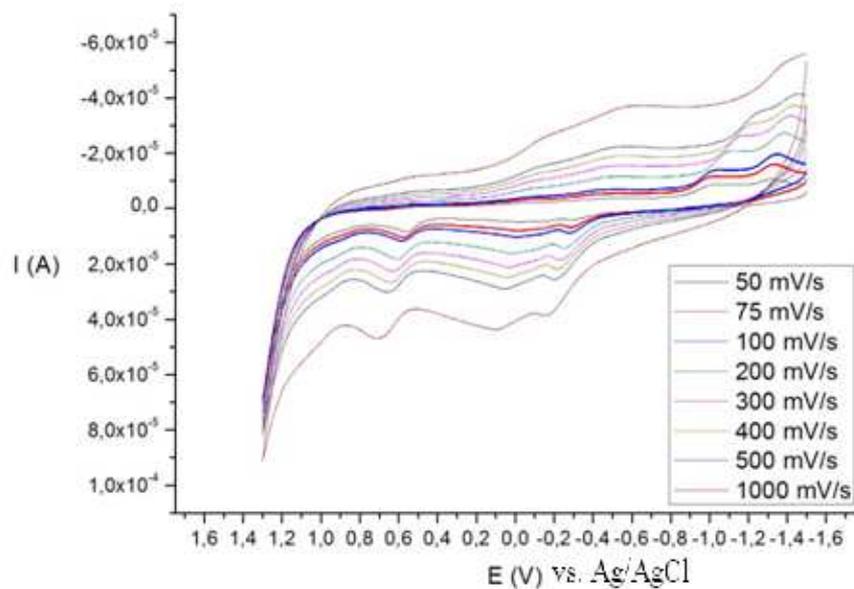
Obtained cyclic voltammograms of Schiff base copper(II) complexes are shown on Figures 23-27.



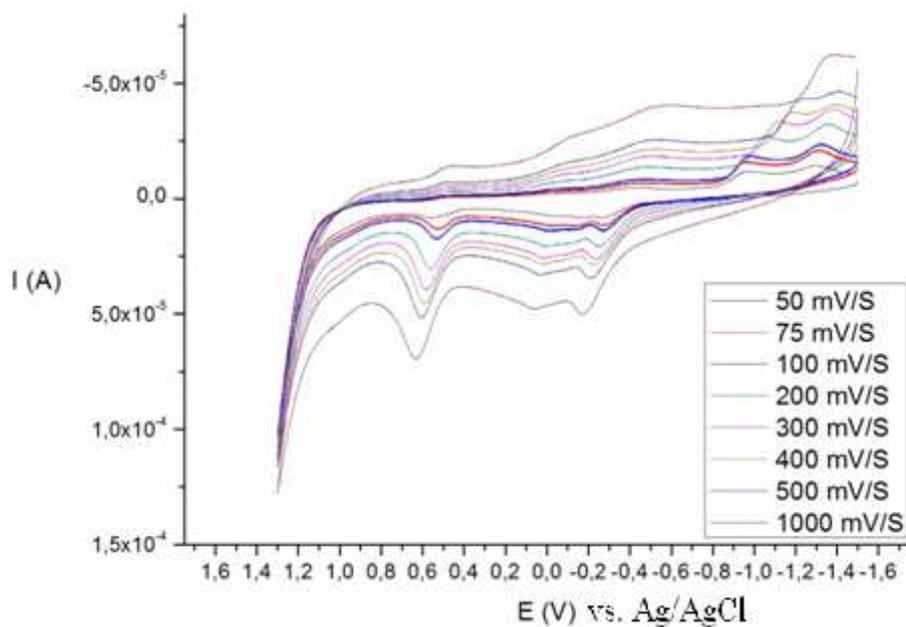
**Figure 23.** Cyclic voltammograms of  $[\text{Cu}(\text{acacphacac en})]$



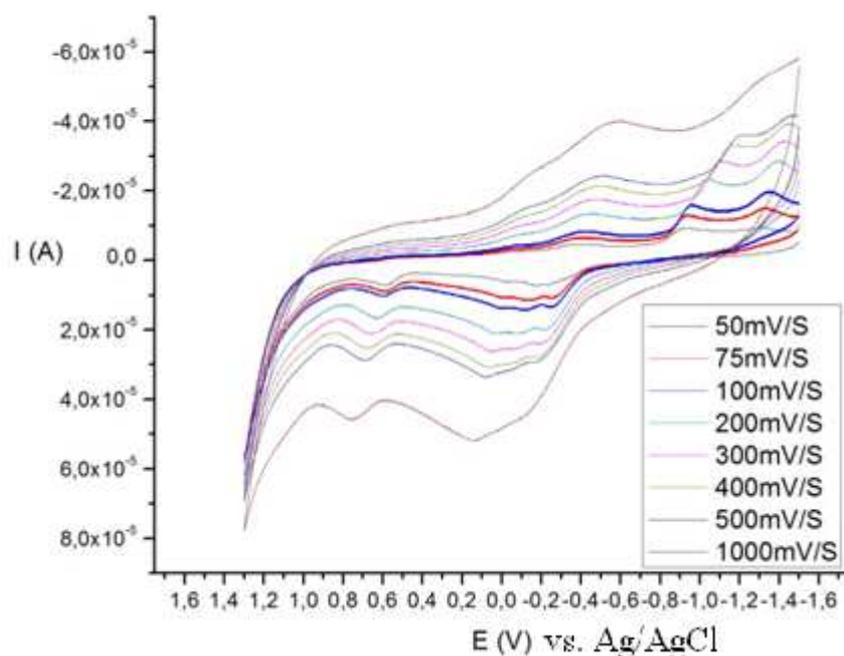
**Figure 24.** Cyclic voltammograms of  $[\text{Cu}(\text{acac}_2 \text{ en})]$



**Figure 25.** Cyclic voltammograms of [Cu(*phacac*<sub>2</sub> *en*)]

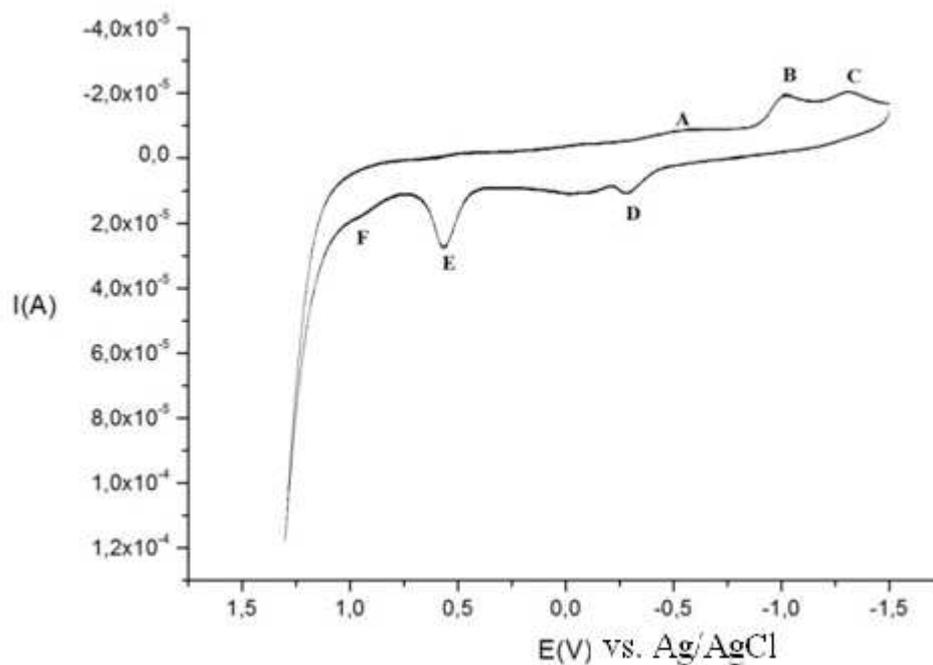


**Figure 26.** Cyclic voltammograms of [Cu(*acac*<sub>2</sub> *pn*)]

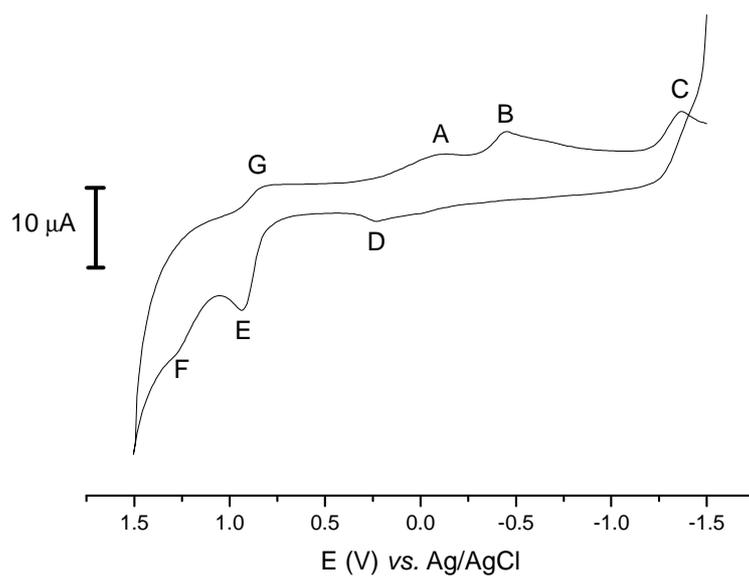


**Figure 27.** Cyclic voltammograms of  $[\text{Cu}(\text{phacac}_2 \text{pn})]$

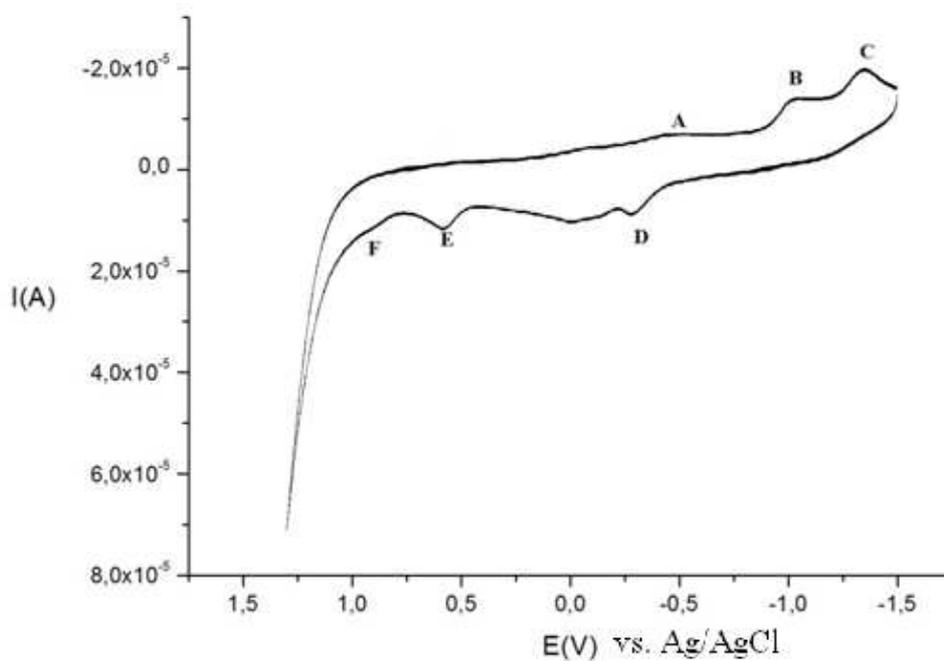
The best peaks are obtained on scan rate of 100 mV/s. On Figures 28-32 are shown cyclovoltammograms which are recorded on this scan rate. Characteristic peaks are assigned from A to F.



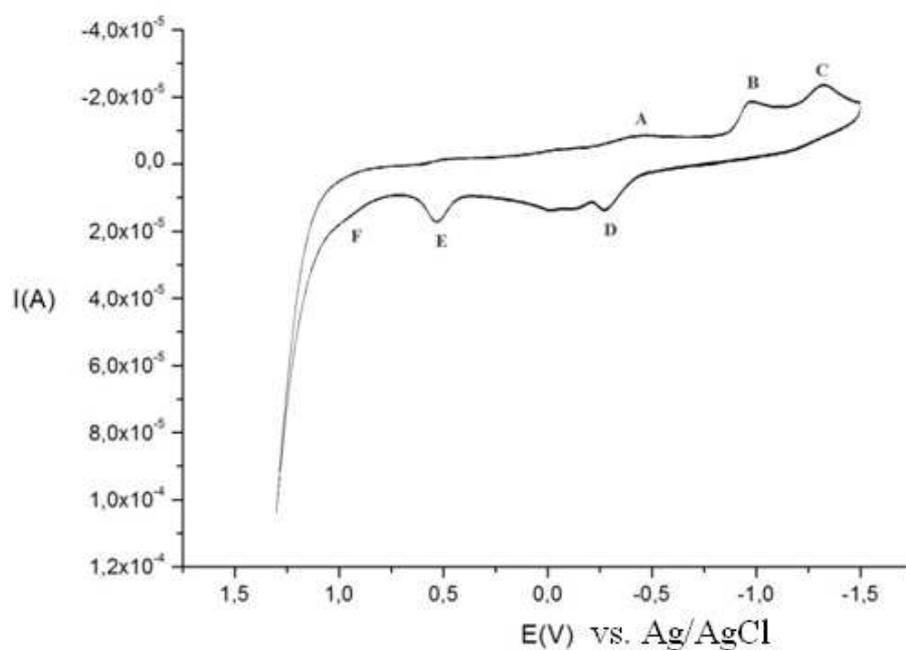
**Figure 28.** Cyclic voltammogram of  $[\text{Cu}(\text{acacphacac en})]$  recorded at  $100 \text{ mV s}^{-1}$



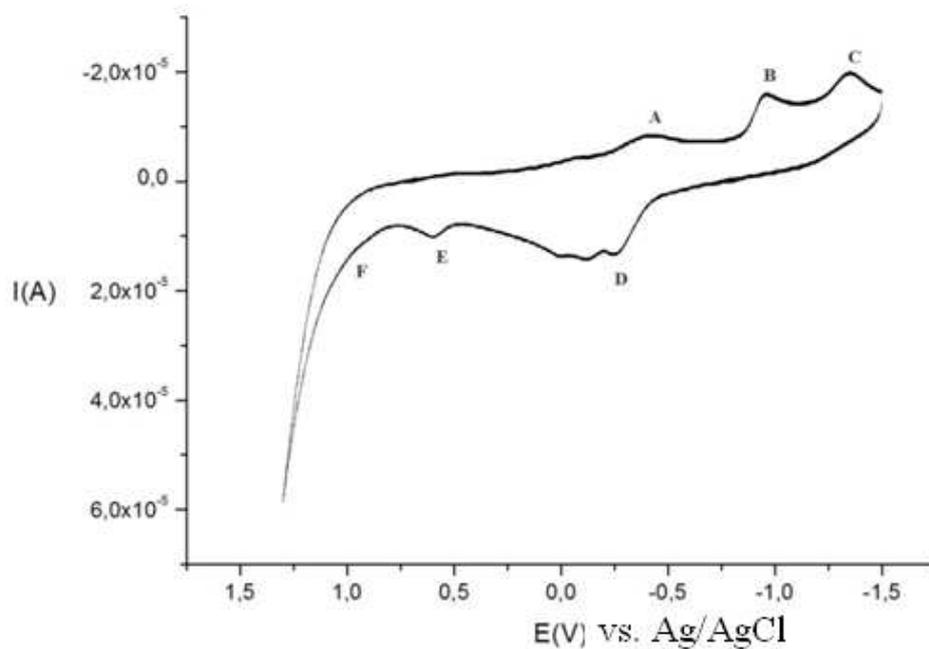
**Figure 29.** Cyclic voltammogram of [Cu(acac<sub>2</sub> en)] recorded at 100 mV s<sup>-1</sup>



**Figure 30.** Cyclic voltammogram of [Cu(phacac<sub>2</sub> en)] recorded at 100 mV s<sup>-1</sup>



**Figure 31.** Cyclic voltammogram of [Cu(*acac*<sub>2</sub>*pn*)] recorded at 100 mV s<sup>-1</sup>



**Figure 32.** Cyclic voltammogram of [Cu(*phacac*<sub>2</sub>*pn*)] recorded at 100 mV s<sup>-1</sup>

As can be seen from Figure 29 on the negative scan of the Cu(*acac*<sub>2</sub>*en*) three anodic peaks (A,B,C) were obtained. The peak A at -0.104 V is quasi-reversible. This peak describes reduction of liberated Cu(II) to Cu(I). Corresponding oxidation peak was

observed at +0.239 V (peak D). This Cu(II)/Cu(I) couple is found to be quasi-reversible with  $\Delta E_p=0.343$  V while the ratio of cathodic and anodic peak currents is corresponding to one-electron process. The sharpness of oxidation peak of copper is due to the stability of Cu(II) in the applied solvent. Since the DMSO has very high donor number this peak is lower than the same peak in AcN. The peaks B and C are irreversible at all sweep rates in the range 10-100 mV s<sup>-1</sup>.

The peak B at -0.448 V can be attributed to the reduction of metal center Cu<sup>2+</sup>(acac<sub>2</sub>en)/Cu<sup>+</sup>(acac<sub>2</sub>en) while peak C at -1.367 V corresponds to irreversible reduction of the imino group [108]. The reversible oxidation peak E of the complex occurs at +0.933 V. Peak E, absent in the CV scan of the ligand alone, can be attributed to the reversible oxidation of the imino groups. These groups do not exist in the free ligand. In the reverse scan they show a small reduction peak G at +0.831 V (Figure 29). The value of E<sub>1/2</sub> is 0.882 V.

Also, the voltammogram shows anodic wave at +1.281 V (peak F). The peak F is observed at nearly the same potential as the corresponding peak in the voltammogram of the free ligand. Based on results for N,N'-bis(acetylaceton)ethylenediimine and copper(II)(ethylenediamine-bisacetylacetonato) complex we used the same, optimized, conditions for investigation of structure influence on chemical behavior of series of Schiff bases and corresponding copper(II) complexes (Table 5) [109]. Under these conditions we were able to detect significant differences in chemical behavior between compounds with fine structural differences. In Table 5 are listed values of potentials for corresponding peaks.

**Table 5.** Values of potentials for corresponding peaks.

Compound	A(V)	B(V)	C(V)	D(V)	E(V)	F(V)
[Cu(acac <sub>2</sub> en)]	-0,56	-1,03	-1,31	-0,27	0,56	0,97
[Cu(acacphacac en)]	-0,54	-1,02	-1,31	-0,29	0,56	0,92
[Cu(phacac <sub>2</sub> en)]	-0,45	-1,03	-1,35	-0,29	0,58	0,94
[Cu(acac <sub>2</sub> pn)]	-0,47	-0,98	-1,33	-0,27	0,53	0,97
[Cu(phacac <sub>2</sub> pn)]	-0,41	-0,96	-1,35	-0,25	0,59	0,93

The difference between potentials of peaks D and B are listed in Table 6. Based on this results can be concluded that oxidation and reduction processes of coordinated copper(II) ions are irreversible. Difference between potentials of peak D and B increase with increasing of scan rate. This indicates the reversibility of oxidation-reduction processes.

**Table 6.** Difference between potentials of peaks D and B

Compound	$E^D - E^B$ (V)
[Cu( <i>acac</i> <sub>2</sub> <i>en</i> )]	1,29
[Cu( <i>acacphacac</i> <i>en</i> )]	1,31
[Cu( <i>phacac</i> <sub>2</sub> <i>en</i> )]	1,32
[Cu( <i>acac</i> <sub>2</sub> <i>pn</i> )]	1,25
[Cu( <i>phacac</i> <sub>2</sub> <i>pn</i> )]	1,22

This is in accordance with ratio values of anodic and cathodic current which are very close to one (Table 7).

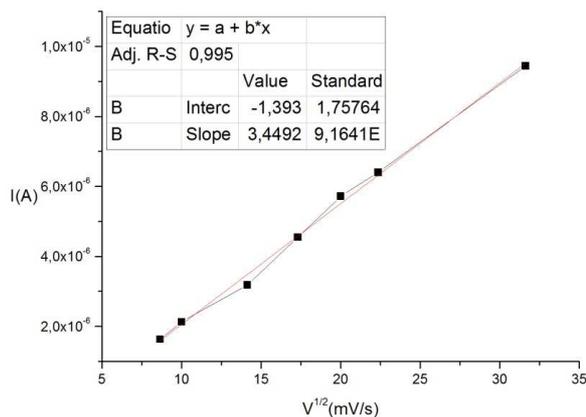
**Table 7.** Ratio values of anodic (D) and cathodic (B) currents

Compound	$I^D$ ( $\mu A$ )	$I^B$ ( $\mu A$ )	$I^D/I^B$ ( $\mu A$ )
[Cu( <i>acac</i> <sub>2</sub> <i>en</i> )]	4.020	2.370	1.700
[Cu( <i>acacphacac</i> <i>en</i> )]	2.130	1.750	1.220
[Cu( <i>phacac</i> <sub>2</sub> <i>en</i> )]	1.120	0.353	3.180
[Cu( <i>acac</i> <sub>2</sub> <i>pn</i> )]	2.310	1.640	1.400
[Cu( <i>phacac</i> <sub>2</sub> <i>pn</i> )]	0.660	1.830	0.360

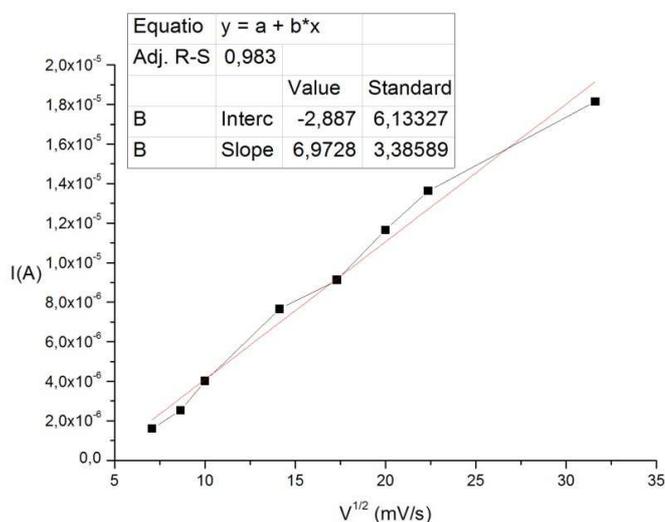
The phenyl groups in comparison to methyl group are stabilised the lower oxidation state of of copper ion as a consequence of inductive effect of these groups. This effect lowered the density of electrons on central metal ion which is center of reduction. This ion started to be more positive and easier capable for reduction. The potentials of cathodic peak are moved to more positive values of potential when the investigated compound contains phenyl groups. The presence of methyl group which have a positive inductive effect we established completely opposite behaviour. The characteristic peaks

of reduction of imino group are moved to negative potentials when the number of withdrawing substituents is increase.

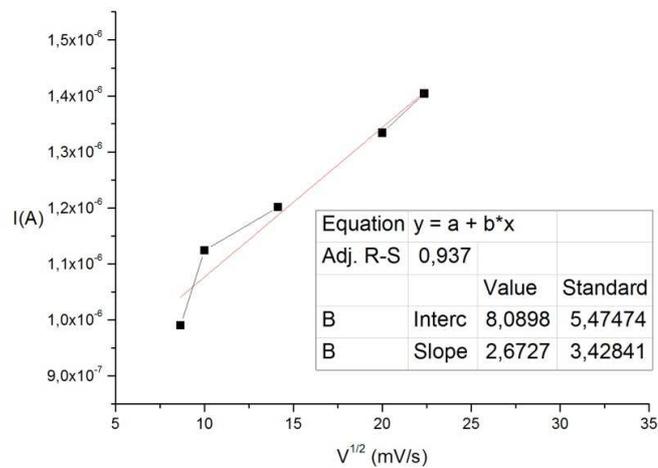
Dependences of current intensity and square root of scan rate are shown on Figures 33-37 (for redox process  $\text{Cu(I)L} \rightarrow \text{Cu(II)L}$ ). Cyclovoltammograms are scanned on different scan rates: 50, 75, 100, 200, 300, 400, 500, and 1000  $\text{mV s}^{-1}$ .



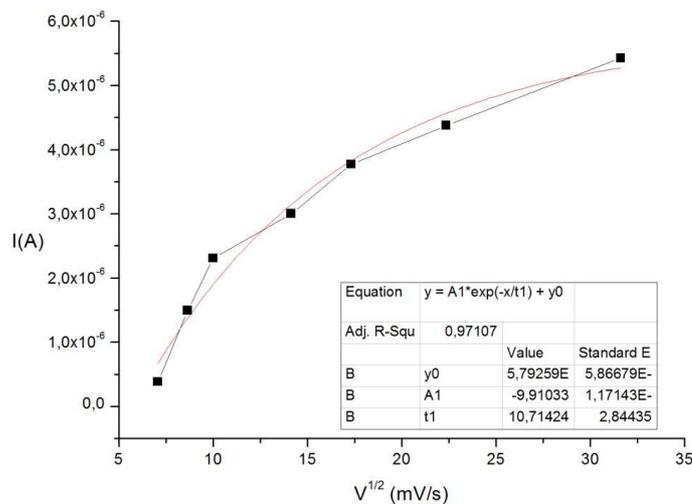
**Figure 33.** Dependence of current intensity and square root of scan rate for  $[\text{Cu}(\text{acacphacac en})]$



**Figure 34.** Dependence of current intensity and square root of scan rate for  $[\text{Cu}(\text{acac}_2 \text{ en})]$



**Figure 35.** Dependence of current intensity and square root of scan rate for  $[Cu(phacac_2 en)]$



**Figure 36.** Dependence of current intensity and square root of scan rate for  $[Cu(acac_2 pn)]$

The electrode processes of complexes  $[Cu(acacphacac en)]$ ,  $[Cu(acac_2 en)]$  and  $[Cu(phacac_2 en)]$  are diffusion controlled, while in the case of  $[Cu(acac_2 pn)]$  and  $[Cu(phacac_2 pn)]$  the processes in electrolytic cell are controlled by adsorption on the surface of electrode.

#### 4.2.2.1. Effect of chelation

Knowledge of electrochemical redox processes of the Schiff bases as free ligands is important in properly assigning the electron transfer processes of corresponding copper(II) complexes. In the voltammograms of investigated copper(II) complexes (compound 7-12) several characteristic peaks were detected. They are the result of the following redox processes: reduction of liberated Cu(II) to Cu(I) (quasi-reversible), reduction (Cu(II)L to Cu(I)L) and oxidation (Cu(II)L to Cu(III)L) of metal center (quasi-reversible), irreversible reduction of the imine group and the oxidation of the imine group [107,108]. Nevertheless, the voltammograms show anodic wave at nearly the same potential as the corresponding peak in the voltammogram of the free ligand.

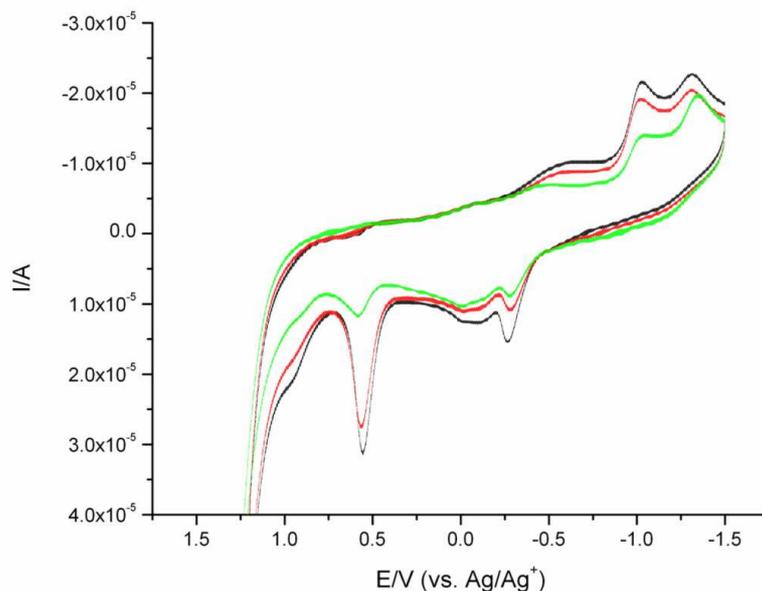
#### 4.2.2.2. Effect of substituents in copper(II) complexes

Analysis of the recorded voltammograms of the complexes indicates that the obtained redox potentials depend on the nature of substituents as well as of the structure of the imine bridge. These effects were found in all of the investigated complexes. Figure 38 shows overlapped voltammograms of [Cu(*acac*<sub>2</sub> *en*)], [Cu(*acac phacac en*)] and [Cu(*phacac*<sub>2</sub> *en*)] complexes as example. The voltammetric parameters obtained for the copper(II) complexes are listed in the Table 8.

**Table 8.** Characteristic peaks obtained in voltammograms of copper(II) complexes.

Comp	$E_{c1}/V^a$	$E_{c2}/V$	$E_{a1}/V$	$E_{a2}/V$	$E_{a3}/V$
7	-1,036	-1,319	-0.270	+ 0,554	+0.968
8	-1,020	-1,329	-0.284	+ 0,563	+0.954
9	-1,010	-1,347	-0.290	+ 0,583	+0.907
10	-0,972	-1,332	-0.278	+ 0,525	+0.974
11	-0,967	-1,349	-0.290	+ 0,550	+0.965
12	-0,958	-1,357	-0.294	+ 0,580	+0.951

<sup>a</sup> vs. Ag/Ag<sup>+</sup>

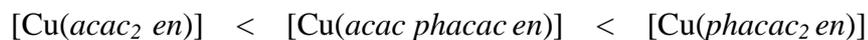


**Figure 37.** Overlapped voltammograms of  $[Cu(acac_2 en)]$  (black),  $[Cu(acac phacac en)]$  (red) and  $[Cu(phacac_2 en)]$  (green) at  $0.1 \text{ V s}^{-1}$

Cathodic peak ( $E_{c1}$ ) is characteristic quasi-reversible peak for reduction of metal center in investigated complexes with corresponding reverse scan anodic peak ( $E_{a1}$ ). Phenyl groups compared to the methyl stabilize the lower oxidation state of the metal ion because of strong electron-withdrawing effect of phenyl group [107,110]. This effect lowers the electron density on central metal ion as reduction center which becomes more positive and thereby more easily reduced. Therefore, the electron-withdrawing group shift the potential of the cathodic peak  $E_{c1}$  to more positive values as can be seen from the Table 7. The presence of electron-donating groups in compounds has a reverse effect [103]. Also, from obtained voltammograms one anodic peak ( $E_{a3}$ ) is observed which is attributed to oxidation of Cu(II) to Cu(III) in complexes without corresponding cathodic wave in the reverse scan [107].

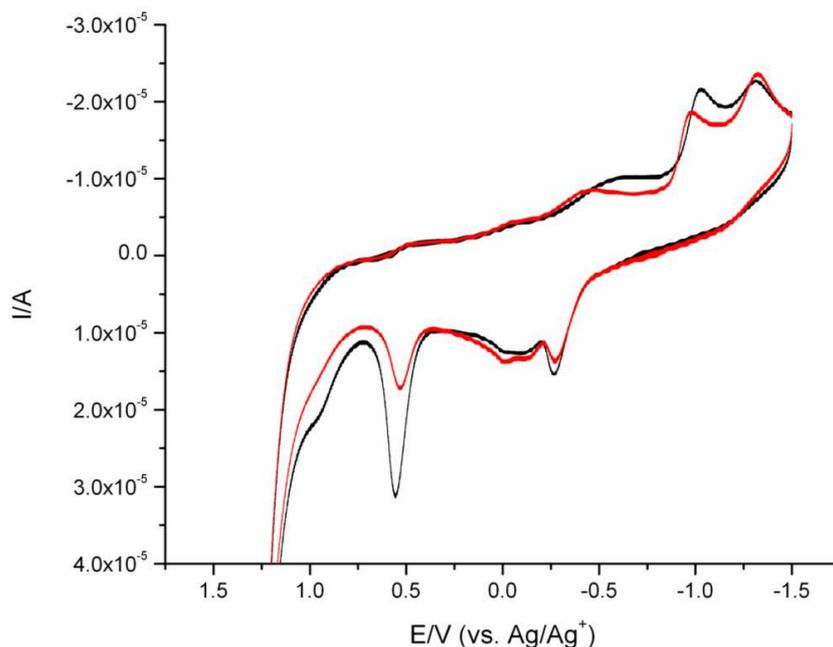
Characteristic peak for the reduction of the imine group, which occurs at the potential  $E_{c2}$  shifts to more negative values with the decrease in the electron-donating ability of the substituents. Also, methyl group may be considered as a  $\pi$ -donor due the hyperconjugation effect of this group [111]. Besides, due to the presence of methyl group in the Schiff base ligands the imine group becomes less resistant to oxidation. It

was found that the anodic peak on  $E_{a2}$  (Table 8) shifts towards more positive values as a result of successive substitution of methyl by phenyl groups. The oxidation potential becomes more positive in the sequence of increasing electron-withdrawing effects of the substituents in the ligand. The potentials increase in following orders:



The phenyl group lowers basicity of nitrogen atoms and therefore its oxidation occurs at more positive values of potentials. This effect is more pronounced by introduction of both phenyl groups. This is in accordance with electrochemical studies of electronic effects of substituents of vanadyl complexes with tetradentate Schiff base ligands derived from 1,2-propylenediamine [112].

Finally, the variety of imine bridge induced the difference of anodic/cathodic peak potentials. The Figure 39 shows overlapped voltammograms of  $[\text{Cu}(\text{acac}_2 \text{ en})]$  and  $[\text{Cu}(\text{acac}_2 \text{ pn})]$ .



**Figure 38.** Voltammograms of  $[\text{Cu}(\text{acac}_2 \text{ en})]$  (black) and  $[\text{Cu}(\text{acac}_2 \text{ pn})]$  (red),  $0.1 \text{ V s}^{-1}$

Steric effect is less pronounced compared to the inductive effect of substituents. Electron density on the copper ion in the complexes with phenyl groups is less and the copper coordination geometry may also be more distorted due to steric effect of the

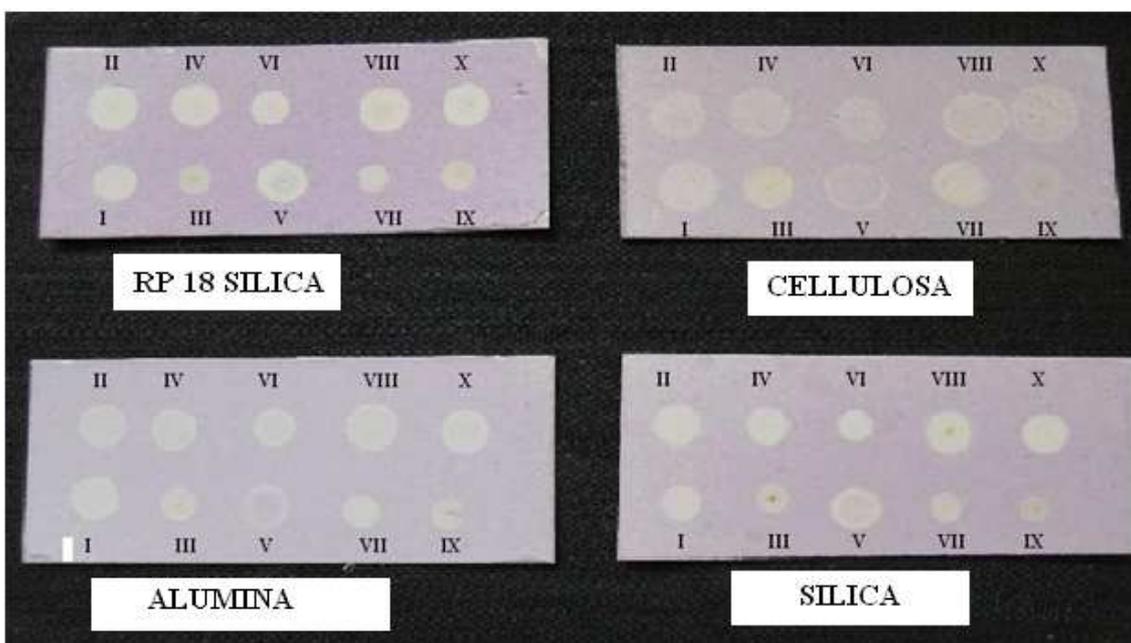
bulky phenyl group substituent [107]. Apparently, by increasing the steric effect of the substituents anodic peak,  $E_a$ , reaches higher values.

### 4.3. Antioxidant activity

#### 4.3.1. Optimization of condition for TLC DPPH assay

##### 4.3.1.1. Stationary phase

When choosing the optimal stationary phase on which will be determined antioxidant activity with TLC DPPH assay is accompanied by changes in purple colour of DPPH to yellow as a result of the antioxidant capacity of tested compounds („Dot-blot“ test). For this optimization process was used four different types of stationary phases: RP 18 silica gel, cellulose, alumina, and silica gel. Zone in which they are applied compounds that possess antioxidant activity are colored yellow (Figure 40).



**Figure 39.** „Dot-blot“ test on different stationary phases

As can be seen from Figure 39, all of the investigated compounds show antioxidant activity. When applied RP-18 silica gel as stationary phase, obtained zones

are sharp, clear and relatively stable compared to the other tested sorbents. Therefore, for the quantification of antioxidant activity is selected RP-18 silica gel as stationary phase.

#### **4.3.1.2. Solubility**

The tested compounds are soluble in various solvents, depending on the structure, and the majority does not dissolve in ethanol and methanol, in which is prepared solution of DPPH. If the determination is done using spectrophotometric DPPH assay, in which it is necessary to mix the solution of the test substance and DPPH reagent in order to reach and response to antioxidant activity was determined, solubility of investigated compounds is a problem. In addition, the solvent in which the dissolved test compounds can interfere with the reaction with DPPH

In the case of application of TLC DPPH assay on a selected stationary phase, i.e. RP18 silica gel were applied small volume of the sample solution (up to 1 mL) in a suitable solvent, which, as a rule, easily vaporized (chloroform), and which evaporate before applying the solution of DPPH. On this way we avoided the problem that often interferes in determination of antioxidant activity in DPPH spectrophotometric assays, and occurs due to the incompatibility of applied solvents to dissolve the compounds and reagents for the determination of antioxidant capacity. Also, in comparison to the spectrophotometric determination of antioxidant activity in RP TLC DPPH assay results are easily comparable and do not depend on the solvent in which the compounds prepared solutions. The applied method is sensitive for the determination of antioxidant activity at low concentrations of the sample, so that the absorption of the complex at 515 nm does not affect the absorbance of DPPH. In this way, i.e. selection of optimal concentrations of Schiff base complexes of copper (II) complexes eliminated the influence of absorption at the wavelength at which absorbs DPPH.

#### **4.3.1.3. Incubation time**

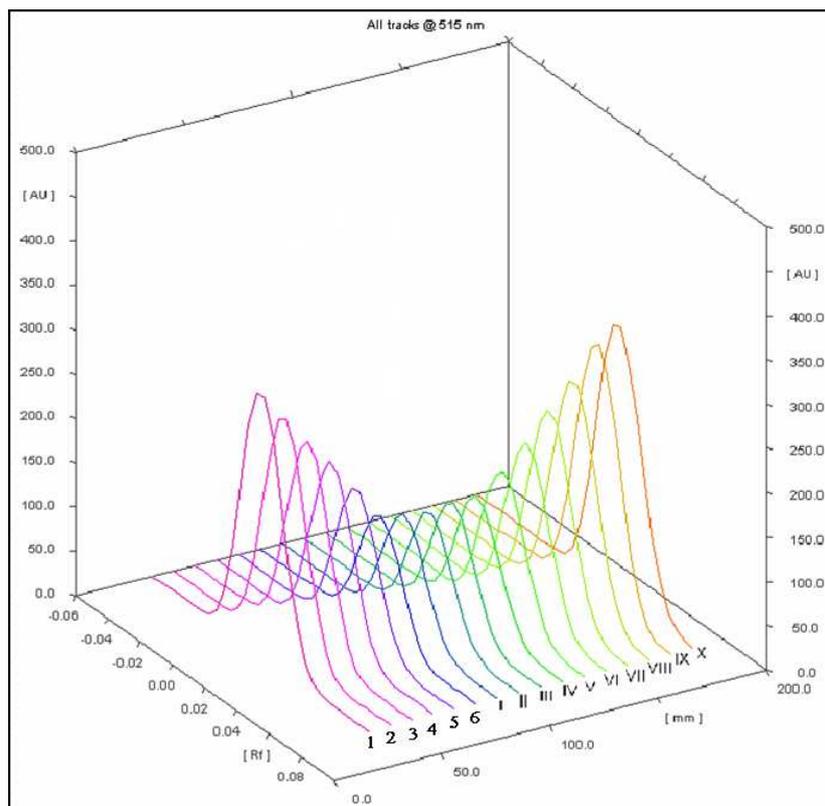
Incubation time is very important parametar in DPPH assay. In order to detreminate the time of incubation (in the dark) we were sccaned TLC plates in the

given incubation time: 5, 10, 15, 20, 25, and 30 minutes. The results of scanning TLC plates 5 minutes after application DPPH are identical to scan results after 10, 15, 20, 25 and 30 minutes. These observations suggest the advantages of RP TLC DPPH essays, as a method of determining the antioxidant activity, compared to DPPH spectrophotometric assays in which the contact time with the DPPH sample is between 20 and 30 minutes. Based on the obtained results, it is obvious that it is necessary scanning RP TLC plates after 5 minutes. On this way significantly reduced the time required for determination of antioxidative activity in comparison with spectrophotometric assay.

#### **4.3.2. Antioxidant activity of Schiff bases and their copper(II) complexes**

Antioxidant activities of the investigated Schiff bases and their Copper(II) complexes were determined by TLC DPPH assay. The relative stable and clear zones on the RP 18 silica gel were observed, enabling the identification of radical-scavenging activity after incubation period. TLC scanner quickly and accurately determines the height and peak areas directly proportional to the absorbance [113].

Chromatograms obtained after scanning using TLC scanner, are shown on Figure 41. Peaks 1-6 belong to standard series of Trolox in the presence of DPPH solution; peaks I, III, V, VII, IX belong to copper(II) complexes 7, 8, 9, 10 and 12; while peaks II, IV, VI, VIII i X correspond to Schiff bases 1, 2, 3, 4 and 6.



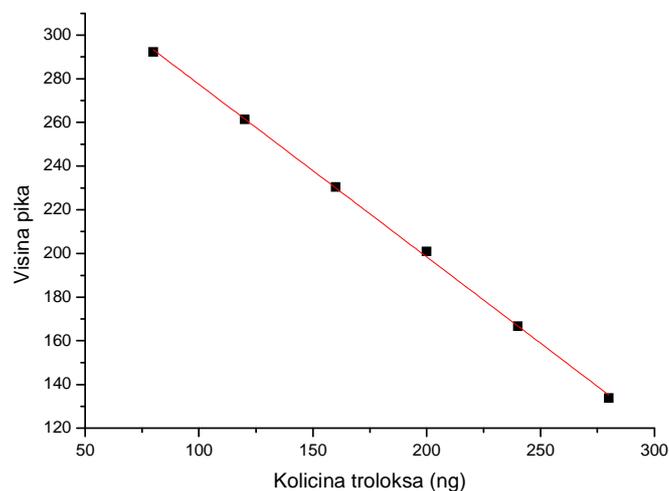
**Figure 40.** Chromatogram obtained by scanning, using TLC scanner

Values of peaks high, as well as, relative antioxidant values of standard solution of Trolox mixed with DPPH are listed in Table 9.

**Table 9.** Peaks heights and relative antioxidant activities of standard series

Track number	Trolox (ng/zone)	Peak high	%RSA
1	80	292.30	18.01
2	120	261.33	26.69
3	160	230.42	35.36
4	200	200.92	43.64
5	240	166.62	53.26
6	280	133.71	62.49

Based on the obtained values, the calibration curve was constructed. The graph shows dependence between peak height and the amount of Trolox (Figure 42). The equation that defines this dependence is:  $y = 365.49 - 0.7904 \cdot x$ ,  $r^2 = 0.9993$ .



**Figure 41.** Calibration curve of standard solution of Trolox

Experimentally obtained values of antioxidant activity of the investigated Schiff bases and their copper(II) complexes expressed as TEAC and % RSA are listed in Table 10.

**Table 10.** Antioxidant activity of the investigated compounds

Compound	TEAC (mmol /g comp)	% RSA
1	1.899	36.80
2	1.846	38.55
3	1.618	46.15
4	1.113	62.83
6	0.543	81.94
7	1.898	36.82
8	1.824	39.28
9	1.388	53.79
10	0.882	70.63
12	0.433	85.58

#### 4.3.2.1. Effect of substituents on antioxidant activity

From the obtained results, several interesting observations can be made. First of all, lower TEAC values (higher antioxidant activity) of copper(II) complexes (7-10 and 11, Table 10) in comparison with corresponding Schiff bases (1-4 and 6, Table 10) are obvious. The coordination of the Schiff bases and the presence of metal ion enhance the antioxidant potential. The presence of the copper(II) ion in the complexes changes the structure of the Schiff base which affects the increase in antioxidant activity of obtained copper(II) complex. Furthermore, it is possible to draw a conclusion on the relationship between the antioxidant activity and the functional groups present in the complexes. It is known, from the literature, that substituents in the Schiff bases have a great influence on the activity of these compounds [114]. As can be seen in Table 1, the antioxidant activity increased by the number of phenyl groups in the complexes containing ethylenediamine as amine part ( $7 < 8 < 9$ , Table 1). The opposite dependence was observed for complexes containing propylenediamine.

Results given in Table 10 shows that the antioxidant activity increases with the introduction of the phenyl group in complexes with ethylene amine as part ( $7 < 8 < 9$ ), while the complex containing propylenediamine determined by reverse dependency. There is obvious effect of structures on antioxidant activity of series of investigated compounds. Substitution of methyl by phenyl groups has effect on TEAC values of ligands and corresponding complexes. Introduction of phenyl group in compounds lowers their antioxidant activity. This is probably due to the electron-withdrawing effect, which is more pronounced by introduction of second phenyl group as seen in results. Also, all compounds containing propane-1,2-diamine as amine part show lower TEAC values in comparison to corresponding compounds with ethane-1,2-diamine. Introduction of two phenyl groups in the structure results in a more pronounced effect on antioxidant activity, probably due to the symmetry of observed compounds. The same trend in the values of antioxidant capacity has been established for series of Schiff bases, with the exception of compound 2, which has an asymmetric structure.

Exactly the same correlation structure and antioxidant activity exists if the mean values of % RSA. Compounds with higher %RSA are stronger antioxidants, which means that the higher the absorbance decrease, is greater antioxidant effect of tested

solutions, i.e. higher percentage of DPPH is react with the observed compounds, either in the Schiff base and its complex with Cu (II).

#### **4.3.3. Correlation between electrochemical behavior and antioxidant activity**

It is known that the antioxidant activity is conceivably related to the electrochemical behavior [115]. Increased both the antioxidant activity and  $E_a$  with an increased number of methyl groups for all of investigated Schiff bases were observed. In the case of copper(II) complexes a different dependence is noticed: increase of the antioxidant activity and decrease of  $E_a$  with an increased number of methyl groups. This is in accordance with rule that complexes with electron-donating groups have lower  $E_a$  than compounds with electron-deficient rings and therefore better antioxidant activity (DPPH assay) and higher reducing power [116].

#### **4.4. Structure Antioxidant activity relationship study**

##### **4.4.1. Structure Antioxidant activity relationship study of tetradentate Schiff bases**

The analysis of calculated descriptors for Schiff bases and complexes revealed clear differences between compounds as a consequence of both substituents and complexation. In the attempt to explain the mechanism of the antioxidant action and the influence of the structure on the antioxidant activity of investigated compounds, Pearson's correlation matrix was carried out. The Pearson's correlation analysis tests the relationship among calculated molecular descriptors (Table 11) and experimentally determined DPPH scavenging activity. DPPH was chosen as free radical because of its ability to be reduced either by electron-transfer or by hydrogen atom transfer. The pairwise method was employed (99% confidence level,  $p < 0.01$ ).

**Table 11.** Descriptors for investigated Schiff bases (Table 1)

		<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>6</b>	
Dipole moment		5.1271	5.0410	4.8587	0.6573	0.5303	
BDE (Scheme 1)		14.0	13.2	13.4	14.5	14.9	
HOMO		-0.2015	-0.2015	-0.2024	-0.2003	-0.2011	
LUMO		-0.0295	-0.0479	-0.0499	-0.0293	-0.0491	
delta		-0.1720	-0.1536	-0.1525	-0.1709	-0.1519	
Atom no	charge NBO						
1	C	-0.7566	C -0.7581	C -0.1175	C -0.7556	C -0.1166	
2	C	0.5192	C 0.5212	C 0.5022	C 0.5170	C 0.5010	
3	O	-0.6447	O -0.6381	O -0.6482	O -0.6518	O -0.6514	
4	C	-0.4663	C -0.4673	C -0.4481	C -0.4661	C -0.4493	
5	C	0.2894	C 0.2864	C 0.2900	C 0.2952	C 0.2945	
6	C	-0.7263	C -0.7257	C -0.7260	C -0.7281	C -0.7277	
7	N	-0.6154	N -0.6178	N -0.6122	N -0.6173	N -0.6153	
8	C	-0.2763	C -0.2762	C -0.2768	C -0.0661	C -0.0662	
9	C	-0.2763	C -0.2767	C -0.2766	C -0.2716	C -0.2721	
10	N	-0.6155	N -0.6120	N -0.6125	N -0.6154	N -0.6122	
16	O	-0.6447	O -0.6480	O -0.6476	O -0.6496	O -0.6521	
17	H	0.2350	C -0.2392	C -0.2391	C -0.7003	C -0.2393	
18	H	0.2350	C -0.2249	C -0.2248	H 0.2354	C -0.2253	
19	H	0.2346	C -0.1946	C -0.1946	H 0.2441	C -0.1956	
20	H	0.2544	C -0.2413	C -0.2412	H 0.2356	C -0.2409	
21	H	0.2551	C -0.2120	C -0.2121	H 0.2548	C -0.2124	
22	H	0.2532	H 0.2345	C -0.2118	H 0.2318	C -0.2122	
23	H	0.2582	H 0.2327	C -0.2412	H 0.2544	C -0.2411	
24	H	0.2445	H 0.2457	C -0.2248	H 0.2577	C -0.2254	
25	H	0.4611	H 0.2467	C -0.1947	H 0.2539	C -0.1955	
26	H	0.2482	H 0.2544	C -0.2391	H 0.2507	C -0.2395	
27	H	0.2327	H 0.2530	H 0.2326	H 0.4576	C -0.7008	
28	H	0.2327	H 0.2580	H 0.2330	H 0.2379	H 0.2331	
29	H	0.2482	H 0.2440	H 0.2538	H 0.2406	H 0.2445	
30	H	0.4611	H 0.4601	H 0.2580	H 0.4581	H 0.2339	
31	H	0.2445	H 0.2484	H 0.2445	H 0.2520	H 0.2574	
32	H	0.2532	H 0.2328	H 0.4626	H 0.2538	H 0.2541	

Continuation of Table 11.

Atom no	charge NBO	L1	L2	L3	L4	L5
33	H	0.2582	H 0.2327	H 0.2491	H 0.2575	H 0.2508
34	H	0.2543	H 0.2485	H 0.2331	H 0.2540	H 0.4586
35	H	0.2550	H 0.4625	H 0.2331	H 0.2544	H 0.2383
36	H	0.2346	H 0.2450	H 0.2490	H 0.2346	H 0.2419
37			H 0.2535	H 0.4625	H 0.2497	H 0.4597
38			H 0.2578	H 0.2448	H 0.2327	H 0.2524
39			H 0.2406	H 0.2537	H 0.2497	H 0.2542
40			H 0.2392	H 0.2579		H 0.2572
41			H 0.2602	H 0.2407		H 0.2403
42			H 0.2393	H 0.2393		H 0.2392
43			H 0.2348	H 0.2601		H 0.2590
44				H 0.2394		H 0.2395
45				H 0.2350		H 0.2358
46				H 0.2349		H 0.2355
47				H 0.2394		H 0.2394
48				H 0.2393		H 0.2391
49				H 0.2601		H 0.2592
50				H 0.2406		H 0.2401
51						H 0.2504
52						H 0.2324
53						H 0.2502

Statistically significant correlation coefficients, between selected descriptors and DPPH scavenging activity for all investigated Schiff bases, obtained by Pearson's correlation matrixes are shown in Table 12. The descriptors used to explain the possible mechanisms of scavenging/antioxidant activity of the studied compounds were selected on the basis of the highest correlation coefficients.

The Pearson's correlation matrix showed a high correlation of TEAC values of Schiff bases with dipole moment, bond dissociation energy and charge on oxygen atoms obtained by the NBO method. The Pearson's correlation matrix shows a high correlation of TEAC values of Schiff bases with Dipole moment (-0.971), Bond Dissociation Energy (0.939), and Charge on oxygen atoms obtained by NBO method (-0.828 for O16, and -0.765 for O3).

**Table 12.** Pearson`s correlation matrix for Schiff bases

	TEAC	Dipole moment	HOMO	LUMO	delta	charge N7*	charge N10*	charge O16*	charge O3*	charge H N7*
Dipole moment	-0.971									
HOMO	0.668	-0.737								
LUMO	0.075	-0.125	0.607							
delta	-0.029	0.075	-0.558	-0.998						
charge N7*	-0.180	0.251	-0.756	-0.372	0.331					
charge N10*	-0.068	0.118	-0.533	-0.988	0.992	0.234				
charge O16*	-0.828	0.843	-0.404	0.412	-0.460	0.143	-0.428			
charge O3*	-0.765	0.780	-0.350	-0.180	0.162	-0.333	0.267	0.542		
charge H N7*	-0.795	0.852	-0.950	-0.332	0.275	0.719	0.254	0.657	0.391	
charge H N10*	-0.839	0.890	-0.896	-0.564	0.521	0.388	0.552	0.514	0.727	0.866

\*charge obtained by NBO analysis

Statistical correlation parameters and descriptors chosen for linear correlations, extracted from Pearson`s correlation matrixes, are shown in Table 13.

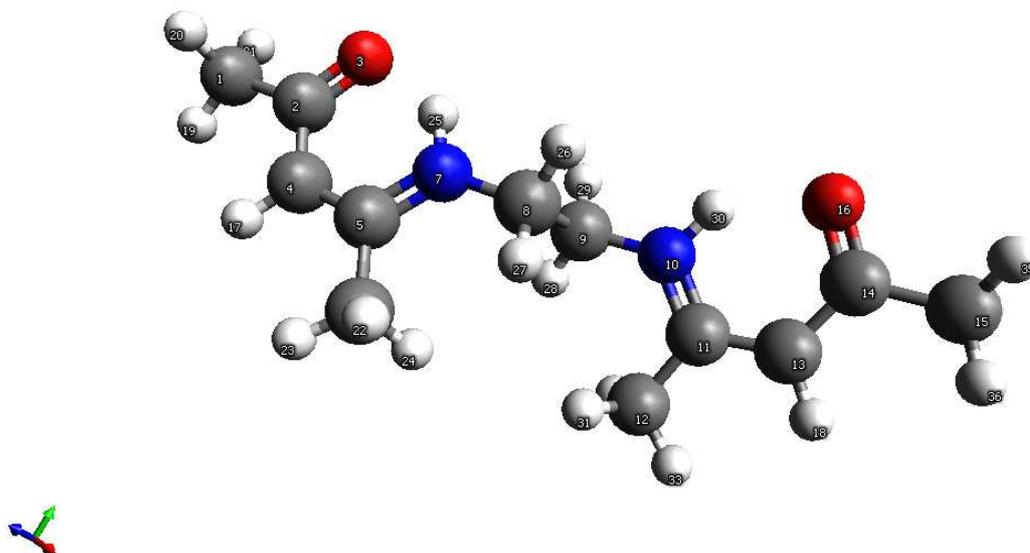
**Table13.** Statistical correlation parameters and descriptors chosen for linear correlations for Schiff bases

	Descriptor	r <sup>a</sup>	R-Squared	Intercept	Slope	SD <sup>b</sup>	P <sup>c</sup>
<b>Schiff bases</b>	Dipole moment	-0.971	0.9424	5.2965	-0.6549	0.4526	0.0060
	BDE <sup>d</sup>	0.939	0.8823	10.1901	0.4034	0.2779	0.0178
	Charge on O16	-0.828	0.6827	-316.173	-492.504	1.0623	0.0846
	Charge on O3	-0.765	0.5885	-139.926	-221.221	1.2096	0.1301
	Charge on H N7	-0.795	0.6294	303.7774	-653.398	1.1480	0.1092
	Charge on H N10	-0.839	0.7042	336.7936	-723.96	1.0257	0.0755

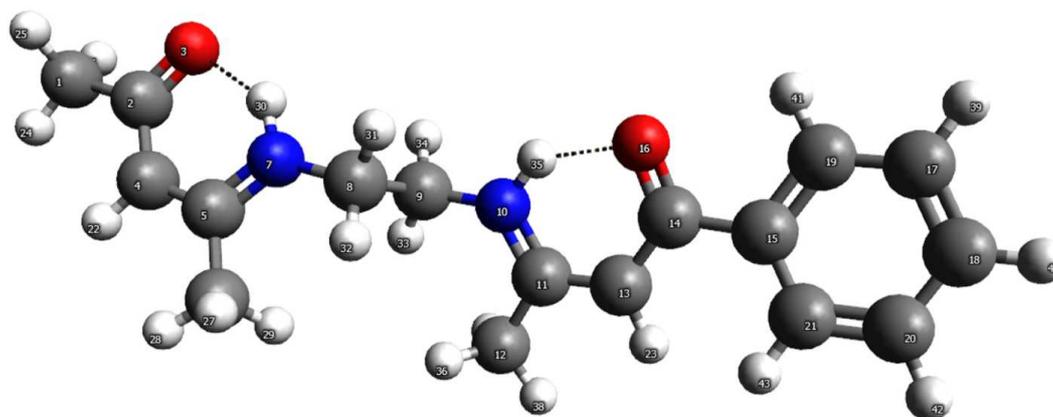
<sup>a</sup> correlation coefficient, <sup>b</sup> standard deviation, <sup>c</sup> significance, <sup>d</sup> Bond Dissociation Energy

Moreover, from the NBO analysis, it was concluded that the acidity of the hydrogen atoms related to the nitrogen atoms of amine bridge is very significant for the activity. Antioxidant activity is in good correlation with charge on hydrogen atoms

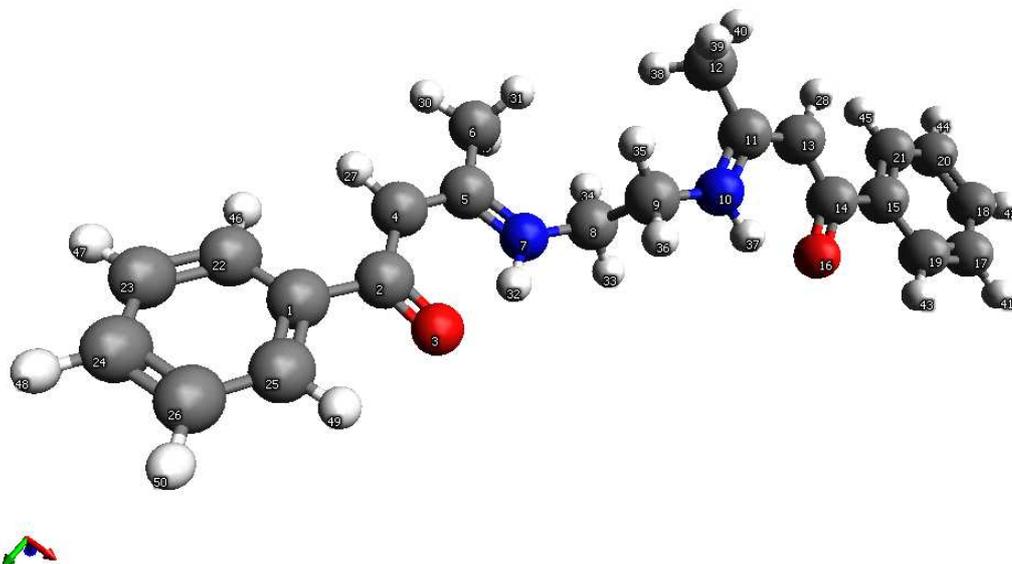
obtained by NBO analysis (H on N7 and H on N10). Fully optimized geometry of investigated Schiff bases (L1-L5) are shown on Figures 42-46.



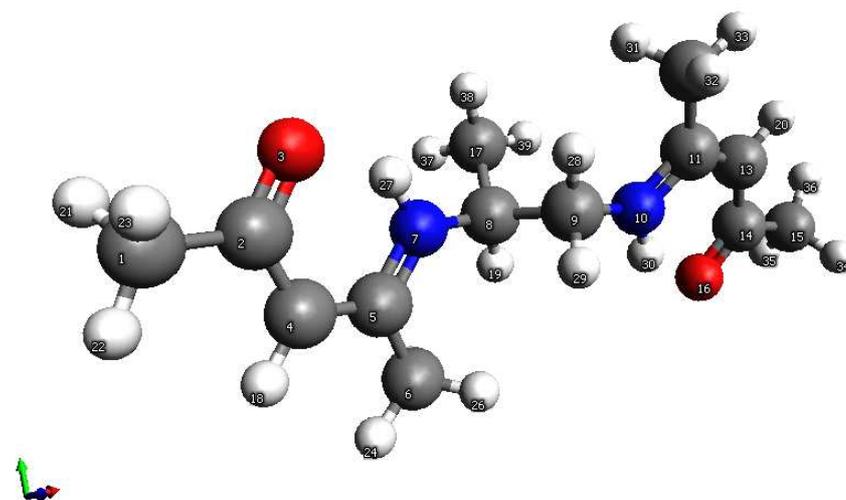
**Figure 42.** The B3LYP/6-31G (d,p) fully optimized geometries of the Schiff base 1



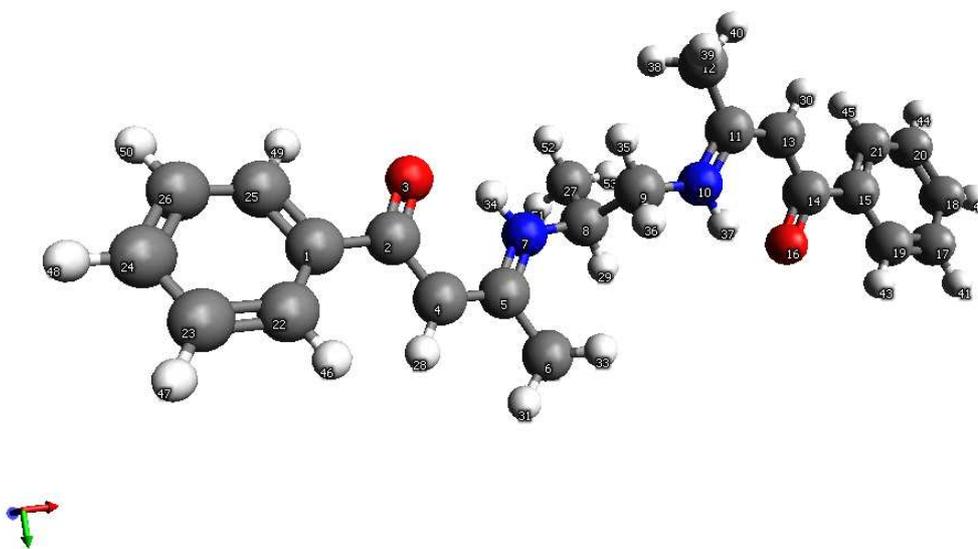
**Figure 43.** The B3LYP/6-31G (d,p) fully optimized geometries of the Schiff base 2



**Figure 44.** The B3LYP/6-31G (d,p) fully optimized geometries of the Schiff base 3



**Figure 45.** The B3LYP/6-31G (d,p) fully optimized geometries of the Schiff base 4



**Figure 46.** The B3LYP/6-31G (d,p) fully optimized geometries of the Schiff base 6

The values of correlation coefficients (Table 12) show that the higher the values of these descriptors, the lower the antioxidant activity. We believe that the mechanism by which Schiff base ligands behave as antioxidants involves the transfer of hydrogen atom from the amino group to the radical DPPH. Schiff bases are capable of forming an intra-molecular H-bond (i.e., between H atom on N7 and atom O3, H atom on N10 and atom O16; dashed line, Figure 43). This weakens N-H bond and H atom is more easily released. Therefore, the donating capacity of hydrogen atom depends on dipole moments, bond dissociation energy, and charge on oxygen and hydrogen atoms. This is confirmed by the obtained results.

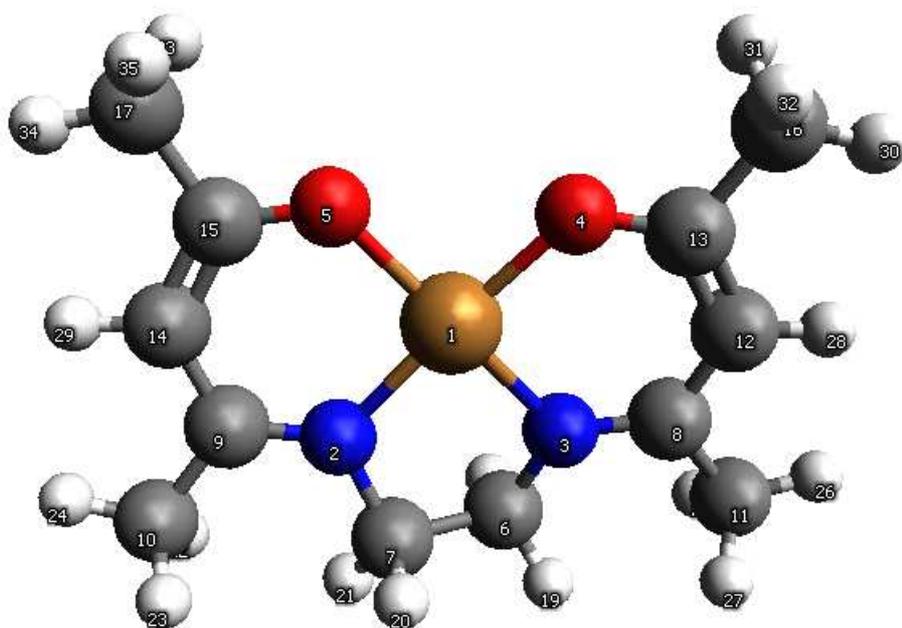
Low BDE values are often attributed to the high antioxidant potential [117,118]. Bond dissociation energy was calculated for N-H bonds on N7 and N10. Difference between BDE for N7-H and N10-H is statistically insignificant. For correlation with antioxidant activity, BDE for N7-H bond was used. It is obviously that the higher the value of BDE, the lower the antioxidant activity (Table 10). This is in accordance with the proposed reaction mechanism. The global minima for Schiff bases containing propylenediamine bridge (L4, L5) (Figures 46, 47) are close in geometry to the centrosymmetric conformation (not true centrosymmetric geometry due to the presence of chiral carbon atom). This leads to very low dipole moment values due to cancellation

of the existing dipoles from two halves of the molecule. Unlike them, Schiff bases containing ethylenediamine bridge (L1, L2, L3) have different conformations of the global minima (Figures 43-45) and higher dipole moment values (Table 11)

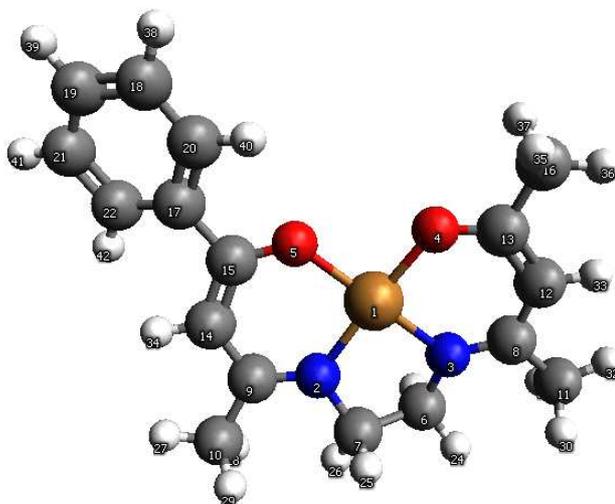
Also, the effects of substituents markedly modify intramolecular hydrogen bonding properties and thus directly affect the antioxidant activity of compounds. TEAC values (Table 10) indicate the electron-donating substituent effect on the activity. Electron-donating groups (methyl) strengthen the N–H bond so that H atom is not easily transferred to the DPPH radical (the hydrogen atom is less acidic). Based on the above mentioned descriptors and their correlation with antioxidant activity, the proposed mechanism of Schiff base action is hydrogen atom transfer.

#### 4.4.2. Quantitative Structure Antioxidant activity relationship study of Copper(II) complexes

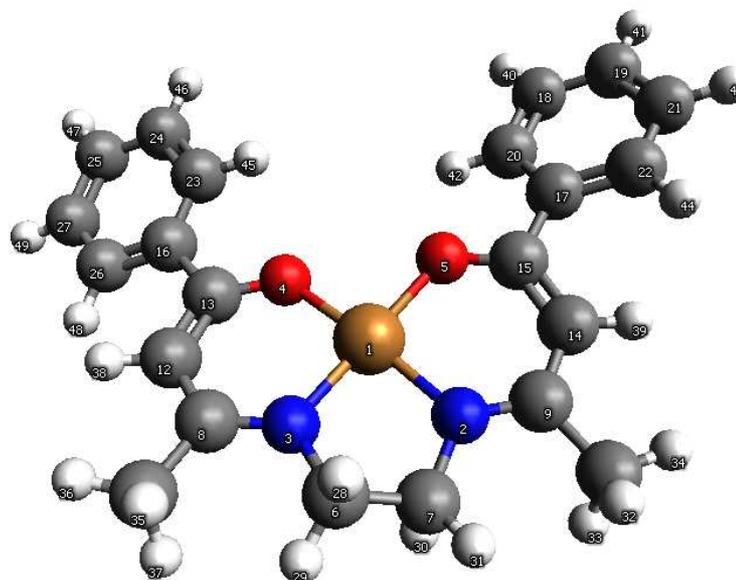
Coordination of the Schiff bases and the presence of the copper(II) ion has significant effect on the increase of antioxidant activity. All copper(II) complexes possess a square-planar geometry around the metal. Angle between best planes of two chelate rings in complex 1 is only  $8.99^\circ$ , indicating square-planar geometry (Figures 48-52).



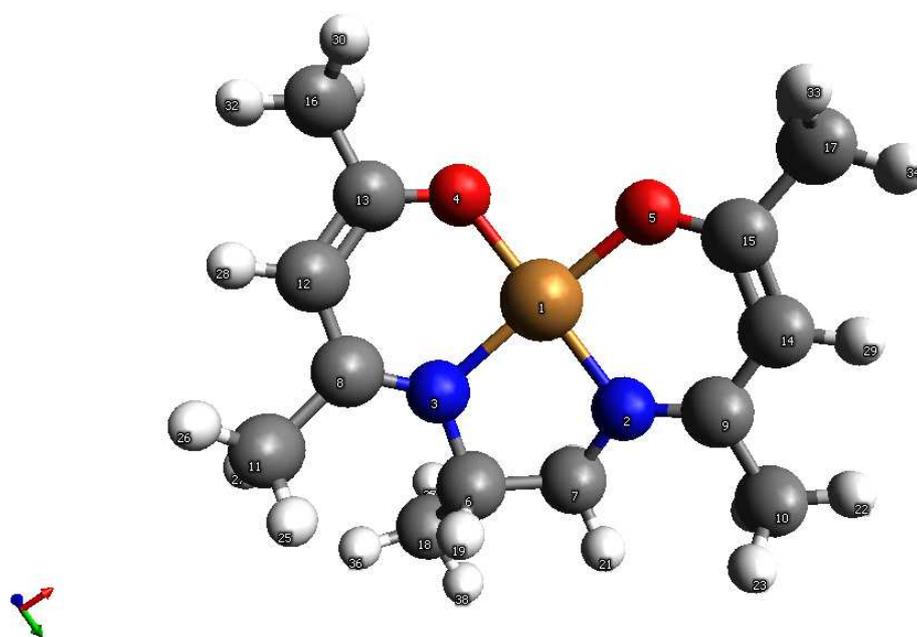
**Figure 47.** The B3LYP/6-31G (d,p) fully optimized geometries of copper(II) complex 1



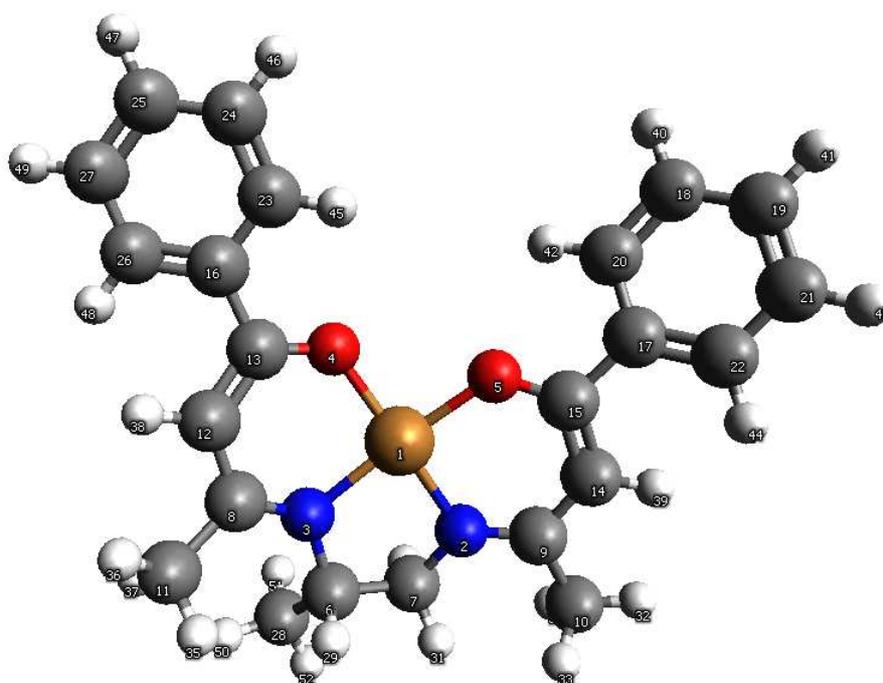
**Figure 48.** The B3LYP/6-31G (d,p) fully optimized geometries of copper(II) complex 2



**Figure 49.** The B3LYP/6-31G (d,p) fully optimized geometries of copper(II) complex 3



**Figure 50.** The B3LYP/6-31G (d,p) fully optimized geometries of copper(II) complex 4



**Figure 51.** The B3LYP/6-31G (d,p) fully optimized geometries of copper(II) complex 5

The substitution of one or both methyl groups with phenyl groups (complexes 2 and 3) introduces new steric hindrance into complex molecule increasing the angle between chelate rings ( $10.86^\circ$  and  $11.71^\circ$ , respectively). In the complexes 4 and 5, new enantiomeric center on carbon atom of methyl group in diamine bridge causes further deviation from square-planar geometry due to steric clashing between this methyl and methyl group from chelate ring.

Calculated descriptors for investigated Copper(II) complexes are listed in Table 14. From Pearson's correlation matrix it is easy to find descriptors that are highly correlated with antioxidant activity of the investigated complexes are chelate plane angle (0.906) and sum spin density ( $-0.887$ ), being an indicative that the presumed mechanism of antioxidant activity is based on the electron-transfer (Table 15).

**Table 14.** Descriptors for investigated copper(II) complexes (Table 1)

Atom no		7		8		9		10		12
	Spin density									
1	Cu	0.541488	Cu	0.544047	Cu	0.545634	Cu	0.539366	Cu	0.543073
2	N	0.122008	N	0.122716	N	0.124117	N	0.119456	N	0.121146
3	N	0.121994	N	0.123428	N	0.124122	N	0.11877	N	0.121038
4	O	0.093561	O	0.093655	O	0.090063	O	0.093211	O	0.090532
5	O	0.093547	O	0.089545	O	0.090066	O	0.097222	O	0.09319
6	C	0.001611	C	0.001803	C	0.001748	C	0.001755	C	0.001754
7	C	0.001611	C	0.001681	C	0.001748	C	0.003127	C	0.003152
8	C	-0.009671	C	-0.009705	C	-0.00948	C	-0.008069	C	-0.008047
9	C	-0.009668	C	-0.009343	C	-0.00948	C	-0.009734	C	-0.009619
10	C	0.011315	C	0.011052	C	0.011199	C	0.011139	C	0.011008
11	C	0.011313	C	0.011433	C	0.0112	C	0.010953	C	0.010912
12	C	0.004183	C	0.004144	C	0.00409	C	0.004372	C	0.004423
13	C	-0.007624	C	-0.007686	C	-0.007565	C	-0.006182	C	-0.006384
14	C	0.004175	C	0.003972	C	0.00409	C	0.004419	C	0.004371
15	C	-0.007618	C	-0.007442	C	-0.007566	C	-0.007923	C	-0.007912
28	H	-0.000569	H	-0.000796	H	0.000187	H	-0.000606	C	0.000994
29	H	-0.000568	H	-0.00062	H	0.003646	H	-0.000589	H	0.002092
30	H	0.00199	H	-0.000804	H	0.000186	H	-0.000296	H	0.000124
31	H	-0.000548	H	-0.00066	H	0.003647	H	-0.000749	H	0.003916
32	H	-0.000713	H	0.001015	H	-0.000807	H	0.00192	H	0.000999
33	H	-0.000712	H	-0.000567	H	-0.000639	H	-0.000711	H	-0.000743
34	H	0.00199	H	-0.000537	H	0.001081	H	0.002059	H	-0.000651
35	H	-0.000549	H	-0.000721	H	-0.000639	H	-0.000567	H	-0.000516
36			H	0.001988	H	0.001081	H	-0.000108	H	0.001288
37			H	-0.000557	H	-0.000807	H	-0.000085	H	-0.000984
38			H	0.000024	H	-0.000544	H	0.000973	H	-0.000601
39			H	0.000036	H	-0.000544			H	-0.000562

## Continuation of Table 14.

		Energy of orbitals				
	<b>alfa homo</b>	-0.19058	-0.19108	-0.1917	-0.19051	-0.19153
	<b>alfa lumo</b>	-0.02441	-0.04208	-0.04413	-0.02481	-0.0442
	<b>beta homo</b>	-0.1878	-0.18845	-0.18912	-0.1878	-0.18901
	<b>beta lumo</b>	-0.07368	-0.07557	-0.07732	-0.0753	-0.07893
	<b>Delta alfa</b>	-0.16617	-0.149	-0.14757	-0.1657	-0.14733
	<b>Delta beta</b>	-0.11412	-0.11288	-0.1118	-0.1125	-0.11008
	<b>Dipole moment</b>	3.9769	3.9655	3.9226	3.9735	3.917
	<b>Chelate plane angle</b>	8.994	10.86	11.711	22.634	22.868
		charge by NBO				
		1	2	3	4	5
1	Cu	1.24399	Cu 1.24968	Cu 1.25275	Cu 1.24248	Cu 1.25059
2	N	-0.67401	N -0.71144	N -0.67133	N -0.6687	N -0.66584
3	N	-0.67403	N -0.71367	N -0.67133	N -0.6735	N -0.67092
4	O	-0.73414	O -0.71272	O -0.73562	O -0.73323	O -0.73351
5	O	-0.73416	O -0.70973	O -0.73561	O -0.73113	O -0.7329
28	H	0.2328	H 0.25037	H 0.23062	H 0.23235	C -0.69605
29	H	0.23279	H 0.24874	H 0.22783	H 0.2328	H 0.23148
33	H	0.25374	H 0.22982	H 0.25043	H 0.2535	H 0.24814
34	H	0.23273	H 0.22632	H 0.25099	H 0.23281	H 0.25128
38			H 0.24117	H 0.23303	H 0.23592	H 0.2336
39			H 0.239	H 0.23303		H 0.23278

1 **Table 15.** Pearson`s correlation matrix for copper(II) complexes

	TEAC	Spin density Cu	Spin density N2	Spin density N3	Spin density O4	Spin density O5	Sum Spin desity	Alfa HOMO	Alfa LUMO	Beta HOMO	Beta LUMO	Delta alfa	Delta beta	Dipole moment	Chelate plane angle	Charge Cu*	Charge N2*	Charge N3*
spin density Cu	-0.414																	
spin density N 2	-0.700	0.915																
spin density N 3	-0.702	0.915	0.985															
spin density O 4	-0.287	-0.589	-0.377	-0.278														
spin density O 5	0.582	-0.937	-0.916	-0.964	0.273													
sum spin desity	-0.887	0.734	0.930	0.942	-0.018	-0.850												
alfa HOMO	-0.056	-0.867	-0.632	-0.599	0.880	0.662	-0.313											
alfa LUMO	-0.023	-0.867	-0.594	-0.619	0.677	0.756	-0.329	0.935										
beta HOMO	-0.109	-0.841	-0.585	-0.557	0.878	0.635	-0.259	0.998	0.945									
beta LUMO	-0.550	-0.491	-0.131	-0.103	0.862	0.233	0.229	0.850	0.801	0.880								
delta alfa	0.021	0.864	0.590	0.618	-0.663	-0.758	0.329	-0.928	-1.000	-0.939	-0.795							
delta beta	0.698	0.306	-0.072	-0.098	-0.793	-0.046	-0.420	-0.727	-0.682	-0.767	-0.979	0.677						
Dipole moment	-0.345	-0.657	-0.385	-0.326	0.966	0.380	-0.021	0.941	0.811	0.948	0.940	-0.801	-0.869					
Chelate plane angle	0.906	-0.468	-0.761	-0.784	-0.265	0.656	-0.937	-0.010	0.011	-0.068	-0.527	-0.012	0.684	-0.278				
charge Cu*	-0.121	0.948	0.745	0.742	-0.741	-0.818	0.481	-0.969	-0.970	-0.963	-0.738	0.966	0.590	-0.831	-0.167			
charge N2*	0.546	-0.314	-0.336	-0.476	-0.518	0.599	-0.518	-0.057	0.256	-0.055	-0.285	-0.273	0.363	-0.356	0.510	-0.181		
charge N3*	0.440	-0.255	-0.236	-0.384	-0.523	0.535	-0.407	-0.070	0.263	-0.058	-0.233	-0.281	0.290	-0.347	0.395	-0.159	0.991	
charge O4*	-0.350	0.230	0.175	0.329	0.505	-0.500	0.330	0.056	-0.288	0.038	0.173	0.306	-0.218	0.314	-0.310	0.163	-0.974	-0.995

2 \*charge obtained by NBO analysis

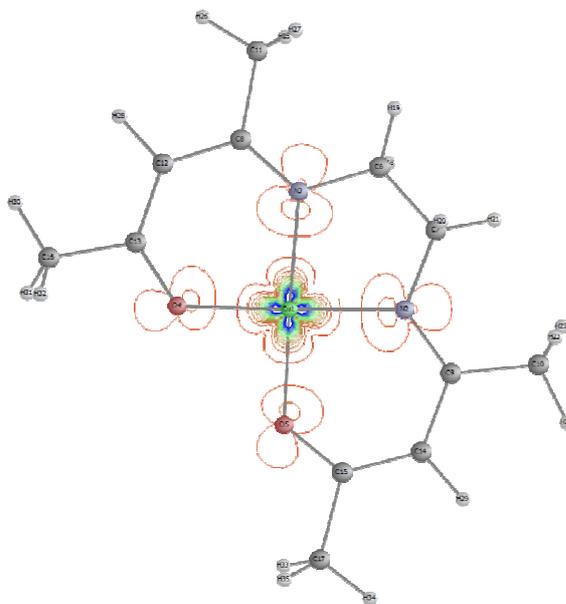
Statistical correlation parameters and descriptors chosen for linear correlations, extracted from Pearson's correlation matrixes, are shown in Table 16.

**Table 16.** Statistical correlation parameters and descriptors chosen for linear correlations for copper(II) complexes

	<b>Descriptor</b>	<b>r<sup>a</sup></b>	<b>R-Squared</b>	<b>Intercept</b>	<b>Slope</b>	<b>SD<sup>b</sup></b>	<b>P<sup>c</sup></b>
<b>Comp</b>	Chelate plane angle	0.906	0.8211	0.1500	0.1375	0.5016	0.0340
	Sum spin density	-0.887	0.7873	331.9138	-339.344	0.5470	0.0447

<sup>a</sup> correlation coefficient, <sup>b</sup> standard deviation, <sup>c</sup> significance, <sup>d</sup> Bond Dissociation Energy

Sum spin density is spin density on the atoms of the inner sphere of complex (N, N, Cu, O, O). This is an important factor which influences the antioxidant potency of the corresponding compound. The higher values of the sum spin density of complex results in stronger ability of the compounds to scavenge free radicals, i.e., lower TEAC values. Based on the values of these descriptors, the influence of substituents on this process can be monitored. Electron-withdrawing effects of phenyl groups affect on the value of sum spin density. Gradual substitution of methyl by phenyl groups increases the sum spin density which leads to the increase of antioxidant activity. The proposed mechanism of antioxidant activity of studied complexes is reduction via electron-transfer. Electron localization is shown in plot of spin density (Figure 53) and it is obvious that spin density is highest at Cu atom.



**Figure 52.** Plot of spin density for copper(II) complex 1

Chelate plane angle describes deviation from square-planar structure of complexes. Higher value of this geometrical descriptor indicates a greater deviation from square-planar structure. From statistical parameters shown in Table 16, it is obvious that increase in chelate plane angle descriptor value leads to increase of the TEAC values, i.e., decrease of antioxidant activity of these complexes. Values of chelate plane angle descriptor of complexes with ethylenediamine bridge are significantly different compared with propylenediamine bridge complexes. The transfer of unpaired electron from compound to DPPH radical is more difficult if the chelate plane angle has a higher value. Therefore, complexes with propylenediamine in the amine bridge have significantly lower antioxidant activity (comp 10 and 12) in comparison with those with ethylenediamine ligand (comp 7, 8 and 9). The influence of chelate plane angle is in accordance with the proposed mechanism of the DPPH scavenging activity for studied complexes.

## 5. CONCLUSION

To optimize the conditions for electrochemical studies of Schiff bases and their copper(II) complexes by cyclic voltammetry we observed the behaviour of N,N'-bis(acetylacetonate)ethylenediimine and copper(II)-(ethylenediiminebis(acetylacetonato)) complex. We analyzed the cyclic voltammograms recorded using different electrodes and solvents as well as different concentrations of solutions at different scan rates. Based on recorded voltammograms we determined the conditions for the electrochemical investigation of different Schiff bases and their copper(II) complexes by cyclic voltammetry. Under these conditions we were able to detect differences in chemical behavior between structurally similar compounds.

Substituents have a detectable effect on the electrochemical behavior of the Schiff bases and their copper(II) complexes. Their inductive and steric effects influence the change in electron density on metal ion and basicity of nitrogen. Therefore, there is the difference in electrochemical behavior, which is detected by shifting of anodic and cathodic potentials values strongly influenced by the nature of the Schiff base ligands. Significant correlations have been observed between redox potentials and antioxidant properties. It is found that compounds with strong scavenging capabilities are oxidized at relatively low potentials and therefore the oxidation potentials can be used as a general indicator of radical scavenging ability. Knowledge of the electronic and steric effects that control redox processes of these compounds offers very interesting research opportunities and may be critical in the design of new one.

Determination of antioxidant activity using thin-layer and TLC scanner provides an opportunity to contact and reaction of the compounds and free radicals independent of the solvent used to prepare the solution. This allows the determination of antioxidant activity of compounds that are not soluble in the solvent used for preparation of stable free radicals (the same solvents are needed in the spectrophotometric determination). Most of the studied Schiff base is sparingly soluble in methanol. In addition, TLC scanner quickly and accurately determines the height and peak area, which is directly proportional to the absorbance. This method can simultaneously determine the antioxidant activity of a large number of compounds, whether they are in a mixture or

individually. In examining the compounds in the mixture, it would be first necessary to develop the chromatogram in a chromatographic system with appropriate solvent.

The method is sensitive enough for the determination of antioxidant activity at low concentrations of the sample. In most cases the absorbance of compounds at low concentrations almost not measurable. Accordingly, if the compound absorbs at the same wavelength as the free radical, its absorbance does not affect the overall absorbance of compounds with free radicals. Reaction mechanism defined in this way, for each group of compounds, contributes to understanding the difference in the antioxidant activity of strength structural related compounds. In this way is explained the impact of the presence of various substituents in the molecule on antioxidant activity. This test and the results allow the design of new structures with desired properties, and their synthesis. Examination of the impact structure on the antioxidant capacity and the results provide us useful information to target the synthesis of new compounds with improved antioxidant activity. Using these methods can also construct a model that can predict the antioxidant activity of new compounds that are structurally similar tested. The structural characteristics of molecules are expressed through molecular descriptors and definitions are those molecular parameters that are responsible for the antioxidant activity. The advantage and importance of this approach lies in the fact that, once in place the appropriate QSAR models, based only on information about the chemical structure can make a conclusion about the antioxidant activity of compounds. This allows selecting those compounds that have a strong potential antioxidant activity and which can be synthesized and tested in the laboratory, thereby reducing the number of required experiments in the process of finding new biologically active compounds.

For better understanding the reactivity patterns and mechanisms of activity of antioxidants, the DFT-based quantum-chemical descriptors were calculated. Quantum-chemical descriptors are the numerical representation of the molecular structures. Dipole moment, bond dissociation energy, charge on oxygen atoms and charge on hydrogen atoms on N7 and N10 show high correlation with antioxidant activity of the investigated Schiff bases. Antioxidant activity of copper(II) complexes is highly correlated with chelate plane angle and sum spin density descriptors. Selected descriptors, which have the best correlation with antioxidant activity, shed light on the reaction mechanism of scavenging/antioxidant activity of the studied compounds.

DPPH may be neutralized either by direct reduction via electron-transfer or by radical quenching via hydrogen atom transfer. The H-atom transfer mechanism was defined as the most important for the antioxidant activity of the investigated Schiff bases while the proposed mechanism for the complexes was single electron transfer. The knowledge of the descriptors that affect the antioxidant activity of investigated and structurally similar compounds and possible mechanisms of antioxidant activity offer very interesting research opportunities and may be critical in the design of new ones.

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## **Author's Biography**

**Najat (Mohamed) Aburas** was born on March 30<sup>th</sup> 1972 in Suk Alkhamisu Libya.

She graduated at the Faculty of Science in the field of Chemistry at Naser University in Alkhomsu, Libya, in 1997.

In 2003 she finished Master studies in Chemistry at the Faculty of Art and Science, Almagreb University, Libya.

**Prilog:**

Bibliografija kandidata kategorisana prema kriterijumima Ministarstva prosvete, nauke i tehnološkog razvoja Republike Srbije.

**M22 - Radovi objavljeni u istaknutom časopisu međunarodnog značaja**

**Najat Aburas**, Aleksandar Lolić, Nikola Stevanović, Tatjana Tripković, Snežana Nikolić-Mandić, Rada Baošić, Electrochemical behavior and antioxidant activity of tetradentate Schiff bases and their copper(II) complexes, *Journal of Iranian Chemical Society* 9(6) (2012) 859-864, DOI: 10.1007/s13738-012-0102-7

**Najat Aburas**, Nikola Stevanović, Miloš Milčić, Aleksandar Lolić, Maja Natić, Živoslav Tešić and Rada Baošić, Influence of structure on antioxidant activity of tetradentate Schiff bases and their Copper(II) complexes: Possible mechanisms, *Journal of the Brazilian Chemical Society*, 24 (8), 1322-1328, 2013, DOI: 0103-5053.20130167

**M23 - Radovi objavljeni u časopisima međunarodnog značaja**

Rada Baošić, Ana Radojević, Tatjana Tripković, **Najat Aburas**, Živoslav Tešić, Quantitative Retention-Property Relationships Studies of some Schiff Base Ligands and their Complexes by Reversed Phase Thin Layer Chromatography, *Chromatographia* 72 (5) (2010) 545- 549 DOI: 10.1365/s10337-010-1664-0

**M34 - Radovi saopšteni na skupu međunarodnog značaja štampan u izvodu**

A.Lolić, **N.Aburas**, T.Tripković, S.Nikolić-Mandić, R.Baošić, Effect of structure on electrochemical behavior of Schiff bases and their complexes with copper(II) by cyclic voltammetry, 16<sup>th</sup> European Conference of Analytical Chemistry, Euroanalysis, 2011, Belgrade, Serbia, Book of abstracts, Session A, EC28.

Прилог 1.

## Изјава о ауторству

Потписана Najat Mohamed Aburas

број уписа 37/2008

### Изјављујем

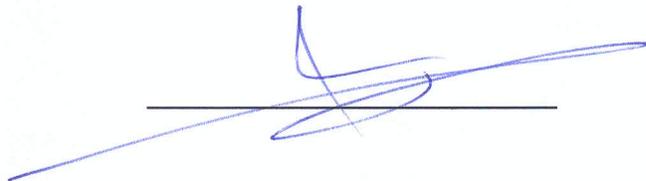
да је докторска дисертација под насловом

“Electrochemical behavior and antioxidant activity of tetradentate Schiff bases and their copper(II) complexes”

- резултат сопственог истраживачког рада,
- да предложена дисертација у целини ни у деловима није била предложена за добијање било које дипломе према студијским програмима других високошколских установа,
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио интелектуалну својину других лица.

Потпис докторанда

У Београду, 27.12.2013.



Прилог 2.

## Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора Najat Mohamed Aburas

Број уписа 37/2008

Студијски програм doktor hemijskih nauka

Наслов рада "Electrochemical behavior and antioxidant activity of tetradentate Schiff bases and their copper(II) complexes"

Ментор dr Rada Baošić, docent

Потписана Najat Mohamed Aburas

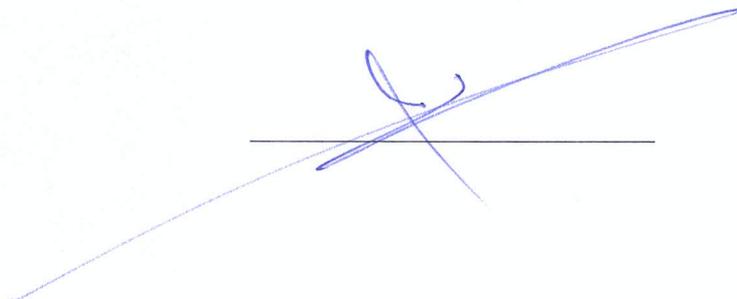
изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла за објављивање на порталу **Дигиталног репозиторијума Универзитета у Београду**.

Дозвољавам да се објаве моји лични подаци везани за добијање академског звања доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

Потпис докторанда

У Београду, 27.12.2013.



### Прилог 3.

## Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

“Electrochemical behavior and antioxidant activity of tetradentate Schiff bases and their copper(II) complexes”

Дисертацију са свим прилозима предала сам у електронском формату погодном за трајно архивирање.

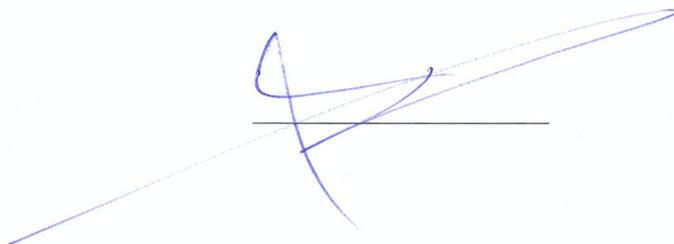
Моју докторску дисертацију похрањену у Дигитални репозиторијум Универзитета у Београду могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучио/ла.

1. Ауторство
2. Ауторство - некомерцијално
3. Ауторство – некомерцијално – без прераде
4. Ауторство – некомерцијално – делити под истим условима
5. Ауторство – без прераде
6. Ауторство – делити под истим условима

(Молимо да заокружите само једну од шест понуђених лиценци, кратак опис лиценци дат је на полеђини листа).

У Београду, 27.12.2013.

**Потпис докторанда**



1. Ауторство - Дозвољаваате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце, чак и у комерцијалне сврхе. Ово је најслободнија од свих лиценци.
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3. Ауторство - некомерцијално – без прераде. Дозвољаваате умножавање, дистрибуцију и јавно саопштавање дела, без промена, преобликовања или употребе дела у свом делу, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца не дозвољава комерцијалну употребу дела. У односу на све остале лиценце, овом лиценцом се ограничава највећи обим права коришћења дела.
4. Ауторство - некомерцијално – делити под истим условима. Дозвољаваате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца не дозвољава комерцијалну употребу дела и прерада.
5. Ауторство – без прераде. Дозвољаваате умножавање, дистрибуцију и јавно саопштавање дела, без промена, преобликовања или употребе дела у свом делу, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце. Ова лиценца дозвољава комерцијалну употребу дела.
6. Ауторство - делити под истим условима. Дозвољаваате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца дозвољава комерцијалну употребу дела и прерада. Слична је софтверским лиценцама, односно лиценцама отвореног кода.