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Synthesis and A Suggestion Mechanism on Biological Evaluation of Amino Acid-Schiff Base Ligands and Co(II), Cu(II) and Ni(II) Complexes

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ABSTRACT

This study aimed to investigate of the antimicrobial activities of some amino acid-Schiff bases complexes as theoretical aspects. Co(II), Cu(II) and Ni(II) complexes of N,N'-(1,4-phenylenedimetiliden)bis DL-Alanine and N,N'-(1,4-phenylendimetiliden)bis DL-Glisine were been prepared and characterized. The antibacterial and antifungal activities were measured by Disc diffusion and MIC method against gram-positive bacteria i.e. Psydomamonas aeruginosa ATCC 29212, Bacillus subtilis RSKK 244, Bacillus megaterium(clinical isolate), gram-negative bacteria Micrococcus Luteus NRRLB and as fungus Candida albicans. The antibiogram tests of amino acid-Schiff bases complexes showed better results than some known antibiotics. Especially Cu(II) complexes were more potent bacteridal than all of the substances synthesized. Furthermore a mechanism of reaction was offered in the explanation of these observation. Some of the compounds exhibited activity comparable to Ketoconazole, Ampicillin, Tetracycline, Penicillin, Gentamisin and Chloroamphenicol.

Keywords: *Schiff base, Amino acid, Metal complexes, Antimicrobial activity, Radical*

1. INTRODUCTION

B Schiff bases and their complexes have a variety of applications in biological, clinical and analytical fields ([1-3]. Recently there has been a considerable interest in the chemistry of amino acid-Schiff bases compounds because of their potential nuclear medicine applications [4]. The imine group of the amino acid-Schiff bases is involved in many different biological processes: decarboxylation, transamination, electron transfer, etc. Salicylaldehyde–amino acid Schiff base complexes are used as non-enzymatic models for the metal-pyridoxal

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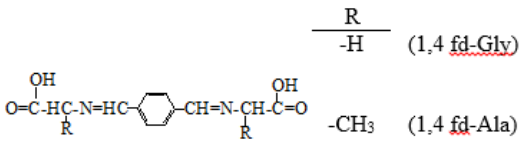
(vitamin B6) amino acid Schiff base systems which are the key intermediates in many metabolic reactions of amino acids catalyzed by enzymes which require pyridoxal as a cofactor [5]. Furthermore, the chemistry of transition metal complexes of amino acid-Schiff bases has received special attention due to their importance in a variety of pharmaceutical and biological process [6,7]. It is well known that the human body contains essential metaloelements which play important roles and interact with many biological molecules [8]. Copper plays a crucial role in enzymes that catalyze oxidation/reduction reactions related to antioxidant systems and is found in many metalloproteins [9]. Nickel plays numerous roles in the biology. For example urease (an enzyme which assists in the hydrolysis of urea) contains nickel. Cobalt is a central component of vitamin B12 (it has a key role in the normal functioning of nervous system) [10].

Active oxygen species have a wide potential for causing cell injury and even death. Nucleic acids, enzymes and membranes are all at the risk of suffering from the attack of active oxygen species. Hydroxyl radical is one of the active oxygen species and it is capable of injuring DNA [11]. The hydroxyl radical is thought to attack both the deoxyribose sugars arrayed along the surface of DNA and the bases which are constructed by the DNA molecule. So, studies on amino acid-Schiff bases and their complexes may be important. Because, -OH radicals may be produce results when fragments of amino acid-Schiff bases [11]. If this radical is more effective in the presence of transition metal ions [12].

The aim of this study was to investigate the antibacterial properties against various pathogenic bacteria of amino acid-Schiff bases (Scheme 1) and their Co(II), Ni(II) and Cu(II) complexes. Later, was to propose a mechanism for these observation. The structures of Schiff bases and their complexes were determined using elemental analyses, 1H-NMR, 13C-NMR electronic spectra, FT-IR, LC-MS and magnetic moment measurements.



Scheme 1 Structure of Amino acid-Schiff bases

2. EXPERİMENTAL

2.1. Materials and Methods

All chemicals used in the study were reagent grade and were purified when it was necessary. All organic solvents used in this study were purified according to standard methods. Elemental analyses were carried out with a LECO-CHNS-9320 instrument. Metal contents were determined by using a Philips PU 9285 atomic absorption instrument. 1H and 13C-n.m.r spectra were recorded with a Bruker DPX-300 MHz and 100 MHz using TMS as an internal standard and d6-DMSO as solvent. Mass spectra were recorded on a Micro Mass-UK Platform II mass spectrometer at The Scientific and Technological Research Council of Turkey (Tubitak), Ankara, Turkey. Electronic spectra were recorded on a Unicam-UV2-100 spectrophotometer in DMF. IR spectra were recorded on a Mattson-5000 FT-IR instrument in KBr pellets. Melting points were determined with a Gallenkamp melting point apparatus. The molar conductivities were measured with a Siemens WPACM 35 conductivity meter (10-3 mol L-1 in DMF solution). Magnetic measurements were carried out with a Sherwood Scientific magnetic susceptibility balance (Model No: MK 1) at 18 0C with Hg[Co(NCS)4] as a calibration.

2.2. Synthesis of Amino acid-Schiff Bases

B A solution of terephthalaldehyde (0.134 g, 0.001 mol) in MeOH (20 ml) was added dropwise to the 10 ml hot aqueous solution of the 0.001 mol DL-amino acid (0.079 g for DL-Glycine; 0.0891g for DL-Alanine) and heated under reflux for 2h. After cooling, the mixture was filtered, evaporated under reduced pressure to produce an oily syrup. This was dissolved in EtOAc:MeOH (3:1, v/v) and then stirred magnetically for two hours at 15°C filtered and allowed to stand. Yellow solid formed were collected by filtration, and then, dried.

2.3. Preparation of the Complexes

All complexes were prepared by the following general method. A sample of M(NO3)2/Cl2·nH2O (n: 6 for Co(II) and Ni(II); n: 0 for Cu(II)) (0.134 g for CuCl2, 0.291 g for Co(NO3)3, 0.237 g for NiCl2, 0.001 mol) was dissolved in methanol (15 ml). To the solution Amino acid-Schiff bases (0.0013 mol) in methanol (15 ml) was added, solution was stirred magnetically and heated at 50°C for 2h. The mixture was kept for a week at the room temperature. The solid product was filtered, washed with acetone solutions and then, dried in a desiccator over calcium chloride.

2.4. Test Microorganisms and Medium and Screening of Antimicrobial Activity

In this study, Pseudomamonas aeruginosa ATCC 29212, Bacillus subtilis RSKK 244, Bacillus megaterium ATCC 6633 Gram-negative bacteria 7644, Micrococcus luteus NRRLB and Candida albicans as fungus were used. Bacterial strains were cultured overnight at 37 °C in Mueller-Hinton broth and the yeasts were cultured overnight at 30 °C in YEPDE Agar for antibacterial and antifungal activity tests. Test strains were suspended in Nutrient agar to give a final density of 5x 105 cfu/ ml.

Minimum inhibitory concentrations (MICs) were determined by macrodilution broth method following the procedures recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1997). MICs were defined as the lowest concentrations of the antimicrobial. Antimicrobial activity of compounds was determined by the disc diffusion method. For testing antifungal activity of the compounds, Micrococcus luteus NRRLB were used (NCCLS, 1997).

Nutrient agar (20 ml) was poured into each sterile Petri dish after injecting cultures (100 µl) of microorganisms and distributing medium in Petri dish homogeneously. Compounds were filtered with a pore size of 0.45µm. All of the compounds were dissolved in DMSO of 5 mg/ml. Empty sterilized discs of 6 mm (Schleicher and Schuell,

No. 2668, Germany) were impregnated with 50 µl of compounds. Discs were placed on agar plates, and the plates were incubated at 37 oC for 24 h for bacteria. The culture suspensions were prepared and adjusted by comparing against 0.3 Mc Farland turbidity standard tubes. Inhibition zones formed on the medium were evaluated in mm. The solvent control (DMSO) did not show any antimicrobial activity. Studies performed in duplicate and the inhibition zones were compared with those of reference discs. Reference discs used for control were as follows: Ketoconazole, Ampicillin, Tetracycline, Penicillin, Chloroamphenicol and Gentamisin.

3. RESULTS AND DISCUSSION

The molar conductance values of the Schiff bases and the complexes found to be 14 - 28 Ω-1cm2mol-1 in 10-3 M in DMF solutions indicated the nonelectrolytic nature of the compounds [13,14]. Schiff bases are yellow, soluble in acetone, methanol and other polar solvents. The complexes are only soluble in DMF and DMSO but insoluble in other common organic solvents.

$[M(SB)_2] \leftrightarrow [M(SB)_2]$ M: Co(II), Cu(II) and Ni(II)

SB: (1,4 fd-Gly), (1,4 fd-Ala)

3.1. IR, UV-Visible and NMR Spectra of Ligands and Their Complexes

Analytical, physical electronic and characteristic IR spectral data of the Schiff bases are given in Tables 1 and 2. The strong absorptions at 1496-1589 cm-1 and 1381-1394 cm-1 are attributed to the asymmetric and symmetric νCOO bands. The azomethine stretching bands are observed in the range 1695- 1696 cm-1. The observation of strong bands 2869-2875, 2819-2824, 2767-2772 cm-1 may be attributed to the νCHaromatic and νCHaliphatic stretching vibration [15]. The electronic spectra of the Schiff bases in DMF show two bands at 261-279 nm assignable to the p-p* transitions of the imine group [16].

Table 1 Analytical and physical data of the Schiff bases and their metal complexes

Compound Empirical Formula	Formula Weight Colour, Yield (%)	m.p.(°C), µeff (BM) Λ (ohm^{-1}cm^{-1}mol^{-1})	Elemental Analysis Found (Calcd) % C	H	N	M
(1,4 fd-Gly) $C_{12}H_{12}N_2O_4$	248, yellow, 32	130-131, - , 08	0.53 (0.58)	0.07 (0.05)	0.15 (0.11)	
(1,4 fd-Ala) $C_{14}H_{16}N_2O_4$	276, yellow, 28	130-131, - , 12	0.66 (0.61)	0.11 (0.06)	0.13 (0.10)	
[Co(1,4 fd-Gly)$_2$] $C_{24}H_{22}N_4O_8Co$	552.9, violet, 62	247, 4.50, 24	0.55 (0.52)	0.05 (0.04)	0.12 (0.10)	0.08 (0.11)
[Co(1,4 fd-Ala)$_2$] $C_{28}H_{30}N_4O_8Co$	608.9, vivolet, 67	248, 3.62, 29	0.60 (0.55)	0.09 (0.05)	0.13 (0.09)	0.07 (0.09)
[Ni(1,4 fd-Gly)$_2$] $C_{24}H_{22}N_4O_8Ni$	552.7, l. green, 48	220, D, 15	0.51 (0.52)	0.02 (0.04)	0.09 (0.10)	0.09 (0.11)
[Ni(1,4 fd-Ala)$_2$] $C_{28}H_{30}N_4O_8Ni$	608.7, l. green, 42	220, D, 17	0.50 (0.55)	0.06 (0.05)	0.09 (0.09)	0.10 (0.10)
[Cu(1,4 fd-Gly)$_2$] $C_{24}H_{22}N_4O_8Cu$	557.5, green, 56	298, 1.82, 27	0.51 (0.52)	0.05 (0.04)	0.13 (0.10)	0.06 (0.11)
[Cu(1,4 fd-Ala)$_2$] $C_{28}H_{30}N_4O_8Cu$	613.5, green, 61	220, 3.21, 12	0.59 (0.54)	0.08 (0.05)	0.13 (0.09)	0.07 (0.10)

Table 2 Major IR absorption bands and electronic spectral data (nm) (ε_{max}, mol^{-1} cm^{-1}) of the compounds.

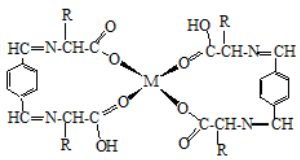
Compound	IR. absorption bands (cm^{-1}) ν OH ν CH=N	ν (-COOH) (asym/ sym)	ν CH$_{(arom)}$ / (ali) ν M-O, ν M-N	Electronic Spectral Data (λ,) (ε_{max})
(1,4 fd-Gly)	3420 1696	1496 / 1394	2870 / 2820, 2770 -,-	266 (2.3 x10^4), 277 (6.4 x10^3)
(1,4 fd-Ala)	3424 1695	1495 / 1391	2871 / 2820, 2773 -,-	268 (3.3 x10^4), 276 (2.4 x10^3)
[Co(1,4 fd-Gly)]	3420 1696	1589 / 1381	2869 / 2821, 2770 532, 462	266 (1,2x10^4), 279 (6.31x10^3), 496 (98)
[Co(1,4 fd-Ala)]	3423 1696	1589 / 1381	2869 / 2819, 2772 529, 458	265 (2,2x10^4), 277 (4.3x10^3), 439 (101)
[Ni(1,4 fd-Gly)]	3425 1696	1562 / 1389	2872 / 2823, 2767 531, 468	259 (1.2x10^4), 275 (6.31x10^3), 754 (16)
[Ni(1,4 fd-Ala)]	3427 1696	1562 / 1389	2874 / 2824, 2769 545, 463	267 (1.1x10^4), 278 (0.32 x10^3), 405 (12)
[Cu(1,4 fd-Gly)]	3425 1696	1580 / 1387	2875 / 2824, 2769 547, 461	267 (2.1x10^4), 278 (5.32 x10^3), 387 (31), 601 (23)
[Cu(1,4 fd-Ala)]	3418 1696	1580 / 1387	2870 / 2823, 2767 545, 463	261 (1.1x10^4), 275 (3.1x10^3),384 (51), 596 (17)

In the IR spectra of complexes no shifts were seen to lower or higher wave numbers of imine protons. This result may be due to having no coordination with the metal ion of the atoms imine (Scheme 2). The carboxylate bands in the IR spectra of the complexes appear in the 1589-1562 cm-1 and 1389-1381 cm-1 range somewhat higher than observed for the free ligands. These indicate that the oxygen of the carboxylate group is coordinated to metal ion [14]. The appearance of new bands in the 531-545 and 458-461 cm-1 regions due to ν(M-O) and ν(M-N), respectively [17]. On the basis of the i.r. spectral results, it may be deduced that the anion of the amino acid Schiff base is coordinated to the metal ion as bidentate ligand (Scheme 2).

The 1H NMR data of the Schiff bases and Ni(II) complexes are presented in Table 3. Since other complexes are paramagnetic, the 1H-NMR spectra could not be obtained. In (1,4 fd-Gly), the doubled observed at 8.36 ppm and the singlet at 9.30 ppm are assigned to ring protons and imine protons respectively. In (1,4 fd-Ala), the doubled observed at 8.23 ppm and the singlet at 9.39 are assigned to ring protons and imine protons respectively. The protons of methyl groups of alanine in the amino acid residues of the Schiff bases are also observed as expected. Furthermore, in the 1H-NMR spectra of the Schiff bases, the peak at 12.31–11.62 ppm is protons attributed to the -OH proton of -COOH group. The 13C-NMR spectra data of the Schiff bases (Table 3) are also in accordance with the proposed structures.

The 1H-NMR spectrum of Ni(II) complexes showed significant shift in the signals of

-OH protons of carboxyl group. Interestingly the imine proton signals did not show a shift in the Ni(II) complexes. These indicated that the carboxyl oxygen is coordinated to metal ion [18].



Scheme 2 Structure of suggestion for complexes (R: -H, -CH_3)

3.2. Electronic Spectra and Magnetic Susceptibilities

The electronic spectra of the Cu(II) complexes shows two d-d bands at ca. 690 nm and ca. 380 nm, indicating a square-planar stereochemistry. The absence of any bands below 800 nm eliminates the possibility of tetrahedral

geometry around the Cu(II) ion (Misra and Soni, 2008). The molar magnetic moment values of the Cu(II) complexes vary in the range 1.80-1.81 BM. This indicates that the complexes are monomeric in nature and metal–metal interactions are absent.

Co(II) complexes gave more intense bands in the d-d electronic spectra and high magnetic moment values between 4.48-4.53 B.M. which support a tetrahedral configuration [19]. The electronic spectra of the Ni(II) complexes do not show d-d bands because of diamagnetic properties.

Mass spectra provide evidence for the molecular formula of the synthesized complexes. LC-mass spectra for the complexes are [M+2H]+ 554.1 (m/z: % 9.3), [M+3H]+ 611.9 (m/z: % 8.1), [M+H]+ 553.0 (m/z: % 5.2), [M-2H]+ 606.1 (m/z: % 11.8), [M+3H]+ 560.0 (m/z: % 6.4), [M]+ 613.1 (m/z : % 1.6), for (LH-Fe), [Co(1,4 fd-Gly)2], [Co(1,4 fd-Ala)2], [Ni(1,4 fd-Gly)2], [Ni(1,4 fd-Ala)2], [Cu(1,4 fd-Gly)2], [Cu(1,4 fd-Ala)2] respectively. The results indicate the dimeric nature of the complexes.

3.3. Biological Results

The biological activity of the ligands and their complexes (table 4) was screened simultaneously with metal salts, and standards, against three gram-positive bacteria (Psydomamonas aeruginosa ATCC 29212, Bacillus subtilis RSKK 244, Bacillus megaterium(clinical isolate), gram-negative bacteria (E. coli ATCC-1280) and the fungus (Candida albicans Y-1200-NIH, Tokyo) by the filter paper disc method.

The susceptibilities of certain strains of bacteria and fungus to the Amino acid-Schiff bases and their complexes cause the inhibition of a visible growth of the microorganism. The MIC of Ketoconazole, Ampicillin, Tetracycline, Penicillin, Chloramphenicol and Gentamisin was individually determined in parallel experiments in order to control the sensitivity of the test organisms. MIC values of the compounds and the standards are presented in Table 5. Antimicrobial results obtained this study were more effective than the ones obtained in our previous studies on the amino acid-Schiff bases [20].

None of the compounds were found to be significantly effective against C. albicans, except for [Cu(1,4 fd-Gly)2] and [Ni(1,4 fd-Gly)2]. NiCl2 6 H2O and Co(NO3)2 6 H2O has the greatest inhibitory effect against Gram (+) and Gram (-) while Ni(II) and Co(II) complexes of aminoacid-Schiff base has the lowest inhibitory effect. NiCl2 6 H2O and it's (1,4 fd-Gly) complexes are effective on B.subtilis, P. aeruginosa, B. megaterium and M. Luteus (Table 4).

The results of antifungal and antibacterial screening indicated that all of the complexes of (1,4 fd-Gly) showed more activity than the (1,4 fd-Ala) complexes. As seen in Table 5, the compound [Cu(1,4 fd- Gly)] showed a significant activity against B.subtilis, P. aeruginos B. Megaterium, M. Luteus; but, [Cu(1,4 fd- Ala)] moderate activity against them. This situation may cause difficult to bacteria to penetrate into the cell wall due to a steric factor of molecules including methyl (-CH3) group.

Today it is clear that some metals have specific affinity for DNA and can bind and disorder helical structures by crosslinking within and between strains. Copper is one of the metal specific affinities [21]. Copper may be denaturing the structure of DNA with hydrogen bonding present within the DNA molecule [22]. This influence of Cu (II) may be increases in the available of active oxygen species such as -OH• [23]. Hydroxyl radical (-OH•) may form free carboxyl group in the amino acid-Schiff bases and their complexes as given below. H2O2 may be from the fragment of group –COOH [11].

$$R\text{-}COOH \rightarrow RCO\bullet + \bullet OH \qquad \text{for Schiff bases}$$
$$2\bullet OH \rightarrow \bullet O_2^- + 2H^+ \leftrightarrow H_2O_2$$

$$Complex(M^{+n})\text{-}COOH \rightarrow Complex(M^{+n-1})\text{-}CO\bullet + \bullet OH \qquad \text{for Cu(II)}$$
$$Complex(M^{+n})\text{-}COOH + \bullet OH \leftrightarrow Complex(M^{+n-1})\text{-}CO\bullet + \bullet O_2^- + 2H^+ \leftrightarrow H_2O_2$$

It is well known that radical groups are toxic ([24,25]. Radical ions or molecules come into existence in the structure having weak bond. Therefore, radical groups in (1,4 fd-Gly) may be formed more easily those in (1,4 fd-Ala). (1,4 fd- Gly) and its complexes are more active than other synthesized molecules against studied bacteria and fungi. The MIC values indicated that the (1,4 fd-Gly) including Schiff bases are more effective than others for Bacillus megaterium and Candida Albicans.

According to the results given in Table 4, only [Ni(1,4 fd-Gly)2] and [Cu(1,4 fd- Gly)2] show activity against Candida Albicans. However, [Cu(1,4 fd-Gly)2] MIC value is better than [Ni(1,4 fd- Gly)2] (Table 5), so we can say that, [Cu(1,4 fd-Gly)2] is the most effective complex against Candida Albicans. Also, [Cu(1,4 fd-Gly)2] shows better activity against Candida Albicans than Ketoconazole. [Cu(1,4 fd- Gly)2] has more activity than all antibiotics against all bacteria except Penicilin against Micrococcus Luteus. [Ni(1,4 fd-Gly)2] has more activity than Ampicillin against Bacillus megaterium. [Cu(1,4 fd-Gly)2] is the most effective compound against all the bacterial we tested and fungi, according to both disc diffusion method and MIC results while the other compounds inhibit some bacterial activity. The antibacterial activity of these compounds was also compared with seven commercial antibiotics, namely, Penicilin, Chloramphenicol, Tetracycline, Ampicillin, Gentamisin, Ketoconazole. It was seen that the synthesized compounds were effective as antibiotics. Antimicrobiyal of these substance results are much better than former our study [15,19]. This reason may be caused by having two aminoacid-Schiff bases groups.

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CONFLICT OF INTEREST

No conflict of interest was declared by the authors.

REFERENCES

[1] R. Eglof, P. Piotr, B. Bogumil, B. Franz, Curr Org. Chem. 13 (2009) 241-249.Weber, C.A., Current, J.R., Benton, W.C., "Vendor selection criteria and methods", European Journal of Operational Research, Cilt 50, 2-18, 1991.

[2] G. Ellis, L. Chang, B. Cogionis, D. Daneman, Clin. Chem. 43 (1997) 2437-2439. Dickson, G.W., "An Analysis of Vendor Selection Systems and Decisions", Journal of Purchasing, Feb, 2(1):5-17, 1996.

[3] A.R. Fakhari, A.R. Khorrami, H. Naeimi, Talanta 66 (2005) 813-817.Wind, Y., Saaty T.L.,"Marketing Applications of the Analytic Hierarchy Process", Management Science, 26(7), 641-658, 1980.

[4] U. Abram, R. Alberto, J Braz Chem Soc 17 (2006) 1486-1500.Babic, B., "Axiomatic design of flexible manufacturing systems", International Journal of Production Research, Cilt 37, No:5, 1159– 1173, 1999.

[5] M. Gharagozlou, D.M. Boghaei, Spectrochim Acta A 71 (2008) 1617-1622.

[6] R.M. Wang, C.J. Hao, Y.P. Wang, S.B. Li, J Mol Catal A-Chem 147 (1999) 173-178.

[7] W.K. Jung, H.C. Koo, K.W. Kim, S. Shin, S.H. Y.H Kim, Park Appl Environ Microb 74 (2008) 2171-2178.

[8] A.P. Mishra, M. Soni Metal-Based Drugs 2008 ID 875410 (2008) 1-7.

[9] S.A. Manley, S. Byrns, A.W. Lyon, P. Brown, J. Gailer, J Biol Inorg Chem 14 (2009) 61-74.

[10] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, CA, USA, (1994) p 64-276.

[11] A.A. Abd El-Raady, T. Nakajima, Ozone-Scı Eng 27 (2005) 11-18.

[12] S. Kobayashi, K. Ueda, T. Komano, Agric Biol Chem 54 (1990) 69-76.

[13] D. Nartop, P. Gürkan, N. Sarı, S. Çete,. J Coord Chem 61 (2008) 3516-3524.

[14] N. Sarı, J Macromol Sci A 43 (2006) 1609-1618.

[15] N. Sari, P. Gürkan, S. Arslan, Trans Metal Chem 28 (2003) 468-474.

[16] J.R. Anacona, I. Osorio, Transit Metal Chem 33 (2008) 517-521.

[17] I. Şakiyan, Trans Metal Chem 32 (2007) 131-135.

[18] I. Kaya, A. Bilici, M. Gul, Polym advan technol 19 (2008) 1154-1163.

[19] N. Sarı, P. Gürkan, S. Çete, I. Şakiyan, Russ J Coord Chem 32 (2006) 511-517.

[20] N. Sarı, P. Gürkan, Trans Metal Chem 28 (2003) 687-693.

[21] N.S. Agar, J.R. Mahoney, J.W. Eaton, Biochem-Pharmacol 41 (1991) 985-993.

[22] H. Zhang, C. Andrekopoulos, J. Joseph, J. Crow, B. Kalyanaraman, Free Radical Bio Med 36 (2004) 1355-1365.

[23] S.I. Liochev, I. Fridovich, J Biol Chem 277 (2002) 34674-34678.

[24] Z.H. Zhao, Y. Sakagami, T. Osaka, Can. J. Microbiol 44 (1998) 441-447.

[25] S.P. Denyer, G.S.A.B Stewart, Int Biodeter Biodegr 41 (1998) 261-268.