Antibacterial activity of Schiff base complex *in vitro*

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Abstract

Schiff base was prepared by adding (5-(4-Methoxy phenyl azo)-2-hydroxybenzaldehyde) to ethanolic solution and o-aminobenzioc acid, Ni (II) Schiff base complex derived from Schiff base and Ni(II) salt were refluxed . The physical properties were studied. Infrared of Schiff base and the complex were measured. The Ni(II) Schiff base complex have been also tested for their antibacterial activities against several human pathogenic bacteria *in vitro* .The bacterial species tested included: *Staphylococcus aureus Streptococcus pyogenes* and Gram-negative, *Escherichia coli* , *Enterobacter aerogenes* , *Vibrio cholerae*, *Proteus mirabilis* , *Salmonella typhi* , *Serratia marcescens*, *Pseudomonas aeruginosa*.

دراسة فعالية معقد شيف المضادة للبكتريا خارج الجسم الحي

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مفتاح البحث: فعالية قواعد شيف

الخلاصة

حضر معقد شيف بإضافة (5-(4-Methoxy phenyl azo)-2-hydroxybenzaldehyde)إلى الايثانول وحامض O - aminobenzioc، و معقد شيف النيكل (II)مشتقة من معقد شيف و ملح النيكل (II)، ودرست الخواص الفيزيائية وتم قياس الأشعة تحت الحمراء لمعقد شيف ومعقد النيكل . وقد تم اختبارمعقد النيكل الثنائي ضد العديد من الانواع البكتيرية المسببة للأمراض البشرية في المختبر المتضمنة الموجبة لصبغة كرام وكانت النتيجة موجبة:

Streptococcus pyogenes. *Staphylococcu aureus*

والسالبة لصبغة كرام: *Escherichia coli , Enterobacter aerogene, Proteus mirabilis , Salmonella typhi ,, Serratia marcescens, Pseudomonas aeruginosa*. *Vibrio cholera*e.

Introduction

Eventhough the discovery and development of antibiotics are among the most powerful and successful achievements of modern science and technology as control tool for infectious diseases, however, the increasing microbial resistance to conventional antibiotics in use necessitates the search for new compounds with improved effects against pathogenic bacteria The most spectacular advances in medicinal chemistry have been reached when heterocyclic compounds played an important role in regulating biological activities Extensive investigations in the field of Schiff bases have been reported [1,2]

Schiff bases and their metal complexes play an important role in the development of coordination chemistry, resulting in an enormous number of publications, ranging from pure synthetic work to physicochemical [3], and biochemical relevant studies of metal complexes and found wide range of applications [4,5].

The compounds having antimicrobial activity may act either by killing the microbe inhibiting multiplication of the microbe, or by blocking their active sites[6].

The aim of this study to determinate the antibacterial study of the synthesized Schiff base *in vitro* using disc diffusion method .

Materials and methods

Preparation of (5-(4-Methoxy phenyl azo)-2-hydroxybenzaldehyde) (MPAHB):

This compound was prepared by adding ethanolic solution (0.01 mol, 1.22gm) salicylaldehyde in basic media to diazonium chloride prepared from coupling reaction to p-nitro aniline at 0ºC (Figure1).

Preparation of Schiff base:

The Schiff base was prepared by adding 25cm^3 (0.01 mol,) from (5-(4-Methoxy phenyl azo)-2-hydroxybenzaldehyde) (MPAHB) to 25cm^3 from ethanolic solution ,(0.01mole-2.7gm) from o-aminobenzioc acid .The mixture was refluxed and stirred for 2 hrs. then precipitate was collected by filtration, washed several time with ethanol and recrystallized from hot ethanol hot and then dried in air and stored in a desiccators over anhydrous $CaCl_2$ under vacuum .the melting point of product found to be (246 **°C**) , the color of the product redly brown .

Preparation of the complex:

A mixture of Schiff Base under investigation(0.003mole-1.167 gm)in 30cm^{3}ethanol and 25cm^3 of the same solvent of Ni(ll) (o.oo15mole -0.225gm) was refluxed for two

hours the dark brown complex was collected by filtration and then washed several time dried in air and stored in a desiccators over anhydrous $CaCl_2$under vacuum.

Antibacterial Testing by Disc Diffusion method:

Compounds were evaluated for their *in vitro* antibacterial activity against nine pathogenic bacteria obtained from Department of Microbiology /Al-Hussein Hospital /Kerbala Province. The bacterial isolates identified with api 20E kit ,api staph, api strept.(BioMerieux).The synthesized Schiff base complex was screened *in vitro* for their antibacterial activity against Gram-positive: *Staphylococcus aureus Streptococcus pyogenes* and Gram-negative: *Escherichia coli , Enterobacter aerogenes , Vibrio cholera*e, *Proteus mirabilis , Salmonella typhi , Serratia marcescens, Pseudomonas aeruginosa* using disc diffusion method[7].

Bacterial inocula were prepared from overnight growth cultures (24 h) in Nutrient broth (Difco) and turbidity was adjusted equivalent to 0.5 McFarland units (approximately 10^8 cfu/mL). Aliquots (100 µL) of inocula were spread over the surface of Mueller Hinton Agar (Difco) plates with a sterile glass spreader.
The plates were then incubated 24 h at 37 °C. The formed inhibition zones were measured in mm .

Results and discussion :

Physical properties of Schiff complex were indicated in the Table .1

IR data of Schiff base and the complex were indicated in the Figures2,3 respectively.

Schiff base ligands were synthesized following the preparative route illustrated in Figure 1. Table 2 was showed antibacterial activity of Schiff base complex .

The study of the growth inhibition zone values of schiff base complexe was indicated that metal complexe exhibit higher antibacterial activity than the free ligand . This is probably due the greater lipophilic nature of the complexes. Such increased activity of the metal chelates can be explained on the basis of Overtone's concept and Tweedy's chelation theory [8]. According to Overtone's concept of cell permeability, the lipid membrane that surrounds the cell favors the passage of only lipid soluble materials due to which liposolubility is considered to be an important factor that controls the antimicrobial activity. On chelation, the polarity of the metal ion will be reduced to a greater extent due to the overlap of the ligand orbital and partial sharing of positive charge of metal ion with donor groups [9, 10] Further, it increases the delocalization of the π electrons over the whole chelate ring and enhances the lipophilicity of the complex. This increased lipophilicity enhances the penetration of the complexes into lipid membrane and thus blocks the metal binding sites on enzymes of microorganisms [11]. These metal complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts further growth of the organism The variation in the activity of different complexes against different organisms depend either on the impermeability of the cells of the microbes or difference in ribosomes of microbial cells [12].

The scope for future research in this field is to subject the synthetic compounds to *in vitro* cytotoxic studies on cancer cell lines, the compounds can be subjected to *in vivo* animal studies to establish their chemotherapeutic efficacy. Mode of action of Schiff base on gram-positive bacteria was by affecting both, the lecithin and phosphate groups on the cellular membrane and DNA[13].

Conclusion:

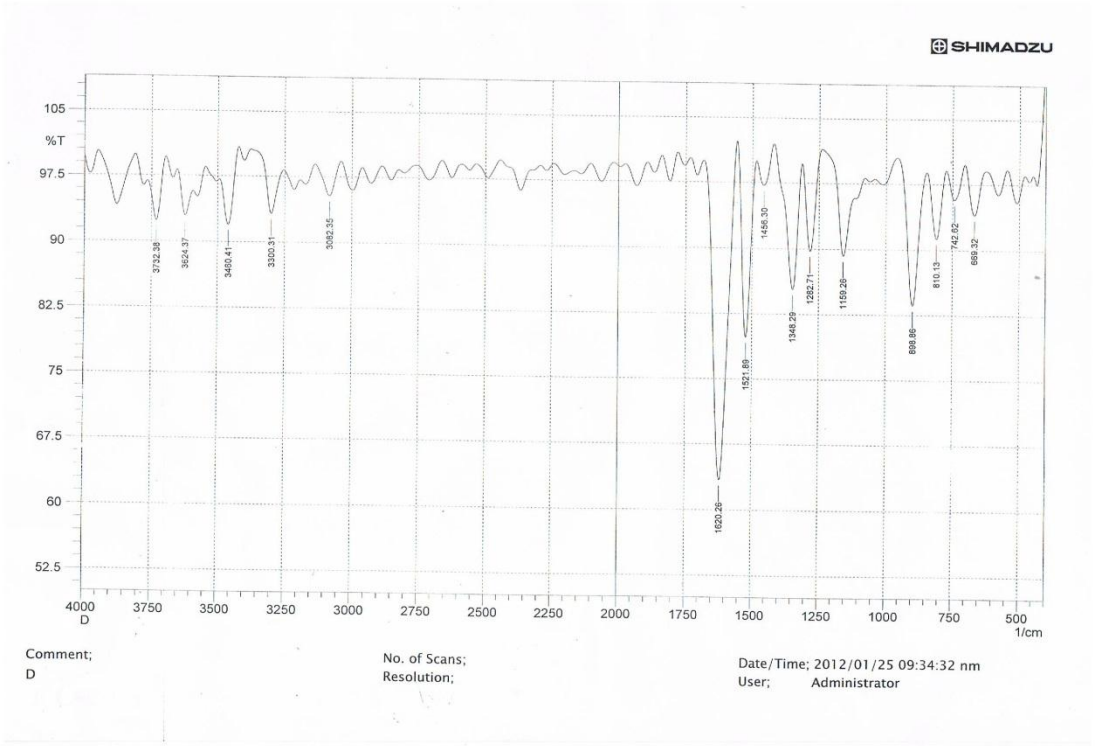
The antibacterial data show that the Schiff base complexes to be more biological activity to some gram-positive and gram-negative bacteria..

Table 1:Physical properties of Schiff base complex.

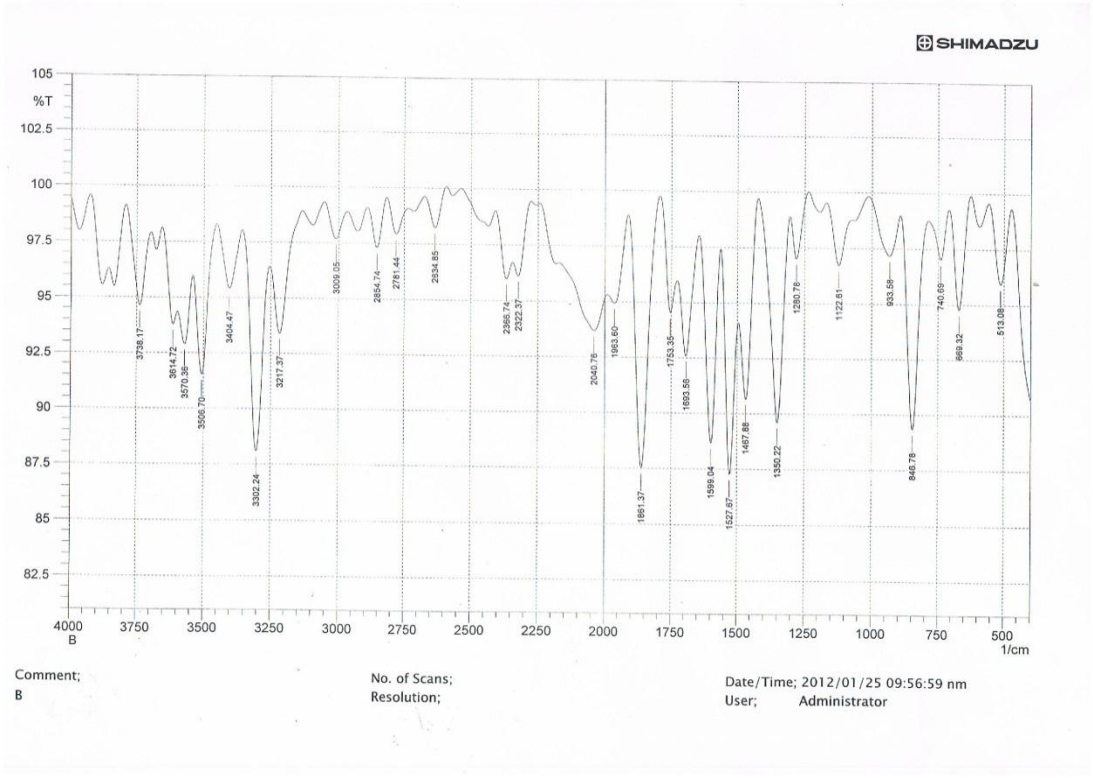
Complex	Color	λ max (cm^{-1})	M.P (°C)	Λ (s)	M.W. (g/mol)
$C_{40}H_{24}N_8O_{10}Ni.3H_2O$	orange	434	290 dec	3.3	959.69

λ max=Wave length max, M.P=melting point, Λ =Conductivity,

M.W.=molecular weight



Figuer 2.IR spectra of Schiff base.



Figuer 3.IR spectra of complex.

Table 2:*In vitro* antibacterial activities of Schiff base complex

Gram negative bacteria	**Inhibition zone (mm)**	**Gram positive bacteria**	**Inhibition zone (mm)**
Escherichia coli	7	*Staphylococcus aureus*	8
Enterobacter aerogenes	8	*Streptococcus pyogenes*	10
*Vibrio cholera*e	8		
Proteus mirabilis	7		
Salmonella typhi	10		
Serratia marcescens	12		
Pseudomonas aeruginosa	8		

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