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Under the title:

The synthesis and evaluation of antioxidant activity of Schiff bases ligands: investigation of their ion extraction capability from aqueous media

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In front of the jury composed of:

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To all my paternal and maternal families

Siham

Abstract:

In this study, two Schiff bases were prepared by reacting salicylic aldehyde with two aniline derivatives. The structures of these compounds were confirmed through different spectroscopic methods such as UV-Visible and Infrared FT-IR spectrometry. The antioxidant activity of prepared compounds was evaluated *in vitro* with both DPPH radical scavenging activity and inhibition of Hydroxide peroxide. These compounds showed best antioxidant activity with respect to use of Hydrogen peroxide scavenging method, compared to the inhibition of DPPH. In addition, the first Schiff base ligand L1 gave excellent chelation and extraction efficiency for lead (II), therefore, considered as a promising antioxidant agents and a good chelator of Pb^{2+} to purify polluted water in this metal.

Keywords: Schiff bases, DPPH, lead (II), antioxidant activity

Résumé :

Dans cette étude, deux bases de Schiff ont été préparées en faisant réagir l'aldéhyde salicylique avec deux dérivés d'aniline. Les structures de ces composés ont été confirmées par différents méthodes spectroscopique telles que la spectrométrie UV-Visible et l'infrarouge FT-IR. L'activité antioxydante des composées préparées ont été évalués *in vitro* avec l'activité de piégeage des radicaux DPPH et l'inhibition du peroxyde d'hydroxyde. Ces composés ont montré une activité antioxydante mieux en ce qui concerne l'utilisation de la méthode de piégeage du Peroxyde d'hydrogène, par rapport à l'inhibition du DPPH. De plus, le premier ligand de base de Schiff L1 a donné une excellente efficacité de chélation et d'extraction pour le plomb (II), donc c'est considéré comme un agent antioxydant prometteur et un bon chélateur de Pb^{2+} pour purifie l'eau pollué en ce métal.

Mots de passe : Bases de Schiff, DPPH, Plomb (II), activité antioxidante.

الملخص:

في هذه الدراسة، تم تحضير قاعدتي شيف عن طريق تفاعل الديهيد ساليسيليك مع مشتقين من الانيلين. تم تأكيد صيغ هذه المركبات من خلال طرق طيفية مختلفة مثل: الأشعة فوق البنفسجية والأشعة تحت الحمراء، قدر النشاط المضادة للأكسدة للمركبات المحضرة مخبريا من خلال نشاط الكسح الجذري DPPH وتثبيط بيروكسيد الهيدروجين. أظهرت هذه المركبات أحسن نشاطا مضادا للأكسدة بخصوص تثبيط بيروكسيد الهيدروكسيد، مقارنة بتثبيط DPPH. علاوة على ذلك أعطت قاعدة شيف الأولى تمخلبا وكفاءة استخلاص ممتازة لمعدن الرصاص (II)، لذلك فهي تعتبر عاملا واعدا مضادا للأكسدة و ممخلب جيد Pb^{2+} لتنقية المياه الملوثة بهذا المعدن.

الكلمات الدالة: قواعد شيف، DPPH، الرصاص (II)، النشاط المضاد للأكسدة

List of abbreviations:

L: Ligand

L1: Schiff base ligand 1

L2: Schiff base ligand 2

Abs: Absorbance

I %: Inhibition percentage

$IC_{50:}$ Inhibitory concentration of 50%

KBr: Potassium Bromide

M: Metal

pH: Hydrogen potential

DPPH: 1,1-diphényl-2-picryl-hydrazyl

FT-IR: Infrared Fourier Transform

IR: Infrared

UV: Ultraviolet

NMR: Nuclear Magnetic Resonance

λ_{max} : Maximum wavelength

λ : Wavelength

μ : Chemical Potential

nm: Nanometer

mL: Milliliter

M.P: Melting point

°C: Degree Celsius

TLC: Thin Layer Chromatography

D : Distribution coefficient

E : Extraction efficiency

TC : Toxicity Class

LD_{50} : Median Leathl Dosage

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ChapterII: Liquid-liquid extraction

ChapterIII: Experimental work

ChapterIV: Results and discussion

General introduction:

Nitrogen chemistry has long been a diversified and important field of research. The chemical element "N" is present in many molecules constituting natural substances, heterocyclics and pharmaceutical product. Many methods have been developed to achieve the synthesis of such compounds[1].

Schiff base ligands are considered as privileged ligands as they are simply synthesized by condensation of primary amines and carbonyl compounds, aldehydes or ketones. Schiff bases are named for Hugo Schiff, a German chemist, Nobel Prize winner, who discovered them in 1864[2]. The formation of carbon–nitrogen double bond plays important role in organic synthesis[3]. At this stage, research on this type of compounds is constantly evolving, thanks to the diversity of their structures, which result from this. It should be noted that the synthesis of Schiff's bases remains a topical research topic that continues attract many research teams around the world, whether at the university or industrial level.

Schiff bases have attracted considerable attention of organic chemists due to their significant biological activities. They are used as substrates in the preparation of a number of industrial and biologically active compounds. Moreover, Schiff bases are also known to have biological activities such as antimicrobial, antifungal anti-bacterial[4] anti-virus agents, antioxidants, radical inhibitors, anti-tumor agents [5], antimalarial, anti-proliferative, anti-inflammatory, antiviral and antipyretic, anti-hypertensive, herbicidal[6].

Schiff bases have also been employed as ligands for complexation of metal ions. On the industrial scale, they have wide range of applications[4]. Schiff bases resulted from aromatic aldehydes ortho-substituted with a hydroxyl group have initially arouse the researchers' interest because of their ability to act as bidentate ligands for transitional metal ions[7] and has taken an important place in the field of complexation associated with the extraction of metal cations[8]. The Schiff bases remain the ligands of choice, capable of coordinating a whole myriad of various metal cations (alkali metals, transition metals….etc.) as well as their ease of implementation on any kind of scale[9]. This ligand may also have wide application in the field of water treatment. Because of their high ability to complex transition metals, the possibility of forming complexes in which a ligand is associated with a transition metal raises the hope of

obtaining new properties, which vary according to the metal center and the ligands. These ligands can be diversified in order to obtain the desired characteristics[10].

Our modern way of life imposes severe constraints on our environment in addition to requiring increasingly efficient health care. Heavy metals (cadmium, lead, mercury, chromium, copper, etc.) constitute a worrying problem when they are involved in the pollution of water resources.

Our work aims are the synthesis of two Schiff base ligands and research an application of those compounds in antioxidant activity and in extraction of lead (II) from aqueous media.

The plan of study consists of two main parts; each part divided into two chapters.

The first part:

The first chapter is devoted to bibliography on the Schiff bases, and their physical and chemical properties and their applications in the biological and industrial aspects.

In the second chapter, a bibliography study on liquid-liquid extraction and its types. We devote the study to extraction by chelation and characterizing the transition metal to be extracted.

The second part:

In the third chapter, we will present the experimental methods used to conduct this work, starting with how these compounds are synthesized, and their biological study and finally presenting the extraction methods.

Finally, in the fourth chapter, we present the results of synthesizing Schiff bases and the results of extracting transition metals by synthesized compounds and its effect on some factors.

The manuscript ends with a general conclusion and perspectives.

ChapterI:

Generality of Schiff bases

Schiff bases are a vast group of compounds characterized by the presence of a double bond linking carbon and nitrogen atoms, the versatility of which is generated in the many ways to combine a variety of alkyl or aryl substituents. Compounds of this type are both found in nature and synthesized in the laboratory. For years, Schiff bases have been greatly inspiring to many chemists and biochemists. In this chapter, we attempt to present a new take on this group of compounds, underlining of the importance of various types of Schiff bases. Among the different types of compounds that can be classified as Schiff bases[11].

A variety of biological activities are reported for Schiff bases and discovery of a new chemotherapeutic Schiff bases have been receiving the attention of medicinal chemist for the discovery and development of new chemotherapeutic agents[12].

Schiff bases represent an important class of organic compounds and have often been used as chelating ligands in coordination chemistry. Due to presence of an azomethine (C=N) group, these compounds have structural similarities with neutral biological systems and are utilized in elucidating the mechanism of transformation of racemization reaction in biological systems[13]. Schiff base ligands are essential in the field of coordination chemistry, especially in the development of complexes of Schiff bases because these compounds are potentially capable of forming stable complexes with metal ions[14].

I.1 Definition of Schiff bases:

Schiff bases are chemical compounds crystalline solids ,that were first obtained in the condensation reactions of primary amines and aldehydes in (1864) by Hugo Schiff[15] .Containing carbon-nitrogen double bond (**C=N**) functional group, called azomethine [16]. These compounds are also knows as imines and aniles if one of the substitutes is a phenyl or substituted phenyl [1].

The common structural feature of these compounds is the azomethine group with a general formula RH**C=N**-R1, where R and R1 are alkyl, aryl, cyclo alkyl or heterocyclic groups which may be variously substituted. Several studies showed that the presence of a lone pair of electrons in an $\mathbf{sp^2}$ hybridized orbital of nitrogen atom of the azomethine group is of considerable chemical and biological importance[17].

A Schiff base behaves as a Flexi-dentate ligand and commonly co-ordinates through the O atom of the de-pronated phenolic group and the N atom of azomethine group[18]. Giving an imine function according to the following reaction scheme:

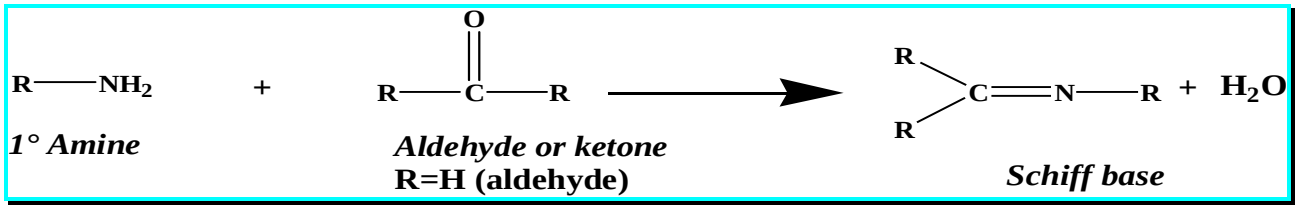


Figure I. 1: General Mechanism of synthesis Schiff bases

In general this type of compounds obtained by the condensation reaction of a ketone or an aldehyde on a primary amine and an aldehyde which involves the use of organic solvents such as Ethanol, tetrahydrofuran (THF), and 1,2-dichloroethane[19]and leads to molecule comprising an imine function.

I.2 Synthesis of Schiff bases:

The first preparation of imines was reported by Schiff[20]. Since then, there are several reaction methods to synthesis Schiff bases[21]. Originally, the classical synthetic route for synthesis of Schiff bases was reported which involves condensation of primary amines with carbonyl compounds under azeotropic distillation[3]. Molecules sieves and Dean-Stark apparatus are then used to remove water formed in the system a conventionally[22]. The most common is an condensation reaction amine with an aldehyde or ketone under refluxing conditions [21](**Figure I.2**):

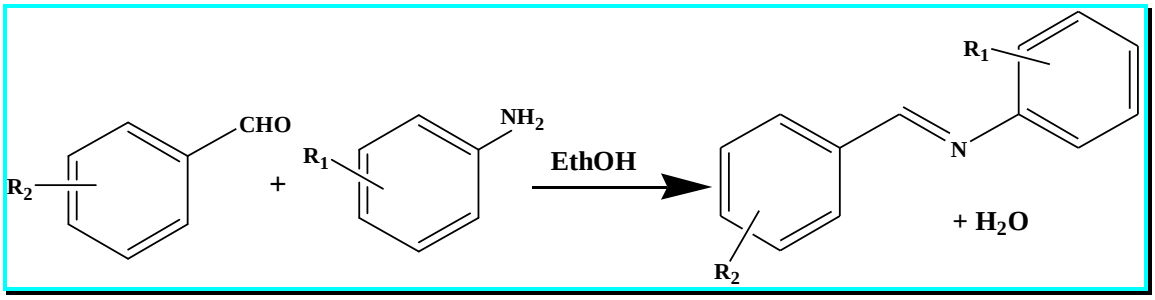


Figure I. 2: Synthesis of Schiff bases

If we focus on the mechanism of transformation of aldehydes and amines in to Schiff bases, two step are possible which are mechanized in (**Figure I.3**)[3]. The first step in this reaction is an attack of nucleophilic nitrogen atom of amine on the carbonyl group, resulting in a normally unstable carbinolamine intermediate. The formation of Schiff bases in the second step largely depends upon the rate of removal of water from reaction mixture[3]. A (**C=N**) bond is formed and the product is called imine[21].

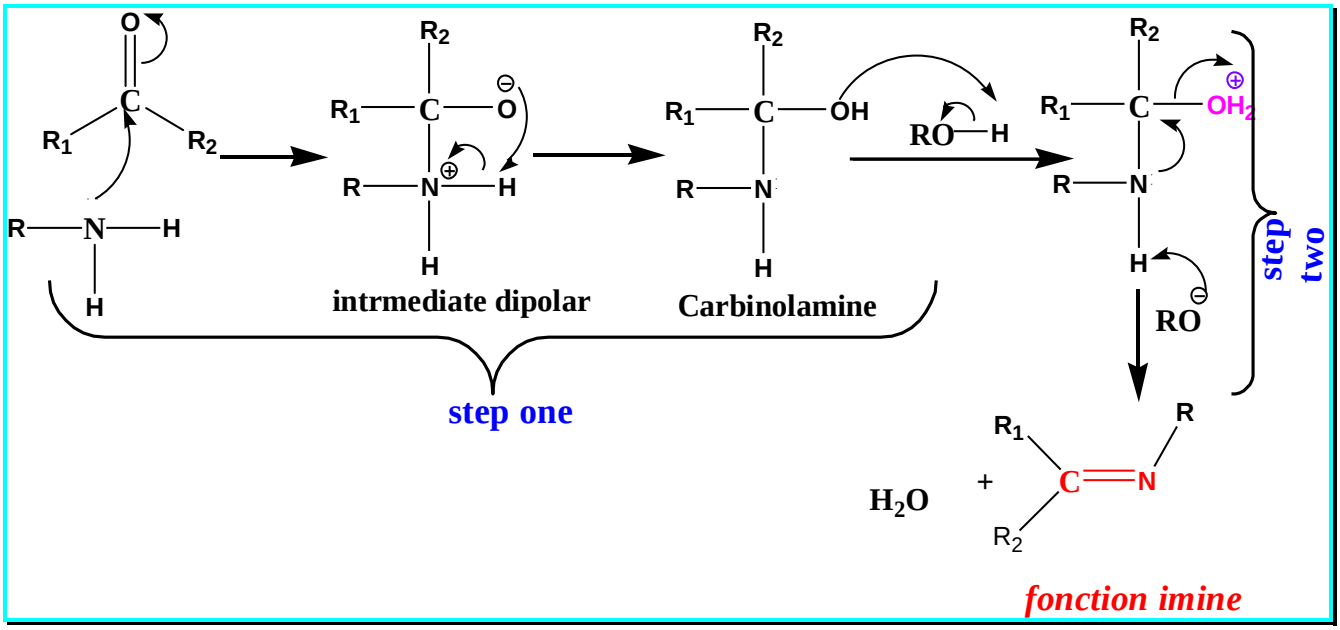


Figure I. 3: Mechanism preparation of Schiff bases

In the 1990s an *in situ* method for water elimination was developed, using dehydrating solvents such as tetra methyl ortho silicate or tri methyl ortho format. In 2004, demonstrated that the efficiency of these methods is dependent on the use of highly electrophilic carbonyl compounds and strongly nucleophilic amines. They proposed as an alternative the use of substances that function as Brönsted-Lowry or Lewis acids to activate the carbonyl group of aldehydes, catalyze the nucleophilic attack by amines, and dehydrate the system, eliminating water as the final step. Examples of Brönsted-Lowry or Lewis acids used for the synthesis of Schiff bases include $ZnCl_2$, ,$TiCl_4$, $MgSO_4$-PPTS, $Ti(OR)_4$, alumina, H_2SO_4, $NaHCO_3$, $MgSO_4$, $Mg(ClO_4)_2$, H_3CCOOH, $Er(OTf)_3$, P_2O_5/Al_2O_3, HCl [22].

This synthesis prepared by nucleophilic addition, forming a hemi-aminal group followed by dehydration generating imine compounds. A variety of methods for synthesis of Schiff bases have been described[2]. They are as follows:

I.2.1 Solvent free synthesis by microwave irradiation:

Microwave assisted synthesis of Schiff bases is rapid and efficient with no use of solvent. Purification procedures are simple and it gives good yields within a few minutes[23].

I.2.2 Solvent free synthesis by using catalyst:

Synthesis of Schiff bases is done efficiently at room temperature by using catalyst e.g. $SnCl_2$, acetic acid when the reaction mixing.

I.2.3 Solvent and catalyst free synthesis:

A mixture of amines and aldehydes/ketones is ground in a mortar and pestle. The completion of reaction takes 2-3 minutes[2].

I.2.4 Solvent based synthesis:

Schiff base synthesis generally requires appropriate solvent, either ethanol or methanol, when the mixture is refluxed in acidic, basic or neutral medium. Products are purified by recrystallization or by TLC and column chromatography. Schemes of the reaction mechanism in different media. Basic medium speeds nucleophilic attack making rapid formation of Schiff bases [2].

Many factors affect the condensation reaction, for example the pH of the solution as well as the steric and electronic effects of the carbonyl compound and amine. In acidic solutions the amine is protonated, thus cannot function, as a nucleophile and the reaction cannot proceed. Furthermore, in very basic reaction conditions the reaction is hindered as sufficiently protons are not available to catalyst the elimination of the carbinolamine hydroxyl group[21].

In general, aldehydes react faster than ketones in Schiff base condensation reactions as there action center of aldehyde is sterically less hindered than that of ketone. Furthermore, the extra carbon of ketone donates electron density and thus makes the ketone less electrophilic compared to aldehyde. In which aromatic aldehydes especially with an effective conjugation system, form stable Schiff bases, where as those aliphatic aldehydes are unstable and readily polymerize[19].

I.3 Schiff bases Classifications :

A Schiff base is a nitrogen analogue of an aldehyde or ketone [24]. They can be classified as follows:

- Classification according to the aldehyde or ketone bond: Aldimine, ketamine.
  - **Aldimine:** is an imine in which the carbon bonded to nitrogen bears an alkyl group and a hydrogen.

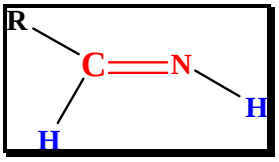


Figure I. 4: Aldimine

  - **Ketamine:** an imine in which the nitrogen bonded carbon is attached to two-alkyl group.

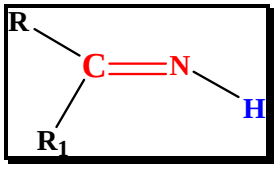


Figure I. 5: Ketimine

- Classification according to the number of heteroatoms contained in their structures:mono, bi ,tri ,tetra and poly dentate[25] (**Figure I.7**and **Figure I.8**).

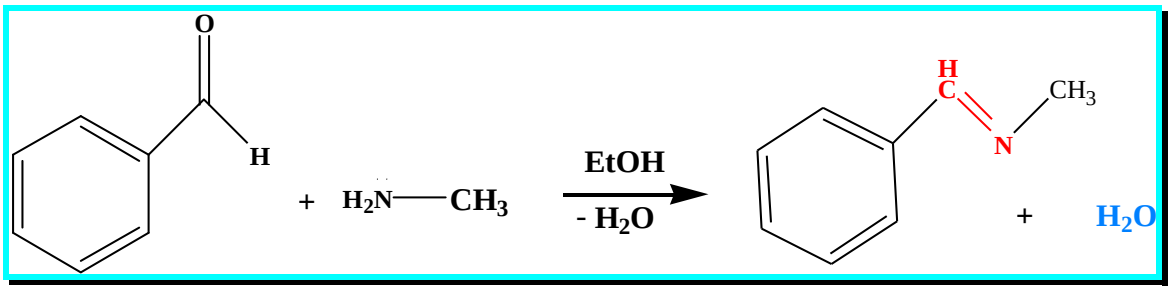


Figure I. 6: Synthesis of Schiff base mono dentate

Table I.1: Summarizes the different of chelating sites

	TYPE	Nature of the coordination
01	N	Coordination site mono-dentate 1N
02	NN- NO	Coordination sites bi-dentate 2N or NO
03	NON	Coordination sites mixed tri-dentate 2N 1O
04	NONO	Coordination sites mixed tetra-dentate 2N 2O
05	NONON	Coordination sites mixed penta-dentate 3N 2O
06	NONONO	Coordination sites mixed hexa-dentate 3N3O
07	NONONON	Coordination sites mixed poly-dentate 4N 3O

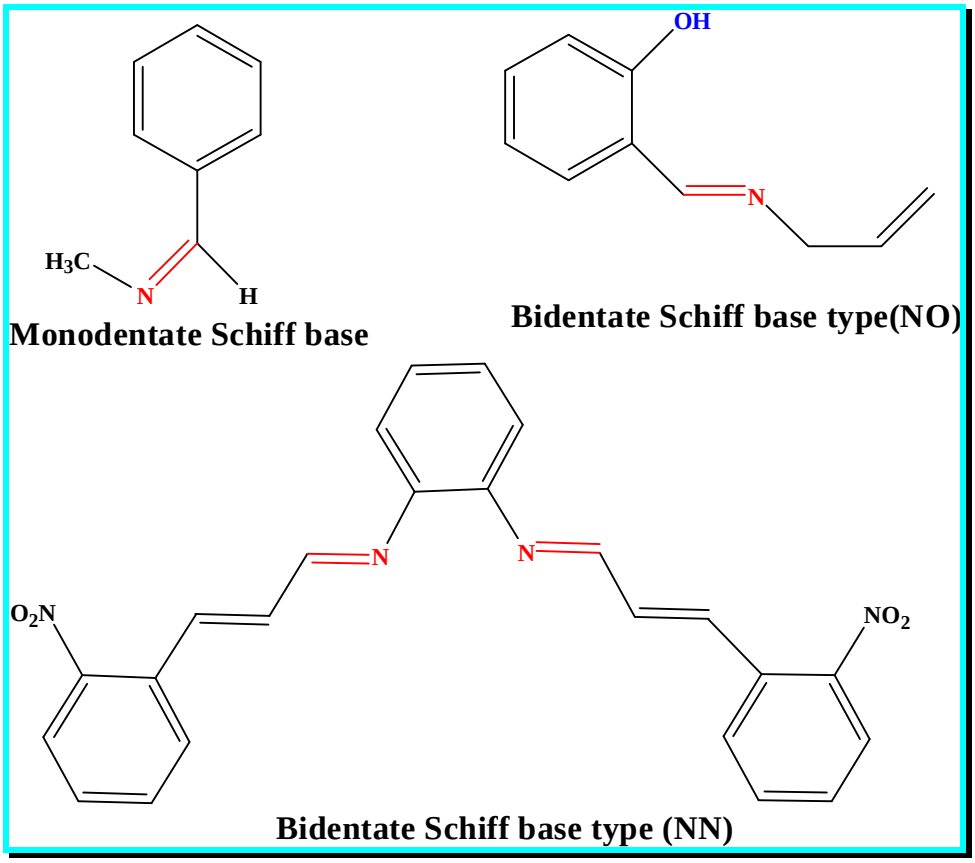


Figure I.7: Example of Schiff bases, mono dentate, bidentate (NN-NO)

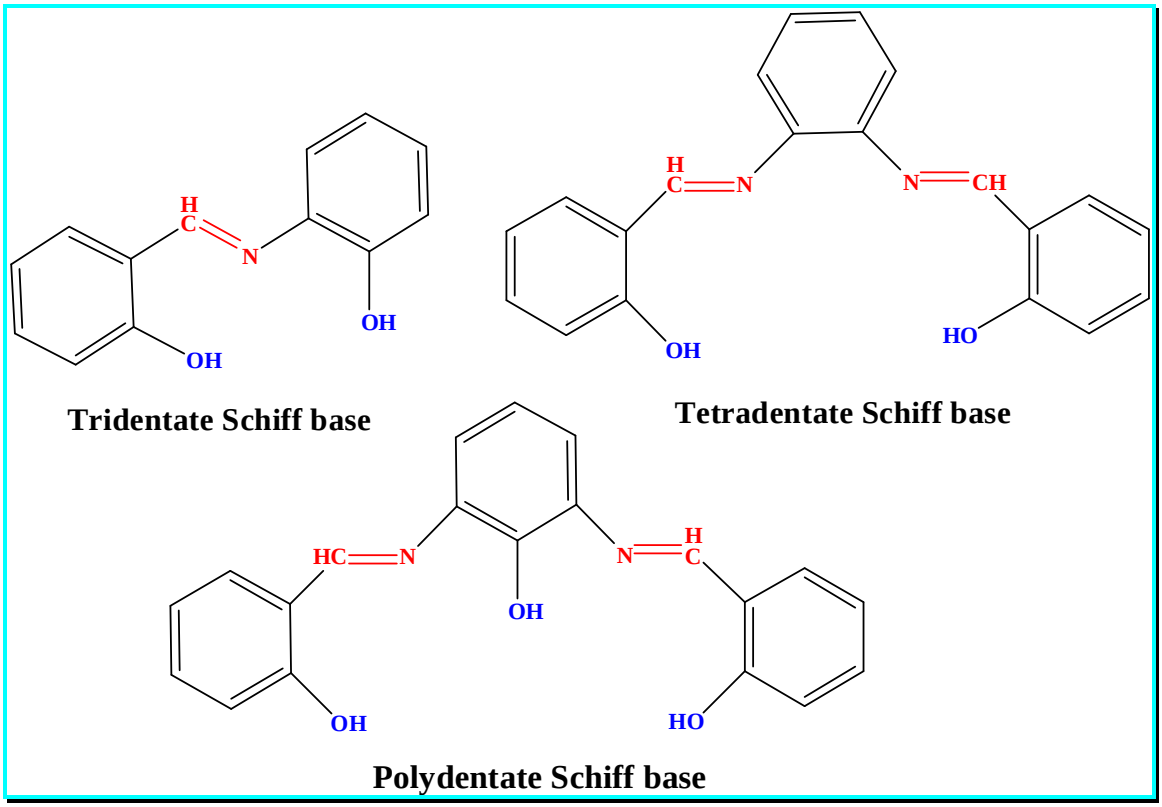


Figure I. 8: Example of Schiff bases, tridentate, tetra dentate, polydentate

- Classification according to their linear or cyclic structure[9]:
  - **Linear Schiff bases:** Depending on the nature of the aliphatic, aromatic or mixed radicals, acquire various behaviors.
    - ✓ Aliphatic linear Schiff bases.
    - ✓ Aromatic linear Schiff bases.
    - ✓ Mixed linear Schiff bases.

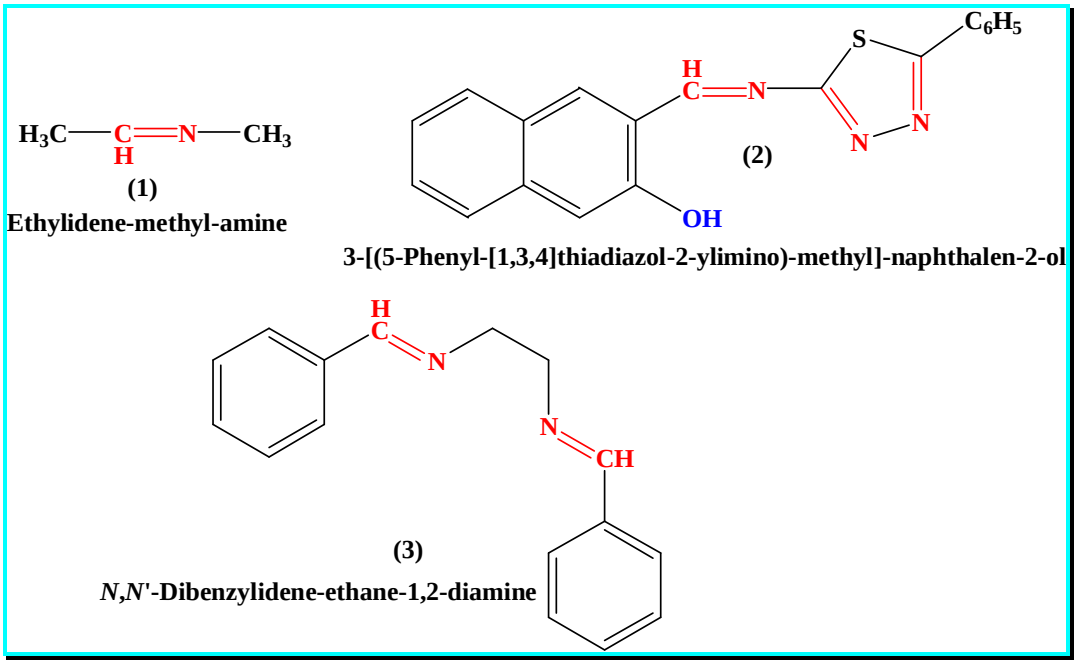


Figure I. 9: Example of linear Schiff bases

- **Cyclic Schiff bases**:
  - ✓ Aliphatic cyclic Schiff bases: They have radicals of an aliphatic nature.

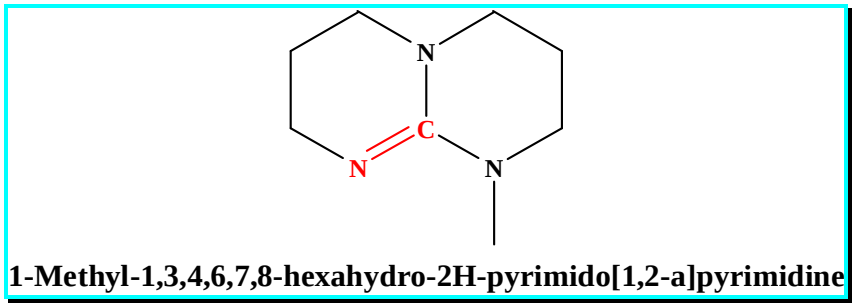


Figure I. 10: Example of aliphatic cyclic Schiff bases

  - ✓ Aromatic cyclic Schiff bases: This compounds are the most exploited in the biological filed.

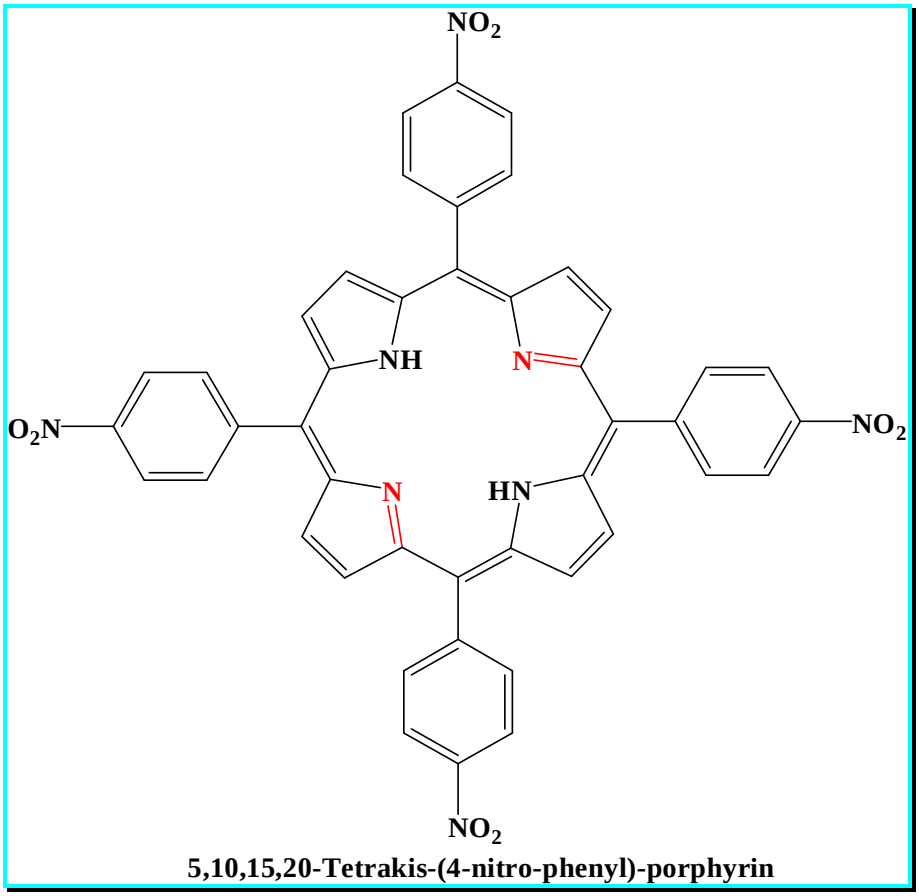


Figure I. 11: Example of aromatic cyclic Schiff base

- ✓ Mixed cyclic Schiff bases: They have radicals of an aliphatic nature and others of an aromatic nature.

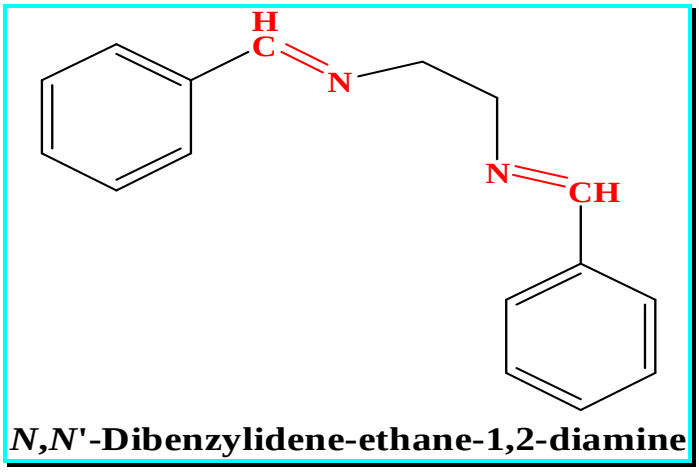


Figure I. 12: Example of mixed cyclic Schiff base

I.4 Chemical and Physical properties :

Most of Schiff bases are yellow crystalline compounds, whose hues generally grow darker with the presence of the substituents leading to orange derivatives and red derivatives. However, no correlation between the aldehyde moiety and the compounds color could be established[7, 26].with a melting point high up to **232 °C** in often[27] , but there some compounds its melting point between **51-94°C**[28, 29],its mass molar is high , it is soluble in organic solvents such as ether, ethanol and Toluene as well as it isn't soluble in water.

I.5 Spectral properties of Schiff bases:

I.5.1 UV-Vis Spectrum:

The UV-Vis spectra of Schiff bases show two major bands to prove existence an azomethine function. This bands at fields 389-467nm[30]and 302-371nm[18, 30, 31]. The first band can be attributed to $\mathbf{n \rightarrow \pi^*}$ transition within **-C=N** group, while the second band would be due to$\mathbf{\pi \rightarrow \pi^*}$ transition within **-C=N** group[30]. In addition to$\mathbf{\pi \rightarrow \pi^*}$transition within the aromatic chromophore[32]ring has wavelengths within the field limits 254-350nm .

The UV-Visible of compounds containing an unconjugated chromophore are characterized by type $\mathbf{n \rightarrow \pi^*}$ transition spectra in the 235-272nm[25].

In general, intermolecular hydrogen bonding causes a large change in band intensity in the UV-visible absorption[29]depending on the nature of radicals.

I.5.2 Infrared spectrum analysis (FTIR):

The IR spectra of the Schiff bases show a strong band in the region 1580-1680 cm^{-1}, which is characteristic of azomethine (stretching frequency **C=N**) group[24]. The **C=N** in an open chain system characterizes by a sharp band definitely assigned in the region of 1690–1640 cm^{-1}.

Aryl conjugation causes a shift towards longer wavelength less of the substituent is located on N or C. Furthermore, the **C=N** band of Schiff bases is mostly overlapped from the aromatic bands *ν*(**C=C**) and therefore difficult to assign[33].

In the 1500–1600 cm^{-1} region, the observed bands were attributed to aromatic **C=C** vibrations, and the **C-N** stretching frequency has been reported in the 1350–1410 cm^{-1} region[26, 34].

In the compounds containing the O-H group ,The O–H stretching frequency of the ligand is expected in the 3300–3800 cm^{-1}region, however, this frequency is generally displaced to the 2840–2960 cm^{-1}region due to the internal hydrogen bridge OH---**N=C**[35]. As the hydrogen bond becomes stronger, the bandwidth increases, and this band sometimes is not detected.

Hydrogen bonds in these Schiff bases are usually very strong. Electron-donating groups on the phenolic ring increase the electron density on the hydroxyl oxygen making the O-H bond stronger, the absorption usually appears as a broad band in the IR spectrum[26].

I.5.3 Nuclear magnetic resonance spectra analysis:

I.5.3.1 ^{1}H NMR spectrum*:*

The ^{1}H NMR spectrum is also a powerful means for the elucidation of the structural characteristics of Schiff bases in the solution[36] the solvent used such as DMSO,$CDCl_3$ .The chemical shifts, expressed in ppm downfield form tetra methyl silane (TMS)[26].

The ^{1}H NMR spectra of the Schiff bases exhibits signals at about 8.3-9.50 ppm [17, 37], this explains the presence of protons in the imine group(CH=N).In addition the region of 7.6–6.8 ppm were assigned chemical shifts for hydrogen of the aromatic ring (Ar-H).

The Schiff bases synthesized from Salicylic aldehyde in the ^{1} H NMR spectra exhibit a broad peak between 14 and 12.5 ppm, which is due to hydrogen-bonded phenolic protons and the integration is generally less than 2.0 thus confirming its involvement in a intermolecular hydrogen bond with the neighboring nitrogen atom[26].

The free NH_2 protons usually show a broad singlet peak in a region at 4-6ppm. If this signal is absent in spectra of Schiff bases, this indicates the formation of the Schiff bases[17].

I.5.3.2 ^{13}C NMR spectra*:*

The ^{13}C NMR spectra of Schiff bases ligand provide further support for the structural characterization of the Schiff bases, The ^{13}C NMR spectra exhibit a specific signal at **165.00 ppm** due to azomethine carbon C=N[2].in addition to the region of 121.5-155.8ppm[37] due to aromatic ring carbon(Ar-C).

I.6 Applications of Schiff bases:

Schiff bases are considered as a very important class of organic compounds [33] and has a wide range of applications in many fields [14] including biological activities and their industrial application [1].

I.6.1 Biological activities of Schiff bases:

Therapeutically, Schiff bases and their metal complexes have gained importance in medicinal and pharmaceutical due to the **-C=N-** imine bond in Schiff bases plays a unique role in conferring broad-spectrum biological activities to these compounds. The electrophilic carbon and nucleophilic nitrogen in **-C=N-** imine bond provides excellent binding opportunities with different nucleophiles and electrophiles, thereby inhibiting targeted diseases, enzymes, or DNA replication[38].

In Schiff bases azomethine linkage provides much flexibility in the design ligands for metal ion coordination. Among metal ion based therapy, metalloantibiotics occupy a prominent position. Metalloantibiotics require the presence of metal ions along with the main synthetic (semi-synthetic) or microbial derived substrate such as *β*-lactams, amino glycosides, quinolones *etc*. The metal ion in these metalloantibiotics is responsible for interaction with various biomolecules such as proteins, membranes, and nucleic acids (RNA/DNA)[38].

Schiff bases have been shown to be promising leads for the design of efficient antimicrobial agents because of their broad range of biological activities. These biological activities include[6]:antifungal, antibacterial[39], anti-malarial, anti-proliferative, anti-inflammatory, antiviral and antipyretic, anti-hypertensive, herbicidal, anti-convulsion anti-oxidant, anti-tumor, anti-depressant and cytotoxic activities[6], anti-Alzheimer, anti-cancer, urease inhibition, pesticidal activity detoxification etc. [38].Among these medical applications the most important are as follows:

I.6.1.1 Antibacterial activity*:*

The vast molecular diversity accessible via Schiff base formation is a viable option to search for new, even more effective antibacterial including anti mycobacterial agents[38].The development of new antibacterial agents with novel and more efficient mechanisms of action is definitely an urgent medical need.

Schiff bases have been pointed to as promising antibacterial agents. For example, N-(salicylidene)-2-hydroxyaniline (**Figure I.13**) is effective against *Mycobacterium tuberculosis* H37Rv, exhibiting an MIC value of **8** µg/mL[22].

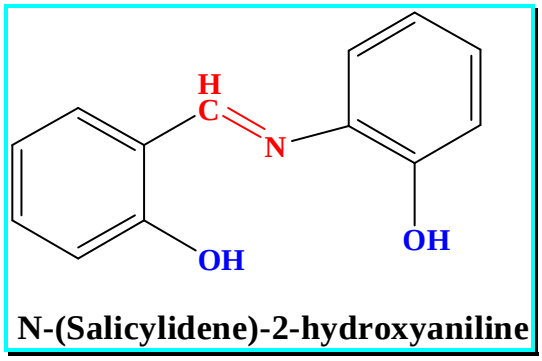


Figure I. 13: Example of antibacterial Schiff base

ÖzlenAltun *et al*[31],assessed the antibacterial activity of Schiff's base L^1 drived from L-phenyl aniline and furfur aldehyde its complex with Pt(II), their Schiff base synthesized (L^1)showed moderate antibacterial activity against *S. aureus*, *L. monocytogenes*, and *C. albicans*at **12.5**, and **6.25µg/mL** concentrations, respectively.

The synthesized complex shows increased activity compared with Schiff bases (L^1). The high activity of the Pt (II) complex may be due to the effect of (Pt^{2+}) ion on the normal cell membrane. The higher antibacterial activity of Schiff base with metal complexes (Pt(II)) than the free ligands(L^1) can be explained by chelation of the Schiff base (L^1) with metal ion (Pt^{+2}) since metal chelates display both polar and non-polar properties[31, 40, 41]. These properties make them suitable for permeation into cells. The polarity of the metal ion is reduced due to partial sharing of the positive charge of the metal ion with the donor groups, such as nitrogen and oxygen on the Schiff base (L^1)[31].

Therefore, the obtained so the metal complex Pt(II) can become more active than the free Schiff base ligand (L^1) [41]and may be preferable to other inorganic complexes of platinum(II) due to its high effectiveness in preventing infection[31].

Although the exact mechanism is not understood biochemically, mode of action of antimicrobials (antibacterial) may involve various targets in microorganisms are:

A. Interference with the cell wall synthesis, damage as result of which cell permeability may be altered or they may disorganize the lipoprotein leading to the cell death.
B. Deactivate various cellular enzymes.
C. Formation of a hydrogen bond through the azomethine group with the active center of cell constituents, resulting in interference with the normal cell process[40].

The higher inhibition of microbial growth is due to uncoordinated heteroatoms and carboxylic moieties.

Effect of azomethine **(>C=N)** group in the inhibition of bacterial. The mode of action of the compounds may involve formation of a hydrogen bond through the azomethine group (>**C=N**-) with the active centers of cell constituents resulting in interferences with the normal cell process[40].

I.6.1.2 Antioxidant activity*:*

Free radicals, which are included in the process of lipid peroxidation, are considered to play a major role in medicine. A compound with radical reducing power may serve as a potential antioxidant.

Antioxidants are free radical scavengers that may prevent, protect, or reduce the extension of oxidative damage[31], antioxidants thus play an important role to protect the human body against damage by ROS.

The ability of Schiff bases and their metal complexes to scavenge free radicals is an important property. Different modes of action such as being free radical terminators, chelators of metal ions involved in catalyzing lipid oxidation or oxygen scavengers that react with oxygen closed systems have been used in categorizing antioxidants[41].

The study of antioxidant activities of Schiff bases and the metal complexes were assessed by Ikechukwu P *et al*. [41] and ÖzlenAltun *et al*. [31]using the DPPH radical scavenging method at different concentrations. They showed the IC_{50}values of Schiff bases and the metal complexes were calculated as **3.2**, **3.6**and **2.3 µM**, respectively. The antioxidant activity was significantly increased as a result of the electron withdrawing effect of the metal ion(Pt^{2+}), which facilitates hydrogen release to reduce the DPPH radical [31, 41].

Schiff bases compounds are capable of donating electrons to neutralize free radicals and thus, could be promising therapeutic agents for the treatment of pathological diseases and conditions caused as a result of excessive radicals or stress.

I.6.1.3 Anti-Alzheimer activity*:*

Alzheimer's disease (AD) is a most common neurodegenerative disorder of the central nervous. AD is associated with memory loss, difficulties in thinking, and many other cognitive disorders. The current available therapies are only effective transiently in the early stage of disease. In search of new therapeutic agents. The excessive build-up ofβ-peptides is a characteristic marker for progression of Alzheimer's disease. Such as 1,4-oxazepine analogues

have been patented as anti-*β*-secretases inhibitors. *β*-Secretase(BACE1 and BACE2) are transmembrane aspartic acid proteases. An elevated level of *β*-secretase is evident in AD patients.

The bioactivity assay of compound in (**Figure I.14**) showed potent inhibitory activity with **IC_{50}** value of **0.070 µM** and **0.024 µM** for BACE1 and BACE2, respectively.

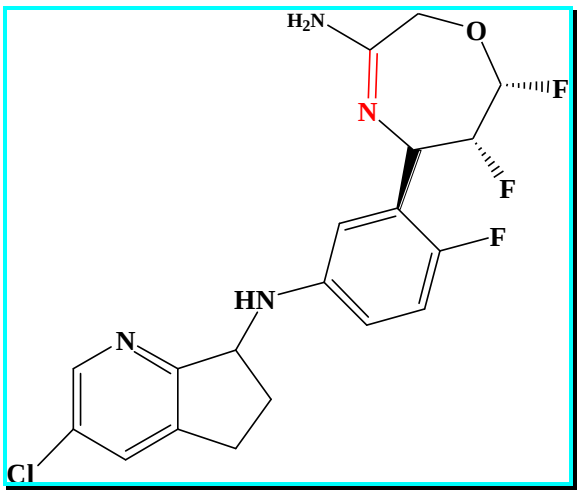


Figure I. 14: Example of Anti-Alzheimer Schiff base

In addition, BACE2 inhibitors play an important role in β-cell proliferation, which leads to improved type 2 diabetes complications. The compound (**Figure I.14**) revealed IC_{50} of **11.06 µM**in BACE inhibition assay by measuring cellular transmembrane protein 27 (TMEM27) cleavages.

Thus, inhibition of BACE2 proposed a remedy for type 2 diabetes by restoring*β*-cell mass to induce insulin secretion in individuals before and after diabetes[38].

I.6.1.4 Antifungal activity*:*

Fungal infection is usually not limited to superficial tissues. Research and development of the most effective treatments of antifungal agents is needed and some of Schiff 's bases are considered promising antifungal drugs[1]. The presence of methoxy, halogen, and naphthyl groups enhances the fungicidal activity of the ligands. Sulfur-containing Schiff bases derived from thiazole and benzothiazole derivatives possess effective antifungal activity[2].Compounds (**Figure I.15**) are examples of chitosan-derived Schiff bases with antifungal activity[22].

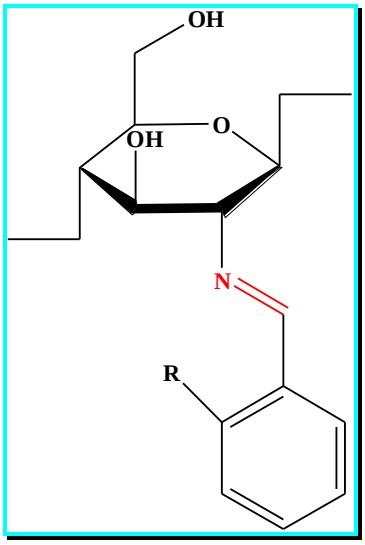


Figure I. 15: Example of Antifungal Schiff base

I.6.1.5 Anticancer activity:

There is an ever-increasing interest in the quest for anticancer therapies. This interest lies in the design of effective anticancer agents based on Schiff base compounds and her metal complexes are capable of intercalating in-between the DNA base pairs thereby causing DNA damage, which ultimately leads to cell death. DNA intercalating anticancer drugs are already in clinical use, however, development of tumor resistance necessitates the development of new potent anticancer drugs[38].

Throughout the years, many Schiff bases have showed their potential as anticancer agents via different mechanism and pathways. This remains an increasingly popular area for research, and Schiff bases certainly provide access to a vast pool of diverse molecules to be screened for their anticancer potential.

Hydrazine Schiff bases are also a proper candidate in physiological and biological studies due to their importance in biological processes, the hydrazine can serve as linkers for drug release to enhance the therapeutic effects against cancer and decrease the side effects[42].

I.6.1.6 Detoxification:

Schiff bases are used to counter the hazardous effects of chemical toxic substances. A general detoxification mechanism of the Schiff base is given in (**FigureI.16**) the same mechanism is claimed to be applicable to other Schiff bases containing an electron acceptor atom near nitrogen atom of the imine. Similarly, the leaving group of the toxic agent can be halide, as well as thiolate, amine or alcohol. Thus a variety of chemical toxins can be neutralized and rendered in-effective by treatment with these Schiff bases[38].

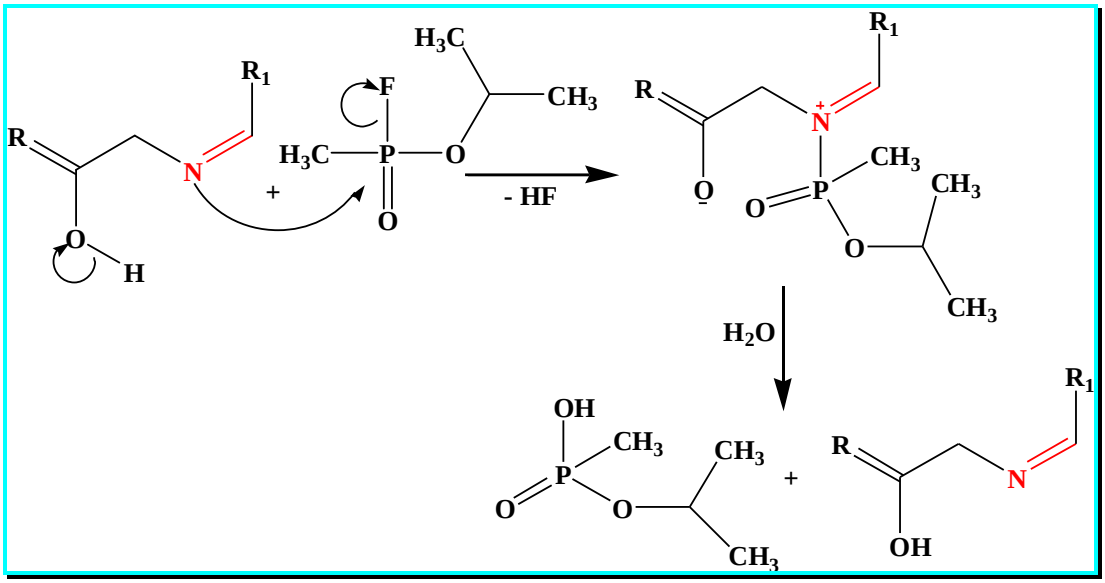


Figure I. 16: General detoxification mechanism of Schiff base

I.6.1.7 Antiviral activity*:*

Viral diseases are life threatening for immune compromised patients and prompt treatment is required to overcome this problem. Although there are many therapeutic options for viral infections, currently available antiviral agents are not yet fully effective, probably due to the high rate of virus mutation. They may also present any of a number of side effects. In fact, from a set of different 1-amino-3-hydroxyguanidine tosylate-derived Schiff bases, such as compound in (**Figure I.17**) was shown to be very effective against mouse hepatitis virus (MHV), inhibiting its growth when employed at concentrations as low as IC_{50} value **3.2 µM**[22].

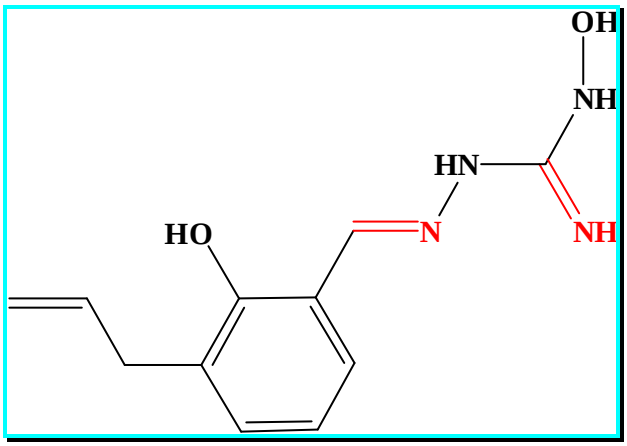


Figure I. 17: Example of Antiviral Schiff base

Kumar and al [43],assessed their cytotoxicity and antiviral activity. Compounds containing hydroxyl group in their structure showed better antiviral activity[43].

I.6.1.8 Antimalarial activity*:*

Malaria is a neglected disease that still causes serious public health problems. Malaria is currently found in more than 100 countries throughout Africa, Latin America, Asia, and Oceania.

Human malaria is mainly caused by four species of *Plasmodium* (*P. falciparum, P. vivax, P. ovale,* and *P. malariae*). The female mosquito of the Anopheles genus is the vector of *Plasmodium*. The search for new drugs, vaccines, and insecticides to prevent or treat this disease is clearly a priority[22].

Schiff bases have been shown to be interesting moieties for the design of anti-malarial agents[22].Among them are natural and synthetic compounds, the first of which, such as Ancistrocladidine (**Figure I.18**) is a secondary metabolite produced by plants from the families Ancistrocladaceae and Dioncophyllaceae. This Compound has been shown to be active against *P. falciparum* K1 and 3D7. The minimum inhibitory concentrations (MIC values) of ancistrocladidine necessary to completely abolish *P. falciparum* K1 and 3D7 growth were **0.3** and **1.9 µg/mL**, respectively.

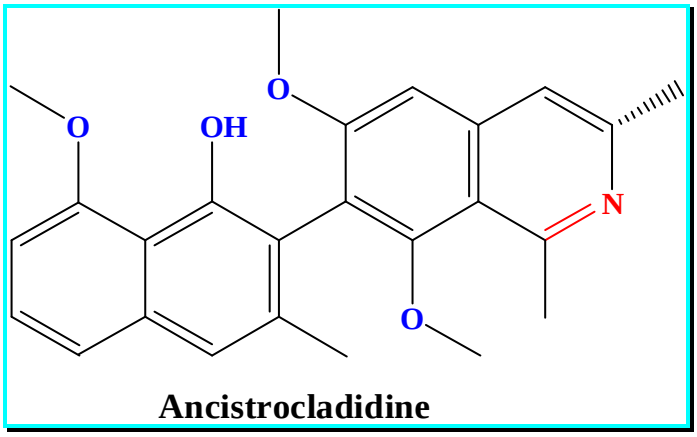


Figure I. 18: Example of Antimalarial natural Schiff base

As for the synthetic compounds, Rathelot *et al*[44],described the synthesis of Schiff base functionalized 5-nitroisoquinolines and investigated the in vitro activity of these compounds against.

Schiff base (**Figure I.19**) was the most effective anti-malarial agent among the synthesised5-nitroisoquinoline derivatives. (In the aldimine series, aromatic ring substitution by halogen groups (Cl or Br) increases the antimalarial activity).

The concentration of compound synthetic necessary to inhibit *P.falciparum*growth by 50% (IC_{50}) was **0.7 µg/mL**[44].

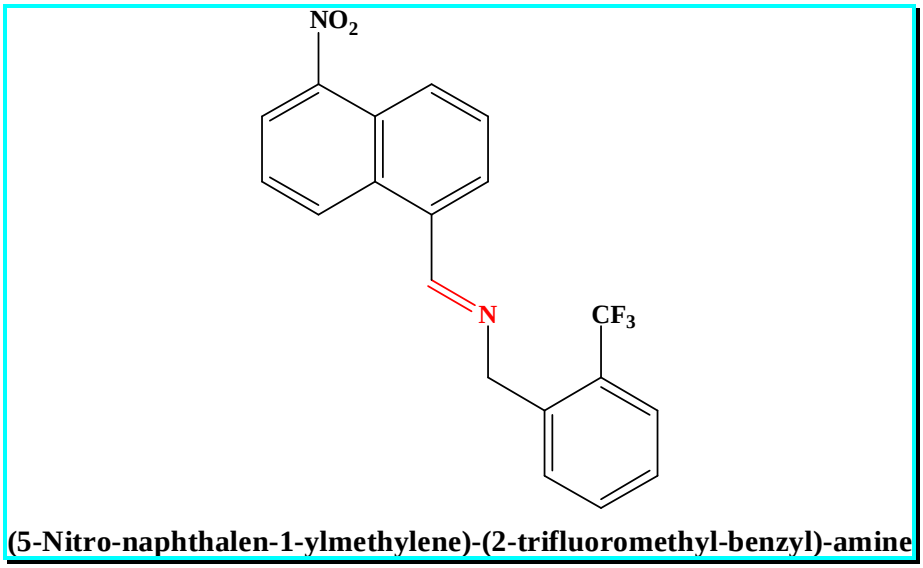


Figure I. 19: Example of Antimalarial synthetic Schiff base

I.6.1.9 Urease inhibition:

Urease is a nickel-containing metalloenzyme (*urea amindohydrolase* EC 3.3.1.5) that is found in bacteria, plant and fungi. Over expression of urease is a virulence factor that provides a feasible environment for *H. pylori.* Accordingly, urease is an important drug target for treatment of *H. pylori* infection. A number of remedies have been reported to inhibit the accelerated urease activity of *H. pylori*. Recently, a series of Schiff bases of thiazole has been patented as effective urease inhibitors.

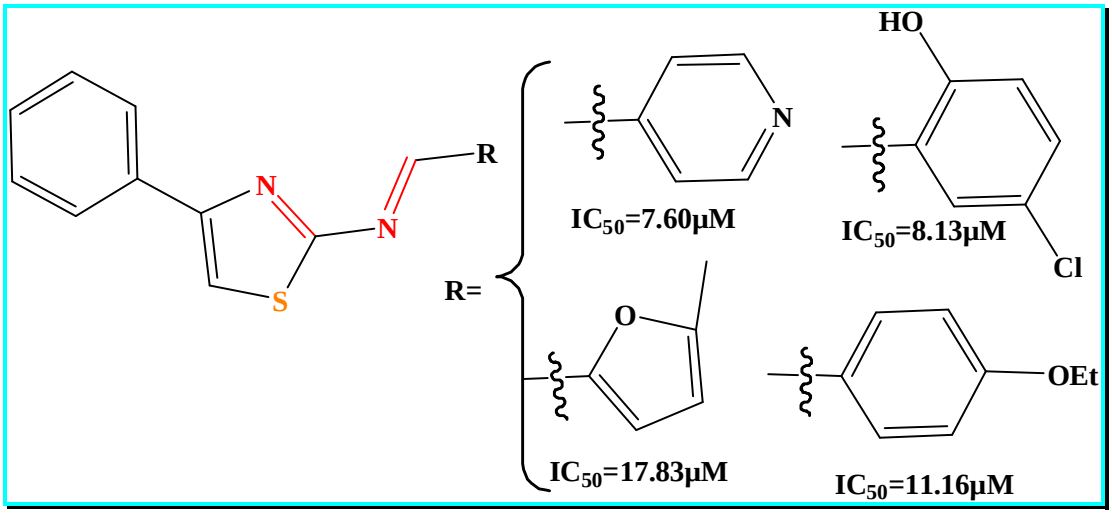


Figure I. 20: Example of thiazole Schiff bases as urease inhibitors

These application are not limited to the laboratory scale only ,but they have been extrapolated to the industrial scale, being among the most exploited innovative systems in the field research[9].

I.6.2 Industriel applications of Schiff bases :

The importance of Azomethins also lies in their use, widely used for industrial field as corrosion inhibitors, as well as their ability to capture metal ions (extraction of the transition metals) [11], intermediates in organic synthesis, polymer stabilizers[2].

a) **As corrosion inhibitors:** Application of corrosion inhibitors is the most economical and practical method to mitigate electrochemical corrosion. From the standpoint of safety, the development of nontoxic and effective inhibitors is very important and desirable[13].

Many heterocyclic compounds containing heteroatoms like N, O and S have been reported to be effective inhibitors for the corrosion[13]and this an interesting application of Schiff bases, 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.

The principal interaction between the inhibitor and the metal surface is chemisorption. The inhibitor molecule should have centers capable of forming bonds with the metal surface by electron transfer. In such cases, the metal acts as an electrophile and the inhibitor acts as a Lewis base. Nucleophilic centers, such as oxygen and nitrogen atoms, of the protective compound have free electron pairs, which are readily available for sharing. Together with the atoms of the benzene rings they create multiple absorption sites for the inhibitor thus enabling stable monolayer formation[17].

b) Extraction transition metal:

In fact , Schiff bases are also fascinating compounds capable of complexing transition metals and stabilizing many different metals in their oxidation states particularly , that shield metal ions from the chemical environment by creating kinetically inert complexes[14, 45]. This is why they are important compounds in:

- Industrial water purification.
- Recovery of heavy metals harmful industrial waste[8].

c. As catalysis:

Azomethine or their complexes with metals are used in several types' reactions for example: epoxidation reaction, hydrolysis and decomposition reaction as catalysts including acid catalysts, reduction or oxidation catalysts.

Schiff bases are used intra alia in catalytic reactions, in crystal engineering , also as photo-or chemo detectors in biological system (e.g., Al^{3+} ion *in vivo*)[11] .

d. As Polymer:

Schiff base are used as polymer stabilizers, particularly in the degradation of natural resins (gum), as initiator of polymer emulsion and the copolymerization of dienyl and vinyl monomers[8].

ChapterII:

Liquid-liquid Extraction

Industrial development has worsened the contamination of surface water and groundwater resulting from heavy metal ions, and has increased concern for the high toxic effect of such contamination to animals, plants, and human beings. Heavy metal ions could accumulate in living bodies and cause serious diseases even at very low concentration. Therefore, the effective removal of heavy metal ions from water is very important and has attracted considerable research and practical interest. Various methods or technologies have been used to remove heavy metal ions from aqueous solutions. Among these methods or technologies, liquid-liquid extraction with chelating compounds (Schiff bases) has received more attention in recent years because this method is easy to handle, relatively low cost, and effective in removing heavy metal ions[46].

Liquid–liquid extraction is one of the most promising techniques for separation of base metals, and such extraction processes have recently attracted attention for selective recovery of metal ions from industrial wastes[47] .However, the success of this recovery process is still limited by the extraction selectivity for the targeted metal using a specific extractant. Actually, despite the large number of extractants that have been investigated and developed to efficiently separate metal ions, the design of selective extractants for the separation of heavy metals particularly bivalent metal cations has remained a great challenge in wastewater treatment.

Recently, researches have been directed towards Schiff-base derivatives as the main extractant candidates[48]. Liquid-liquid extraction of metal ions with chelating compounds is becoming increasingly important as a method for separation in analytical chemistry[49].

II.1 Definition of liquid-liquid extraction:

Liquid-liquid extraction or solvent extraction is a separation technique a compound from an aqueous solution, or separate a substance suspended in a solution. The extraction is done, for example, by agitation the aqueous solution with an organic solvent that does not mix water, and then allowing the two liquid layers to separate from each other. During this process, the dissolved substance to be extracted is distributed between the aqueous and organic layers with degree of concentration dependent on the degree of substitution ability of the water and organic solvents. The organic solvent is generally called the extracted solvent.

II.2 Evaluation of the extraction power:

To assess the degree of extraction of a species for a given extraction system, the distribution coefficient''D'' is used. On a practical level, and use the extraction efficiency "E".

II.2.1 Distribution coefficient :

The distribution coefficient (D) is defined as an experimental parameter. It expresses the ratio of the contents (mass or molar) of a solute between the two phase when the equilibrium.

To characterize the extraction ability, the distribution ratio D between the two phases was examined on the ligand concentration. Moreover, the distribution ratio between the organic and aqueous phases is defined as[48]:

$$M^{2+} + 2HL_{Org} \overset{K_{ex}}{\longleftrightarrow} ML_{2\ Org} + 2H^{+}$$

Which has the constant:

$$K_{ex} = \frac{[ML_2]_{Org}\ [H^+]^2}{[M^{+2}]\ [HL]^2_{Org}} \Rightarrow \frac{ML_2}{M^{+2}} = \frac{K_{ex}[HL]^2}{[H^+]^2}$$

The metal distribution coefficient is defined by:

$$D = \frac{C_{Org}}{C_{aq}} = \frac{[ML_2]_{org}}{M^{+2}} = \frac{K_{ex}[HL]^2}{[H^+]^2}$$

From where:

$$\log D = \log K_{ex} + 2\log[HL]^2{}_{org} + 2pH$$

For extraction of metal ions, it is preferable that the chelating agent used has high distribution coefficient[50].

II.2.2 Extraction yield or efficiency :

Liquid-liquid extraction can be expressed by its efficiency or metal ratio extract:

$$E(\%) = 100 \times \frac{[ML_2]_{Org}V_{Org}}{[ML_2]_{Org} + [M^{+2}]_{aq}V_{aq}}$$

Dividing by $[M^{+2}]\ V_{Org}$ we find :

$$E(\%) = 100 \times \frac{\frac{[ML_2]_{Org}V_{Org}}{[M^{+2}]_{aq}V_{aq}}}{\frac{[ML_2]_{Org}V_{Org}}{[M^{+2}]_{aq}V_{aq}} + \frac{V_{aq}}{V_{Org}}} = 100 \times \frac{D}{D + \frac{V_{aq}}{V_{Org}}}$$

The efficiency E (%) highlights the ratio of the organic and aqueous phases. When the volume of the two phases is equal ($V_{eq} = V_{Org}$), we obtain:

$$\mathrm{E}(\%) = 100 \times \frac{\mathrm{D}}{\mathrm{D}+1}$$

II.3 Extraction mechanisms :

Liquid-liquid extraction is the most common process for the separation and extraction of metals. The type of solvent extractants and innovative methods used in various literatures observes the evolution in this process. Extractants are classified into cation exchanger, anion exchange, chelating, and solvating (neutral). Each of the extractant has its own advantages and disadvantages in extraction[51].

II.3.1 Extraction by chelation :

'Chelation' is defined as formation of stable metal ligand complexes, which are soluble in water. Though, 'chelation' term defines the mobilization of metal ions from contaminated sites using multidentate ligands (chelators) as reagents, however, some authors assumed hydration of metal ion as an initial step (reaction takes place in aqueous solution) and then, multidentate ligand tend to substitute water molecules from metal-water complexes in order to form metal-ligand complex.

Chelation concept is based on coordinative incorporation of a metal ion into a heterocyclic ring structure. Metal ions in solution always make bonds with ligands and form complex ions.

Recovery of these metals from industrial waste is an essential issue to consider for the aid of industrial and economic benefits. This will be done through chelation extraction. Formation of metal-chelate complex may be considered a heterogeneous chemical reaction, which depends on certain process parameters such as reaction pH, molar concentration of chelating agents, etc.

The effect of various important factors will be discussed on metal-ligand complexation process, which play a dominant role to increase the extraction efficiency.

- **Effect of reaction pH:**

Extraction of metal-ligand complex compounds is represented by:

$$\mathrm{M^{+n}} + \mathrm{nHL} \rightleftharpoons \mathrm{ML_n} + \mathrm{nH^{+}}$$

Through the expression of the logarithm of the previous distribution coefficient, it is clear that the curve between log D (distribution coefficient) and pH of the solution should be a straight line with slope n, which indicates that if molar concentration of chelating agent [HL] is constant, then extraction of heavy metals will be completely dependent on reaction pH.

- **Effect of molar concentration of chelating agent:**

Molar concentration of chelating agent also plays a significant role in metal extraction process. Distribution coefficient (D) largely depends on reagent concentration in solution at constant pH as shown in eq:

$$\log D = \log K_{ex} + n \log[HL]org + npH$$

In addition, a straight line with a slope of charge n of the metal cation is obtained by plotting a curve between log D and log [HL].

II.4 Applications of Chelation technology for metal extraction:

Chelation is used for:

- Metal extraction from spent catalysts.
- Metal extraction from soil.
- Metal extraction from water (water purification).

II.5 Metals:

Metals, simple bodies, crystalline solids, characterized by high thermal conductivity. I can classify metals into several categories alkali metals, alkaline earth metals, transition metal, the latter are important in our work:

II.5.1 Transition metals :

The elements of the “d” block of the periodic table or transition elements take this name from their location in the periodic table, placed between the elements of the “s” block and the “p” block. One of the most striking characteristics of transition elements is their ability to possess variable valences. Transition elements from many coordination compounds unlike “s” and “p” block elements.

Indeed, these elements have small ions, very charged with empty orbitals and the energies of these orbitals have values, which are able to receive electronic pairs, brought by other groups called ligands or coordinates.

Specific properties of transition metal complex, such as the stability of several redox states, the flexibility of the coordination sphere are privileged factors for making them catalysts[52].

II.5.1.1.1 Definition of transition metals :

Transition metals were so called because they seemed to assume the transition between elements with very pronounced metallic characters and non-metals (formerly metalloids).in Mendeleieff's table, they constitute a bridge between the two classes of elements.

In the periodic table, the transition metals are inserted between columns 2 to 13 of the representative elements. They correspond to group IB to VIIB and include the lanthanides and actinides. Their outer electronic configuration remains in principle the same, with a saturated "s" orbital; however, there are a few exceptions (Chromium, cooper etc.….) for which the "s" orbital is occupied by only one electron, to satisfy Hund's rule. The inner shells are gradually completed by filling the "d" orbitals with 10 electrons and the "f" orbitals with 14 electrons[53].

II.5.1.1.2 Transition metals properties:

Transition metals have interesting properties for industry, construction and everyday life:

- Transition metals have several valences or oxidation numbers: from +1 to+8.
- Transition metals are generally harder.
- Transition metals are characterized by the presence of "d" states. They break down into three series: the 3d, 4d and 5d series.
- It has ability to from coordination compounds(complex), and the due to:
  - ✓ The high charge of most ions.
  - ✓ The presence of empty "d" orbitals that allow the entry of electronic pairs that are present on the ligands.
- In general, the melting and boiling point of most transition elements are very high.

II.5.2 The transition metal studied :

II.5.2.1 Lead :

Lead when freshly cut is shiny gray with a hint of blue. It tarnishes to a dull gray color (silver gray) when exposed to air.

Lead is a silvery gray metal extracted mainly from minerals containing zinc, silver and minimal amounts of copper, the earth's crust contains an average of 16 mg/kg.

It can also be found in water and in the air, but in very small quantities. Lead is also recovered from recycled materials; more than 50% of global production comes from recycling, making lead one of the most recycled metals in the world. Its attractive properties, such as high density and malleability, corrosion resistance, etc. Give rise to a whole range of applications. However, at higher levels (concentration above 50 mg/kg in soils), lead has a toxic effect on living beings and the environment[54, 55].

II.5.2.2 Physical and chemical Properties of lead metal:

Of lead. There are four natural isotopes ^{204}Pb, ^{206}Pb, ^{207}Pb and ^{208}Pb. Their relative abundance in nature is 1.48%, 23.6%,22.6%, 53.6% respectively, but their proportion in materials vary, depending on the source, which can be used to identify source of lead contamination[55].

Lead belongs to group IVB of the periodic table, its electron configuration is $[X_e]4f^{14}5d^{10}6s^26p^2$ with the oxidation states (+2) and (+4) in addition to the metallic from. In natural environments, minerals incorporate this element under the degree of oxidation (+2), the degree (+4) is only represented in very oxidizing conditions and is found mainly in organic compounds whose source is human activity. **Table II.1** presents some physico-chemical properties of the element lead[54].

Table II. 1: Physico-chemical properties of the element lead

Properties	**Values**
Atomic mass	207.2g/mol
Atomic number	82
Melting point	327 °C
Boiling point	1740 °C
Density	11.35 g /cm^3
Valences	+2 ,+4
Atomic radius	1.20 A°
Electronic configuration	$[X_e]4f^{14}5d^{10}6s^2 6p^2$

II.5.2.3 Toxicity :

Lead can enter the body by three routes:

- Inhalation of lead vapor or dust.
- Ingestion from dust, contaminated soil or lead paint present in old homes directly put in the mouth mainly by children.
- Cutaneous absorption.

After ingestion, lead rapidly diffuses via the bloodstream into the various organs such as (teeth and bones). Lead has a toxic cumulative effect; the person affected may be subject to many disorders: great fatigue, lack of concentration, memory loss, behavioral and sleep disorders, brain damage. Its main target organs are the nervous system, kidneys and bloods[55].

To avoid all this toxicity resulting from lead metal and its effect on living organisms, we resort to one of the above-mentioned methods of protection among them liquid-liquid extraction of transition metals.

ChapterIII:

Experimental part

This chapter is devoted to describing chemical production, experimental techniques for synthesis and spectroscopic techniques used to characterize our obtained products.

We also try in this part to present the results of the study of Schiff's bases and their application to confirm their biological effectiveness and confirm their chelating power with respect to the transition metal lead (II).

III.1 Analytical techniques and apparatus:

a. Melting point:

It is possible to determination the nature of substance (identification) by measuring its melting point. This is why the melting point is a characteristic property of matter. However, this information is not sufficient to allow formal identification, as several molecules may have a very close melting point. On the other hand, it makes it possible to eliminate from the field of possibility molecules having a melting temperature different from that measured.

The melting temperatures are measured in a capillary tube on a SMP50 melting point apparatus (automatic melting point).

b. Chromatography:

TLC using silica gel plates with product development using a 254nm and 365nm multi-band UV lamp checked the purity of synthesized ligands and starting materials.

c. Ultra violet spectroscopy:

The UV-visible spectra of the ligands (Schiff bases) were recorded in solvent between 200-800 nm at room temperature, using glass cuvettes 1 cm thick.

d. Infra-Red Spectroscopy:

The infrared spectra of ligands were recorded on an FTIR Fourier transform apparatus. Solid products are analyzed as KBr pellets. Wavenumbers are expressed in cm^{-1}. Through it, the functions in the compounds are detected.

III.1.1 Products and materials used :

Table III. 1: Used products, their properties and materials

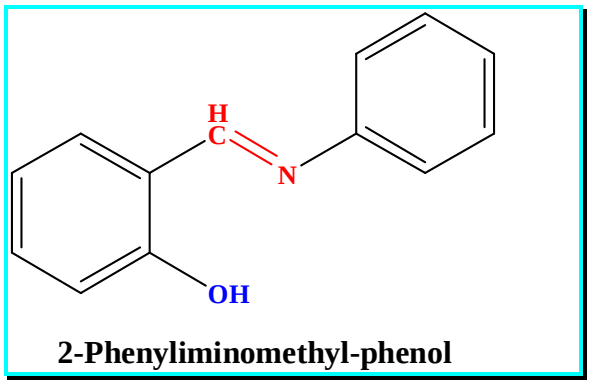
Material	Formula	Molar mass (g/mol)	Purity (%)	company
Aniline	C_6H_7N	93.13	99.9	**PROLABO**
m-Nitro aniline	$C_6H_6N_2O_2$	138.14	98	**Alfa chemical**
Salicylic Aldehyde	C_6H_4CHO	122.12	99	**ACROS ORGANICS**
DPPH	$C_{18}H_{12}N_5O_6$	394.32	/	**ALDRICH**
Lead(II) nitrate	$Pb(NO_3)_2$	331.21	99.5	**Riedel-deHaen**
Potassium iodide	KI	166	99	**BIOCHEM**
Hydrogen peroxide	H_2O_2	34.01	35	**HONEYWELL**
Sulfuric acid	H_2SO_4	98.07	97	**BIOCHEM**
Sodium thiosulfate	$Na_2S_2O_3$	248.19	99	**BIOCHEM**
Ammonium molybdate	$(NH_4)_2MoO_4$	196.01	98	**BIOCHEM**
Ascorbic acid	$C_6H_8O_{35}$	176.12	99	**BIOCHEM**
Ethanol	C_2H_5OH	46.08	97	**MERCK**
Toluene	$C_6H_5CH_3$	92.14	99	**BIOCHEM**
Hexane	C_6H_{14}	86.18	95	**BIOCHEM**
Methanol	CH_3OH	32.04	99.7	**HONEYWELL**
Chloroform	$CHCl_3$	119.38	100	**PROLAB**
Diethyl ether	$(C_2H_5)_2O$	74.12	100	**PROLAB**
pH meter HANNA	Conductivity meter HANNA	Spectrometer SHIMADZU IR A20913700919	spectrophotometer	SMP50 melting point apparatus (automatic melting point)

III.2 Experimental methods :

III.2.1 Synthesis of Schiff bases ligands :

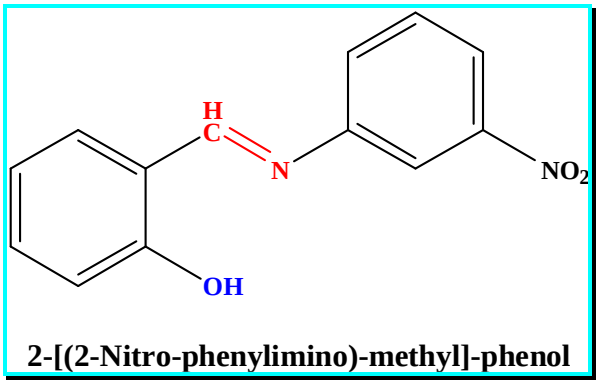
The aromatic aldehyde/substituted aldehyde in solvent are mixed with of amine/substituted amine, and the mixture is stirred for a certain period.

Two compounds were synthesized from Schiff's bases ligands of type NO and ONN, and **Figure (III-1)** shows that:



2-Phenyliminomethyl-phenol

(L1-SA)



2-[(2-Nitro-phenylimino)-methyl]-phenol

(L2)

Figure III. 1: structures of synthesized compounds

Before the synthesis of these compounds, we tested the toxicity of several of Schiff bases and according to the results of toxicity class and the other toxicity parameters, we chose two Schiff bases L1 and L2.

In silico toxicology is one type of toxicity assessment that uses computational methods to analyze, simulate, visualize, or predict the toxicity of chemicals. *In silico* toxicology aims to complement existing toxicity tests to predict toxicity, prioritize chemicals, guide toxicity tests, and minimize late-stage failures in drugs design

Toxicity is a measure of any undesirable or adverse effect of chemicals. Specific types of these adverse effects are called toxicity endpoints, such as carcinogenicity or genotoxicity, and can be quantitative (e.g., LD_{50}: lethal dose to 50% of tested individuals) or qualitative, such as binary (e.g., toxic or non-toxic) or ordinary (e.g., low, moderate, or high toxicity)[56].

The *in silico* toxicity study of the derivatives was performed using ProTox-II webserver. It aims to predict the hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity; also, it predicts the median lethal dose (LD_{50}) and the toxicity class (TC).

Table III. 2: *In silico* toxicity profiles of the two Schiff bases and Paracetamol

	Paracetamol	**L1**	**L2**
Hepatotoxicity	Active	Inactive	Active
Carcinogenicity	Inactive	Inactive	Active
Immunotoxicity	Inactive	Inactive	Inactive
Mutagenicity	Inactive	Active	Active
Cytotoxicity	Inactive	Inactive	Inactive
LD_{50} (mg/Kg)	338	500	1345
TC	4	4	4

ProTox-II webserver successfully predicted the hepatoxicity of Paracetamol, which is known[57] L1 and L2 were predected non-toxic derivatives with LD_{50} equal to 500 and 1345 mg/Kg respectively and their TC were 4. The toxicity class of L^2 was detected to be equal to 4 and was predicted as non-toxic in immunotoxicity and cytotoxicity, L2 possess the highest LD_{50}, compound L2 was predicted as toxic in mutagenicity, carcinogenicity and hepatotoxicity. Finally, compound L1 did not show any hepatotoxicity, immunotoxicity, carcinogenicity or cytotoxicity.

The *in-silico* toxicity study predicted the toxicity of the derivatives to put in mind their toxicity in order to select the best candidates for synthesis.

III.3 Synthesis of L1:

The synthesis reaction is carried out by adding aniline to the salicylic aldehyde in the presence of the solvent according to the following reaction:

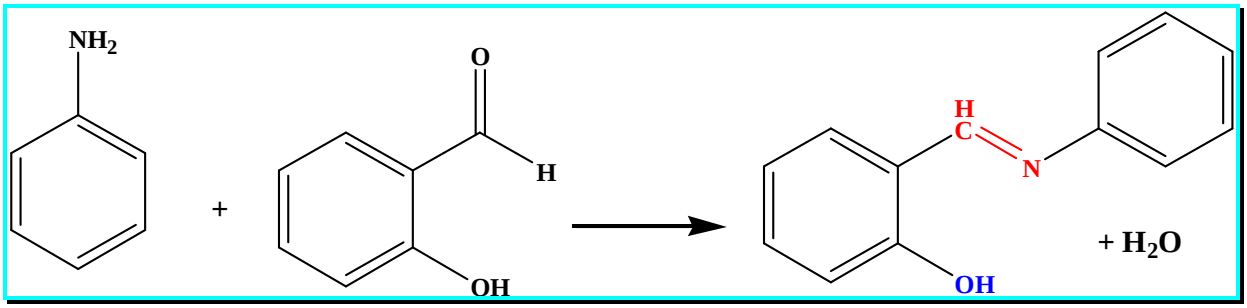


Figure III. 2: General reaction of synthesis of ligand 1 (L1)

This ligand was synthesized by three different experimental methods and are as follows:

A. The first protocol:

Schiff bases ligand (L1a) are prepared by condensation of salicylic aldehyde (0.1mol) with aromatic amine (aniline) (0.1mol) in ethanol (20 mL) and the mixture was stirred at room temperature for 15 min, the solution turned yellow after about 5 min of stirring. TLC (Toluene/Hexane (15/1) monitored the progress of reaction. On completion of reaction the product was separated as yellow-colored amorphous product, the mixture is placed in an ice bath for \ hr to obtain yellow crystals of the Schiff base. Which the filtered, dried, and recrystallized from ethanol. Further recrystallization may be made from ethanol[58].

B. The second protocol:

Schiff bases (L1b) were prepared by condensation of 0.01 mol of amine (aniline) in 10 mL of water and 0.01 mol of salicylic aldehyde. The mixture was stirred at room temperature for 10 min. The yellow precipitate formed was filtered after cooling, washed with water and dried. The ligand was prepared using the same methods as in Shamly *et al*[24].

C. The Third protocol:

The Schiff base ligand (L1c) was being prepared by mixing (0.01 mol) salicylic aldehyde with equivalent amount of aniline (0.01 mol) in 15 mL of ethanol. The resulting mixture was left under reflux for 2 h at 70°C monitored by TLC (Toluene/ Hexane (15/1)). At the end of this period, the solvent was removed by evaporation. After the cooling the obtained yellow crystals, was washed with hot ethanol. Were purified by recrystallization from ethanol, washed with ethanol, and dried[18, 59] with slight modifications.

III.4 Synthesis of L2:

Schiff bases ligand (L2) are prepared by condensation of salicylic aldehyde (1mol) with 3-Nitro-phenyl amine (1 mol) in 50 mL ethanol. The resulting mixture was left under reflux for 1 h 30 min at 70°C monitored by TLC (Toluene/ Hexane (15/1)). At the end of this period, filtered the solution to get the ligand as orange crystals powder and wash it by using diethyl ether. The obtained at the end of the reaction appeared in two types, yellow to orange and orange. To purify and separate these two types, recrystallization was carried out on hot ethanol, were the yellow to orange crystals dissolved in hot ethanol but the orange crystals are few to almost insoluble in ethanol. Then filtering, we get the orange crystals in filter paper, then it is dried. As for the second crystals dissolved in the filtrate, it is crystallized and filtered again, then dried. The reaction is given in **Figure (III.3)**.

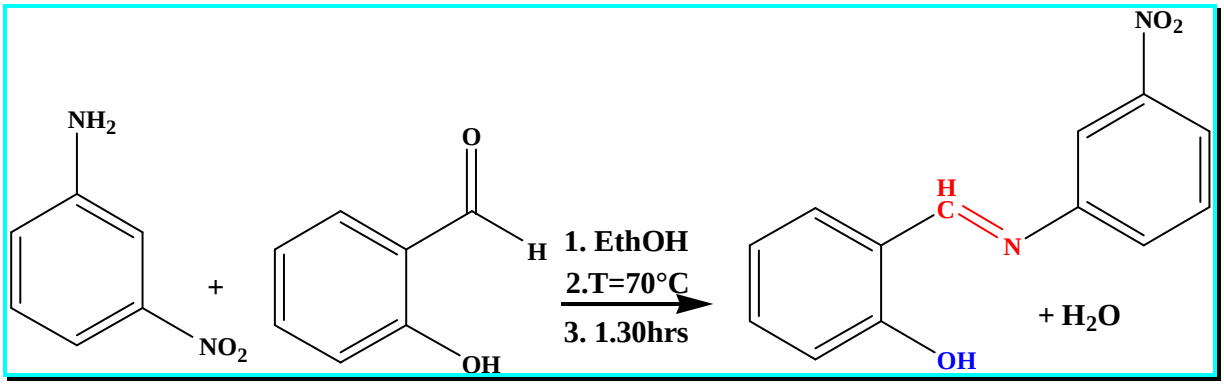


Figure III. 3: The synthesis of ligand 2 (L2)

III.5 Applications of Schiff bases ligands:

III.5.1 Evaluation of the biological activity:

To evaluate the biological activity of Schiff bases antioxidant activity was selected.

- **DPPH free radical scavenging test:**

DPPH free radical scavenging assay is among the most frequently used methods and offers the first approach for evaluating antioxidant activity. DPPH is a stable chromogen radical with a deep purple color. It is commercially available and does not need to be generated prior to the assay. The DPPH scavenging assay is based on hydrogen donation of antioxidants to neutralize DPPH radical (Figure III. 4*: Reduction of free radical DPPH*). The reaction is accompanied with color change of the DPPH measured at 517 nm, and the discoloration acts as an indicator of the antioxidant efficacy. The antioxidant activity by DPPH scavenging method is often reported as IC_{50} that is defined as the inhibition concentration of the antioxidant necessary to decrease the initial DPPH concentration by 50%. [60]. The antioxidant activity of our Schiff bases was determined using the DPPH free radical scavenging test according to Mansouri *et al*[41] with slight modifications.

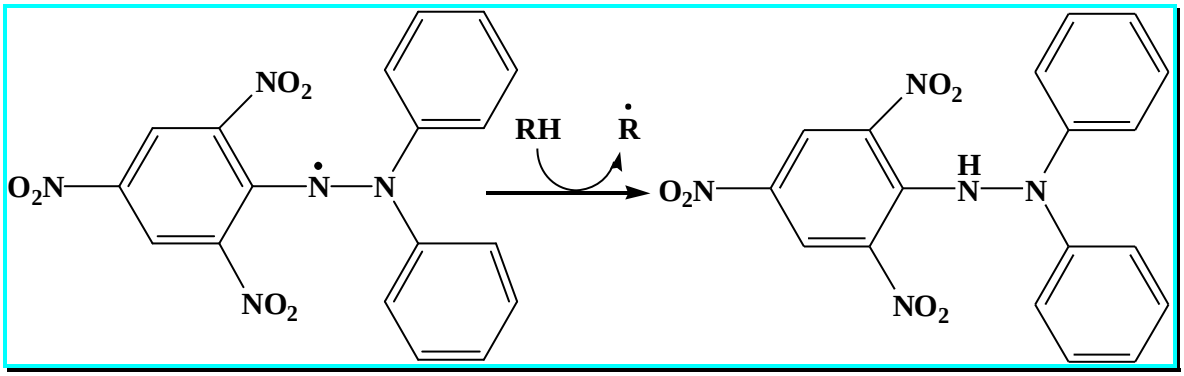


Figure III. 4: Reduction of free radical DPPH

Protocol:

1.5 mL of Schiff base ligand 1 (L1), diluted in ethanol is added to 1.5 mL of a solution of DPPH (100µM) prepared in ethanol, the mixture is left in the dark for 30 min and the discoloration compared to the negative control containing the DPPH solution and 1.5 mL of ethanol, the absorbance was measured at 517 nm.

Same procedure is repeated, replacing the ligand 1 (L1) with ligand (L2) diluted in the chloroform and ascorbic acid as reference.

The anti-radical activity is estimated according to the equation below:

$$\textbf{\textit{Antiradical activty}} = \frac{\textbf{\textit{Abs}}\ \mathbf{517}_{\textbf{\textit{negative control}}} - \textbf{\textit{Abs}}\ \mathbf{517}_{\textbf{sample}}}{\textbf{\textit{Abs}}\ \mathbf{517}_{\textbf{\textit{negative control}}}} \times \mathbf{100}$$

- **Hydrogen peroxide scavenging activity assay:**

Protocol:

This titration is done by mixing the following in order aliquot of:

- 1.0 mL of 0.1 mM H_2O_2.
- 1.0 mL of various concentrations of Schiff bases.
- Followed by 2 drops of 3% ammonium molybdate.
- 10 mL of 2 M H_2SO_4.
- 7.0 mL of 1.8 M KI.

The mixed solution was titrated with 5.09 mM $Na_2S_2O_3$ until yellow color disappeared.

Percentage of scavenging of hydrogen peroxide was calculated as:

$$\textbf{\textit{Inhibition}}\ \% = \frac{\boldsymbol{V}_{\mathbf{0}_{\textbf{\textit{negative control}}}} - \boldsymbol{V}_{\mathbf{1}_{\textbf{sample}}}}{\boldsymbol{V}_{\mathbf{0}_{\textbf{\textit{negative control}}}}} \times \mathbf{100}$$

Where V_0 was volume of $Na_2S_2O_3$ solution used to titrate the control sample in the presence of hydrogen peroxide (without Schiff base), V_1 was the volume of $Na_2S_2O_3$ solution used in the presence of the Schiff bases.

Same procedure is repeated, replacing the ligands with ascorbic acid as reference.

III.5.2 Evaluation of the ion extraction capability of Schiff bases:

There are many transition metals, lead was selected and extracted by chelation:

- **Extraction of lead by Schiff base ligand 1(L1):**

The metal ion solution of lead (II) are prepared in distilled water. The initial concentration of metal in the aqueous phase in all experiments was $[Pb^{+2}] = 4 \times 10^{-4} M$.

The liquid-liquid extraction of lead (II) by chelation in nitrate medium was carried out from an aqueous phase of $Pb(NO_2)_3$ in contact with an organic phase containing the extractants (L1) diluted in chloroform.

Extraction procedure:

- ✓ The pH and the conductivity were measured before the beginning of the extraction for volume 10mL of the aqueous phase containing lead metal by a pH meter and conductivity mete respectively.
- ✓ 10 mL of an aqueous phase was stirred with 10 mL of organic solution containing different concentrations of extractant (L1). The agitation of the two phases is ensured by a magnetic stirrer
- ✓ The ionic strength was maintained at μ= 0 with absence Na_2SO_4.The variation in pH of the aqueous phase is done by adding NaOH 0.1M or HCl 0.1M.
- ✓ After 30 min, the extraction equilibrium having been largely reached. After phase separation, the metal concentration in the aqueous phases was determined using a conductivity meter. The determination of the distribution coefficient of the metal at the considered pH.

ChapterIV:

Results and discussion

IV.1. Synthesis:

The Schiff bases derived from salicylic aldehyde and aniline was prepared as illustrated in Figure (III.2) and Figure (III.3).

After performing the purification process of Schiff base ligand 1 (L1), the follow of synthesized compounds with TLC confirmed their purity as shown in the Figure IV.1**.**

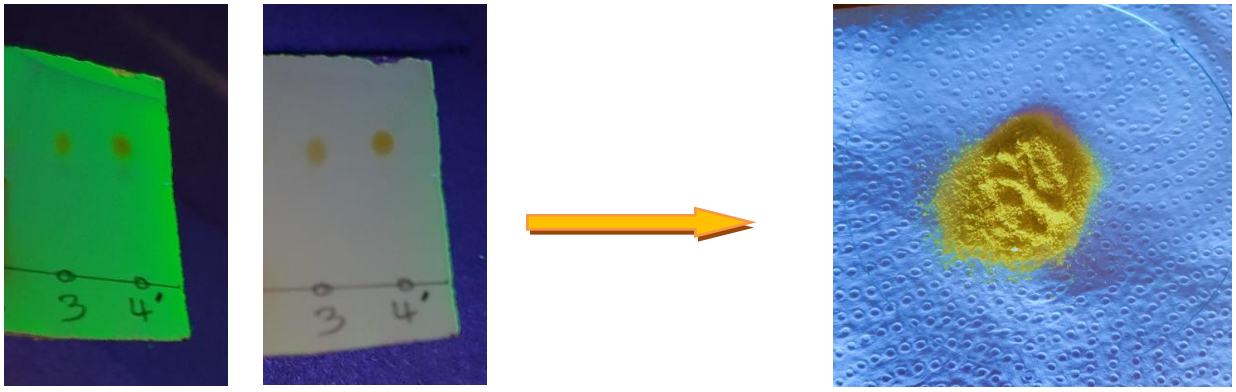


Figure IV. 1: Schiff base N-Salicylideneaniline L1a-L1c

As for Schiff base ligand (L2), follow the purity by TLC showed two spots indicating the presence of two compounds as shown in the Figure (IV. 2).

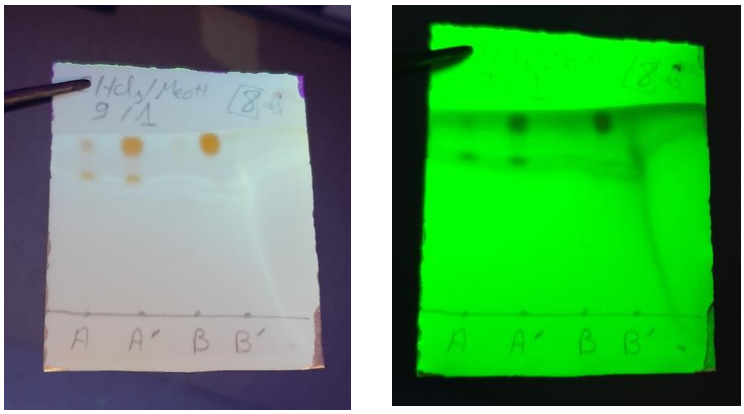


Figure IV. 2: Results TLC of Schiff bases ligand 2

The two resulting compounds were purified and separated by recrystallization procedure by the different solubility between them. The separation results showed that there are two types of compounds. The compound L2 is purified as orange crystals dissolved in chloroform and showed under wavelength 365 nm as orange spot, as for wavelength 254 nm as brown spot. The compound L2a is purified as yellow to orange crystals dissolved in hot ethanol TLC

showed a single spot, from as orange spot under wavelength 365nm and as light brown spot under wavelength 254 nm. The Figure (IV. 3) shows that:

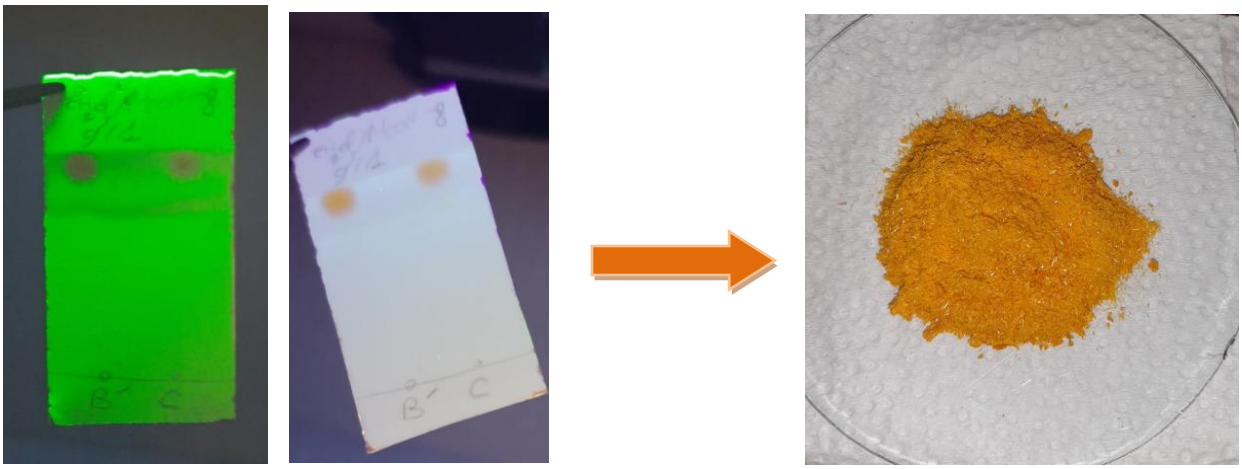


Figure IV. 3: Results of TLC for L2 – L2a

IV.1 Physical characterization of the Ligands:

The physical appearance, percent yield and melting point are listed on (Table IV.1).

Table IV. 1: Physical parameters and percent yield of synthesized compounds

Schiff base	Color	Yield%	M.P(°C)
L1a	Yellow	38.19	48.5
L1b	Yellow	30.64	52.5
L1c	Yellow	96.30	52.4
L2	Orange	69.89	130.2
L2a	Yellow to orange	/	139.7

The table shows the Schiff bases synthesized in different methods with the physical properties of each of them:

Ligand L1:

Schiff base L1 was synthesized by three experimental methods and the result of each them are shown in the table above. We found that all methods gave the same yellow color.

Regarding the yield, we notice that the product L1c has a better yield, and this is due to the difference in the method used in temperature and it was done in reflux compared to methods 1and 2 at room temperature. This indicates that the temperature is a strong catalyst.

As for comparison between methods 1and 2 both without reflux and the difference between them lies in the solvent used and the time, the first was in an ethanol medium for a period of 15 min with a yield 38.19% while the second method had a yield of 30.04% in an aqueous medium. This explains that the solvent and time has a great importance in obtaining a better yield. And the difference between them is about 8.15% which is not significant, which confirms that the interest in green chemistry in organic synthesis and moving away from volatile organic solvents may be better with the increase in temperature T, time and the use of reflux.

Moreover, looking at the melting point of the Schiff base ligand L1 that has been synthesized in different methods, were it becomes clear to us that the melting point of each of them is within the filed (48-52.5 °C) corresponds to Arod *et al* [28]. The convergence of melting points or perhaps almost the equality is explained by obtaining the same compound despite the different experimental methods.

Ligand L2:

The reaction of the salicylic aldehyde with m-nitro aniline gave two products L2 and L2a. As the results of the **table IV.1** showed there is a slight difference in color and the results also showed that L^2 has a higher yield compared to L2a, and this explains that ligand L2a is a secondary compound, or perhaps isomer an analogue to L2, and we will prove this by other methods of analysis.

With regard to the melting point of ligands L2 and L2a, through the results it was noted that melting point of ligand L2 is 130.2 °C is close to the melting point of the same compound synthesized by Dalal. M.Ibrahim *et al*. is 134 °C. Through this we can interpret the saying that L2 may be the same compound and this is not sufficient to rule that completely. As for L2a, its melting point was higher by 5 °C degrees with respect to [61].

IV.2 Spectral Characterization:

The structural characterization of the Schiff base was based on UV-Vis and FT-IR spectroscopy; the results are shown in the following tables and spectra.

IV.2.1 UV-spectrum:

We have illustrated the UV-data in table IV.2 and the UV-spectra of Schiff bases under study in Figure IV.4 and Figure IV.5.

Table IV. 2: The UV-Vis spectral data of Schiff bases

Schiff base	λ_{max} value (nm)	Assignment
L1a	368	π→π*
	383	π→π*
	434	n→π*
L1b	368	π→π*
	392	π→π*
	434	n→π*
L1c	368	π→π*
	387	π→π*
	434	n→π*
L2	368	π→π*
	401	n→π*
L2a	368	π→π*
	389	n→π*

a- Ligand (L1a):

The UV-vis spectrum of ligand (L1a) shows three absorption bands . First band at 368 nm due to $\boldsymbol{\pi \rightarrow \pi^*}$ transition within the aromatic ring[62]. the second band at 383 nm is attributed to the $\boldsymbol{\pi \rightarrow \pi^*}$ transition of the azomethine group, the band at 434 nm can be also attributed to $\boldsymbol{n \rightarrow \pi^*}$ transition in the **C=N** group.These transitions are consistent with most previous studies[31, 63].

b- Ligand (L1b):

The UV-visible spectra of (L1b) in ethanol showed absorption bands between 200-800 nm.While the UV-Vis spectra of this Ligand contains three bands. The first band at 368 nm are assigned to $\boldsymbol{\pi \rightarrow \pi^*}$transition of the aromatic rings. The shoulder signal at 392 nm can be also attributed to the $\boldsymbol{\pi \rightarrow \pi^*}$ transitions of the azomethine groups. The thrid band at 434 nm may be ascribed to the long pair electrons of nitrogen and carbon atoms (C=N).

c- Ligand (L1c):

In the UV-visible spectra of to the Schiff base L1c, an bound at around 368nm is indicative of the $\pi \rightarrow \pi^*$ transitions of the aromatic rings. The shoulder signal at 387 nm can be also assigned to the $\pi \rightarrow \pi^*$ transitions of the imine groups. On the other hand, an bound at around 434 nm can be also attriuted to the $n \rightarrow \pi^*$ transition which correspond to the group HC=N.

The λ_{max} values of ultraviolet –visible absorption of Schiff bases are genrally observed at 250-450nm depending on the structure. From the foregoing, it was found that all the bounds indicating the transitions explained within this field as well as previous studies[6, 24]. Which proves this, in addition to the melting point of these three compounds, that the three structures also have the same electronic transitions, which leads us to say that Schiff bases salicylideneaniline was obtained by three experimental methods.

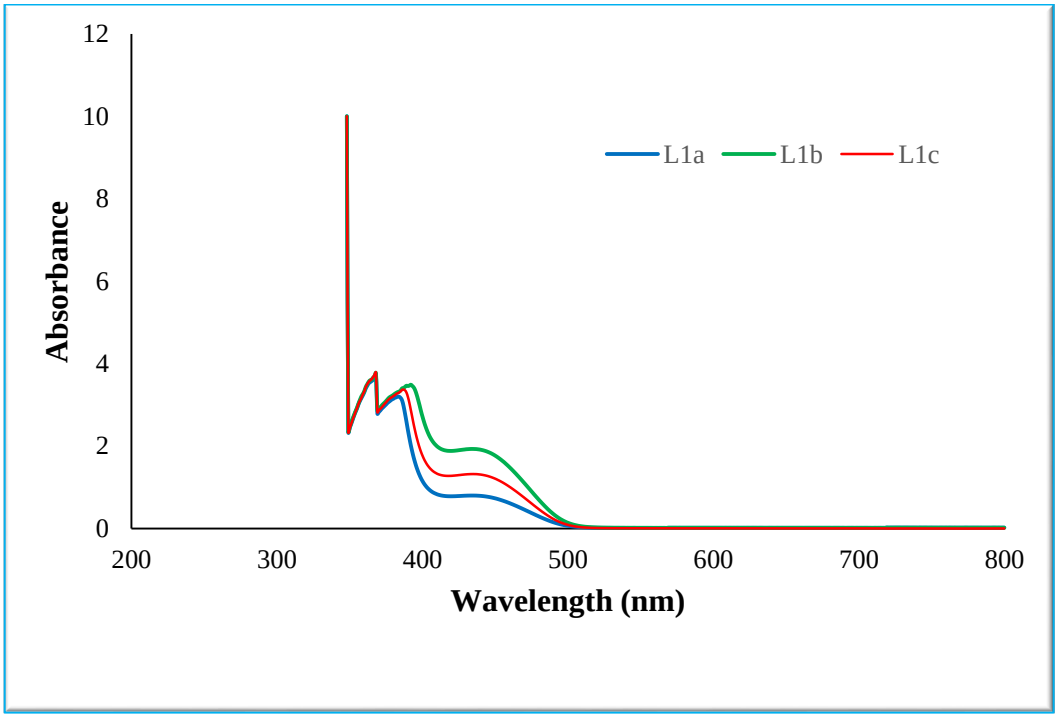


Figure IV. 4: UV-Visible spectra for ligand L1a-L1b-L1c

d- Ligand (L^2):

Within the UV–Vis spectrum of the ligand-L2, two high intensity absorption bands were displayed at 368nm and 401 nm which were assignable to intra ligand **π→π*** and **n→π*** transitions, which correspond to **C=C** aromatic and to the imine **C=N** group respectively [39].

e- Ligand (L^2a):

In the UV-Vis spectrum two bands are observed , the first at 368 nm, correspond to the $\pi \rightarrow \pi^*$ transition band of the C=C aromatic systems, and the last located at 389 nm corresponding to the transition $n \rightarrow \pi^*$ which correspond to C=N imine group[30].

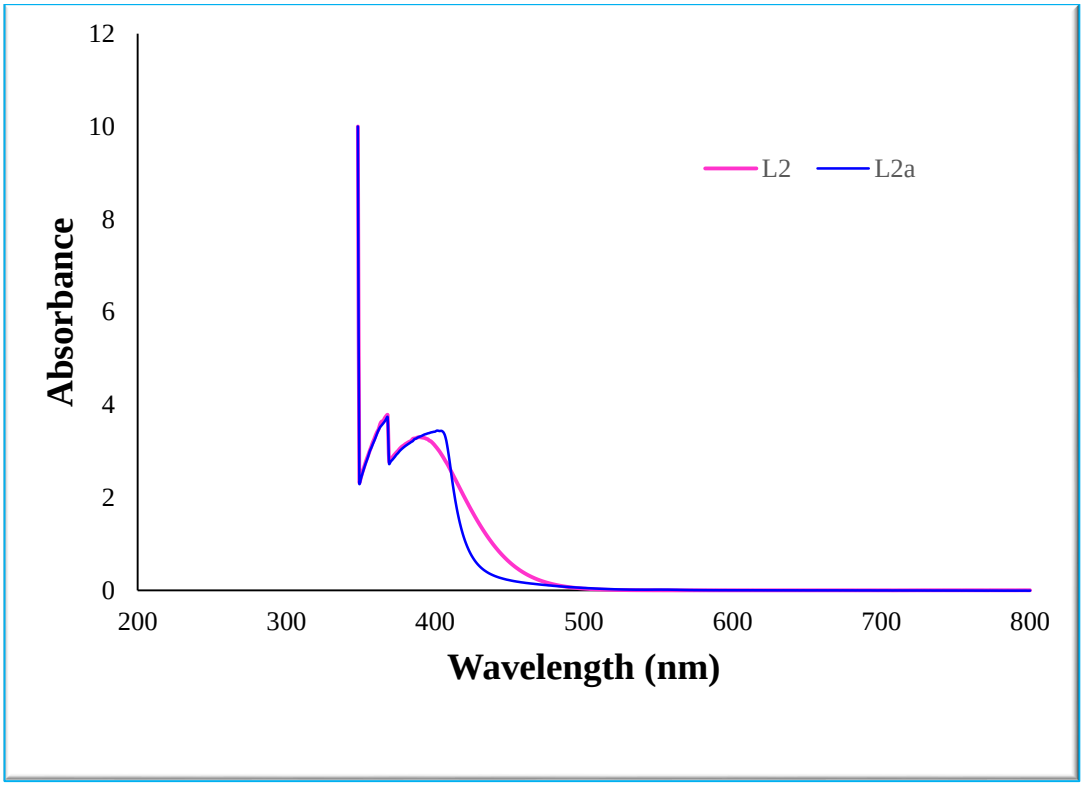


Figure IV. 5: UV-Visible spectra for Ligand L2-L2a

IV.2.2 FT-IR spectra:

The infrared spectrum of Ligands (L1a, L1b, L1c, L2, L2a) shows the main characteristic vibration absorption of the group: C=N (υ(C=N) = 1580-1680 cm^{-1}, forte); and a broad band at 3400cm^{-1} due to the phenolic –OH; disappearance of the salicylic aldehyde C=O band and the NH_2 band of amine[63].

By comparing the absorption bands of the latter with those found on the infrared spectrum of the ligands L1-L2, we find:

Among bands characterization the Schiff bases is that corresponding to the imine group, the vibration of the value of the imine function is characterized by a sharp and intense band at 1616 cm^{-1} for (L1a, L1b, L1c) and 1625 cm^{-1} for (L2- L2a).

In addition to these two important bands, there are relative absorptions to the structure for each of them:

Ligands L1a, L1b, L1c:

- The presence of the phenolic hydroxyl (OH) group in the imine is characterized by the appearance of an elongation vibration band at 3400 cm^{-1}.

A relatively strong single strain band at 3080 cm^{-1}, reflecting vibration of adjacent C-H sp^2 group in substituted aromatic rings.

- A medium- intensity elongation band, located at 1589.2 cm^{-1}, which characterizes the vibration of aromatic C=C.
- A strong band centered at 1375.8 cm^{-1}, which is attributed to the C-N.
- A broad centered at 1272.9 cm^{-1} for ligand L1b, L1c and at 1270cm^{-1} for ligand L1b, characteristic of the vibration of the C-O alcohol.

These results explain that the ligands (L1a, L1b, L1c) contain the same functions, which indicates the possibility of obtaining the same compound Salicylideneaniline. Also, the identification of these bands was consistent with the analysis of most the previously manufactured Schiff bases.[16, 45].

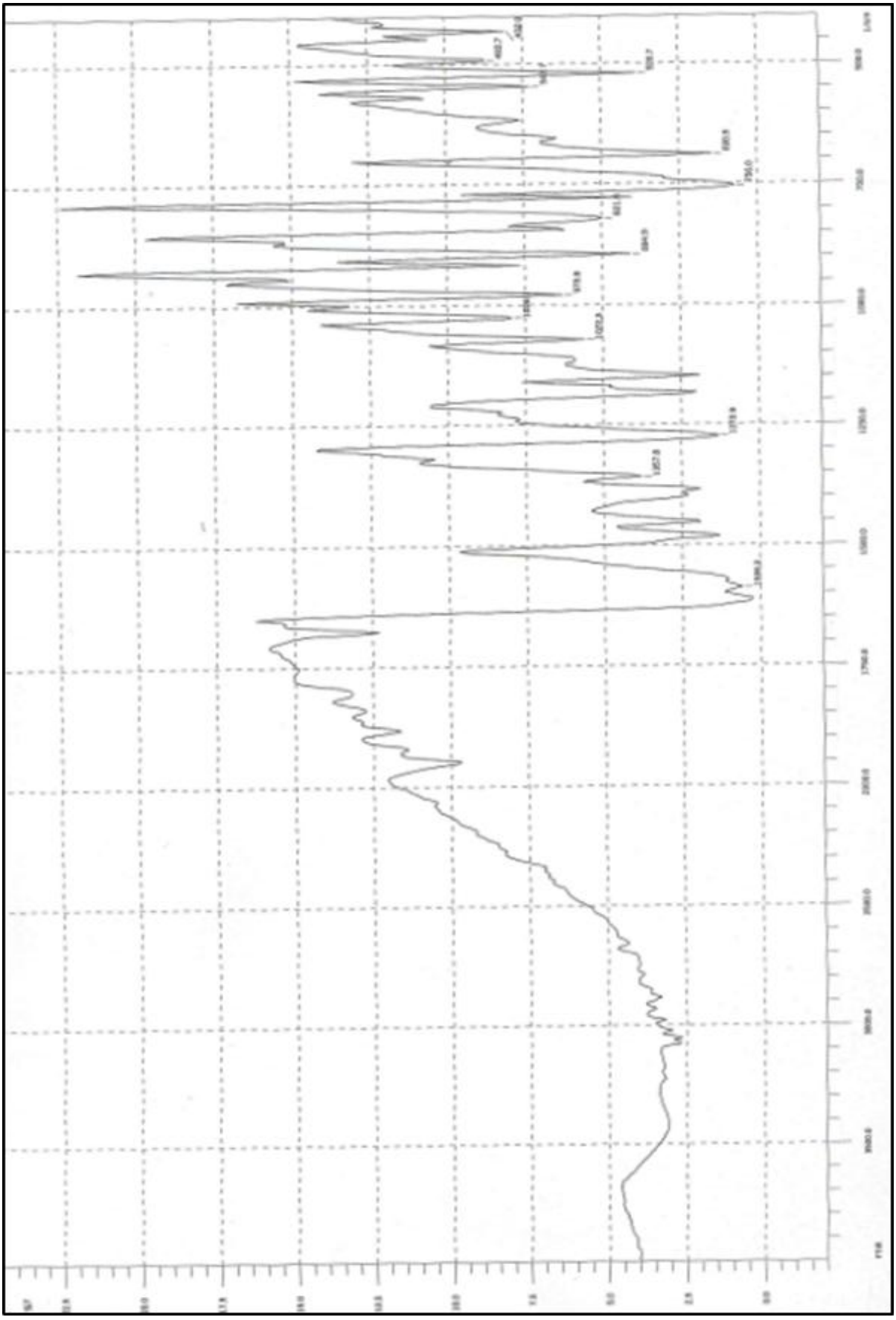


Figure IV. 6: IR spectra of salicylic aldehyde-aniline Schiff base L1a

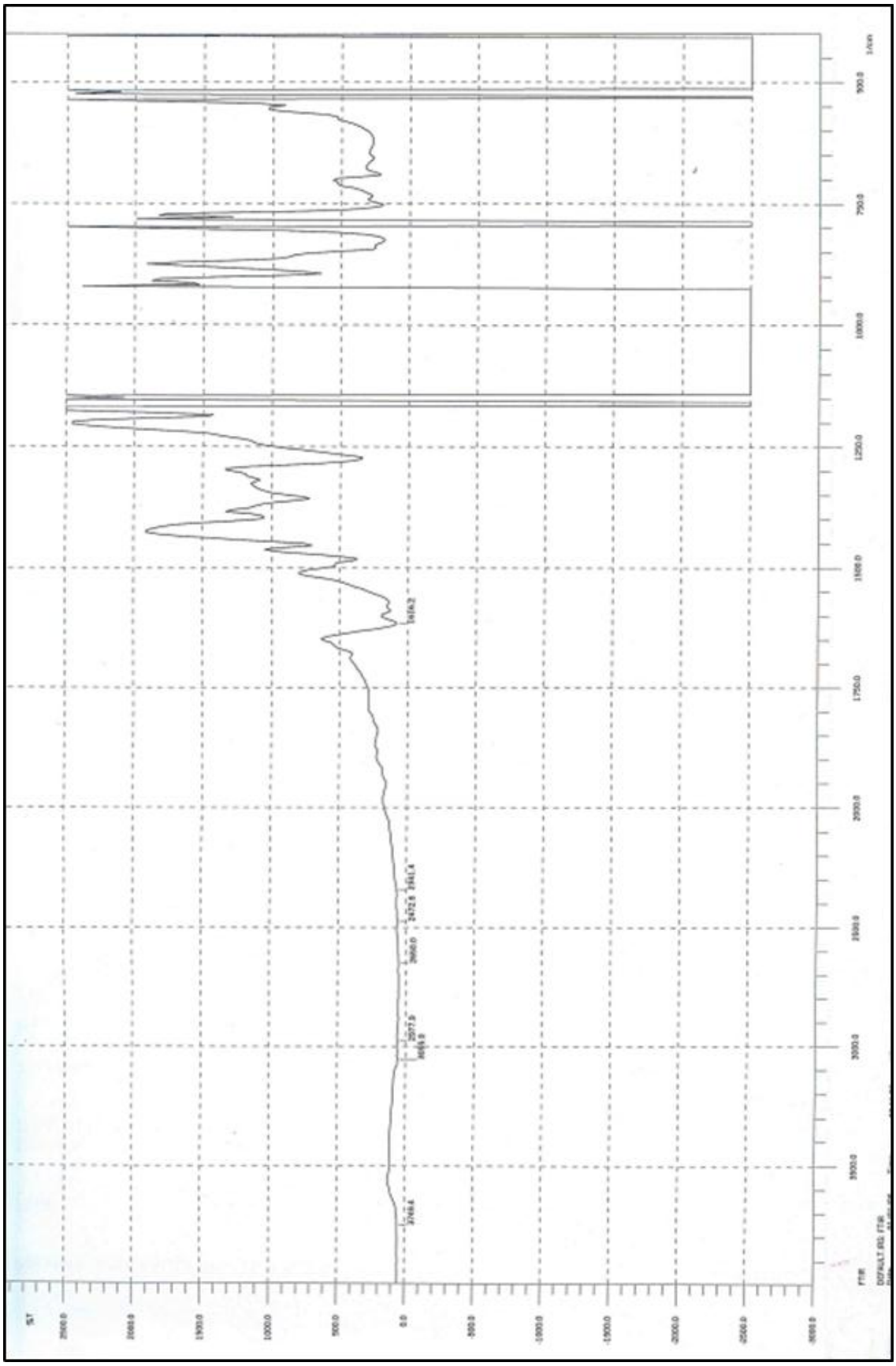


Figure IV. 7: IR spectra of Schiff base ligand L1b

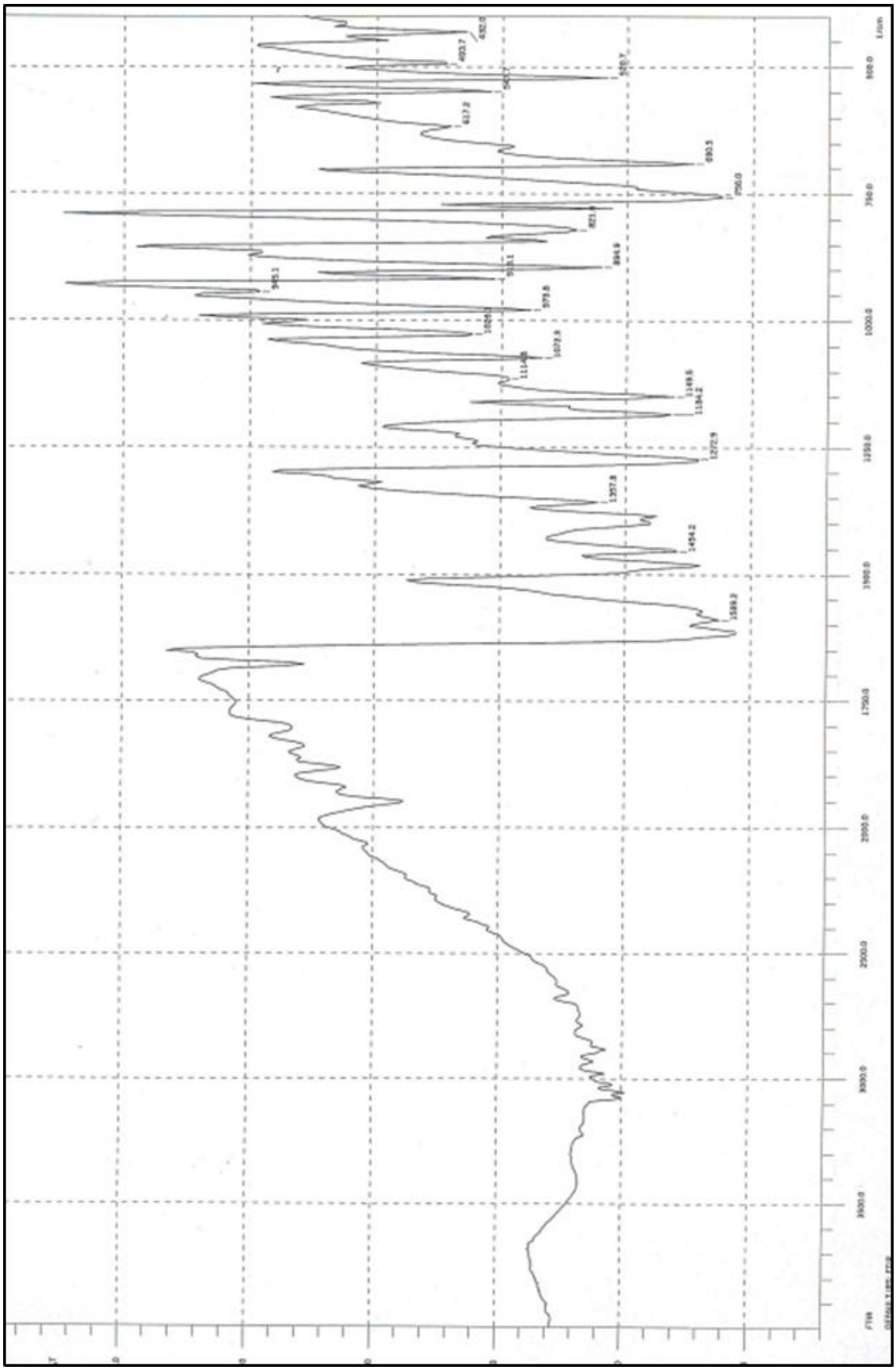


Figure IV. 8: IR spectra of salicylic aldehyde-aniline Schiff base L1c

In addition, **table IV.3** summarizes the most characteristic bands of the corresponding Schiff bases in the following figures.

Table IV. 3: Infrared spectral data of Schiff bases ligands L1a-L1b- L1c

Schiff base	L1a	L1b	L1c
υ(O-H) Elongation	3400	3400	3400
υ(C-H $_{sp2}$) Elongation	3080	3080	3080
υ(C=N)	1616	1616	1616
υ(O-H)Deformation	1484.2	1484.2	1484.2
υ(C=C)	1589.2	1589.2	1589.2
υ(C-O)	1272	1270	1272.9
υ(C-N)	1357.8	1357.8	1357.8
υ(C-C)	1072.3	/	1072.3
υ(C-H $_{sp2}$) Deformation	979.8	950	979.8
υ(C-H $_{sp2}$)substituted at ortho position	756	750	756

Ligands L2- L2a:

The results of the infrared analysis of compound L2 and L2a show on the spectrum the characteristic bands of the main group, we note Figure IV.9 and Figure IV.10and table IV.4.

- The presence of two bands corresponding to the –OH chromophore which appear in the form of a wide elongation band at 3400 cm^{-}.
- Actual band at 3080.cm^{-1} is assigned to stretching vibration of adjacent C-H sp^2 group in substituted aromatic rings.
- The IR spectra of the Schiff bases showed a strong band at 1523.7cm^{-1}, which is characteristic of nitro (stretching frequency N-O) group.
- The spectrum shows a medium- intensity elongation band around at 1573.8 cm^{-1}, attributed to the stretching vibration of the band C=C aromatic.
- We note the presence of elongation vibration band at 1270 cm^{-1} corresponding to the vibration of the C-O alcohol.
- The characteristic band of the(C-N) is observed at υ(C-N) =1350.1 cm^{-1}.
- The ligands display a band at 1188.1cm^{-1}, which is assigned to υ(C-N) (starching frequency C-N nitro).

Through the results, we conclude that we are on the right path to obtaining the compound 2-(2-nitrophenylimini)-methyl phenol, this is by proving the existence of all its constituent functions according to some previously synthesized Schiff bases[26, 29, 39, 64].

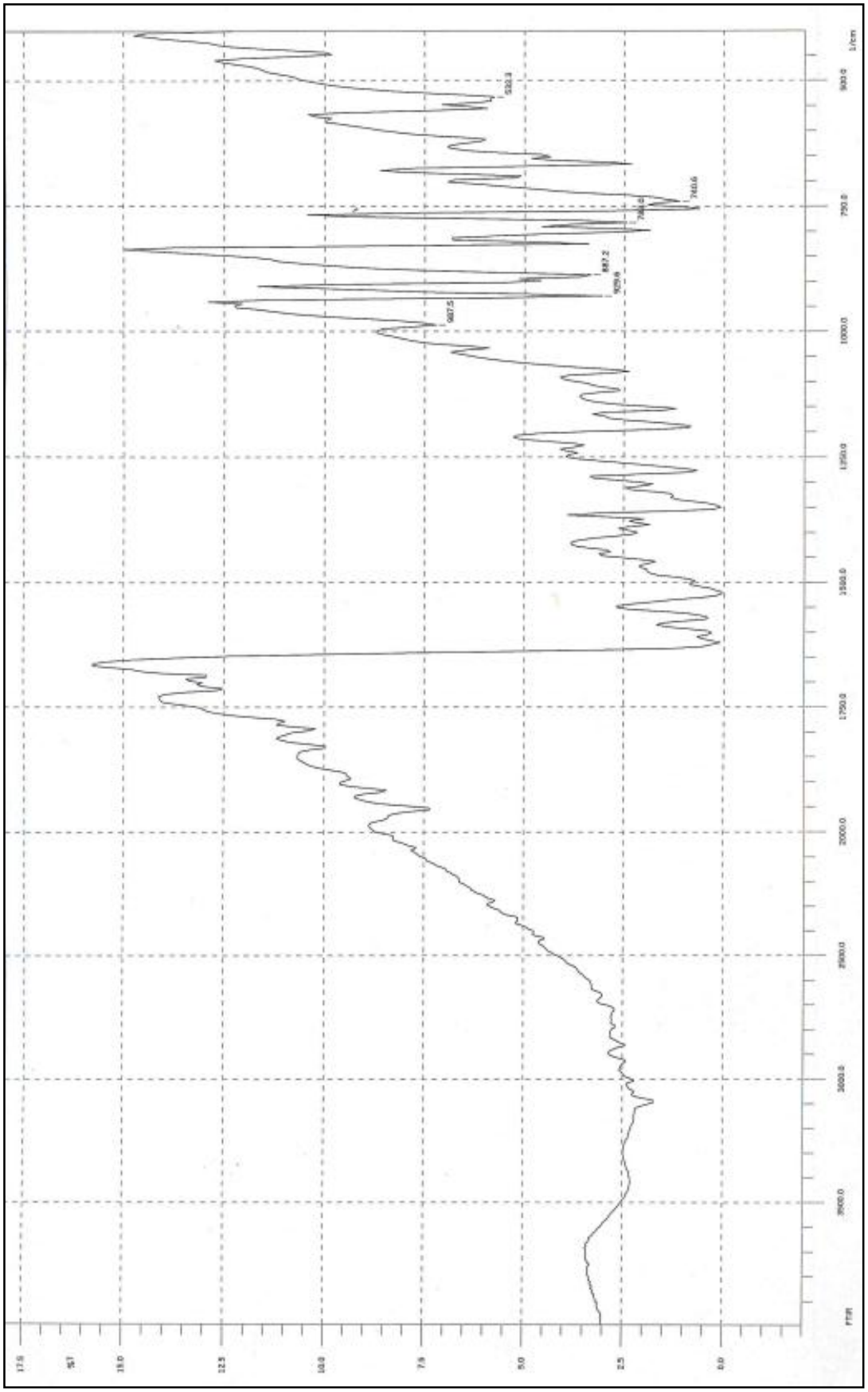


Figure IV. 9: IR spectra of Schiff base ligand L2

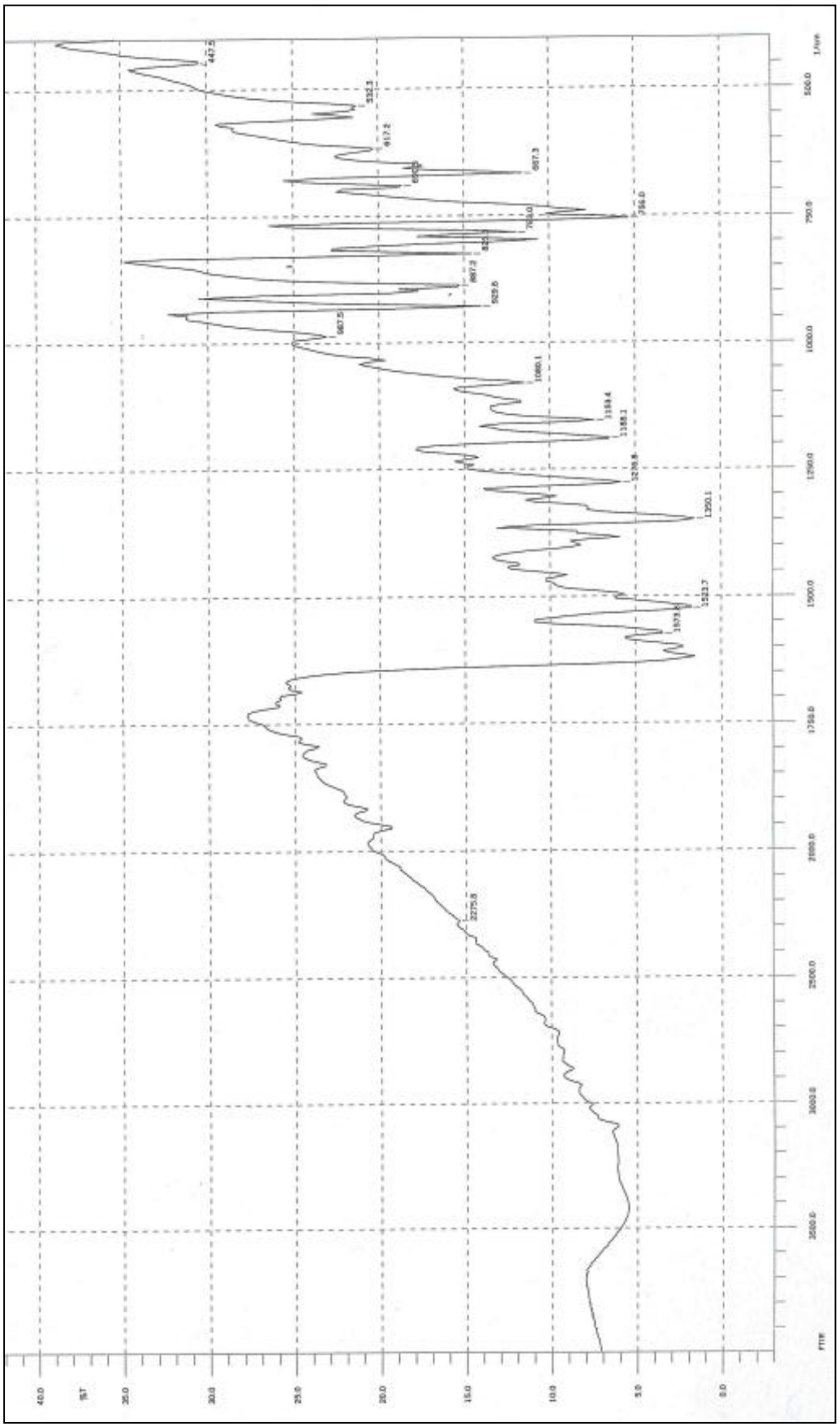


Figure IV. 10: IR spectra of Schiff base ligand L2a

Table IV. 4: IR spectral of Schiff bases ligands L2, L2a

Schiff base	L2	L2a
υ(O-H) Elongation	3400	3400
υ(C-H_{sp2}) Elongation	3080	3080
υ(C=N)	1625	1625
υ(C=C)	1573.8	1573.8
υ(C-O)	1276.8	1276.8
υ(C-N)	1350.1	1350.1
υ(C-N_{nitro})	1188.1	1188.1
υ(N-O)	1523.7	1523.7
υ(C-C)	1080	1080
υ(C-H_{sp2})	987.5	987.5
υ(CH_{sp2})substituted at meta position	783	783
υ(C-H_{sp2}) substituted at ortho position	756	740.6

IV.3 Biological study:

The ability of Schiff bases to scavenge free radicals is an important property. Different modes of action such as being free radical terminators, chelators of metal ions involved in oxygen scavengers that react with oxygen closed systems have been used in categorizing antioxidants.[41].

In this study, we present the DPPH and H_2O_2 scavenging ability of the Schiff bases L1 (L1) and L2. The antioxidant assay was carried out using different concentrations of the test samples, while ascorbic acid (vitamin C) was used as standard.

The evaluation of the anti-radical activity of our ligands via the DPPH and Hydrogen peroxide H_2O_2 test led to results illustrated by Figure IV.11 to Figure IV.14.

➢ DPPH radical scavenging activity:

All of the obtained compounds were subjected to evaluation of their antioxidant activity in DPPH test, **Table IV.5**.

These results show that not all Schiff bases synthesized have an anti-radical power towards DPPH. It was found that compounds L2 are poor radical scavengers, while ligand L1 turned to be more active.

Figure IV.11 shows the dose-response curve of DPPH radical scavenging activity of the Schiff base L1. Compared with ascorbic acid Schiff base L1 interact with DPPH radical and exhibit, slightly lower, than the reference compound ascorbic acid (IC_{50}=6.694 g/L). It was observed that vitamin ascorbic acid had higher activity with IC_{50} in the range of 0.01g/L.

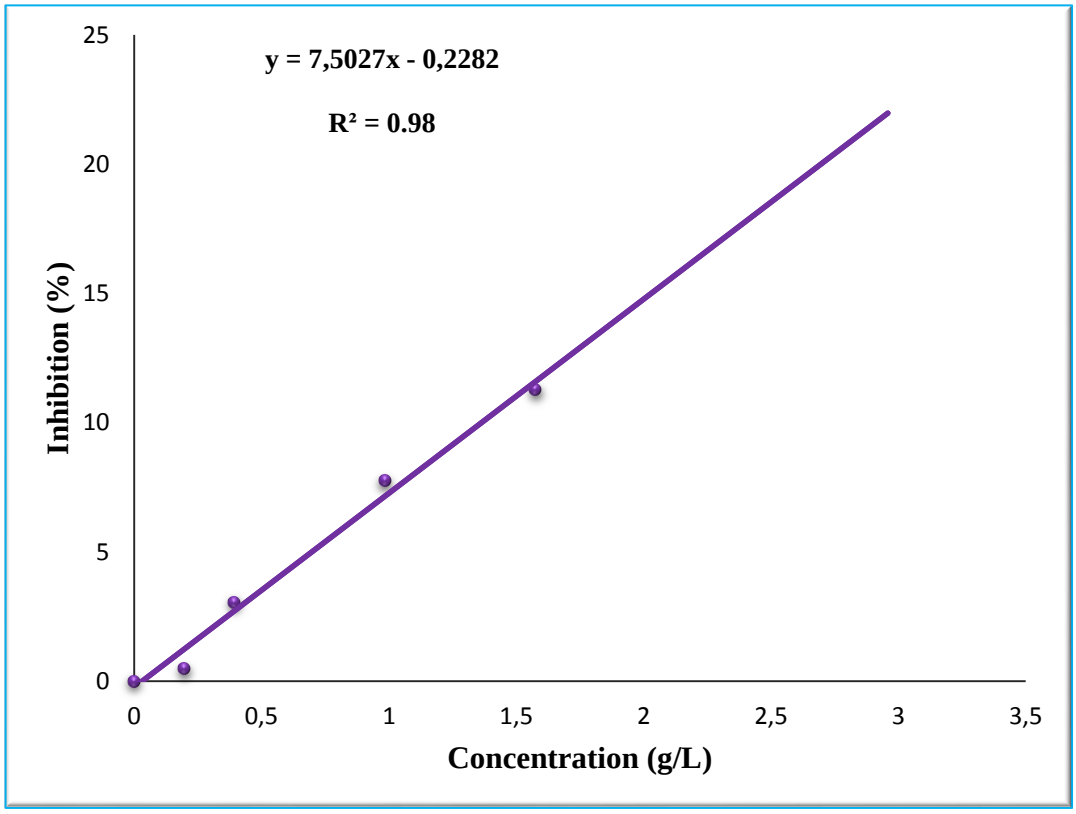


Figure IV. 11: DPPH scavenging activity of Schiff base L1

Table IV. 5: Results of antioxidant activity evaluated by the DPPH and H_2O_2 tests

Test	DPPH Radical Scavenging Activity	H_2O_2 Radical Scavenging Activity
	IC_{50}(g/L)	IC_{50} (g/L)
L1	6.694±0.394	4.531±0.197
L2	-	7.986±3.872
Vc	0.010	0.012±0.0004

The enhanced inhibition displayed on the DPPH radical by the test samples shows that Schiff base L1 are capable of donating hydrogen or electron to neutralize free radicals and thus, could be a promising therapeutic agent for the treatment of pathological diseases and conditions caused as a result of excessive radicals or stress.

Finally, we say that results obtained in the DPPH radical scavenging activity of Schiff base ligand 1 were in contrast to the results of Zorica D[15]. Based on this, compound salicylideneaniline (ligand 1) can be considered as good antioxidants.

- **Hydrogen peroxide scavenging activity:**

Volumetric methods of hydro peroxide determination, such as titrations, have been in use for more than 50 years. Titration methods are very simple, rapid, and require only unsophisticated equipment. Many titrations have 1:1 stoichiometry and are suitable for evaluation of large number samples. Iodometric titration of hydro peroxides was developed and standardized, and official methods were published by AOCS in 1997[65].

The reaction chemistry of the titration is very straightforward, developed from observations that all peroxidic compounds react with iodide ions, and are reduced to hydroxyl derivatives while at the same time, iodide ions are oxidized further to free iodine

The hydro peroxide reduction proceeds as:

$$H_2O_2 + 2H^+ + 2e^- \rightarrow 2H_2O$$

The electrons for this reduction are provided by the saturated KI, which dissociates in solution:

$$2KI \rightarrow 2\ K^+ + 2\ I^-$$

$$2\ I^- \rightarrow I_2 + 2\ e^-$$

This is an equilibrium reaction. When the electrons are removed, e.g. by reaction with H_2O_2, the reaction is driven to the right. Otherwise, I^- is favored.

In the net reaction, reduction of one H_2O_2 releases one I_2 for reaction with thiosulfate.

$$H_2O_2 + 2\ H^+_{(clear)} + 2\ KI \rightarrow I_2(yellow) + 2H_2O + 2\ K^+$$

In presence of excess iodide, a complex ion that reacts in same way as free iodine is formed. The released iodine is then titrated, usually with standardized sodium thiosulfate, which becomes oxidized into a tetrathionate (reduction of free iodine with thiosulfate).

$$I_2\,(yellow) + 2\ Na_2S_2O_3 \rightarrow Na_2S_4O_6\,(colorless) + 2\ NaI$$

This reaction requires the presence of acid and molybdate catalyst. Therefore, the presence of antioxidant, which can donate an electron, leads to the reduction in the volume of thiosulfate necessary to titrate the iodine I_2, that is interpreted by the reduction in the concentration of H_2O_2 (inhibition of H_2O_2).

The Schiff bases were also screened for inhibition of H_2O_2 method. The percentage inhibition results of the test samples are shown in table IV.5, the Schiff bases resulted in a lower activity comparable to those of the standard ascorbic acid.

The hydrogen peroxide H_2O_2 scavenging ability of the test samples can be ranked in the order:

Vitamine C > L1 > L2

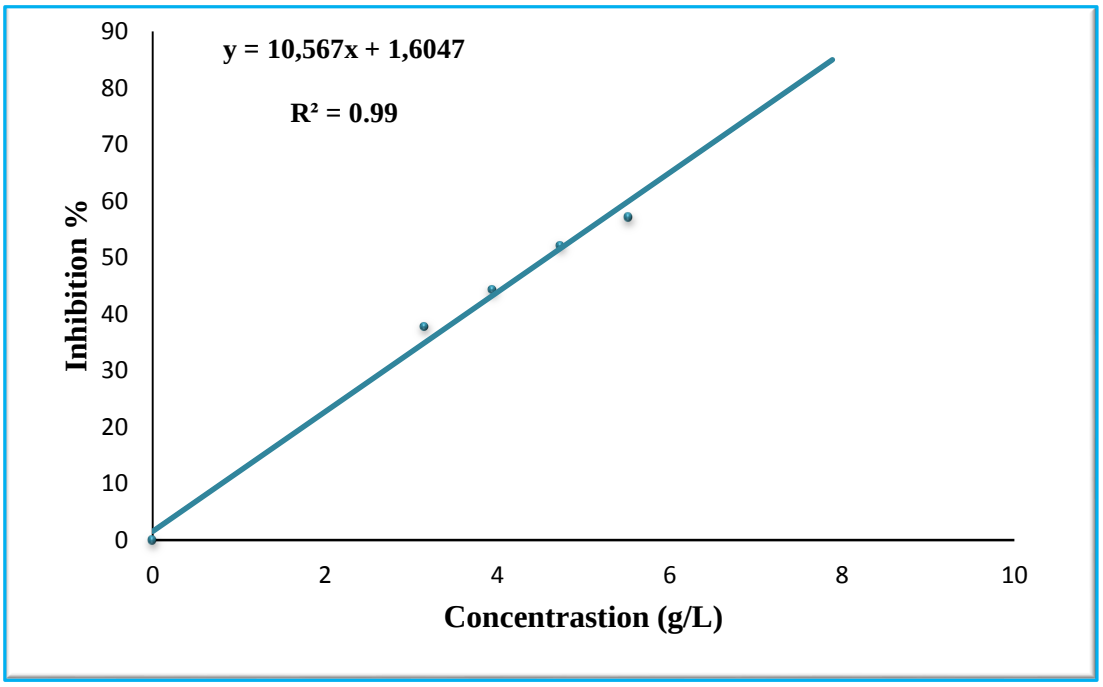


Figure IV. 12: H_2O_2 scavenging ability of Schiff bases L1

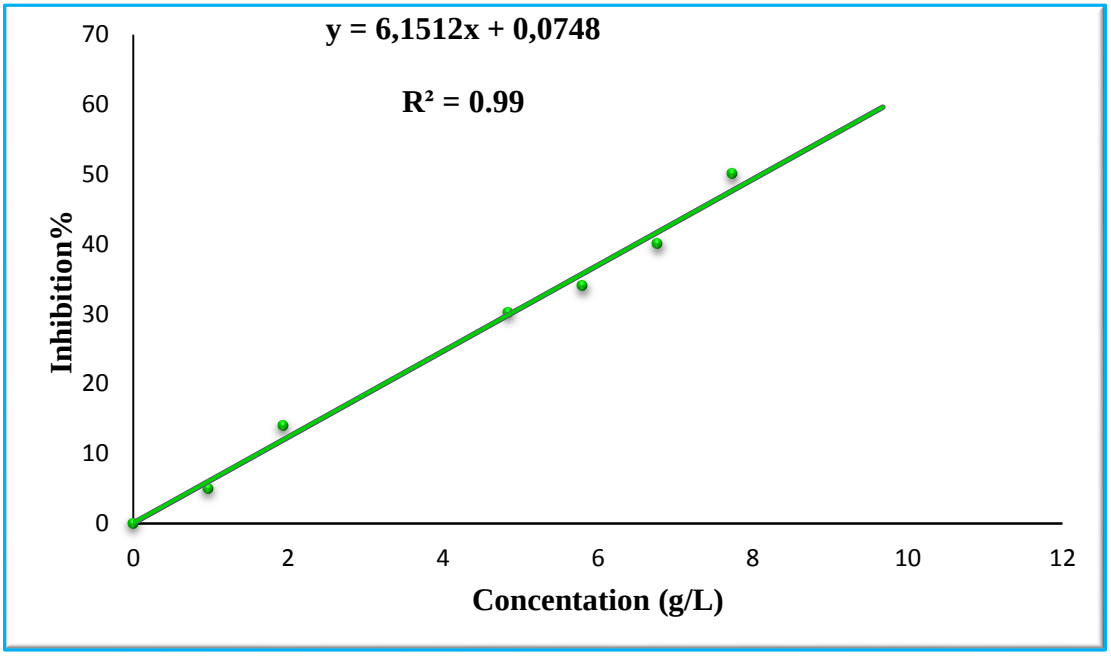


Figure IV. 13: H_2O_2 scavenging ability of Schiff bases L2

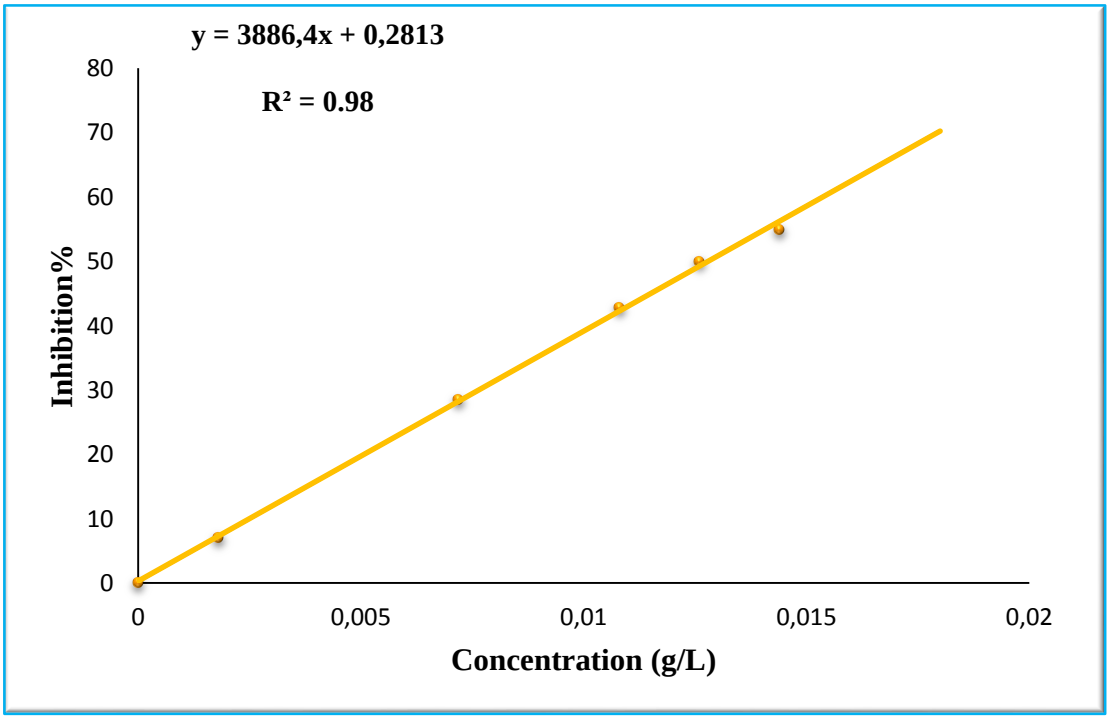


Figure IV. 14: H_2O_2 scavenging ability of Vitamin C

Hydrogen peroxide is not very reactive itself, but it can cross the cell membrane and produce the hydroxide radical, which is highly reactive. Therefore, scavenging of hydrogen peroxide is very important for protection of pharmaceuticals and food systems.

It seems that the ortho OH group in L1 and L2 can form a fairly stable intramolecular hydrogen bond with the C=N group. This intramolecular hydrogen bond may contribute to the greater stability of the Schiff base functional group and higher biological activity.

The product L1 revealed a greater antioxidant power than the product L2 (IC_{50}=4.531±0.197 and 7.986±3.872 respectively).

The difference between the values of the product L1, L2 is due to the presence of the nitro group in L2. According to the values of HOMO energy for the two Schiff bases L1 and L2 (E_{HOMO} =-5.955 eV and E_{HOMO} = - 6.159 eV respectively)[66].

The ionization potential is calculated according to the relation:

$IP = - E_{HOMO}$

In general, the interpretation of this variance between the values of IC_{50} of the compounds studied is attributed to the ionization potential of each compound; one can find an inverse relationship between the values of IC_{50} and the ionization potential IP (see **Table IV.5**)

In conclusion, the newly synthesized Schiff bases are promising potential antioxidant agent candidates for the scavenging of ROS, which cause damage in humans.

IV.4 Liquid- liquid Extraction of lead by chelation:

IV.4.1 Liquid-liquid extraction by Salicylideneaniline Schiff base L1:

In recent years, the process of extraction of transition metals by salicylideneaniline L1 has been performed by many researchers. They used the spectrophotometer to measure the concentration in certain experimental conditions [65, 67-70].

In the present work, we have developed the process of extraction by chelation in chloroform medium with some modification in experimental studies and changing the method of measuring concentration.

❖ pH effect:

The study of the influence of pH on the extraction of lead (II) by L^1in chloroform medium, was carried out by establishing lead Log D=f (pH) at 25 °C, for various concentration of extractant. The results are collected below:

Table IV. 6: pH effect on extraction for different concentration

		[L1]=0.005M		
pH	**3.89**	**4.35**	**4.41**	**5.01**
Log(D)	-0.631	-0.499	0.954	1.389
		[L1]=0.01M		
pH	**4.4**	**4.63**	**5**	**5.7**
Log(D)	-1.935	-1.924	-1.921	-0.267
		[L1]=0.02M		
pH	**2.55**	**2.65**	**2.8**	/
Log(D)	-0.886	-0.677	-0.517	/

A study of the variation of Log D as a function of pH, while keeping the ligand concentration constant, was carried out for the extraction of lead (II) in chloroform medium. FigureIV.15 shows the evolution of the curves Log D=f (pH) obtained in the chloroform/water mixture, for different concentrations of extractant.

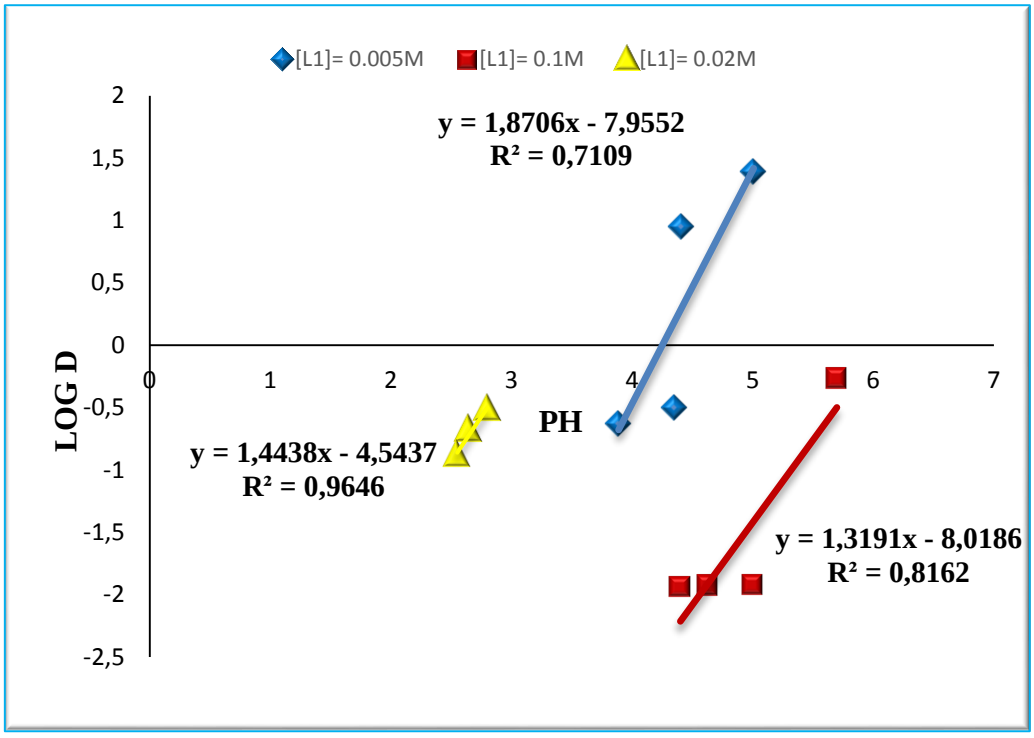


Figure IV. 15: Variation of Log D as a function of pH, during the extraction of lead (II) by L1, for different extractant concentration

The three curves are in the form of straight lines with slopes ≈2, indicating the exchange of two protons between the extractant and the metal cation.

A ligand molecule would therefore be involved in the coordination of the central lead (II), through is phenol group. We further notice that the lead (II) distribution increases with increasing pH and extractant concentration.

❖ Effect of extractant concentration:

We have also studied the variation of Log D as a function of (Log[$L1$]) at constant pH=2.88 during the extraction of lead (II) by L1. The results are collected in the table below:

Table IV. 7: Effect of chelates concentration on extraction

[L]	0.005	0.01	0.02
Log[L1]	-2.3	-2	-1.69
Log(D)	-4.213	-4.22	-0.88

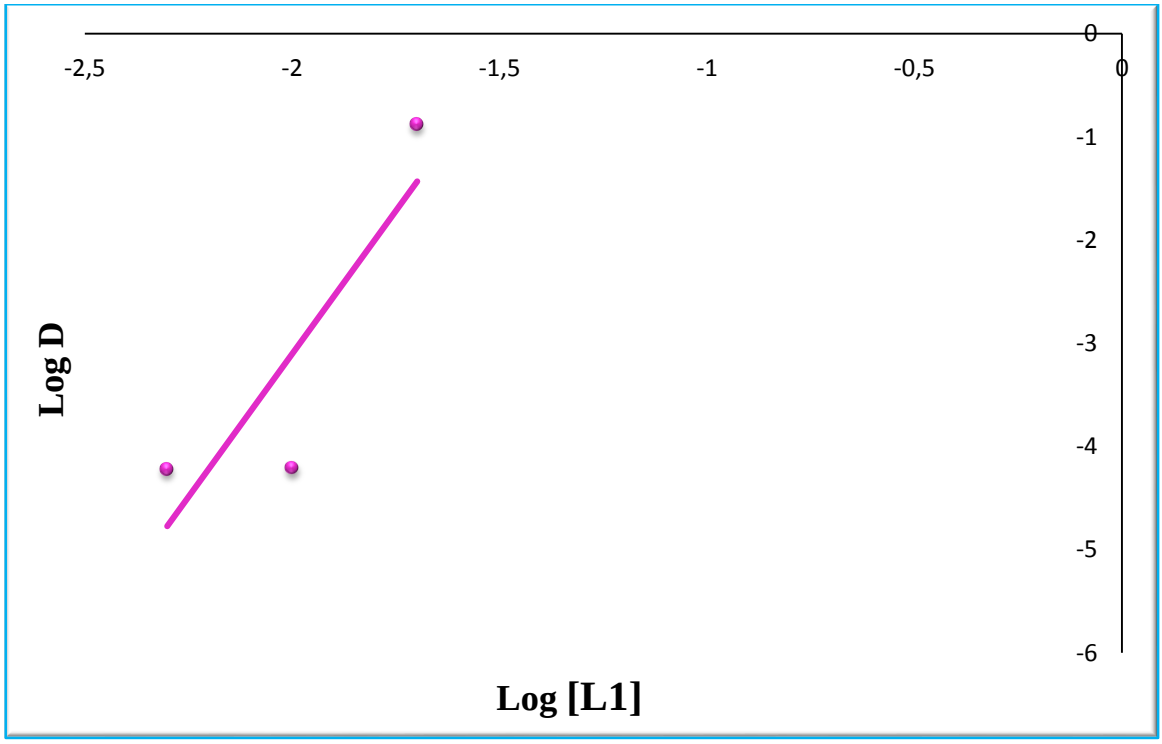


Figure IV. 16: The effect of the concentration ligand on extraction lead (II) by L1

The curves obtained turn out to be straight lines, indicating that the distribution coefficient increases with the extractant concentration.

❖ Extraction efficiency:

As mentioned in the theatrical part, the quantification of an extraction is generally revealed through its extraction efficiency, defined as follows:

$$E(\%) = \frac{D}{D+1} \times 100$$

Its establishment makes it possible to evaluate and quantify the extracting properties of an organic ligand, in order to use it on an industrial scale.

In our case, we used D values obtained to determine the different extraction percentage, (E %), for various concentration.

Table IV. 8: Variation in the extraction efficiency of lead, (E %) by L1 at different pH

$[\mathbf{L1}] = \mathbf{0.005M}$				
pH	3.89	4.35	4.41	5.01
E%	18.96	24.07	89.99	96.08
$[\boldsymbol{L1}] = \mathbf{0.01}\boldsymbol{M}$				
pH	4.4	4.63	5	5.7
E%	1.15	1.17	1.18	35.06
$[\boldsymbol{L1}] = \mathbf{0.02}\boldsymbol{M}$				
pH	2.55	2.65	2.88	/
E%	11.50	17.35	23.31	/

From the above results, we conclude that:

- ✓ The extraction efficiency increases with pH of the aqueous medium.
- ✓ L1 seems to extract lead in a chloride medium, in the moderately acidic pH 2.5 and 5.7.
- ✓ An extraction efficiency of 96.08% is reached at a pH equal to approximately 5.01 with [L1] =0.005M.

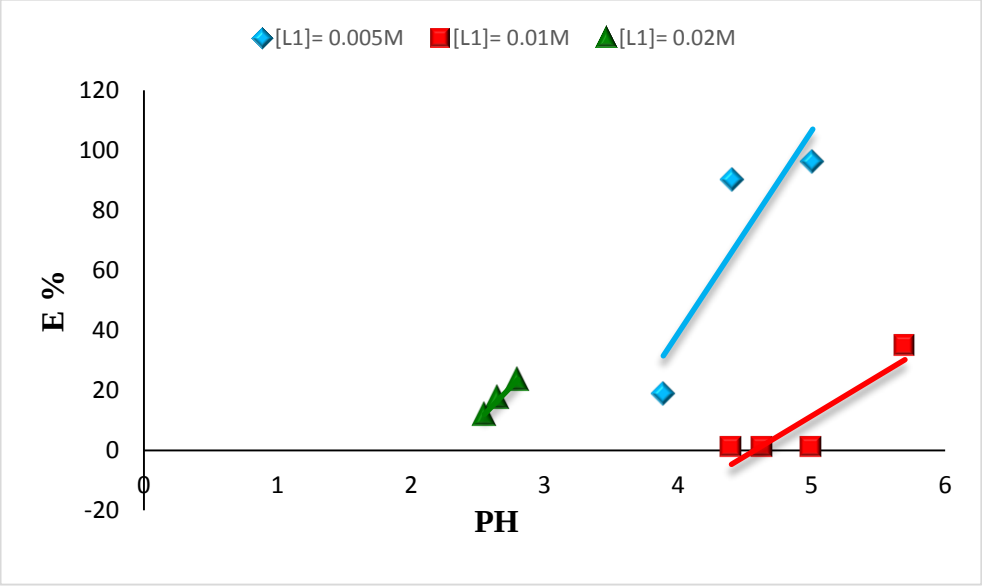


Figure IV. 17: Variation of E% as a function of pH, during the extraction of lead (II) by L1, for different extractant concentration

General conclusion

General conclusion

This work, aimed to search for high-performance extractants capable of extracting the metals (transition metals). The generic idea is to seek to improve the performance of extractant. After an in-depth examination and a detailed understanding of its extraction mechanisms. For this purpose, Schiff bases, especially those containing hydroxyl substituents or substituents with free electron pairs, appear to provide the best profile in terms of biological activity and metals chelation.

Our experimental study, based on the synthesis of chelates capable of extracting transition metals, aimed to test the extraction of lead metal and deduce the optimal extraction conditions.

During this work we set out synthesize specific Schiff bases of the type NNO and NO. We were able to achieve the synthesis of two imine Schiff bases that would strongly confer coordination due to their content of donating electron pairs. The chelation force was studied through its interaction with lead (II) metal.

All the obtained compounds were characterized by implementing infrared and ultraviolet techniques available in the laboratory. The characterization method allowed us to confirm the function of the imine.

In another part of the work carried out, we were interested in evaluating the biological properties of our Schiff bases, those compounds exhibit a lower antioxidant activity compared to ascorbic acid.

The study of the extraction of lead (II) by L^1 made it possible to illustrate the extraction yield increases at pH>5 of the aqueous phase. From this result, we could contributed to purification of water and environmental protection through the extraction of heavy metals like lead.

The perspective envisaged of this work is to use these molecules to extract other metals such as Zn (II), Fe (II), Fe (III), Cd (II), Cr (III), Mg (II), Ag (I) etc. and to improve the extractant power of the Schiff base molecule by changing the extraction medium or by adding other synergistic agents.

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