Spectroscopic studies and Analysis of Complexes via the Reaction of Selected Ligands with Some Metal Ions

A Thesis
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2011  1432
بسم الله الرحمن الرحيم

وَفَوْقَ كُلّ ذِي عِلِمٍ عَلِيمٌ

صدق الله العلي العظيم

سورة يوسف الآية (67)
الإهداء

الي خاتم المرسلين ونورا للمهتدين .......
المصلح محمد (صلى الله عليه وآله وسلم)
الي التي راني قلبها قبل عينيها ومنبع الآمال .......
والدتي العزيزة
الي نبراسي الذي ينير دربي .......
والدي العزيز
الي أخي الغالي .......
علي
الي أخواتي الغاليات .......
(زينب... ولمى... ونضال)
هدى
شكر وتقدير
أشكر الله سبحانه وتعالى وأحمده الذي وفقتني لإنجاز هذا البحث، ويسعدني أن أتقدم بخلاص الشكر والتقدير إلى من تعجز كلمات الشكر عن الوفاء بحقه إلى أستاذي المشرف الدكتور عصام محمد علي الهاشمي الذي منحنى الوقت والجهد والتقنية التي وضعها بين يدي فجزاه الله عني كل الخير.

كما يسعدني أيضا أن أتقدم بخلاص الشكر والامتنان إلى أستاذي المشرف الدكتور باسم إبراهيم العبدلي لما أبداه لي من المساعدة والتوجيه قبل وأثناء فترة البحث فحفظه الله وسدد خطاه.

وأتوجه بالشكر للاساتذة الدكتور نغم شاكر العوادي لمساندتها العلمية والمعنوية فلها مني كل الحمية والتقدير.

كما أتوجه بالشكر إلى كل من الاستاذ المساعد الدكتور سعاد محمد حسين والدكتور محمد العماري والست زينب عبد الزهرة لما أبدوه من عون ومساعدة لأنجاز البحث.

كما وأتقدم بشكري لكل من زملائي الاعزاء الست غادة فاضل والست ندى علي جواد والست صفاء حسين والست زينب زاهد والست كفاح حسن والاستاذ على سعد والاستاذ مازن عبد الله والاستاذ منتظرب عبد الباري.

وأتقدم بشكري وتقديري إلى عمادة كلية العلوم / جامعة بغداد، ورئاسة قسم الكيمياء للتسهيلات والمساعدات لي خلال فترة الدراسة والبحث.

هدي
Abstract

Newly developed spectrophotometric method was used in the present research project for the determination of metformin drug (MTF), via the complexation of the drug with copper(II). The colored product was measured at 530 nm. The optimization of all chemical and physical parameters for MTF-OH-Cu(II) system are described. A linear range of 94.04% for 0.0-100 mM was obtained with a Limit of Detection (L.O.D) 662 ng. The newly developed system was applied for the analysis of pharmaceutical preparation. A comparison was made using paired t-test shows that, the newly developed method can be used as an alternative analytical method for the analysis of metformin (MTF). All this project work was based on on-line determination via Continuous Flow Injection Analysis (C.F.I.A).

The research project also include, mixed ligand complexes of Cu(II), Ni(II) and Co(II) derived from metformin(MTF) as primary ligand and cysteine as secondary ligand have been prepared and characterized by elemental analyses, atomic absorption, FTIR, electronic spectra, NMR spectra, molar conductivity and magnetic susceptibility measurements.

The elemental analysis, atomic absorption data reveal the formation of [1:1:1] [M:MTF:Cys] complexes .The electronic spectra and magnetic moment measurements reveal the presence of the complexes in an octahedral geometry and molar conductivity studies of the complexes indicate their non-electrolytic nature. The infrared and NMR spectral data showed that the chelation behaviour of the ligands towards transition metal ions is through two imino groups of MTF, whereas the amino acid coordinates through sulphur atom and the amino nitrogen.
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<td>Big</td>
<td>Biguanides</td>
</tr>
<tr>
<td>NIDDM</td>
<td>Non-insulin-dependent diabetes mellitus</td>
</tr>
<tr>
<td>MTF</td>
<td>Metformin</td>
</tr>
<tr>
<td>L-Cys</td>
<td>L-Cysteine</td>
</tr>
<tr>
<td>UV-Vis</td>
<td>Ultraviolet-Visible</td>
</tr>
<tr>
<td>IR</td>
<td>Infrared</td>
</tr>
<tr>
<td>$\nu_{sy}$</td>
<td>symmetric stretching vibration</td>
</tr>
<tr>
<td>$\nu_{asy}$</td>
<td>asymmetric stretching vibration</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethyl formamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>FT-IR</td>
<td>Fourier Transform Infrared</td>
</tr>
<tr>
<td>FIA</td>
<td>Flow Injection Analysis</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>$\mu$g</td>
<td>Microgram</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>$\mu$L</td>
<td>Microliter</td>
</tr>
<tr>
<td>mV</td>
<td>Millivolt</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar</td>
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<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>sec</td>
<td>Second</td>
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<tr>
<td>$\Delta t_B$</td>
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<td>$\nu$</td>
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<td>h</td>
<td>Hour</td>
</tr>
<tr>
<td>B.M</td>
<td>Bohr magneton</td>
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</table>
1.1 Biguanides

Biguanides are class of compounds that has been known for quite long time\(^{(1-4)}\). Their structures are of two guanidine molecules Fig (1-1) (that are linked together through common (-NH-) link\(^{(5,6)}\)), which carry three nitrogen atoms of two different functional groups, the first one is of imine type and the other are of two amine units. This special assembly is the origin of the most significant characteristic of guanidines- their extreme high basicity\(^{(7)}\). Guanidines show one of the strongest Bronsted basicities under the organic neutral bases which are comparable to the hydroxyl ion (OH\(^-\)) and therefore they can be categorised as organic superbases\(^{(8)}\).

![Guanidine structure](image)

**Fig (1-1) Structure of guanidine, biguanides.**

The Biguanides are very strong diacid bases characterized by strongly basic, as well as strongly acidic, dissociation constants (pKa values ~ 11.5 and 2-3, respectively)\(^{(9)}\). These compounds are stronger bases than ammonia (pKa = 9.2) and approach similar basicity values to alkyl amines.

Biguanides can be drawn in many resonance forms as illustrated in Fig (1-2). There is a conjugated double bond system stabilized as in an intramolecular hydrogen – bonded six membered ring\(^{(10)}\).
INTRODUCTION

Fig (1-2): Resonance structures of biguanides.

Hydrogen bonding giving the cyclic structures is supposed to influence the physiological properties of biguanides\(^{(11)}\).

In fact, X-ray crystallographic analysis\(^{(12,13)}\), N-NMR spectroscopy\(^{(14)}\), molecular modeling\(^{(15)}\) and tautomer stability studies\(^{(16)}\) have confirmed that biguanides should be represented as shown in Fig (1-3).

Fig (1-3): Represented structure of biguanides.

Biguanides have a conjugated -C=\(\text{N}-\text{C}=\text{N}\)- chromophore and additional N atoms with lone pairs capable of forming a delocalized \(\pi\) electron system\(^{(17)}\). Both structures of the basic (BigH) and normal salt (BigH\(^+\)) forms possess a conjugated single and double bond system whereby biguanides are capable of complex formation with transition elements\(^{(10,18)}\). In particular, it is noteworthy that the behaviour of (BigH),
which in the free state gives crystals containing an unsymmetrical tautomeric form (1)\(^{(19)}\), assume symmetrical tautomeric forms when coordinated to a metal, which are either deprotonated (2)\(^{(20)}\) or neutral (3)\(^{(21,22)}\).

Since the early 1900’s, biguanides and substituted biguanides have attracted considerable attention for their hypoglycemic activity\(^{(23,24)}\) the relationship of structure to hypoglycemic activity was observed from studying a series of N’-alkyl and arylalkyl biguanides and pharmacologically testing them in the guinea pig\(^{(11)}\). The data suggest that hypoglycemic activity is associated with selected biguanides in the form of an intramolecularly hydrogen bonded cation Fig (1-4).

Fig (1-4): Requisites for effective hypoglycemic activity in biguanides.

These compounds have proven also to be useful for the treatment of malaria\(^{(25,26)}\), fileria\(^{(27)}\), influenza,\(^{(28)}\) antitumor\(^{(29,30)}\) and more recently for therapeutic treatment of pain, anxiety, and memory disorders\(^{(31)}\). Moreover, biguanides are also valuable catalysts for some organic synthesis\(^{(32,33)}\). Table (1-1) shows some types of biguanides with biological activities.
Table (1-1): Some types of biguanides with biological activities

<table>
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<tr>
<th>Compound</th>
<th>activity</th>
<th>Ref</th>
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<tr>
<td><img src="image.png" alt="Structure" /></td>
<td>Hypoglycemic</td>
<td>(4)</td>
</tr>
<tr>
<td><img src="image.png" alt="Structure" /></td>
<td>Antimalarial</td>
<td>(34)</td>
</tr>
<tr>
<td><img src="image.png" alt="Structure" /></td>
<td>Spasmolytic</td>
<td>(35)</td>
</tr>
<tr>
<td><img src="image.png" alt="Structure" /></td>
<td>Antiseptic</td>
<td>(36)</td>
</tr>
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</table>

1.1.1 Complexes of Biguanides and its N-substituted derivatives

Biguanides and its N-substituted derivatives are bidentate ligands which contain nitrogen donor atoms\(^{(37,38)}\).

As a species containing the Y-shaped CN\(_3\) unit, in neutral or anionic form, is capable of exhibiting a variety of coordination modes and a range of donor properties\(^{(39)}\).

These compounds are considered as a strong \(\sigma\)- and \(\pi\)-donating ligands which form highly colored stable complexes with transition metal ions either in high or usual oxidation states utilizing the availability of vacant d-orbitals of the metal which may overlap with the filled \(\pi\) orbitals of the ligand\(^{(17,40)}\) and their color Varies with nature of the metal ion and its
oxidation state, as well as with the number of ligands in the complex\(^{(10)}\). High stabilities of metal biguanides have been attributed to the presence of strong chelate ring current\(^{(41-43)}\).

The modes of metal ion coordination by biguanide were extensively investigated by many workers\(^{(20,44,45)}\) and (N,N) bidentated chelation by the N\(^2\) and N\(^4\) atoms were established to be the most acceptable mode of coordination\(^{(21,43)}\)(\(^{46-48}\)).

While the protonated and nonprotonated forms of these ligand complexes may be represented as (4) and (5)\(^{(49)}\) respectively.

Accordingly, the complexes can be classified into two groups:

1. Uncharged metal biguanides complex \([M^n \text{ (Big)}_n]\), \([M^n \text{ (Big)}_n]. m\text{H}_2\text{O}\).
2. Charged cationic metal biguanides complex \([M^n \text{ (BigH)}_n]X_n\). (Where \(X=\text{Cl}^-, \text{Br}^-, \text{HCO}_3^-, \text{etc.}\) ) according to the ligand basicity\(^{(50)}\).

Vanadyl biguanide complexes were first reported by Banerjee and Ray in 1959\(^{(51)}\) and complex compounds of biguanides with various transition metals such as Cu(II), Ni(II), Co(II), Co(III), Cr(III), Mn(III), Mn(IV), V(IV), Re(V), Os(VI), Ag(III), Pd(II) and Zn(II) were reviewed by Ray in 1961\(^{(10)}\).

The electronic and infrared spectra of the uncharged biguanide complexes of the 3d-transition metals (M(II)=V, Cr, Mn, Co, Ni, Cu and Zn) have been studied and assignments of the chief bands are made,
spectral data conform to distorted octahedral structures for the V, Cr, Mn and Co complexes, square planar structures for Ni and Cu and a tetrahedral configuration for Zn\(^{(52)}\).

Some cationic complexes of Ag(I):[Ag(BigH)(H\(_2\)O)\(_2\)]\(_2\)SO\(_4\) (6), [Ag(BigH)(H\(_2\)O)\(_2\)]HSO\(_4\) (7) and Ag(III):[Ag(BigH)\(_2\)]X\(_3\) (8) with biguanides have been prepared and characterized\(^{(53)}\) by elemental analysis and IR spectral studies. Only thermal analysis were performed for Ag(I) complexes.

![Diagram of complexes (6) and (7)]

Complexes of Cr (III), Mn (IV), Co (III) and Cu (II) with biguanide and its derivatives have shown square planar or octahedral structures involving two or three ligand molecules\(^{(20,22,54,55)}\). In all these structures, the metal ion closes the quasiplanar pseudoaromatic rings due to the extensive
\( \pi \)-electron delocalization along the N-C-N skeleton, resulting in sp\(^2\) hybridization of the amine groups\(^{56}\).

Oxorhenium (\(\text{V}\)) complexes of biguanide and its derivatives have been formulated as [ReO (Big)\(_2\) X], (X=Cl\(^-\) or OH\(^-\)) on the basis of elemental analysis and IR spectra\(^{57}\).

Recently, it was shown that Vanadyl-biguanide complexes are potential synergistic insulin mimics (9)\(^{18}\), while the complexes of Technetium isotope (\(^{99}\text{Tc}\)) with biguanide derivatives could be used as renal imaging agents\(^{58}\).

![Structure of Biguanide Derivatives](image)

\( R^1=R^2=\text{H}, \text{CH}_3, (\text{CH}_2)_2\text{C}_6\text{H}_5 \)

### 1.2 Metformin Hydrochloride (MTF. HCl)

Among the various substituted biguanides, Metformin.HCl(10) (chemically, it is known as N,N-dimethyl biguanide)\(^{59,60}\) is one of the interesting compound that chelate metals.

In 1949 a preparation of dimethyl biguanide (known as flumamine) was used against influenza in Philippines\(^{61}\), this results obtained during the 1940s while the antimalarial hydrochloride was found to have a weak glucose-lowering effect\(^{62}\). The latter prompted research worker to investigate the glucose-lowering activity of dimethyl biguanide, they selected dimethyl biguanide for clinical development, were the name was proposed 'Glucophage' (glucose eater), then this results were published in 1957\(^{63}\).
Metformin is characterized by the nonpolar side chain (-CH$_3$) and the strong basic character of the polar guanidine moiety. Like biguanide, it is a moderately strong base, forming well defined salts. Table (1-2) shows the major properties of Metformin. HCl.

![Chemical structure of Metformin](image)

**Table (1-2): General properties of MTF.HCl**

<table>
<thead>
<tr>
<th>Name of drug</th>
<th>Metformin. HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Name</td>
<td>N,N-dimethylbiguanide hydrochloride.</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C$<em>4$H$</em>{11}$N$_5$.HCl</td>
</tr>
<tr>
<td>Molecular weight</td>
<td>165.63</td>
</tr>
<tr>
<td>Appearance</td>
<td>White crystalline powder</td>
</tr>
<tr>
<td>Melting point</td>
<td>222-226°C</td>
</tr>
<tr>
<td>Solubility</td>
<td>Water, 95% Ethanol</td>
</tr>
<tr>
<td>Practically</td>
<td>Acetone, Ether, Chloroform</td>
</tr>
<tr>
<td>insoluble</td>
<td></td>
</tr>
<tr>
<td>pKa$_1$, pKa$_2$</td>
<td>11.53, 2.73</td>
</tr>
</tbody>
</table>

**1.2.1 Mechanism of action**

Metformin.HCl is an antidiabetic agent which used in the treatment of non-insulin-dependent diabetes mellitus (NIDDM).
It enhance insulin sensitivity and is not effective in the absence of insulin\(^ {67} \). It lowers blood glucose level in (NIDDM) patients by decreasing hepatic glucose production, decreasing intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization\(^ {68,69} \).

Besides decreasing the glucose level, Metformin also acts as antimicrobial, analgesic, anti-malarial\(^ {70} \) and antimetabolite for organisms that inhibit the metabolism of folic acid\(^ {71} \).

### 1.2.2 Side effect of toxicity

Metformin.HCl is safe\(^ {72} \) and not teratogenic\(^ {73} \) in many of the species studied. The most frequent adverse effects of MTF.HCl are\(^ {65,74} \).

1. Diarrhea.
2. Epigastric discomfort.
3. Nausea.
4. Weight loss.
5. Abdominal bloating.

These side effects are transitory, self-limiting and can reduced by starting with a low dose, titrating up slowly of taking the medication with food\(^ {75} \).

### 1.2.3 Complexes of Metformin

Metformin have two imine groups in cis position thus acting as a chelating agent\(^ {64} \), it is possessing excellent capacity for coordination with transition metals\(^ {18,57} \).

There was evidence of it versatility in coordination mode, it was shown that it could coordinate as:

1. Unidentate through N\(^3 \) atom in cationic form\(^ {76,77} \).
2. N², N⁴ chelate both in neutral as well as in anionic form and also it can be found in some complexes as a discrete cation.

Coordination of metformin with many elements of the transition series giving highly colored chelate complexes, especially Cu (II), Ni (II), Co(II) and Pt (II).

Bis(1,1-dimethyl biguanido) copper (II) and nickel (II) have been prepared as square planar configuration, X-Ray crystal structure was proved that the deprotonation of the ligand causes an increase in the π-conjugation in the C-N-C system, reducing the bond angle at the central N atom to nearly 120°, 118.46° for Cu (II) and Ni (II) respectively.

Complexes of metformin present a broad spectrum of biological activity. Recently, it was found that vanadyl complex with metformin shows potential synergistic insulin mimics, while platinum (IV) complex reveals antitumor activity.

Co(II), Zn(II) and Pt(II) complexes have been prepared of unidentate and chelate coordination mode for the ligand respectively. Moreover all these complexes possess antimicrobial activity and also have an interesting thermal behaviour.

The new complexes [M(N,N-dimethylbiguanide)₂(ClO₄)₂] (M(II): Mn, Ni, Cu and Zn) have been synthesized and characterized by IR, ¹H, ¹³C NMR, electronic spectroscopy data and display low cytotoxicity as potential wide spectrum antimicrobial agents.

In order to improve the biological activity of Ni(II) and Cu(II) complexes with biguanide moiety, new complexes with units incorporated in a macrocycle have been synthesized and characterized by microanalytical, IR, UV-Visible data and thermal behaviour of these derivatives was
investigated, the ligands obtained by its one pot condensation with ammonia/hydrazine and formaldehyde.

Two Fe(III) complexes with therapeutically active biguanides-N,N-anhydrobis-(beta-oxyethyl) biguanide (MBigH) and N,N-dimethyl biguanide, respectively-[Fe(MBigH)_3]Cl_3.9H_2O and [Fe(MBigH)(MTF)]Cl_3.3H_2O were synthesized and characterized by elemental analysis, electrical conductivity, IR and UV/Vis spectroscopy and thermal analysis. Table (1-3) shows structures of some metal complexes of metformin, which were cited above.

**Table (1-3): Some metal complexes of Metformin**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Diagram" /></td>
<td>(84)</td>
</tr>
<tr>
<td><img src="image2" alt="Diagram" /></td>
<td>(64)</td>
</tr>
<tr>
<td><img src="image3" alt="Diagram" /></td>
<td>(76) and (77)</td>
</tr>
</tbody>
</table>

M=Co(II), Zn(II)
1.3 L-Cysteine

L-Cysteine (2-amino-3-mercapto propionic acid) is a sulphur-containing amino acid, Fig(1-5). It is known that L-cysteine is nonessential\(^{(90)}\) and an important amino acid due to its determinant roles in
biological systems and presence in a large number of biological materials and pharmaceutical preparations\(^{91}\).

![Structure of Cysteine](image)

**Fig (1-5): Structure of Cysteine.**

The sulphydryl (-SH) group of cysteine plays a key role in the biological activity of protein and enzymes\(^ {60,90}\). It has been used in some antibiotic preparation used for the treatment of skin damages and as a radio-protective agent\(^ {92,93}\), supplement in some foods\(^ {94-96}\), cancer indicator, and implicated in a number of pathological conditions, including Alzheimer's and Parkinson's disease as well as autoimmune deficiency syndrome\(^ {97-99}\). On the other hand, deficiency in L-cysteine is associated with a number of clinical situations such as liver damage, skin lesions, slowed growth, and AIDS\(^ {100-104}\). Also, L-cysteine is widely used in the food industry as an antioxidant and in the pharmaceutical industry in drug formulation and as biomarker\(^ {105}\).

### 1.3.1 Complexes of Cysteine

Amino acids, especially sulphur-containing α-amino acids and their esters are known as coordinating agents for metal ions due to the presence of amino (NH\(_2\)) and carboxylate (COO) groups in their structures\(^ {106-108}\).

Many transition metal-amino acid complexes have considerable biological activity, such as antitumor properties\(^ {109,110}\). Amino acids usually increase the diffusibility of complexes and enhance their biological action inside the cell. Such systems are widely used in the field of chemotherapy\(^ {111}\). Studies have shown that cysteine may be considered a biologically important ligand manifested in the formation of chelate rings.
involving various coordination (N,S; O,S; or N,O) sites\textsuperscript{(112-115)}. In view of its biological and chemical properties, cysteine is a suitable ligand for the synthesis of complexes involving bioactive metals as cobalt, nickel and copper\textsuperscript{(116,117)}.

On the other hand, sulphur compounds have been the subject of interest in coordination chemistry. The activity of these compounds\textsuperscript{(118,119)} is due to their ability to form stable complexes with essential metal ions.

1.3.2 Mixed-ligand complexes of divalent transition metal with Cysteine

Mixed ligand complexes are well known to play a significant role in biological system\textsuperscript{(120)}, and has been well recognized\textsuperscript{(121,122)}. Many researchers have studied characterization, antimicrobial and toxicological activity of mixed ligand complexes of transition metal ions and actinide metal ion\textsuperscript{(123-127)}. Antitumor activity of some mixed ligand complexes have also been reported\textsuperscript{(128,129)}, further mixed ligand complexes are established to be biologically active against pathogenic microorganisms\textsuperscript{(130,131)}.

In 1965 O. Szazuchin studied the synthesis of complex combinations of Zn(II) and Ni(II) with amino acids: D-pencilamine and L-cysteine. These complex combinations have biological and therapeutical activities\textsuperscript{(132)}. Cysteine has been used as a co-ligand to form mixed ligand complexes of ranitidine which is the most usful drug in the management of peptic and duodenal ulcer\textsuperscript{(91)}.

Recent results on the mixed ligand complexes of Zn (II) and Co (II) with cysteine, histidine, cysteine methylester and histidine methylester were reported\textsuperscript{(133)}, and the structure of these complexes are shown in Fig(1-6).

14
Fig (1-6): Mixed ligand complexes of A- Cystein, Histidine ;B- Cysteine methylester and histidine methylester.

Mixed ligand complexes of Co(II), Ni(II) and Cu(II) with cysteine and 4-substituted thio semicarbazides have been synthesized\(^ {134} \), which cysteine behaves as dinegative bidentate ligand via cysteine-SH and COOH groups, as is shown in Fig (1-7).

Fig (1-7): Ternary metal complexes with cysteine and 4- substituted thiosemicarbazides.
1.4 Methods used for the determination of Metformin Hydrochloride

Literature survey reveals that methods like UV-spectrophotometry\(^{(135,136)}\), HPLC\(^{(137-139)}\), Gas Chromatography\(^{(140,141)}\) and Liquid Chromatography, \(^{(142)}\) have been reported for determination of metformin. HCl in pharmaceutical formulations of biological fluids.

J. Brohon\(^{(143)}\) reported a determination method for metformin. HCl by developing it to the substituted triazine derivative and analysed by Gas Liquid Chromatography.

Table(1-4) tabulated some methods for the determination of metformin. HCl.

**Table (1-4): Some methods for the determination of Metformin.HCl**

<table>
<thead>
<tr>
<th>Type of method</th>
<th>Comments</th>
<th>Calibration range</th>
<th>Application</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorimetric titration</td>
<td>Titrated in nonaqueous medium with HClO(_4) using different indicators</td>
<td>-</td>
<td>Pharmaceutical</td>
<td>(144)</td>
</tr>
<tr>
<td>Potentiometric titration</td>
<td>Titrated with HClO(_4) in glacial acetic acid &amp; mercury (II) acetate</td>
<td>-</td>
<td>-</td>
<td>(145)</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>Reacted with NaOCl followed by NaOH &amp; ZnSO(_4) to give yellow color</td>
<td>-</td>
<td>Pharmaceutical</td>
<td>(70)</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>Reacted with biacetyl &amp; I-naphthol in 50% alkaline ethandiol</td>
<td>-</td>
<td>Biological fluids</td>
<td>(69)</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>Extracted from bromothymol blue &amp; was determined at 232nm</td>
<td>(10^{-5}-2\times10^{-4}) mol.L(^{-1})</td>
<td>Biological fluids</td>
<td>(146)</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>Oxidized by hydrogen peroxide to form a yellow color &amp; was determined at 400nm</td>
<td>4-26(\mu)g.ml(^{-1})</td>
<td>Tablet dosage forms</td>
<td>(147)</td>
</tr>
<tr>
<td>Type of method</td>
<td>Comments</td>
<td>Calibration range</td>
<td>Application</td>
<td>Ref.</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>---------------------------------------------------------------------------</td>
<td>-------------------</td>
<td>---------------------</td>
<td>------</td>
</tr>
<tr>
<td>Spectrophotometry</td>
<td>A primary amino group of metformin reacts with ninhydrin in alkaline medium to form violet color which is determined at 570nm</td>
<td>8-18µg.ml⁻¹</td>
<td>Tablet dosage forms</td>
<td>(148)</td>
</tr>
<tr>
<td>Reversed phase High Performance Liquid Chromatography RP-HPLC</td>
<td>Simple &amp; sensitive RP-HPLC method with UV-Vis detection include a mixture of acetonitrile &amp; ammonium acetate buffer (42:58) as a mobile phase</td>
<td>0.5-50µg.ml⁻¹</td>
<td>Pharmaceutical</td>
<td>(149)</td>
</tr>
</tbody>
</table>

1.5 Determination as metal chelates

The formation of colored complex is one of the key chemical steps used in the qualitative analysis in aqueous solutions. In addition, the absorption of UV–Vis radiation by these colored complexes is often used for the quantitative analysis of chelate compounds or metal ions by spectrophotometry.

A chelate complex which was formed disturbs the electronic state of the organic molecule to produce, as a rule of bathochromic shift, through some hyposochromic shifts are known. Most transition metals with an incompletely- electron subshell have chromophoric properties, these metals may occur in various oxidation states. They can give color reactions with colorless compounds containing no chromophoric groups.

There is a close relation between the color of a substance and its electronic structure. A molecule exhibits absorption in the visible or Ultra-violet range, when radiation causes an electronic transition, raising the molecule (ion) from the ground state to an exited state. The production
or change of a color is connected with deformation of the normal electronic structure of the molecule.

Table (1-5) tabulate application of some drugs which was determined as chelated with metal ion in pharmaceutical formulations.

### Table (1-5): Application for some selected spectrophotometric determination of some drugs using metal chelation

<table>
<thead>
<tr>
<th>Drug as chelate</th>
<th>Function group</th>
<th>Metal ion</th>
<th>M:L ratio</th>
<th>λ&lt;sub&gt;max&lt;/sub&gt; (nm)</th>
<th>Calibration range</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atenolol</td>
<td>Hydroxyl &amp; amino groups</td>
<td>Cu(II)</td>
<td>1:2</td>
<td>694</td>
<td>(4.1-12)×10⁻³ mol.L⁻¹</td>
<td>(153)</td>
</tr>
<tr>
<td>Coftazidime</td>
<td>Sulphur atom of β- lactam &amp; thiazol ring</td>
<td>Pd(II)</td>
<td>1:2</td>
<td>354</td>
<td>8-28 µg.ml⁻¹</td>
<td>(154)</td>
</tr>
<tr>
<td>Tetracycline. HCl</td>
<td>1,3- diketone groups</td>
<td>Fe (III)</td>
<td>1:2</td>
<td>423</td>
<td>(10-200)³ µg.ml⁻¹</td>
<td>(155)</td>
</tr>
<tr>
<td>Acobutolol. HCl</td>
<td>Hydroxyl &amp; amino groups</td>
<td>Co (II)</td>
<td>1:2</td>
<td>613</td>
<td>(1.1-5.1)×10⁻³ mol.L⁻¹</td>
<td>(153)</td>
</tr>
<tr>
<td>Ciprofloxacin.HCl</td>
<td>Carboxylic &amp; carbonyl groups</td>
<td>Fe (III)</td>
<td>1:2</td>
<td>435</td>
<td>20-200µg.ml⁻¹</td>
<td>(156)</td>
</tr>
<tr>
<td>Carbocisteine</td>
<td>Two carboxylic groups</td>
<td>Cu (II)</td>
<td>1:1</td>
<td>280</td>
<td>5-70µg.ml⁻¹</td>
<td>(157)</td>
</tr>
<tr>
<td>Penicillamine</td>
<td>Sulphur atom &amp; amino group</td>
<td>Ni (II)</td>
<td>1:2</td>
<td>270</td>
<td>1-20µg.ml⁻¹</td>
<td>(157)</td>
</tr>
<tr>
<td>Risedronate sodium</td>
<td>-</td>
<td>Cu (II)</td>
<td>1:1</td>
<td>264</td>
<td>2-40µg.ml⁻¹</td>
<td>(158)</td>
</tr>
</tbody>
</table>

1.6. Flow Injection Analysis (FIA)

Flow injection analysis (FIA) was developed in the mid-1970s as a highly efficient technique for the automated analysis of samples<sup>(159,160)</sup>. FIA allows for the rapid, sequential analysis of an unlimited number<sup>(161)</sup> of samples, it is one member of a class techniques called continuous flow
analyzers, in which samples are introduced sequentially at regular intervals into a liquid carrier stream that transports the samples to the detector\textsuperscript{(162-164)}.

FIA is generally\textsuperscript{(165)}, a simple and non expensive technique employing common instrumentation such as peristaltic pumps and low pressure injection valves. Compared to batch methods, it offers increased sampling, lower reagents consumption, automation sample preparation and detection\textsuperscript{(166)}; better precision and high versatility, since a variety of detectors may be employed\textsuperscript{(167,168)}, such as UV-Vis spectrophotometry\textsuperscript{(169)}, atomic absorption spectroscopy\textsuperscript{(170)}, ion selective electrodes\textsuperscript{(171)}, or with chemiluminescence and fluorescence system\textsuperscript{(172-175)}.

The above mentioned advantages of FIA have led to a continuously increasing interest in pharmaceutical analysis and quality control application\textsuperscript{(176)}.

Table (1-6) tabulates the application of FIA through coupling with different techniques.

**Table (1-6): Application of FIA assays with different detection system**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Coupled technique with FI</th>
<th>Calibration range</th>
<th>Application</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrogen peroxide</td>
<td>Chemiluminescence reaction</td>
<td>-</td>
<td>-</td>
<td>(177)</td>
</tr>
<tr>
<td></td>
<td>Chemiluminescence reaction</td>
<td>0.02-6.8 µg.ml(^{-1})</td>
<td>-</td>
<td>(178)</td>
</tr>
<tr>
<td>Oxonium ion &amp; substituted acetic acid</td>
<td>Chemiluminescence reaction</td>
<td>0-3804 µg.ml(^{-1})</td>
<td>-</td>
<td>(179)</td>
</tr>
<tr>
<td>Selenium (IV)</td>
<td>Spectrophotometry</td>
<td>1-5 µg.ml(^{-1})</td>
<td>Blood &amp; Urine</td>
<td>(180)</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>Chemiluminescence reaction</td>
<td>1.25µg.ml(^{-1})(LOD)</td>
<td>-</td>
<td>(181)</td>
</tr>
<tr>
<td>4-Amino antipyrine</td>
<td>Spectrophotometry</td>
<td>2.5-400 µg.ml(^{-1})</td>
<td>Serum &amp; Urine</td>
<td>(182)</td>
</tr>
</tbody>
</table>
### Analyte | Coupled technique with FI | Calibration range | Application | Ref.  
--- | --- | --- | --- | ---  
Paracetamol | Spectrophotometry | 180-300 μg.L⁻¹ | Pharmaceutical | (183)  
Amoxicillin | Spectrophotometry | 1-120 μg.ml⁻¹ | Pharmaceutical | (184)  
Bismuth(III) | Chemiluminescence | 30-110 μg.ml⁻¹ | Pharmaceutical | (185)  
Ascorbic acid | Spectrophotometry | 0.1-10 mmol.L⁻¹ | Pharmaceutical & urine | (186)  
Phenothiazine | Flourescence reaction | 0.01-4.00 μg.ml⁻¹ | Pharmaceutical | (187)  
Pipemidic acid | Chemiluminescence reaction | 0.1-80 μg.ml⁻¹ | Tablets | (188)  
Furosemide | Liquid Chromatography | - | Urine | (189)  
Nicotine | Infrared spectrometry | - | Tobacco | (190)  

#### 1.6.1 Flow Injection analyzer

A typical flow injection setup Fig (1-8)(191) involves injection of a defined volume of the sample (S) into a moving stream of a solution which serves as a carrier and propelles the sample zone to a flow-through detector (D), at a constant flow rate through channel (narrow tubing) by a peristaltic pump; the fluid dynamics of flow mixes sample and reagent, leading to chemical reaction to form detectable species. This species are sensed by the detector as a transient peak; the height and area of the peak are proportional to the concentration of the analyte(163).
Fig (1-8): (a) The simplest single line FIA manifold utilizing a carrier stream of reagent; S is the injection port, D is the detector and W is the waste. (b) The analog output has the form of a peak, H is the peak height, T is the residence time corresponding to the peak height measurement and A is the detector output signal.

Additional components may include a flow through heater to increase the speed of chemical reaction, columns for sample reduction, debubblers and filters for particulate removal.

1.6.2 Principles

A FIA curve occurs due to two processes, one involving the simultaneous, physical process of zone dispersion and the second involving the chemical process resulting from reaction between sample and reagent species. A difference in the concentration gradient is thus generated.

Immediately after injection with a sampling valve, sample zone concentration profile is rectangular shown in Fig (1-9a)\(^{192}\) as it moves through the tubing, band broadening or dispersion takes place. The shape of the resulting zone is determined by two phenomena. The first is convection arising from laminar flow in which the center of the fluid moves more rapidly than the liquid adjacent the walls, thus creating the parabolic
shaped front and the skewes zone profile had shown in Fig(1-9b)\(^{(192)}\). Broadening also occurs as a consequence of diffusion.

Two types of diffusion can in principle, occur:

1. Radial (perpendicular) to the flow direction.
2. Longitudinal (parallel) to the flow.

The latter is of no significance in narrow tubing, whereas radial diffusion is always important under this circumstances. In fact, at flow rates it may be the major source of dispersion. Here, the radial dispersion from the walls toward the centre serves the important function of essentially freeing the walls of analyte and thus eliminating cross-contamination between samples.

Fig (1-9): Effect of convection on concentration profiles of analytes at the detector, (a) no dispersion, (b) dispersion by convection, (c) dispersion by convection and radial diffusion, (d) dispersion by diffusion.

Where:

\(C_0\) = Concentration in injection volume.
\(C\) = Peak concentration at detector.
1.6.3 Flow Injection methods based on metals-drugs interactions

A considerable group of FI methods for the determination of active pharmaceutical ingredients is based on the interactions between metals and drugs in which formation of coloured complexes between them.

The advantages of using metal ions as complexation agents in FI include simple manifolds, readily available and cost effective reagents, while the sampling rate is generally very high ranging between $60 \text{h}^{-1}$\(^{(193)}\) and $210 \text{h}^{-1}$\(^{(194)}\).

Table (1-7) tabulate some of FI – spectrophotometric determination of some drugs based on metal chelation.

**Table (1-7): FIA–Spectrophotometric determination of some drugs by chelation with metal ion**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Metal ion as chelation</th>
<th>Carrier stream</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>Calibration range</th>
<th>Application</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxytetacycline</td>
<td>Fe (III)</td>
<td>Sulphuric acid (0.01ml.L(^{-1})) with Fe ion</td>
<td>435</td>
<td>-</td>
<td>Drug formulations</td>
<td>(195)</td>
</tr>
<tr>
<td>Metyldopa</td>
<td>Mo (VI)</td>
<td>Water</td>
<td>410</td>
<td>50-200mg.L(^{-1})</td>
<td>pharmaceutical formulation</td>
<td>(194)</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>Cu (II)</td>
<td>Acetate buffer (pH=5.9)</td>
<td>330</td>
<td>$5\times10^{4}$-$2\times10^{3}$ mol.L(^{-1})</td>
<td>Pharmaceutical &amp; urine</td>
<td>(193)</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Fe (II)</td>
<td>Sodium hydrogen sulphite</td>
<td>530</td>
<td>5-200mg.L(^{-1})</td>
<td>Pharmaceutical formulation</td>
<td>(196)</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>Fe (III)</td>
<td>Sulphuric acid (0.05mol.L(^{-1})) with Fe ion</td>
<td>420</td>
<td>1.8-289mg.L(^{-1})</td>
<td>Pharmaceuticals &amp; urine</td>
<td>(197)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Al (III)</td>
<td>Tris - buffer (pH=7.0)</td>
<td>376</td>
<td>-</td>
<td>Pharmaceutical preparation</td>
<td>(198)</td>
</tr>
</tbody>
</table>
1.7 **Aim of the project**

This research work aims to:

1. Create a new approach for the determination of metformin drug based on metal-drug interaction; that will be studied extensively in this research in order to establish a real comparative approach with the other methods for the determination of metformin drug and to create a new line of research in continuous Flow Injection Analysis (CFIA).

2. All the results that will be obtained will be subjected for detailed data treatments and comparison with other available methods. The system that has been tried for the spectrophotometric determination of drug will be involved without using of chemical reagent.

3. Synthesize mixed ligand complexes of Cu(II), Ni(II) and Co(II) derived from metformin (MTF) as primary ligand and cysteine(Cys) as secondary ligand.

4. Characterize and study the cited above compounds by NMR, IR and UV-Vis spectra, elemental analysis, atomic absorption, molar conductivity and magnetic susceptibility measurements.
2. A Chemicals and apparatus used throughout the determination of Metformin.HCl

2. A. 1 Chemicals

- **Hydrochloric acid (100 mM)**
  5 ml of HCl (HCl: d$_{20}^°$=1.16 g.ml$^{-1}$,32%) was taken, diluted to 500 ml which was standardized with sodium carbonate solution. Both chemicals were supplied by BDH.

- **Sodium hydroxide (100 mM)(BDH)**
  Approximately 4.00 g of NaOH was dissolved in pre-boiled distilled water to 1 litter volumetric flask. This solution was standardized with standard hydrochloric acid.

- **Copper(II) nitrate solution (Cu(NO$_3$)$_2$. 3H$_2$O)(BDH)**
  7.6046 g was dissolved and completed to 2 litter volumetric flask with distilled water. This insure that the Cu(II) ion concentration 1000 µg. ml$^{-1}$.

- **Metformin.HCl (200 mM)(SDI)**
  16.563 g of metformin.HCl (C$_{4}$H$_{11}$N$_{5}$.HCl) was dissolved in 500 ml of distilled water to have 200 mM.

2. A. 2 Apparatus used

- **Peristaltic pump. (Switzerland)**
  An ISMATEC type ISM796 peristaltic pump three channel was used.

- **Connection tubes**
  Various manifold parts mode of either from selector rubber or poly propylen or even teflon with inside diameter between 0.5-1 mm were used.
➢ **Rotary six ports injection valve**

Six ports medium pressure (models: V-450) (upchurch scientific Inc.) valve was used with variable sample volume, fixed on the carrier stream (distilled water line). Fig(3-2).

➢ **Junction point**

Liquid junction point made of methylmethacrylate (organic glass) (Y-junction) for the combination of metformin, sodium hydroxide and copper(II) solutions. Fig(3-3, 3-4).

➢ **Electronic measuring and readout system**

The electronic system used throughout this work composed of Zero-3 volts solar cell with an electronic amplification unit and the system includes two high intensity light emission diod (LED) of a maximum wave length 525-530 and 550 nm.

The read out of the system composed of, in voltage output or X,Y-t potentiometric recorder (KOMPENSOGRAPH C1032) SIEMENS (Germany) (Readable voltage) or Digital AVO-meter (auto range) (mV-1000V) DC (ASWAR DT 9202A-china).

➢ **UV-Visible Spectrometer (Cary-Varian El 04103410)**

UV-Visible spectrophotometer used to scan the spectrum for the formation of Metformin-Hydroxide ion-Cu(II)ion complex.
2.B Chemicals and instrumentation used throughout the prepared mixed ligand complexes

2. B. 1 Instrumentation

➢ Infrared spectrophotometer

The IR spectra of the ligands and the prepared mixed complexes were recorded using (KBr)(4000-400) and (CsI)(4000-200) disc on

1. SHIMADZU FTIR-8400S, Fourier Transform, Infrared spectrophotometer.
2. SHIMADZU FTIR-21, Fourier Transform, Infrared spectrophotometer.

➢ Electronic spectra

The electronic spectra of compounds were obtained using:

1. UV-Visible Spectrophotometer (Cary-Varian El 04103410) region (200-800)nm.
2. UV-Visible Spectrophotometer (SHIMADZU-1650PC) region (200-1100)nm.

➢ Melting points

The melting points were obtained using Gallenkamp melting point apparatus.

➢ Metal analysis

The metal content of the prepared mixed complexes were determined by atomic absorption technique using Varian- AA6200 Flame Atomic Absorption spectrophotometer.

➢ Elemental microanalysis. (C.H.N.S)

The elemental microanalyses of the prepared compounds were performed on Eurovector EA 3000A, AL al-Bayt University (Jordan).
EXPERIMENTAL

1. H-NMR and 13C-NMR spectra
   The 1H and 13C-NMR spectra were performed by using Bruker Ultra shield 300 MHz NMR, AL al-Bayt University (Jordan).

2. Conductivity measurements
   The conductivity values of the prepared complexes were measured in DMF solution (10⁻³ M) at room temperature using WTW series Cand 720.

3. Magnetic susceptibility measurements
   Magnetic moment μ_eff (B.M) for the mixed complexes were obtained at room temperature using Magnetic susceptibility balance Model MSB-MK-1.

4. Chloride contents
   The chloride contents for complexes were determined by Mohr's method.

2. B. 2 Chemicals

   a. Inorganic chemicals

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Formula</th>
<th>Purity%</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper(II)chloride dihydrate</td>
<td>CuCl₂. 2H₂O</td>
<td>98.5</td>
<td>BDH</td>
</tr>
<tr>
<td>Nickel(II)chloride hexahydrate</td>
<td>NiCl₂. 6H₂O</td>
<td>97</td>
<td>BDH</td>
</tr>
<tr>
<td>Cobalt(II)chloride hexahydrate</td>
<td>CoCl₂. 6H₂O</td>
<td>99.99</td>
<td>Merck</td>
</tr>
<tr>
<td>Sodium hydroxide</td>
<td>NaOH</td>
<td>96</td>
<td>BDH</td>
</tr>
</tbody>
</table>
2. Organic chemicals

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Formula</th>
<th>Purity</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metformin. HCl (N,N- dimethyl biguanide) hydrochloride</td>
<td>C₉H₁₁N₅. HCl</td>
<td>97</td>
<td>SDI</td>
</tr>
<tr>
<td>Cysteine (amino acid)</td>
<td>C₃H₇NO₂S</td>
<td>98</td>
<td>BDH</td>
</tr>
</tbody>
</table>

3. Solvents

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Formula</th>
<th>Purity %</th>
<th>Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute ethanol</td>
<td>C₂H₅OH</td>
<td>99.9</td>
<td>BDH</td>
</tr>
<tr>
<td>Acetone</td>
<td>C₃H₆O</td>
<td>99.9</td>
<td>BDH</td>
</tr>
<tr>
<td>Methanol</td>
<td>CH₃OH</td>
<td>99.9</td>
<td>BDH</td>
</tr>
<tr>
<td>Chloroform</td>
<td>CHCl₃</td>
<td>98</td>
<td>BDH</td>
</tr>
<tr>
<td>Diethyl ether</td>
<td>(C₂H₅)₂O</td>
<td>99</td>
<td>Fluka</td>
</tr>
<tr>
<td>N,N-Dimethyl formamide (DMF)</td>
<td>C₃H₇NO</td>
<td>99</td>
<td>Fluka</td>
</tr>
<tr>
<td>Dimethylsulfoxide (DMSO)</td>
<td>C₂H₆SO</td>
<td>99.5</td>
<td>Fluka</td>
</tr>
</tbody>
</table>

2.2B.3 Preparation of Cu(II), Ni(II) and Co(II) mixed ligand complexes

Mixed ligands complexes were prepared from metal salts as chloride, metformin. HCl, as a primary ligand and amino acid(cysteine), as a secondary ligand.

To an aqueous solution(10ml) of (1mmol) Cu(II), Ni(II) and Co(II) (0.170g, 0.237g and 0.237g respectively), an aqueous solution(10ml) of metformin.HCl (0.165g, 1mmol) containing NaOH (0.04gm, 1mmol) was added. The reaction mixture was stirred and kept in a boiling water bath for 10 minutes. To this hot solution an aqueous solution (5ml) of cysteine (0. 121g , 1mmol) was added with constant stirring. The mixture was again

29
heated in a water bath; the pH was adjusted to 7.5～8 with NaOH, intense colored precipitate appeared. The stirring was maintained for 1.5 h. The mixture was cooled and the solid complex obtained was filtered, washed with water, then with absolute ethanol and finally with ether and dried over silica gel.

2. B. 4 Nomenclature of the free ligands and their prepared mixed ligand complexes

Table(2-1): Molecular formula and nomenclature of the free ligands and theirs prepared complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Formula</th>
<th>Name</th>
<th>M.wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTF</td>
<td>C₄H₁₁N₅. HCl</td>
<td>N,N-dimethylbiguanide hydrochloride</td>
<td>165.63</td>
</tr>
<tr>
<td>Cys</td>
<td>C₃H₇NO₂S</td>
<td>2-amino-3-mercaptopropionic acid</td>
<td>120.8</td>
</tr>
<tr>
<td>Cu(II)complex</td>
<td>[Cu(MTF)(Cys)(H₂O)]₂</td>
<td>[Diaqua{N,N-dimethylbiguanido}(2-amino-3-mercaptopropionic acid)copper(II)]</td>
<td>347.34</td>
</tr>
<tr>
<td>Ni(II)complex</td>
<td>[Ni(MTF)(Cys)(H₂O)]₂.H₂O</td>
<td>[Diaqua {N,N-dimethylbiguanido}(2-amino-3-mercaptopropionic acid)nickel(II)]monohydrate</td>
<td>360.49</td>
</tr>
<tr>
<td>Co(II)complex</td>
<td>[Co(MTF)(Cys)(H₂O)]₂</td>
<td>[Diaqua {N,N-dimethylbiguanido}(2-amino-3-mercaptopropionic acid)cobalt(II)]</td>
<td>342.73</td>
</tr>
</tbody>
</table>
3.1 Introduction

The study carried out in this chapter will deal with the reaction and its possible product concerning the type and the nature of the species formed, i.e. the color of the species, the clarity, the speed of the formation of the colored species and any other effect example: pH, temperature and order of addition. These factors should be taken into concentration when designing a manifold for the continuous on-line flow injection method for analysis.

3.1.1 Order of addition

It was found that the possible on the combination of the chemicals involved in the formation of the colored species that will be the target of this project. Since we have three chemical reactants which are, the drug the denoted letter A, the hydroxide ion denoted by the letter B and the copper (II) ion denoted by the letter C, these possible combination carried out using a trial concentration of 50 mM for metformin, 50 mM for hydroxide ion and 1000 µg.ml⁻¹ for Cu(II) ion; these possible combination with the trial concentration as shown in table (3-1).

Table (3-1): Order of addition of chemicals used in metformin formation Complex.

<table>
<thead>
<tr>
<th>Order of addition</th>
<th>Color of the reaction product</th>
</tr>
</thead>
<tbody>
<tr>
<td>A+B+C</td>
<td>Pink(faint)</td>
</tr>
<tr>
<td>B+A+C</td>
<td></td>
</tr>
<tr>
<td>A+C+B</td>
<td>Violet turns into pink at an excess OH⁻ ion</td>
</tr>
<tr>
<td>C+A+B</td>
<td></td>
</tr>
<tr>
<td>B+C+A</td>
<td>Violet turns into purple at an excess drug with less intensity of</td>
</tr>
<tr>
<td></td>
<td>the pink color</td>
</tr>
<tr>
<td>C+B+A</td>
<td></td>
</tr>
</tbody>
</table>

* No color is formed when any two combinations is carried out.

The table shows that whether drug treated with alkaline solution followed by the solution of Cu(II) ion gave a clear pink color compared
with other combination of the order for the addition of the chemicals, therefore a reaction system was designed on this basis, i.e. a definite conclusion can be reached shows that Cu(II) ion meet the drug in alkaline solution at a later stage.

3.1.2 Spectrophotometric scanning for the MTF-OH⁻-Cu(II) system

A scanning were carried out from 200-800 nm for three chemicals (Fig.3-1; should be followed) A, metformin drug (50 mM) which is indicated by the yellow absorption spectrum, which is mostly in the UV region. While a scanning for Cu(II) ion shows a maximum at 300 nm (blue), while a scanning for the pink colored species formed shows a maximum at 530 nm, these experiment were very necessary in order to design the detection unit with a light emitting diod (LED) at a certain and selected wave length suitable for the measurement of the colored species.

![Figure (3-1): UV-Vis spectrophotometric of the pink species formed by reaction of metformin.HCl(50 mM) in alkaline medium(50 mM) followed by the reaction with Cu(II) ion(500 µg.ml⁻¹). (distilled water as a reference).](image)
3.1.3 Design of manifold reaction of the Metformin drug in an alkaline medium with Cu(II) ion based on online measurement

The system used is composed of three main parts:

**Part A: Supply of chemicals**

This part composed of three reagent bottles containing, distilled water for carrier stream which is attached to a six-ports injection valve capable of supplying a repeatable fine measured volume of a liquor via two mechanisms: (a) **Load mode**, (b) **Inject mode**; as this valve is a medium pressure injection valve with an external sample loop style injector for application up to 1000 psi (69 bar). Each port is connected through an internal flow path to the adjacent port. In the "**Load**" position, the sample loop is loaded through port 2, while the system flow runs the port 5 and 6, when the valve is switched 60 degrees to the "**inject**" position, the sample loop is included into the system, and flow goes from port 5, through the loop and out port 6 as shown in Fig.(3-2). The solution transferred via three lines system using peristaltic pump of a variable speed which can deliver liquid at a desired flow rate.

**Part B: The manifolds**

It was believed and noticed that a direct mixing of the chemicals at the optimum addition arrived to (section 3.1.1), therefore a block of polymethylmethacrylate 20 mm thickness, was drilled and shaped as shown in Fig.(3-3); where there are two Y-junction, the first junction were the carrier stream ,carrying the metformin drug meet the hydroxide ion and directly mixes with the Cu(II) ion at the second Y-junction Fig.(3-4), the output of this designed reaction mixing cell, have an output leading to the microphotometric unit.
Figure (3-2): Injection valve; Load mode, Injected mode.
Figure (3-3): Mixing-reaction two Y-junction for MTF- OH⁻ - Cu(II).

Figure (3-4): Mixing reaction; first Y and second Y-junction.
Part C: Microphotometric unit

The microphotometric unit is composed of four parts:

1. The flow cell, this is a hexagonal 2 mm path length designed for a continuous flow.
2. Zero-3 volts solar cell with an electronic amplification unit capable of zero-adjacemnt up to six volts, the output can be attached to a digital display or X-t potentiometric recorder or both at the same time.
3. Two high intensity light emission diod (LED) of a maximum wavelength 525-530 and 550 nm. The intensity of these two LED can be monitored through a variable resistance, so it can be used efficiently for low concentration as well as high concentration of the complex formed.
4. Read out system which could be a digital Auto-voltmeter or an X-t potentiometric chart recorder.

The first, second and third parts are closed in block of polymethylmethacrylate and brass metal to ensure zero adjacemtent of any scattered light coming from any source, the whole block are sealed with a black silicon.

Figure (3-5) shows the schematic flow gram of the whole system, while figure (3-6) shows a presentation of the whole system.

The whole system with all conducted parameters of chemicals and physicals or design of the whole manifold system was put into a trial experimental measurement to check the repeatability and sensitivity of the whole system. Figure (3-7) shows repeatable successive measurements of variable concentration of metformin drug, which indicate a reliable measurement; therefore it was used throughout this project.
Fig(3-5): Schematic flow gram of the MTF-OH Cu(II) system.
Fig(3-6): A presentation of the whole system Metformin-OH⁻-Cu(II).

Figure(3-7): Repeated successive measurements of Metformin-OH⁻-Cu(II) system.
CHAPTER FOUR

RESULTS and DISCUSSIONS

4.1 Introduction

It was quite necessary for the determination of metformin via the formation of colored complex with Cu(II) ion in alkaline medium (metformin-OH-Cu(II)), to carry out a preliminary experiments for all physical and chemical parameters, in order to established the optimum parameters for the reaction.

Part I: A detailed study conducted for all chemical and physical parameters, aiming to achieve optimum reaction parameters for the formation of metformin-OH-Cu(II) colored complex. Also to obtain a calibration graph and the Limit of Detection (L.O.D) within the newly microphotometric used.

Part II: The use of arrived reaction mode obtained above and apply it on pharmaceutical drugs containing the metformin using the newly designed microphotometric, using standard addition method. Comparison of the results obtained with the conventional method available.

4.1.1 Mode of working for the Metformin-OH-Cu(II)

A homemade photometric system coupled with Flow Injection manifold was used throughout this research project. The whole schematic diagram for the determination of metformin is shown in Fig(3-6), where three lines flow system were used. The first line transport hydroxide ion where it meet the second line which is either carries distilled water they give base line or transport distilled water plus the sample segment from injection valve via "injected mode". Then these two solutions meet the Cu(II) ion via the third line. All these mixes in a poly methylacrylate block designed to satisfy the reaction mixing speed of the formed complex where directly passes to microphotometric detector working at two different Light
Emitting Diod (LED)\(^{(199)}\) at 530 & 550 nm, with 2 mm path length and a photosilicon detector will complete amplification and control for intensity of the incident light; thus insure the possibility of variation of the power of the incident light with variable of concentration of reactant. The output can be represented digitally or by plotting on X-t recorder or both at the same time.

Extensive preliminary experiments were carried out to establish the start point of mode, order, concentration of reactants and the speed with a selected sample volume.

### 4.1.2 Study of the optimum parameter for the determination of Metformin via Flow Injection Analysis (FIA)

#### 4.1.2.1 Chemicals parameters

Since the chemical parameter has a great deal on the system used for the determination of metformin. It quite necessary to study their effect in order to established the optimum concentration for the material used to form the complex for MTF\(-\)OH\(^{-}\)\(-\)Cu(II) to obtain maximum absorbance for the colored complex, it was noticed that at nil concentration for any individual of the chemicals used for the reaction the absorbance will be zero in another way it means incomplete formation of the colored complex.

#### 4.1.2.1.1 Copper (II) ion concentration effect

Using the preliminary experiment parameters of the chemical variable which was MTF 35 mM, NaOH 50 mM and a variable Cu(II) ion concentration ranging from 0.0-350 µg.ml\(^{-1}\), physical parameters were kept as follows: flow rate 1.0 ml.min\(^{-1}\) with 40µL injected sample volume and using allowed injection time 15 sec. Table (4-1) summarized the data obtained showing the average of three successive readings with the standard deviation, relative standard deviation and the confidence interval of the absorbance at 95% confidence (\(\alpha=0.05\)).
Table (4-1): Effect of the variation of copper(II) ion concentration on the absorbance of MTF-OH-Cu(II) system.

<table>
<thead>
<tr>
<th>[Cu(II)] µg.ml⁻¹</th>
<th>Absorbance yᵢ (mV)</th>
<th>Average $\bar{y}_i$ (mV)</th>
<th>σᵢ⁻¹</th>
<th>RSD%</th>
<th>Confidence interval of the average $\bar{y}<em>i$ ± $t</em>{0.05,n-1} \frac{\sigma_{n-1}}{\sqrt{n}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>50</td>
<td>78,80,82</td>
<td>80</td>
<td>2.0</td>
<td>2.5</td>
<td>80 ± 4.968</td>
</tr>
<tr>
<td>100</td>
<td>128,128,131</td>
<td>129</td>
<td>1.732</td>
<td>1.342</td>
<td>129 ± 4.303</td>
</tr>
<tr>
<td>150</td>
<td>154.5,153,152</td>
<td>153.16</td>
<td>1.258</td>
<td>0.821</td>
<td>153.16 ± 3.125</td>
</tr>
<tr>
<td>200</td>
<td>178,179,180</td>
<td>179</td>
<td>1.00</td>
<td>0.558</td>
<td>179 ± 2.484</td>
</tr>
<tr>
<td>225</td>
<td>184,185,184.8</td>
<td>184.6</td>
<td>0.529</td>
<td>0.286</td>
<td>184.6± 1.314</td>
</tr>
<tr>
<td>250</td>
<td>177,177,176</td>
<td>176.66</td>
<td>0.577</td>
<td>0.326</td>
<td>176.66 ± 1.433</td>
</tr>
<tr>
<td>275</td>
<td>170,168,168</td>
<td>168.66</td>
<td>1.154</td>
<td>0.684</td>
<td>168.66 ± 2.867</td>
</tr>
<tr>
<td>300</td>
<td>160,156,154.8</td>
<td>156.93</td>
<td>2.722</td>
<td>1.734</td>
<td>156.93 ± 6.762</td>
</tr>
<tr>
<td>350</td>
<td>147,142,143</td>
<td>144</td>
<td>2.645</td>
<td>1.836</td>
<td>144 ± 6.571</td>
</tr>
</tbody>
</table>

From the results obtained it was noticed that the optimum Cu(II) ion concentration was 225 µg.ml⁻¹, Fig(4-1A) shows the plot of the results as it was obtained from the microphotometer. It was noticed too an increase in the absorbance of the complex with increasing Cu(II) ion concentration, while at higher concentration above 225 µg.ml⁻¹ there was a decrease in the absorbance of the complex; this might be due to the precipitation of Cu(II) ion as Cu(OH)₂ causing a decrease in the height of the response peak and its irregularities as could be seen in Fig(4-1B). There was also a delay and an increase in the width of the peak due to distraction of the flow rate by the minutes. On this basis the 225µg.ml⁻¹ was the optimum Cu(II) ion that satisfies the MTF-OH-Cu(II).
Figure (4-1-A): Variation of energy transducer response expressed as average peak height in (mV) versus concentration of Cu (II) expressed in (μg.ml⁻¹).

Figure (4-1-B): Energy transducer response concentration of Cu(II) profile.

4.1.2.1.2 Variation of sodium hydroxide solution concentration

A series of sodium hydroxide solution were prepared ranging from 0.0-50 mM using the optimum Cu(II) ion (225 μg.ml⁻¹) and 40 mM metformin using 40 μL as an injected sample volume with a flow rate 1.0ml.min⁻¹, the results shown in table(4-2) which summarizes the
absorbance obtained with the average of three successive readings, standard deviation, relative standard deviation and the confidence interval of the absorbance average at 95% confidence.

Table (4-2): Variation of sodium hydroxide concentration on the absorbance of the complex MTF-OH$^{-}$-Cu(II) formation using 40mM of MTF.

<table>
<thead>
<tr>
<th>[OH$^-$/mM</th>
<th>Absorbance $y_i$ (mV)</th>
<th>Average $\bar{y}_i$ (mV)</th>
<th>$\sigma_{n-1}$</th>
<th>RSD%</th>
<th>Confidence interval of the average $\bar{y}<em>i$ $\bar{y}<em>i \pm t</em>{0.05,n-1} \frac{\sigma</em>{n-1}}{\sqrt{n}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5</td>
<td>101.1, 101.1, 102</td>
<td>101.33</td>
<td>0.577</td>
<td>0.569</td>
<td>101.33 ± 1.433</td>
</tr>
<tr>
<td>7.5</td>
<td>130.1, 130.1, 130.4</td>
<td>130.13</td>
<td>0.230</td>
<td>0.176</td>
<td>130.13 ± 0.571</td>
</tr>
<tr>
<td>8</td>
<td>143.1, 143.1, 143</td>
<td>143</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>145.6, 145.1, 145</td>
<td>145.2</td>
<td>0.346</td>
<td>0.238</td>
<td>145.2 ± 0.859</td>
</tr>
<tr>
<td>20</td>
<td>142.8, 142.1, 142</td>
<td>142.26</td>
<td>0.461</td>
<td>0.324</td>
<td>142.8 ± 1.145</td>
</tr>
<tr>
<td>30</td>
<td>142.1, 142.1, 141.2</td>
<td>141.73</td>
<td>0.461</td>
<td>0.325</td>
<td>141.73 ± 1.145</td>
</tr>
<tr>
<td>50</td>
<td>141.1, 140.1, 140.6</td>
<td>140.53</td>
<td>0.503</td>
<td>0.357</td>
<td>140.53 ± 1.249</td>
</tr>
</tbody>
</table>

The results shows and indicates that the presence hydroxide ion is necessary for the completion of the reaction, it was proved that the elimination of the base (OH$^-$) completely from the reaction does not give any signal for the benefit of the formation of the complex(or any colored species).

It was noticed that the increase in the base concentration(OH$^-$) up to 8mM gave a regular with a sharp maxima Fig(4-2A) with a suitable peak height 143.00 mV comparing with a lower concentration of 8mM the responses were of low sensitivities(low response).This might be due to not reaching the optimum level of the best concentration to the necessary level;
while an increase on the base concentration above 8 mM, it was noticed that there were no significant differences on the height of the responses, this might be attributed to the formation of the precipitate and does not aid in the determination process as it takes a longer time to discharge and evacuate the complex or the formed precipitate in the measuring cell. On this basis 8 mM was chosen as shown in Fig(4-2B) as the optimum concentration of the base medium (OH⁻) to form the ligand the metformin with the Cu(II) ion to form colored complex.

Figure (4-2-A): Variation in energy transducer response in (mV) (peak height $n=3$) versus sodium hydroxide solution concentration.
4.1.2.2 Physical parameters

4.1.2.2.1 Flow rate

Using optimum variable of chemicals for the determination of metformin (c.f., section 4-1-2-1-1,4-1-2-1-2) with metformin concentration 40 mM with an injected sample volume of 40 µL using an allowed injection time 15 sec, the results showed in table(4-3) summarized the results obtained which include the absorbance of three successive readings and the arrival time to the measuring cell in seconds, also the table shows the variable of $\Delta_{IB}$ with flow rate.
Table (4-3): Effect of the variation of the flow rate on the absorbance of the complex formation.

<table>
<thead>
<tr>
<th>Indication approximate of flow rate</th>
<th>Flow rate (ml.min(^{-1}))</th>
<th>Absorbance (y_i) (mV)</th>
<th>Average (\overline{y}_j) (mV)</th>
<th>Arrival time to the measuring cell (sec)</th>
<th>Peak width (\Delta t_B) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>0.3</td>
<td>112,107.2,109</td>
<td>109.4</td>
<td>60</td>
<td>2.75</td>
</tr>
<tr>
<td>7</td>
<td>0.4</td>
<td>130,126.4,128</td>
<td>128.13</td>
<td>45</td>
<td>2.50</td>
</tr>
<tr>
<td>10</td>
<td>0.6</td>
<td>134,132,132</td>
<td>132.66</td>
<td>38</td>
<td>1.25</td>
</tr>
<tr>
<td>15</td>
<td>1.0</td>
<td>140,140,140</td>
<td>140</td>
<td>21</td>
<td>1.10</td>
</tr>
<tr>
<td>17</td>
<td>1.1</td>
<td>146,146,146</td>
<td>146</td>
<td>15</td>
<td>1.00</td>
</tr>
<tr>
<td>20</td>
<td>1.3</td>
<td>132,130.4,130.4</td>
<td>130.93</td>
<td>9</td>
<td>0.85</td>
</tr>
<tr>
<td>25</td>
<td>1.7</td>
<td>108,106,106</td>
<td>106.66</td>
<td>8</td>
<td>0.50</td>
</tr>
</tbody>
</table>

From the results obtained above, it was noticed that an increase in the absorbance with increase of flow rate reaching up to 1.1 ml.min\(^{-1}\), this might be attributed that the physical variable has really no such significant effect, important must of it the dilution of the sample segment due to dispersion region surrounding the central part of the segment and the central dispersion due to diffusion and convection. It was noticed that a flow rate above 1.1 ml.min\(^{-1}\) that a decrease in the absorbance or responses with an increase of the flow rate in spite of obtaining sharp maxima this might be due to incompletion of the reaction to form the complex, or the unavailability of enough time for the absorbance measurement before its departure of the measuring cell at a short time as shown in Fig(4-3A). In addition that the time of the arrival of the sample segment to the measuring cell decrease with the increase of the flow rate as shown in Fig(4-3B). Also in the same time there is a decrease in the width of the response (\(\Delta t_B\)) as shown in Fig(4-3C). On this basis the flow rate for the three lines were
chosen as 1.1 ml.min\(^{-1}\), while the arrival time necessary to reach the measuring cell is 15 sec. with a base width of the response of the 1.00 min, on this basis a regular response with a sharp maximum (undistracted) peaks as shown in Fig.(4-3D).

**Figure (4-3):** Variation in flow rate against:

- A- Energy transducer response expressed as peak height in mV.
- B- Arrival time of sample segment to the measuring cell.

**Figure (4-3C):** Decrease of $\Delta t_B$ (response base width) with the increase of flow rate.
Figure (4-3-D): Effect of the variation of flow rate on energy transducer response for the MTF-OH\textsuperscript{-}Cu(II).

### 4.1.2.2.2 Injected sample volume

Using optimum parameters for the reaction MTF-OH\textsuperscript{-}Cu(II) as it was mentioned in section (4-1-2-1-1,4-1-2-1-2), the metformin concentration 40mM with a variable sample loop volume ranging from 10-80µL, table(4-4) shows the injected sample volume, each was repeated three time, the average, Δ\text{IB} and the time necessary for the sample segment to reach the measuring cell.

Table (4-4): Effect of the variation of injected sample volume on the Absorbance at a selected Metformin concentration of 40mM.

<table>
<thead>
<tr>
<th>Injected sample volume (µL)</th>
<th>Absorbance $y_i$ (mV)</th>
<th>Average $\bar{y}_i$ (mV)</th>
<th>$\Delta$IB (min)</th>
<th>Arrival time to the measuring cell (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>72.84, 84</td>
<td>80</td>
<td>0.6</td>
<td>6</td>
</tr>
<tr>
<td>20</td>
<td>106, 106, 107</td>
<td>106.33</td>
<td>0.75</td>
<td>9</td>
</tr>
<tr>
<td>30</td>
<td>130, 130, 130.4</td>
<td>130.13</td>
<td>0.85</td>
<td>12</td>
</tr>
<tr>
<td>40</td>
<td>145.6, 145.6, 145.6</td>
<td>145.6</td>
<td>1.0</td>
<td>15</td>
</tr>
<tr>
<td>50</td>
<td>148, 148, 148</td>
<td>148</td>
<td>1.5</td>
<td>18</td>
</tr>
<tr>
<td>80</td>
<td>150, 150, 150</td>
<td>150</td>
<td>1.75</td>
<td>28</td>
</tr>
</tbody>
</table>
From the results obtained above, it can be noticed the increase in the response height and an increase in its base width with an increase with the injected sample volume. The increase in the response height at a volume larger than 40 µL has not significant differences as in Fig(4-4A) and for the purpose to compromise with the height of the response and adjustment in the cost in the consumption of the chemicals; 40µL was chosen as the best injected sample volume whereby a smooth profile with a sample maxima with ΔtB of 1.0 min and an arrival time of the sample segment to the measuring cell of 15 sec as shown in Fig(4-4B).

Figure (4-4-A): Variation of injected sample volume on energy transducer response expressed in (mV).
4.1.2.2.3 Allowed permissible time

Allowed permissible time for the sample to be injected via the carrier stream was studied and its effect on the response and its sensitivities was followed using the optimum parameters of chemicals (4,8,11,13,20,24,28,31,35) seconds were used for this study. The obtained result was tabulated in table (4-5), were the average successive measurement expressed in mV, the sample standard deviation, relative standard deviation and the confidence interval of the response average at 95% confidence. It can be seen from the table that there is increase in the response with increasing the allowed permissible time for the sample to injected up to 20 sec, followed by a constancy in the response as in Fig (4-5), with an increase in the width of the base of the response; it can be inference that an increase in the injection time above 20 sec causes to the distraction of the flow as a result of elongated of period of leaving of injected sample volume in the valve in the "Injection" mode which leads to the slow movement of the colored
segment in the measuring flow cell for a longer period of time, causing to have a suitable peak height and a regular profile of the response.

Table (4-5): Effect of allowed permissible time on the response MTF. complex using 40µL of sample volume.

<table>
<thead>
<tr>
<th>Allowed permissible time for sample volume on carrier stream (sec.)</th>
<th>Absorbance (mV)</th>
<th>Average (mV)</th>
<th>σ&lt;sub&gt;n-1&lt;/sub&gt;</th>
<th>RSD%</th>
<th>Confidence interval of the average $\bar{Y}<em>i$ ± 0.05 $\frac{\sigma</em>{n-1}}{\sqrt{n}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>56.8,56.55.2</td>
<td>56</td>
<td>0.80</td>
<td>1.42</td>
<td>56 ± 1.98</td>
</tr>
<tr>
<td>8</td>
<td>111,112.35,112.7</td>
<td>112</td>
<td>0.89</td>
<td>0.79</td>
<td>112 ± 2.21</td>
</tr>
<tr>
<td>11</td>
<td>119.6,119.4,121</td>
<td>120</td>
<td>0.87</td>
<td>0.72</td>
<td>120 ± 2.16</td>
</tr>
<tr>
<td>15</td>
<td>145.145.6,145.4</td>
<td>145.33</td>
<td>0.30</td>
<td>0.20</td>
<td>145.33 ± 0.74</td>
</tr>
<tr>
<td>20</td>
<td>148,148.4,148.4</td>
<td>148.26</td>
<td>0.23</td>
<td>0.15</td>
<td>148.26 ± 0.57</td>
</tr>
<tr>
<td>24</td>
<td>146.4,146.146</td>
<td>146.13</td>
<td>0.23</td>
<td>0.15</td>
<td>146.13 ± 0.57</td>
</tr>
<tr>
<td>28</td>
<td>144.4,144.4,143.2</td>
<td>144</td>
<td>0.69</td>
<td>0.47</td>
<td>144 ± 1.71</td>
</tr>
<tr>
<td>31</td>
<td>142.6,144.4,142</td>
<td>143</td>
<td>1.24</td>
<td>0.86</td>
<td>143 ± 3.08</td>
</tr>
<tr>
<td>35</td>
<td>143.5,144,140</td>
<td>142.5</td>
<td>2.17</td>
<td>1.52</td>
<td>142.5 ± 5.39</td>
</tr>
</tbody>
</table>

Figure (4-5): Variation of energy transducer response expressed as an average peak height in (mV) versus time of injection for sample with (40µL).
4.1.2.3 Study of the variation of Metformin concentration on the response of the complex formed

A serious of metformin solution having the concentration of 0.0-200mM using 40 µL as an injected sample volume with all the chemical and physical variable fixed at its optimum parameters, table (4-6A) tabulate all the results obtained including the average of three successive measurements, standard sample deviation, percentage relative standard sample deviation, confidence interval of the response and the estimated value ($\hat{y}_i$) of the response obtained from linear regression analysis carried out for the data obtained. Using simple linear robustic equation of degree one at 95% confidence interval as shown in Fig(4-6), which shows a correlation coefficient(C.C), coefficient of determination(C.O.D) and the percentage linearity; all these results of the linear regression analysis was summarized in table(4-6B).

![Figure (4-6): Linear calibration graph for the energy transducer response with metformin concentration expressed in mM.](image)

\[ \hat{y}_i = 52.70 \pm 6.80 + 1.92 \pm 0.13 \text{[MTF] mM} \]
\[ \% r^2 = 94.05 \]
Table (4-6A): Variation of the concentration of metformin versus energy transducer response in (mV) of the complex formation.

<table>
<thead>
<tr>
<th>[MTF] $x_i$ (mM)</th>
<th>Absorbance $y_i$ (mV)</th>
<th>Average $\bar{y}_i$ (mV)</th>
<th>$\sigma_{n-1}$</th>
<th>RSD %</th>
<th>Confidence interval of the average $\bar{y}<em>i$ $\bar{y}<em>i \pm t</em>{0.05,n-1} \sigma</em>{n-1} \sqrt{\frac{1}{n}}$</th>
<th>Predict response $\hat{y}_i$ (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>52.70</td>
</tr>
<tr>
<td>0.9</td>
<td>50.4,50.49.6</td>
<td>50</td>
<td>0.40</td>
<td>0.80</td>
<td>50 ± 0.99</td>
<td>54.43</td>
</tr>
<tr>
<td>3</td>
<td>66.8,65.6,65.6</td>
<td>66</td>
<td>0.69</td>
<td>1.04</td>
<td>66 ± 1.71</td>
<td>58.77</td>
</tr>
<tr>
<td>5</td>
<td>70.2,70.2,69.6</td>
<td>70</td>
<td>0.34</td>
<td>0.48</td>
<td>70 ± 0.84</td>
<td>62.32</td>
</tr>
<tr>
<td>8</td>
<td>75,75.3,74.7</td>
<td>75</td>
<td>0.30</td>
<td>0.40</td>
<td>75 ± 0.74</td>
<td>68.09</td>
</tr>
<tr>
<td>10</td>
<td>81.8,82.82.2</td>
<td>82</td>
<td>0.20</td>
<td>0.24</td>
<td>82 ± 0.49</td>
<td>71.93</td>
</tr>
<tr>
<td>20</td>
<td>96, 96, 96</td>
<td>96</td>
<td>0.0</td>
<td>0.0</td>
<td>96 ± 0.0</td>
<td>91.17</td>
</tr>
<tr>
<td>30</td>
<td>132.4,132,131.6</td>
<td>132</td>
<td>0.40</td>
<td>0.30</td>
<td>132 ± 0.99</td>
<td>110.40</td>
</tr>
<tr>
<td>40</td>
<td>148.5,148,147.5</td>
<td>148</td>
<td>0.50</td>
<td>0.33</td>
<td>148 ± 1.28</td>
<td>129.64</td>
</tr>
<tr>
<td>50</td>
<td>152, 152, 152</td>
<td>152</td>
<td>0.0</td>
<td>0.0</td>
<td>152 ± 0.0</td>
<td>148.87</td>
</tr>
<tr>
<td>60</td>
<td>164.44,164,163.56</td>
<td>164</td>
<td>0.44</td>
<td>0.26</td>
<td>164 ± 1.09</td>
<td>168.11</td>
</tr>
<tr>
<td>70</td>
<td>182,180.8,180.2</td>
<td>181</td>
<td>0.91</td>
<td>0.50</td>
<td>181 ± 2.26</td>
<td>187.34</td>
</tr>
<tr>
<td>80</td>
<td>205, 205, 205</td>
<td>205</td>
<td>0.0</td>
<td>0.0</td>
<td>205 ± 0.0</td>
<td>206.58</td>
</tr>
<tr>
<td>90</td>
<td>220.2,220,219.8</td>
<td>220</td>
<td>0.02</td>
<td>0.09</td>
<td>220 ± 0.49</td>
<td>225.81</td>
</tr>
<tr>
<td>100</td>
<td>239.8,240.4,239.8</td>
<td>240</td>
<td>0.34</td>
<td>0.14</td>
<td>240 ± 0.84</td>
<td>245.05</td>
</tr>
<tr>
<td>120</td>
<td>120.8,120,119.2</td>
<td>120</td>
<td>0.80</td>
<td>0.66</td>
<td>120 ± 1.98</td>
<td>—</td>
</tr>
<tr>
<td>140</td>
<td>113.4,111,111.6</td>
<td>112</td>
<td>1.24</td>
<td>1.10</td>
<td>112 ± 3.08</td>
<td>—</td>
</tr>
<tr>
<td>160</td>
<td>96,97,95</td>
<td>96</td>
<td>1.00</td>
<td>1.04</td>
<td>96 ± 2.48</td>
<td>—</td>
</tr>
<tr>
<td>180</td>
<td>120.6,121,118.4</td>
<td>120</td>
<td>1.40</td>
<td>1.16</td>
<td>120 ± 3.47</td>
<td>—</td>
</tr>
<tr>
<td>200</td>
<td>122,119,119</td>
<td>120</td>
<td>1.73</td>
<td>1.44</td>
<td>120 ± 4.29</td>
<td>—</td>
</tr>
</tbody>
</table>
Table (4-6B): Summary of linear regression for the variation of signal response with metformin concentration using first degree equation of known form $y=a+bx$

<table>
<thead>
<tr>
<th>Concentration mM</th>
<th>Linear range mM</th>
<th>Straight line equation $\hat{y}<em>{m} = a\pm s</em>{a} + b\pm s_{b}[x]$</th>
<th>Correlation coefficient ($r$)</th>
<th>Percentage linearity ($r^{2}%$)</th>
<th>calculated $t$-value</th>
<th>$t$-value tabulated at 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.0-200)</td>
<td>(0.0-100)</td>
<td>$\hat{y}=52.70\pm6.80+1.92\pm0.13[x]\text{mM}$</td>
<td>0.9697</td>
<td>94.05</td>
<td>14.33</td>
<td>2.16</td>
</tr>
</tbody>
</table>

$n^{*}=15$

[x]=Concentration of drug in mM.
y= Energy transducer response expressed as average peak height in (mV).
$\hat{y}$=Predict of energy transducer response expressed as average peak height of the linear Robustic equation of the form: $y=a+bx$.

4.1.2.3.1 Limit of Detection (L.O.D)

In general, the limit of detection of analysis may be described as that concentration which gives an instrument signal ($y$) significantly different from the "Blank" or "Background" signal.

A commonly used definition in the literature in analytical chemistry is that the limit of detection is the analyte concentration giving a signal equal to blank signal, $(y_{B})$, plus three standard deviations of the blank, $(S_{B})^{(200)}$.

$$y-y_{B}=3S_{B}$$

Using successive gradual dilution of the minimum concentration of metformin drug that was used in the calibration graph which was 0.9 mM a limit of detection of 662 ng/injected sample volume as is shown in Fig (4-7).
CHAPTER FOUR

RESULTS and DISCUSSIONS

Figure (4-7): Limit of detection for determination of metformin using MTF-OH\textsuperscript{-}Cu(II).

4.1.2.3.2 Repeatability

To study the reality of the measurement and its repeatability where at a constant fixed concentration of metformin drug is injected using the optimum parameters whether its chemical or physical and its coupling with flow injection analysis technique. A repeated measurement of 10,20,50 mM for six successive measurements where measured and the obtained result is tabulated in table(4-7) which shows that the percentage relative standard deviation was less than 2%, while Fig(4-8) shows a kind of response and the profile for the used concentrations.

Table (4-7): The repeatability for the response obtained for the formation of MTF-OH\textsuperscript{-}Cu(II) complex at the optimum reaction for the used reactants.

<table>
<thead>
<tr>
<th>[MTF] mM</th>
<th>Number of injection (n)</th>
<th>Average response y\textsubscript{i} (mV)</th>
<th>σ\textsubscript{n-1}</th>
<th>RSD%</th>
<th>Time for six measurements (min)</th>
<th>( \bar{y}\textsubscript{i} \pm t\textsubscript{0.05,n-1} \frac{\sigma\textsubscript{n-1}}{\sqrt{n}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>6</td>
<td>82</td>
<td>1.28</td>
<td>1.56</td>
<td>13.5</td>
<td>82 \pm 2.25</td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>96</td>
<td>1.13</td>
<td>1.17</td>
<td>13.8</td>
<td>96 \pm 2.06</td>
</tr>
<tr>
<td>50</td>
<td>6</td>
<td>152</td>
<td>1.36</td>
<td>0.89</td>
<td>14.4</td>
<td>152 \pm 2.39</td>
</tr>
</tbody>
</table>
4.1.2.3.3 Application

The used microphotometer throughout this work was put into a test for its efficiency of the measurement in three different metformin drug in three different pharmaceutical preparation from different origin of supplier. Thirteen tablets from each pharmaceutical drug; each tablets was weight and an average of the tablets weight, standard deviation was measured; these tablets were crushed, grinded then was dissolved what was equivalent 30 mM in tiny amount of distilled water then was filtered on a washed filtered paper in order to get rid off the insoluble material what were exist; the residue was washed with distilled water and the volume was completed with 100 ml in volumetric flask.

Metformin in each pharmaceutical drug was determined using Continuous Flow Injection Analysis (C.F.I.A) and the result was expressed in a direct calibration graph, Fig(4-6) and table(4-8) summarizes the results.
obtained at confidence 95% successively and it was noticed that the recovery ranges from 89.17 to 96.66 %.

Table (4-8): Determination of metformin at different manufactures of pharmaceutical drugs in direct calibration graph by using MTF-OH-Cu(II) system.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Pharmaceutical tablet content &amp; manufactures</th>
<th>Confidence interval of concentration of tablets for $n=\infty$ of tablets at 95% $\overline{\mu} \pm 1.96 \frac{sn-1}{\sqrt{n}}$ &amp; confidence interval at 99% $\overline{\mu} \pm 2.58 \frac{sn-1}{\sqrt{n}}$</th>
<th>Confidence interval of concentration of active material for $n=\infty$ of tablets at 95% $\overline{\mu} \pm 1.96 \frac{sn-1}{\sqrt{n}}$ &amp; confidence interval at 99% $\overline{\mu} \pm 2.58 \frac{sn-1}{\sqrt{n}}$</th>
<th>Weight sample equivalent to 30 mM (g)</th>
<th>Average response (mV)</th>
<th>Practical concentration 30mM</th>
<th>Practical weight (mg)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Glyciphage/ Medical Bahri company (Syria) 500 mg</td>
<td>0.75±0.0051 0.751± 0.0068</td>
<td>500±3.39 500±4.52</td>
<td>0.7463</td>
<td>103.20</td>
<td>26.75</td>
<td>443.06</td>
<td>89.17</td>
</tr>
<tr>
<td>2</td>
<td>Metforal/ Laboratori Guidotti S.P.A. (Italy) 500mg</td>
<td>0.5508±0.0012 0.5508±0.0016</td>
<td>500±1.089 500±1.452</td>
<td>0.5473</td>
<td>104.00</td>
<td>27.5</td>
<td>455.48</td>
<td>91.67</td>
</tr>
<tr>
<td>3</td>
<td>Glycophade/ merk (France) 500mg</td>
<td>0.5289±0.0038 0.5289±0.0051</td>
<td>500±3.592 500±4.821</td>
<td>0.5256</td>
<td>108.00</td>
<td>29.00</td>
<td>480.32</td>
<td>96.66</td>
</tr>
</tbody>
</table>

Since the active metformin material is mixed through the process of tablet formation by the addition of additives that might the stability of the drug until the absorbed of the body and since the know how is secret of the manufactures, therefore this leads us to use the standard addition method, which might overcome the difficulty of having different matrices; table (4-9)
tabulated the results obtained which shows percentage recovery of 90.02 up to 101.49, while Fig(4-9) shows calibration graph using this procedure.

![Figure (4-9): Standard addition method for the determination of metformin in pharmaceutical preparation.](image)

**Table (4-9): Determination of Metformin in different pharmaceutical drugs by the standard addition curve for the complex MTF-OH-Cu(II) formation.**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Weight sample equivalent to 10 mM</th>
<th>Practical concentration from addition (mM)</th>
<th>Practical weight (Theoretical weight 500mg)</th>
<th>Intercept (a) &amp; Slope (b)</th>
<th>Correlation coefficient (r)</th>
<th>Percentage linearity (%r)</th>
<th>Calculated t-value</th>
<th>T-value at 95% confidence interval</th>
<th>Tabulated t-value</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.2487</td>
<td>9.00</td>
<td>450.11</td>
<td>(49.68) 1.14</td>
<td>0.9974 (99.49)</td>
<td></td>
<td>24.25</td>
<td></td>
<td></td>
<td>90.02</td>
</tr>
<tr>
<td>2</td>
<td>0.1824</td>
<td>9.50</td>
<td>475.15</td>
<td>(52.32) 1.25</td>
<td>0.9977 (99.54)</td>
<td></td>
<td>25.54</td>
<td>3.18&lt;&lt;</td>
<td></td>
<td>95.03</td>
</tr>
<tr>
<td>3</td>
<td>0.1752</td>
<td>10.15</td>
<td>507.49</td>
<td>(53.76) 1.29</td>
<td>0.9989 (99.79)</td>
<td></td>
<td>38.53</td>
<td></td>
<td></td>
<td>101.49</td>
</tr>
</tbody>
</table>

n = 5 (number of measurements)
Metformin.HCl was determined by the conventional classical spectrophotometric measurement, whereby a series of solution ranging from 0.9-380 mM, using the same experimental conditions that were used for newly developed method for the formation of the colored (pink) complex; the absorbance was measured for the successive measurements at 530 nm, it can be seen from table(4-10) and Fig(4-10) extended of linearity of linear regression equation as percentage linearity at 97.48%, table(4-10) also shows practically measured concentration in the three pharmaceutical drugs.

**Table(4-10): Determination of Metformin in different pharmaceutical drugs using the conventional method.**

<table>
<thead>
<tr>
<th>Concentration range (mM)</th>
<th>Linear range (mM)</th>
<th>$\hat{y}=a\pm s_a t+b\pm s_b t[x]$</th>
<th>Correlation coefficient $r$</th>
<th>Percentage linearity ($r^2$)</th>
<th>Calculated $t$-value</th>
<th>Tabulated $t$-value at 95% confidence interval</th>
<th>Sample number</th>
<th>Found value (mM)</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9-380</td>
<td>5-240</td>
<td>$\hat{y}=0.088\pm 0.0056+0.00087\pm 4.94\times 10^{-5}[x]$ mM</td>
<td>0.98731</td>
<td>97.48</td>
<td>17.61</td>
<td>2.306</td>
<td>1</td>
<td>26.5</td>
<td>88.33</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>27.0</td>
<td>90.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3</td>
<td>28.5</td>
<td>95.00</td>
</tr>
</tbody>
</table>

$n^* =10$ (number of measurements)
Two methods were used for the determination of metformin, the first one was based on spectrophotometer; using homemade microphotometer and the classical spectrophotometric at 530 nm. Using statistical chemometric treatment table (4-11) was obtained and the results of both methods were tested using paired t-test.

Table (4-11): Paired t-test for the comparison of the spectrophotometric method with continuous flow injection method of analysis adopted through this work.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Practical content (mg)</th>
<th>(d) (mg)</th>
<th>$\bar{x}_d$</th>
<th>$\sigma_{n-1}$</th>
<th>Paired t-test</th>
<th>Tabulated t-value at 95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UV-Vis</td>
<td>SP. - FIA</td>
<td></td>
<td></td>
<td>$\frac{\bar{x}_d}{\sqrt{n}}$</td>
<td>$\frac{\bar{x}_d}{\sqrt{n-1}}$</td>
</tr>
<tr>
<td>1</td>
<td>441.67</td>
<td>443.06</td>
<td>1.39</td>
<td></td>
<td>4.04</td>
<td>2.30</td>
</tr>
<tr>
<td>2</td>
<td>450.05</td>
<td>455.48</td>
<td>5.43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>475.00</td>
<td>480.32</td>
<td>5.32</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$n^* =$ number of samples
The final conclusion in table (4-11) shows that there are no significant differences between the newly developed method and the conventional traditional spectrophotometric method at confidence interval 95%, as the calculated value is less than the tabulated value; therefore the newly developed method can be regarded or used as an alternative method for the determination of metformin. Also the speed of analysis, small consumption chemicals and higher sensitivity which lead the method to follow the small concentration pattern.
4.2 Synthesis and characterization of mixed ligand complexes

4.2.1 Physical properties and elemental analysis of the prepared mixed ligand complexes

The elemental analysis (C.H.N.S) and metal determination data of the prepared mixed complexes as shown in table (4-12) were exhibited the formation of (1:1:1) (M:MTF:Cys) ratio; it was found that the calculated values are in a good agreement with the found values. The prepared complexes were found to be insoluble in most common organic solvents such as methanol, ethanol, chloroform and acetone but they were soluble in DMF and DMSO. Physical properties of free ligands and their prepared complexes are given in table (4-12).

Table (4-12): Physical properties of the free ligands and their metal complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Colour</th>
<th>Melting point °C</th>
<th>Yield %</th>
<th>Elemental Analysis Found % (calculated)</th>
<th>Metal found % (calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>C%</td>
<td>H%</td>
</tr>
<tr>
<td>MTF.HCl</td>
<td>White</td>
<td>222-226</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L-Cys</td>
<td>White</td>
<td>240dec.</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu(II) complex</td>
<td>Olive green</td>
<td>268dec.</td>
<td>74</td>
<td>24.35 (24.18)</td>
<td>5.83 (5.75)</td>
</tr>
<tr>
<td>Ni(II) complex</td>
<td>Light orange</td>
<td>285dec.</td>
<td>72</td>
<td>24.34 (24.52)</td>
<td>6.19 (6.10)</td>
</tr>
<tr>
<td>Co(II) complex</td>
<td>Light olive</td>
<td>262dec.</td>
<td>56</td>
<td>23.22 (23.30)</td>
<td>5.79 (5.83)</td>
</tr>
</tbody>
</table>
4.2.2 NMR spectra

$^1H$ and $^{13}C$ -NMR spectra of free L-cysteine, free metformin.HCl and their mixed complexes were identified with the help of literature data.

4.2.2.1 $^1H$-NMR spectra of free L-cysteine, free metformin.HCl and their mixed ligand complexes with Cu(II), Ni(II) and Co(II)

$^1H$-NMR assignments for free ligands and their mixed complexes in DMSO are summarized in table(4-13).

In free L-cysteine, signals appeared at 2.85 ppm and 3.12 ppm were attributed to CH($\alpha$) and CH$_2$(β) protons respectively$^{(201)}$; the signal in $^1$HNMR spectra of all complexes at (2.79-2.81 ppm) is assigned to -CH$_2$- and -CH- protons of coordinated cysteine$^{(201,202)}$, as shown in Figs(4-11,12,13) and a large downfield shift (4.6-4.76 ppm) of NH$_2$ proton as compared to its value 6.1ppm in corresponding amino acid in the zwitterionic form$^{(203,204)}$, this suggests that cysteine is bound to Cu(II), Ni(II) and Co(II) in their complexes through amino group. While in the case of free metformin.HCl, the combined signal was observed at 6.69 ppm due to the NH$_2$ and NH protons$^{(85,205)}$; which were shifted to downfield in the $^1$H-NMR spectra of mixed ligand complexes$^{(85,87,206)}$. Another signal assignable to the methyl protons at 3.3 ppm in the metal complexes spectra (that appears in 2.92ppm in free metformin)$^{(85,205)}$; whereas the peak of ligand assignable to imine protons were shifted from 7.15 ppm$^{(87)}$ to (9.18-9.30ppm) in the metal complexes spectra, indicating their coordination through N$^2$ and N$^4$ atoms$^{(206)}$. 
Table (4-13): $^1$H-NMR chemical shifts $\delta$(ppm) of free L-cysteine, free metformin.HCl and their mixed ligand complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shift of L-Cys $\delta$(ppm)</th>
<th>Chemical shift of MTF.HCl $\delta$(ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH(α)</td>
<td>CH$_2$(β)</td>
</tr>
<tr>
<td>L-Cys</td>
<td>2.85</td>
<td>3.12</td>
</tr>
<tr>
<td>MTF.HCl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu(II) complex</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ni(II) complex</td>
<td>2.7</td>
<td>-</td>
</tr>
<tr>
<td>Co(II) complex</td>
<td>2.8</td>
<td>-</td>
</tr>
</tbody>
</table>

4.2.2.2 $^{13}$C-NMR spectra of free L-cysteine, free metformin.HCl and their mixed ligand complexes with Cu(II), Ni(II) and Co(II)

The $^{13}$C-NMR spectra of these mixed complexes are shown in Figs (4-14, 15, 16) and the assignments of the main signals in the $^{13}$C-NMR of free L-cysteine, free metformin.HCl and their mixed ligand complexes with metal ions (Cu(II), Ni(II) and Co(II)) in DMSO are tabulated in table (4-14).

In L-cysteine, CH$_2$(α) and CH(β) resonances were shifted to higher values in the mixed ligand complexes, comparing with these in the free ligand (56.50, 26.12 ppm for CH$_2$(α) and CH(β) respectively); confirming the involvement of thiol sulphur and amino nitrogen atoms in metal ion coordination$^{(201,202,207)}$. The signal due to cysteine-COO$^-$ group did not undergo significant chemical shift, because it is not involved in coordinating with the metal cations (Cu(II), Ni(II) and Co(II))$^{(201)}$.

The signals of imine carbon atoms were shifted downfield by (157.8, 157.41 ppm) for all mixed complexes towards the corresponding signals of free metformin.HCl, indicating the participation of their in chelation$^{(87,205)}$, while primary carbon atoms peaks were observed at (38.90-40.80 ppm) in
all mixed complexes spectra, which are assigned at (37.42 ppm) in the free ligand\textsuperscript{(87)}.

**Table (4-14):** $^{13}$C-NMR chemical shifts $\delta$(ppm) of free L-cysteine, free metformin.HCl and their mixed ligand complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Chemical shift of L-Cys $\delta$(ppm)</th>
<th>Chemical shift of MTF.HCl $\delta$(ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CH($\alpha$)</td>
<td>CH$_2$($\beta$)</td>
</tr>
<tr>
<td>L-Cys</td>
<td>56.50</td>
<td>26.12</td>
</tr>
<tr>
<td>MTF.HCl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cu(II)complex</td>
<td>57.42</td>
<td>30.95</td>
</tr>
<tr>
<td>Ni(II)complex</td>
<td>57.21</td>
<td>31.15</td>
</tr>
<tr>
<td>Co(II)complex</td>
<td>57.37</td>
<td>31.20</td>
</tr>
</tbody>
</table>

**Fig (4-11):** $^1$H-NMR spectrum of Cu(II) mixed ligand complex.
Fig(4-12): $^1$H-NMR spectrum of Ni(II) mixed ligand complex.

Fig(4-13): $^1$H-NMR spectrum of Co(II) mixed ligand complex.
Fig(4-14): $^{13}$C-NMR spectrum of Cu(II) mixed ligand complex.

Fig(4-15): $^{13}$C-NMR spectrum of Ni(II) mixed ligand complex.
4.2.3 Infrared spectra

4.2.3.1 Infrared spectrum of free L-cysteine

The infrared spectrum of L-cysteine (zwitterionic form) Fig(4-17), table(4-14) exhibited significant features, the peaks at 3456 cm\(^{-1}\) and 3176 cm\(^{-1}\) were assigned to (NH) stretching in protonated amine (NH\(_3^+\)) of L-cysteine\(^{(208,209)}\), while the peaks at 2966 cm\(^{-1}\) and 694 cm\(^{-1}\) were assigned to \(\nu(CH)\) and \(\nu(C-S)\) respectively\(^{(209,210,211)}\). The asymmetric and symmetric vibration (COO) group were observed at 1589 cm\(^{-1}\) and 1392 cm\(^{-1}\) respectively; the peaks due to amino twisting, rocking and carboxylate wagging frequencies were observed in the range(1200-600) cm\(^{-1}\)\(^{(202,207)}\). An intense absorption band was observed at 2550 cm\(^{-1}\), which was assigned to the (SH) stretching mode\(^{(209,211,212)}\).
4.2.3.2 Infrared Spectrum of free metformin.HCl

The infrared spectrum of metformin.HCl Fig(4-18), table(4-15) exhibited an intense absorption bands in the range (3370-3170) cm\(^{-1}\) assignable to stretching vibration of (NH) groups\(^{85,89,213}\). The broadness of the bands is probable due to overlapping of these vibrations with the ones corresponding to the inter or intramolecular hydrogen bonds \(^{89,214}\). The strong bands were observed at 1627,1581 cm\(^{-1}\) which are due to \(\nu(C=\text{N})\) and band at 1570 cm\(^{-1}\) have been assigned for (NH) deformation\(^{52,87,89,213}\).

4.2.3.3 Infrared spectra of mixed ligand complexes with Cu(II), Ni(II) and Co(II).

The spectra of all mixed complexes are shown in Figs(4-19,20,21) and assignments of the frequencies of the vibrational bands (cm\(^{-1}\)) are given in table (4-14).

As regards the chelation of L-cysteine, a new band was observed at 3210 cm\(^{-1}\), 3209 cm\(^{-1}\) and 3207 cm\(^{-1}\) due to stretching vibration of (NH\(_2\)) in the mixed complexes of Cu(II), Ni(II) and Co(II) respectively\(^{202,209,212}\), indicates the coordination through the nitrogen of amino group; an additional evidence of such coordination is the fact that the band commonly observable in the range(2000-2100 cm\(^{-1}\)) does not appear in the spectra of the mixed complexes, this band would be characteristic of a free nitrogen atom of amino acid\(^{210,215}\). No shift group was observed in the asymmetric and symmetric stretching vibration of (COO\(^{-}\)) group of L-cysteine, this supports the non-involved of cysteine-(COO\(^{-}\)) group in the metal coordination\(^{202}\).

The peak due to \(\nu(S-H)\) is lost in the spectra of mixed ligand complexes, due to deprotonation of the (S-H) group on binding with metal\(^{201,202,207,216}\); in addition the corresponding vibration \(\nu(C-S)\) in all mixed complexes was shifted to lower frequencies, also indicating coordination of L-cysteine through the sulphur atom\(^{201,211}\).
Other new (low intensity) bands were observed in the ranges (375-400cm\(^{-1}\)), (445-478 cm\(^{-1}\)) due to \(\nu(M-S)\) and \(\nu(M-N)\) stretching vibration respectively\(^{202,207,217}\).

Whereas in the case of metformin.HCl ligand, the bands due to \(\nu_{\text{asy}}(\text{NH}_2)\) and \(\nu_{\text{sy}}(\text{NH}_2)\) are shifted to higher frequencies comparative with the free ligand one\(^{84,86,213}\). The new strong bands in the range (1685-1650 cm\(^{-1}\)) are assigned to the coordinated imino groups\(^{52,88}\). The formation of a chelate ring is supported by the appearance of a new band at (1365-1200 cm\(^{-1}\)) assigned to ring vibration\(^{85,87,213}\).

The low intensity bands was observed in the range(435-420cm\(^{-1}\)), which are assigned to \(\nu(M-N)\) stretching \(^{85,86}\), and all mixed complexes also show other bands in the range(3550-3350cm\(^{-1}\))region attributed to coordinated or lattice water\(^{89}\).

Table(4-15): Characteristic IR stretching vibration(cm\(^{-1}\)) of free ligands and their mixed ligand complexes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>L -Cys</th>
<th>MTF</th>
<th>Cu(II) complex</th>
<th>Ni(II) complex</th>
<th>Co(II) complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Cys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\nu(N-H))</td>
<td>3456</td>
<td>3176</td>
<td>3210</td>
<td>3209</td>
<td>3207</td>
</tr>
<tr>
<td>(\nu_{\text{asy}}(\text{COO}^-))</td>
<td>1589</td>
<td></td>
<td>1616</td>
<td>1610</td>
<td>1610</td>
</tr>
<tr>
<td>(\nu_{\text{sy}}(\text{COO}^-))</td>
<td>1392</td>
<td></td>
<td>1396</td>
<td>1409</td>
<td>1398</td>
</tr>
<tr>
<td>(\nu(C-S))</td>
<td>694</td>
<td></td>
<td>400</td>
<td>385</td>
<td>375</td>
</tr>
<tr>
<td>(\nu(M-N))</td>
<td></td>
<td></td>
<td>475</td>
<td>478</td>
<td>445</td>
</tr>
<tr>
<td>(\nu(M-S))</td>
<td></td>
<td></td>
<td>675</td>
<td>665</td>
<td>676</td>
</tr>
<tr>
<td>(\nu(S-H))</td>
<td>2550</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTF.HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\nu_{\text{asy}}(\text{NH}_2))</td>
<td></td>
<td></td>
<td>3371</td>
<td>3463</td>
<td>3429</td>
</tr>
<tr>
<td>(\nu_{\text{sy}}(\text{NH}_2))</td>
<td></td>
<td></td>
<td>3170</td>
<td>3266</td>
<td>3271</td>
</tr>
<tr>
<td>(\nu(\text{NH}))</td>
<td></td>
<td></td>
<td>3321</td>
<td>3392</td>
<td>3371</td>
</tr>
<tr>
<td>(\nu(M-N))</td>
<td></td>
<td></td>
<td>3294</td>
<td>3348</td>
<td>3344</td>
</tr>
<tr>
<td>(\nu(\text{C}=\text{N}))</td>
<td></td>
<td></td>
<td></td>
<td>435</td>
<td>438</td>
</tr>
<tr>
<td>(\nu_{\text{chlate ring}})</td>
<td></td>
<td></td>
<td></td>
<td>1627</td>
<td>1685</td>
</tr>
<tr>
<td>H(_2)O Lattice</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(coordinate)</td>
<td></td>
<td></td>
<td>(3550,705,914)</td>
<td>3350</td>
<td>(3525,723,887)</td>
</tr>
</tbody>
</table>
Fig(4-17): FT-IR spectrum of L-Cysteine.
Fig(4-18): FT-IR spectrum of Metformin.HCl.
Fig(4-19): FT-IR spectrum of Cu(II) mixed ligand complex.
Fig(4-20): FT-IR spectrum of Ni(II) mixed ligand complex.
Fig(4-21): FT-IR spectrum of Co(II) mixed ligand complex.
4.2.4. Magnetic susceptibility measurements

Magnetic measurements are widely used in studying transition metal complexes\(^{(218)}\). The magnetic properties are due to the presence of unpaired electrons in the partially filled d-orbital in the outer shell of these elements. These magnetic measurements give an idea about the electronic state of the metal ion in the complex.

The magnetic moment is given by the following equation:

$$\mu_{s+L} = \sqrt{4s(s + 1) + L(L + 1)} \text{ B.M}$$

\(\mu\) = Magnetic moment
\(S\) = Spin quantum number
\(L\) = Orbital quantum number

Although detail determination of the electronic structure requires consideration of the orbital moment, for most complexes of the first transition series, the spin only moment is sufficient, as any orbital contribution is small\(^{(219)}\).

$$\mu_{s+L} = \sqrt{4s(s + 1)} \text{ B.M}$$

\(S = n/2\) (\(n\) = number of unpaired electrons)

The value of magnetic susceptibility of the prepared complexes at room temperature is calculated using the following equations:

$$\mu_{\text{eff}} = 2.828 \sqrt{X_A \cdot T} \text{ B.M}$$

\(X_A = X_m + D\)
\(X_m = X_g \cdot \text{M.wt}\)

Where: \(T\) = Absolute temperature, \(X_A\) = Atomic susceptibility
\(X_m\) = Molar susceptibility, \(X_g\) = Mass susceptibility
\(D\) = Pascal's constant, \(\mu_{\text{eff}}\) = Effective magnetic moment

The magnetic moment value of Cu (II) complex (d\(^9\)) are 1.85 B.M., this value refers to a distorted octahedral Cu(II) complex\(^{(220)}\). The magnetic moment of Ni(II) complex(d\(^8\)) was found to be 3.1 B.M., which is in fair agreement with the predicted value of octahedral geometry\(^{(221,222)}\).
Whereas Co (II) complex (d^7) has $\mu_{\text{eff}} = 4.9$ B.M., which indicates that it is of high spin octahedral type\(^{(223,224)}\).

4.2.5 Study of the electronic spectra and conductivity measurements of the prepared complexes

4.2.5.1 Electronic spectrum of free ligand Cys

Electronic spectrum of Cys in DMSO, Fig(4-22) showed an absorption band at 277 nm ($36101 \text{ cm}^{-1}$), table (4-15), which can be attributed to ($\pi \rightarrow \pi^*$) transition\(^{(225)}\).

4.2.5.2 Electronic spectrum of free ligand MTF.HCl

Electronic spectrum of MTF.HCl in DMSO, Fig(4-23) showed an absorption band at 255 nm ($39215 \text{ cm}^{-1}$), table (4-15), which can be attributed to ($\pi \rightarrow \pi^*$) transition of (C=\(\text{N}\)) in the biguanide group\(^{(226)}\).

Fig(4-22): Electronic spectrum of Cysteine in DMSO.
4.2.5.3 Electronic spectra of mixed ligand complexes of Cu(II), Ni(II) and Co(II)

The electronic spectra and molar conductivity of these complexes are given in table (4-16). The molar conductivity of all complexes in DMF is 14-18 S.mol⁻¹.cm⁻¹, which shows that the complexes were non-electrolytic; these results were confirmed by performing of the chloride content, which proved the unavailability of this ion.

The electronic spectra of all metal complexes recorded in DMSO; the spectrum of Cu(II) complex, Fig(4-24) displayed a band at 669nm (14947 cm⁻¹) that can be assigned to $^2$Eg→$^2$T₂g transition, indicating the Cu(II) complex has distorted octahedral geometry.

Electronic spectrum of Ni(II) complex ,Fig(4-25) showed three bands at 1025nm(9756 cm⁻¹),711nm(14064 cm⁻¹) and 444nm(22522 cm⁻¹) These were assigned respectively to the transitions $^3$A₂g→$^3$T₂g(F)(v₁), $^3$A₂g→$^3$T₁g(F)(v₂) and $^3$A₂g→$^3$T₁g(P)(v₃) of octahedral geometry.
The band at 914nm (10940 cm\(^{-1}\)) was attributed to the forbidden transition \(^3\!\!A_2g\rightarrow ^1\!\!Eg\)\(^{(231)}\). We can be calculated the values of Dq/B', B' and 10Dq from \(\nu_3/\nu_2\) (1.6) ratio and using (Tanabe-Sugano) diagram for \(d^8\), the value of \(\beta(0.634)\) indicated a covalent character\(^{(232)}\).

The electronic spectrum of the Cobalt complex, Fig(4-26) exhibited three bands at 1015 nm (9852 cm\(^{-1}\)), 718nm (13927 cm\(^{-1}\)) and 498nm (20080 cm\(^{-1}\)) that can be assigned to the transitions \(^4\!\!T_1g\rightarrow ^4\!\!T_2g(F)(\nu_1)\), \(^4\!\!T_1g\rightarrow ^4\!\!A_2g(F)(\nu_2)\) and \(^4\!\!T_1g\rightarrow ^4\!\!T_1g(P)(\nu_3)\) respectively, suggesting an octahedral geometry around Co(II) ion\(^{(224,231)}\). By using (Tanabe-Sugano) diagram for \(d^7\) and from \(\nu_3/\nu_1\) (2.0) ratio, we can be calculated the values of Dq/B', B' and 10Dq, the value of \(\beta(0.760)\) indicates that the complex has covalent character\(^{(224)}\).

Table(4-16): The data of electronic spectra, molar conductivity and ligand field parameters of the prepared complexes.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Absorption bands (cm(^{-1}))</th>
<th>Transition character</th>
<th>B</th>
<th>B'</th>
<th>Dq/ B'</th>
<th>(\beta)</th>
<th>10Dq</th>
<th>Conductivity S.mol(^{-1}).cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu(II) complex</td>
<td>14947</td>
<td>(^2!!Eg\rightarrow ^2!!T_2g)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15</td>
</tr>
<tr>
<td>Ni(II) complex</td>
<td>9756 (\nu_1) 14064 (\nu_2) 22522 (\nu_3)</td>
<td>(^3!!A_2g\rightarrow ^3!!T_1g(F)) (\nu_1) (^3!!A_2g\rightarrow ^3!!T_1g(F)) (\nu_2) (^3!!A_2g\rightarrow ^3!!T_1g(P)) (\nu_3)</td>
<td>1030</td>
<td>653.7</td>
<td>1.44</td>
<td>0.634</td>
<td>9414</td>
<td>18.2</td>
</tr>
<tr>
<td>Co(II) complex</td>
<td>9852 (\nu_1) 13927 (\nu_2) 20080 (\nu_3)</td>
<td>(^4!!T_1g\rightarrow ^4!!T_2g(F)) (\nu_1) (^4!!T_1g\rightarrow ^4!!A_2g(F)) (\nu_2) (^4!!T_1g\rightarrow ^4!!T_1g(P)) (\nu_3)</td>
<td>971</td>
<td>752</td>
<td>0.83</td>
<td>0.760</td>
<td>6127</td>
<td>14.4</td>
</tr>
</tbody>
</table>
Fig(4-24): Electronic spectrum of Cu(II) mixed ligand complex in DMSO
Fig(4-24): Electronic spectrum of Ni(II) mixed ligand complex in DMSO
Fig(4-26): Electronic spectrum of Co(II) mixed ligand complex in DMSO.
According to these results and those obtained from IR study, magnetic moment and elemental analysis, the structures of mixed ligand complexes, can be suggested as illustrated below:

\[ \text{[Cu(MTF)(Cys)(H}_2\text{O)]}_2 \]

\[ \text{[Ni(MTF)(Cys)(H}_2\text{O)}_2].\text{H}_2\text{O} \]
[Co(MTF)(Cys)(H₂O)₂]
Conclusion

The work presented in this thesis; present a sample of completeness between the preparation for a complex species from the chelate of one of the most important drug, which is metformin regarding these drug as an inorganic chelate reagent for copper ion via the formation of the color species complex , then using these approach for the formation of the complex through spectrophotometric determination of drug without using of chemical reagent with addition of establishing a complex microphotometer (homemade) for the online and continuous analysis for one of the most important drug. The established method were subjected for the comparison with use of a worldwide used spectrophotometer(UV-Visible Spectrometer (Cary-Varian El 04103410)) for comparison. The homemade system which was built in the laboratory was at a level or even it exceed that level the absorbance of 2, which is the logarithm of two that means complete absorbance of the incident light, which is the maximum limit for the spectrophotometer determination above this limit; deviation can be expected, while the newly design system can exceed that the limit as it contain a completely different approach regarding the source, cell type, its geometry, the detector used the amplification of the energy transducer response. Expressing the response digitally or plotting on x-t recorder or even both can be represented simultaneously. All the results were subjected to data treatments, which show that there were no significant differences between well recognized instrument and the homemade unit.

This thesis also present; three Mixed ligand complexes of Cu(II), Ni(II) and Co(II) derived from metformin(MTF) as primary ligand and cysteine (Cys) as second ligand have been prepared and characterized by elemental analyses, atomic absorption, FTIR, UV-Vis and NMR spectra, molar conductivity and magnetic susceptibility measurements.
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الخلاصة

تم تطوير طريقة طيفية جديدة استخدمت في موضوع البحث لتقدير عقار الميتورمرين من خلال تكوين معقد للدواء مع آيون النحاس الثنائي. تم قياس طيف المعد الملون عند 530 نم. تم وصف مفاضلة الظروف الكيميائية والفيزيائية لنظام تدثير الميتورمرين بتكوين معقد مع آيون النحاس الثنائي في الوسط القاعدي وتم التوصل إلى مدى خطي للتبدير بحدود 94.04% للمدى من 0-100 مللي مول/لتر مع حد كشف 662 نغم.

ثم تم استخدام النظام والطريقة المطورتين لتحليل العديد من التحضيرات الدوائية التجارية ومن ثم استخدام معالجات رياضية مستندة على اختبار المزدوج-1، والذي أظهر بأنه لا يوجد فرق جوهيري للطريقة المستخدمة وإمكانية استخدامها كطريقة تحليلية بديلة لتقدير عقار الميتورمرين. إعتمد مشروع البحث على إجراء كافة التفاعلات والتقييمات باستخدام التحليل بالحفل الجزيئي المستمر.

تضمن البحث أيضا تحضير معقدات مخلبية لأيون النحاس الثنائي، النيكل الثنائي والكوليد الثنائي من مزيج ليكيندي أستخدم فيه عقار الميتورمرين كليكيند أساسي ومادة السيستن كليكيند ثانوي وشخصت هذه المعقدات بواسطة تحليل العناصر، الامتصاص الذري، أطياف الإشعة تحت الحمراء، الرنين البصري النووي، الإطارات الإلكترونية، التوصيلة المولارية وقياسات الحساسية المغناطيسية.

إن تحليل العناصر وبيانات الامتصاص الذري قد أزالت النقاب عن تكوين المعقدات بنسبة مولية مساوية إلى (1:1:1) (M:MTF:Cys). بينما أظهرت قياسات طيف NMR وIR أظهرت السلوك المخلبي للكليكيند نحو إيون الفلزات الانتقالية (Co(II), Ni(II), Cu(II))، وهو من خلال مجموعات الأمينات لعقار الميتورمرين. أما بالنسبة للحامض الأميني (السيستين) فكان من خلال ذرة الكبريت وذرة النتروجين لمجموعة الأمينو.
دراسات طيفية وتحليلية لمعقدات مهددة من خلال تعاملك بين مكونات من بعض الأيونات الفلزية

رسالة مقدمة إلى

كلية العلوم – جامعة بغداد

كجزء من متطلبات نيل درجة الماجستير في الكيمياء اللاعضوية من قبل

هدى مؤيد نافع الكواز

بكالوريوس علوم كيمياء – جامعة بغداد

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