# Synthesis and Characterization of a New Complexo Cobalt (III) Sulfasalazine Hydroxamate

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**Abstract**

Cobalt (III) Sulfasalazine hydroxamate complex was synthesized and characterized by physicochemical and spectroscopic methods. A spectroscopic investigation of its reaction with Co(III) revealed the sole formation of the 1:3 complex at equilibrium. The isolated complex indicated octahedral coordination via the (N) atom of the hydroxamate group. The limiting concentration for interference by Ni(II), Cd(II), AI(III), and Zn(II) cations were reported. Microbial sensitivity test on five microorganisms were tested.

Keywords: Hydroxamic acid, Cobalt(II), spectrophotometry, microbial sensitivity.

## Introduction

The hydroxamic acid functionality, –C(=O)NROH, is a key structural constituent of many biomolecules, some of which such as siderophores (pseudobactin, desferrioxamines, ferrichromes *etc*.) are naturally occurring,[1] and others, such as the peroxidase, matrix metalloproteinase and urease inhibitors,[2] are of synthetic origin. Hydroxamic acids can also act as anticancer, anti-fungal, anti-tuberculous and hypotensive agents.[1–3].Recent studies have shown that hydroxamic acids are nitric oxide donors,[4] and that acetylated hydroxamate derivatives can act as effcetive aspirin analogues by prostaglandin $H_2$ synthase inhibition.[5] The versatile biological activity of hydroxamates is due to their strong metal ion chelating ability,[1,6] their NO-releasing properties,[4] their ability when ionized to form salt linkages in their complexes with proteins,[5] or when unionized to engage in key hydrogen bonding interactions, and to provide sites for acylation[5]. Hydroxamic acids are of major physiological and biomedical importance and many of their roles depend on metal ion binding, which can occur in a variety of ways, particularly with ligands which have other coordinating groups near the hydroxamate group[7.] Hydroxamic acids[8] have been the source of much biochemical interest in recent years due to the fact that they show a wide range of biological activities. Much of their activities are due to their chelating properties with metal ions.[9] Although hydroxamic acids were well known as metal ion chelators, their efficacy data on binding with drug and there synthesis were rare.

The mode of action of sulfasalazine (SSZ) (Fig.(1)) or its metabolites[10], 5-aminosalicylic acid (5-ASA) and sulfapyridine (SP), is still under investigation, but may be related to the anti-inflammatory and/ or immunomodulatory properties that have been observed in animal and in vitro models, to its affinity for connective tissue, and/or to the relatively high concentration it reaches in serous fluids, the liver and intestinal walls, as demonstrated in autoradiographic studies in animals. In ulcerative colitis, clinical studies utilizing rectal administration of SSZ, SP and 5-ASA have indicated that the major therapeutic action may reside in the 5-ASA moiety. Monohydroxamic acid form typical octahedral complexes with transition metal via coordination through the oxygen atoms and formation of reasonably ionic metal oxygen bonds[11].

Antimicrobial activity of hydroxamates includes inhibition of enzymes necessary for the growth of bacteria or yeasts as well as enzymes, the causes of pathogenicity. Urease is a nickel-dependent metalloenzyme very effectively inhibited by hydroxamates[12-15].

In this study we synthesized new hydroxamic acid derivatires containing Cobalt -Sulfasalazine hydroxamate.We aimed at developing a new method for coupling new hydroxamic acid derivatives with cobalt in addition, some physico-chemical properties were investigated and included in this work.

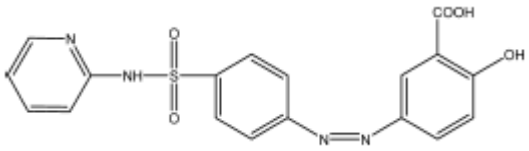


***Fig.(1) Chemical stretcher of Sulfasalazine (SSZ).***
***5-([P-(2-Pyridylsulfamoyl) phenyl] azo) salicylic acid.***

## Experimental

### Apparatus

1. Shimadzu 160-Double beam UV-Visible spectrophotometer.
2. SP3-300 IR spectrophotometer.
3. pH meter type Kent.
4. Melting point type SMPI.

### Chemical Materials

All chemicals used are analytical reagent grade. They include:
ethanol, hydroxylamine hydrochloride, ethylchlorofotmate, tetrahydrofuran, dimethylformamide, N –methylmorpholine, $CoCI_2.6H_2O$. Azulfidine tablets from Pharmacia AB Stockholm (Sweden).

### Procedure

A- Preparation of Sulfasalazine Hydroxamic acid [16]:

Sulfasalazine Hydroxamic acid was prepared as follows: To a solution of 1 gm SSZ and 0.4 gm N- methyl morpholine in 10 ml tetrahydrofuran at 0 $C^o$, 0.423 gm ethylchloroformate was added dropwise and the mixture was stirred for 2 h. The solid was filtered off and the filtrate was added to the solution of 0.229 gm hydroxylamine hydrochloride and 0.394 gm triethylamine in 10 ml dimethylformamide. The reaction mixture was stirred for 3 h at 25 $C^o$. Dimethylformamide was evaporated. The residue was extracted with 50 ml ethyl acetate, washed with water and dried. All of these reactions can be carried out at room temperature or below, this prevents hydrolysis.

B- Preparation of Cobalt-Sulfasalazine Hydroxamate complex:

Co-SSZ Hydroxamate complex was prepared as follows: 0.53 gm $CoCl_2.6H_2O$ was added with stirring to SSZ- Hydroxamic acid (0.5 g) in EtOH (20 ml) at 80 $C^o$, to this mixture 1ml of 1N HCI was added and the solution agitated for one hour until a precipitate appeared .The precipitate was left overnight and removed by filtration, washed with EtOH and dried.

C- Determination of stoichiometry of complex:

Job's method of continuous varations was used to determine the nature of complex. In this method a series of mixtures are prepared which two constituents are present at varying concentrations, but their sum is held constant. 0.01M solutions of SSZ – hydroxamic acid and Co(II) ions were prepared both in 0.1N HCI. To five 50 ml volumetric flasks 1, 3, 5, 7, and 9 ml of SSZ- hydroxamic acid and then 9, 7, 5, 3, and 1 ml of Co(II) ions were transfer and then diluted with 0.1 N HCl. The sum of the concentrations of the SSZ-hydroxamic acid and Co(II) ions in each flask was a constant as required by the method. The reaction was allowed to proceed to equilibrium at least 30 min. The absorbances of the mixtures were measured at 652 nm. Normally, a maximum appears in the curve at amole fraction corresponding to the complex that forms.

D- Interference study of metal ions solutions:

A number of transition metal ions were studied for their possible interference in the determination of Co in optimum conditions. 0.01 M Standard solutions for Ni (II), Cd (II), Al (III) and Zn (II) were prepared following appropriate dilution from 0.1 M metal solutions in distilled water.

E- Qualitative Analysis:

It is sometimes possible to establish the structure of a compound on the basis of spectra alone, these spectra must usually be supplemented with other information about the unknown: physical state, solubility, and confirmatory test for functional groups.
Hydroxamic acid test:

Dissolve about 30 mg of the unknown in 1 ml of ethanol, and add 1 ml of 1 N HCI followed by 1 drop of 10% aqueous iron (III) chloride solution. Ared-blue color is appositive test. The hydroxamic acid forms a red-blue complex with iron (III) ion.

F- Physicochemcial properties of the Hydroxamic acid and complex:

The physicochemcial properties of the Hydroxamic acid and isolated complex are given in Table (1).

***Table (1)***
***Analytical data and physicochemcial properties of the Hydroxamic acid and isolated complex.***

| *Compound* | *M.P* | *M.Wt* | *Color* |
|---|---|---|---|
| Hydroxamic acid | 250 C$^o$ | 413.4 | Yellow |
| Co-SSZ Hydroxamate complex | 260C$^o$ | 438.4 | Rose |

G-Evaluation of the antimicrobial activity:

The antimicrobial activity of the test compound was assayed against five bacteria. *Staphylococcus aureus,* Streptococcus, *Escherichia coli, Streptococcus, and Pseudomonias*. All these organisms, were +ve. All are regarded as pathogenic to humans and animals. All media and bacteria suspensions were prepared using a method adapted from that of Cruickshank (1965)[17]. About 15-20 cm$^3$ of agar was poured into sterile Petri plates about 10cm in diameter. After solidification of the agar, three cups (10 mm in diameter and 5 mm deep) were removed from each agar dish and fresh bacteria suspension was then uniformly spread on each cup. At this point, each of the cups was spotted three times with test solution at concentration of 50, 100 and 200 g/cm$^3$ in dimethylsulphoxide (DMSO). After incubating the plates at 37 C$^o$ overnight, the diameter of the zone of inhibition of the bacteria growth was then recorded. A 5% phenol solution was used as a positive control and DMSO as solvent control each time the experiments were performed.

**Results and Discussion**

**Infrared spectrum Analysises:**

The infrared spectrum of SSZ Fig.(2) show that acid exist as hydrogen-bonded that have characteristically broad hydrogen- bonded stretching bands around 2820 cm$^{-1}$. This broad band (superimposed on C-H stretching) plus a strong carbonyl band at 1676 cm$^{-1}$ suggests-COOH[18]. $SO_2$ group can identified by the apperance of the strong band in the 1200-1120 cm$^{-1}$ regions due to the symmetric vibration Fig. (2), (3) and (4). The spectrum of hydroxamic acid Fig. (3) is characterized by vibration due to N–H group[19]. N–H group is given by the doublet N-H stretching near 3063 cm$^{-1}$ and in plane N-H bending band near 1587 cm $^{-1}$.The IR spectrum of SSZ and hydroxamic acid show abroad band at 3134 cm$^{-1}$ and 3136 cm$^{-1}$, which can attributed to the phenolic group. To ascertain the involvement of OH of phenolic group of SSZ in the coordination, process to be followed the stretching vibration band of C–O in the complex. Examination of the complex found that the νC–O is shifted to lower wavenumber from 1280 cm $^{-1}$ in case of SSZ and hydroxamic acid to 1084 cm$^{-1}$ in case of complex. This result indicates that phenolic group is participated in the complexation and SSZ acts as bidentate. The lower shift of γ(OH) from 1394 cm$^{-1}$ in the free hydroxamic acid to 1359 cm$^{-1}$ in the complex is the another factor confirmation which proves the involvement of OH phenolic group in the coordination process.

The νC=O vibration located at 1676 cm$^{-1}$, 1618 cm$^{-1}$, and 1622 cm$^{-1}$ in the SSZ, hydroxamic acid and complex respectively, which renders it difficult to attribute to the involvement of C=O group in coordination.

The decrease in the νN–H band from 3063 cm$^{-1}$ in the hydroxamic acid to 2360 cm$^{-1}$ in the complex indicates N-bonding of mode that is, suggesting deprotonation of nitrogen atom of the hydroxamic acid group[20]. Conclusive evidence regarding the bonding of oxygen to the metal ions is provided by occurrence of bands at 533 cm$^{-1}$ as the result of ν M-O band. Suggesting deprotonation of phenolic group of the hydroxamic acid. The analytical data and some physico-chemical properties are shown in Table (2). The spectrum of Co-hydroxamate complex shows two bands located at about 2360 cm$^{-1}$, and 1622 cm$^{-1}$ which are due to transition from the ground term to the $^4T_1(P)$ and $^4T_1(F)$ levels respctively[21].This spectrum indicate octahedral cobalt (III) complex[22].

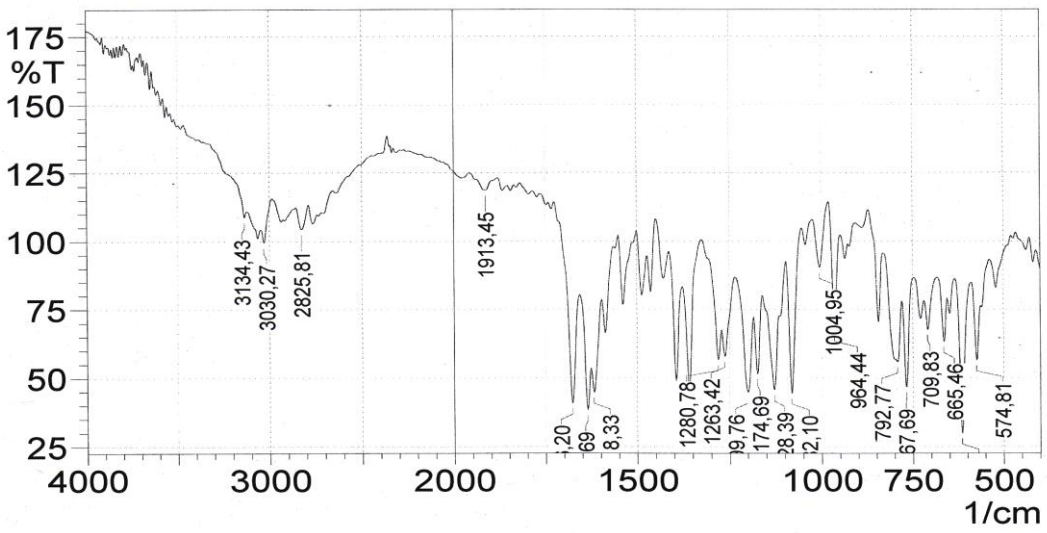


*Fig.(2) IR Spectrum of Sulfasalazine (SSZ).*

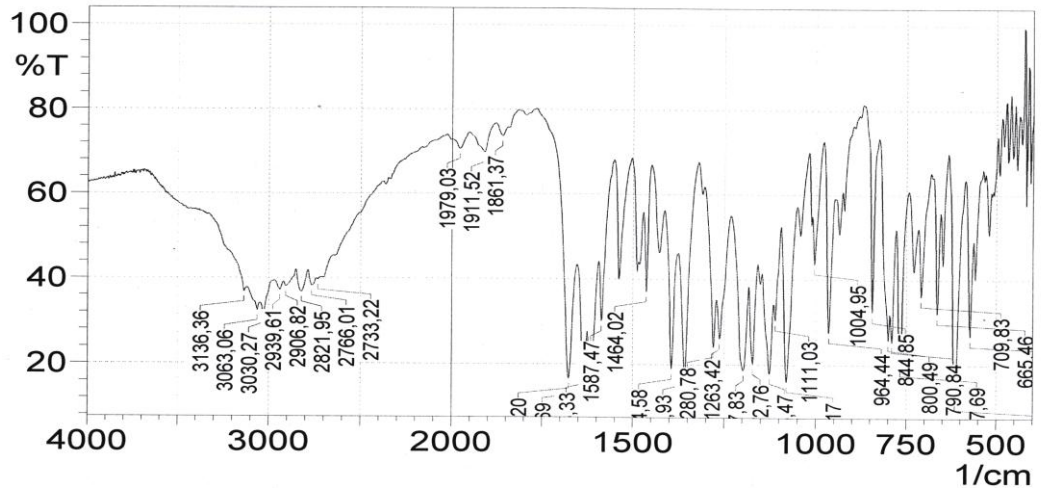


*Fig.(3) IR Spectrum of SSZ - Hydroxamic acid.*

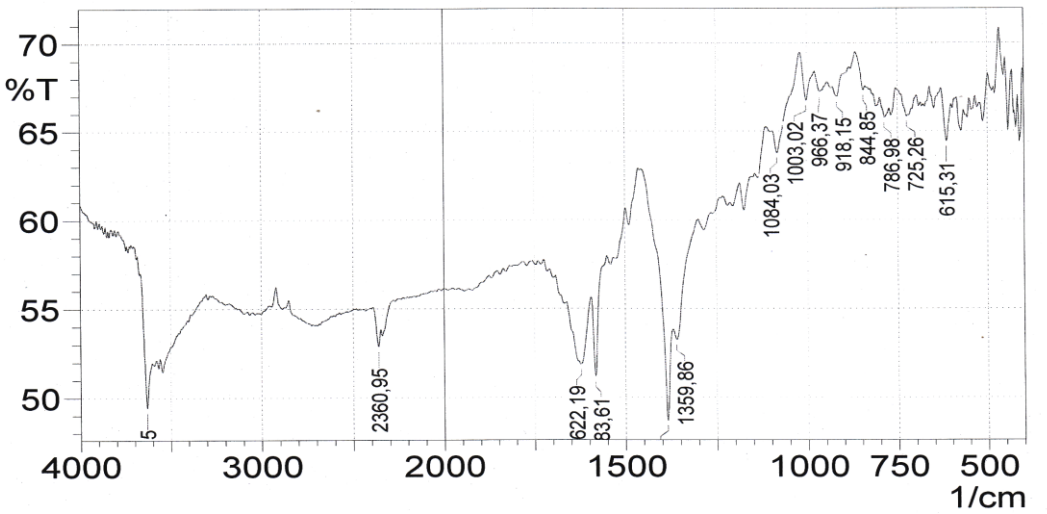


*Fig.(4) IR Spectrum of Co – SSZ - Hydroxamate complex.*

***Table (2)***
***Diagnostic I.R data for the Hydroxamic acid and complex ($cm^{-1}$).***

| *Compound* | *N-H* $cm^{-1}$ | *N-H* $\Delta cm^{-1}$ | *C-O* $cm^{-1}$ | *C-O* $\Delta cm^{-1}$ |
|---|---|---|---|---|
| Hydroxamic acid | 3063 | 703 | 1280 | 176 |
| Co-SSZ Hydroxamate complex | 2360 | | 1084 | |

**Electronic absorption spectra**

Electronic absorption spectra of the complex and reagent were recorded in EtOH solution, Fig.(5), (6). From the electronic spectra, it is observed that, for the Co (III) complex, the UV spectrum shows a band of medium intensity at 652 nm which is assigned to the transition $^4T_1gF \rightarrow {}^4T_1gP$ ($\nu_3$) but the transition $^4T_1gF \rightarrow {}^4A_2gF$ ($\nu_2$) and $^4T_1gF \rightarrow {}^4T_2gF$ ($\nu_1$) of an octahedral cobalt (III) complex can not be observe even with concentrated solution. It may be lost in the low energy tail of the charge transfer transition. The UV spectra of the reagent SSZ show two bands at 362 and 348 nm assignable to $\pi \rightarrow \pi^*$ and a band at 235 nm attributed to $n \rightarrow \pi^*$ transitions respectively. The spectra of the complex shows different compared to the reagent. Moreover, electronic absorption spectra of the complex, show a band at 652 nm which can be seen clearly, improve that formation anew complex of Co(III) Sulfasalazine hydroxamate complex.

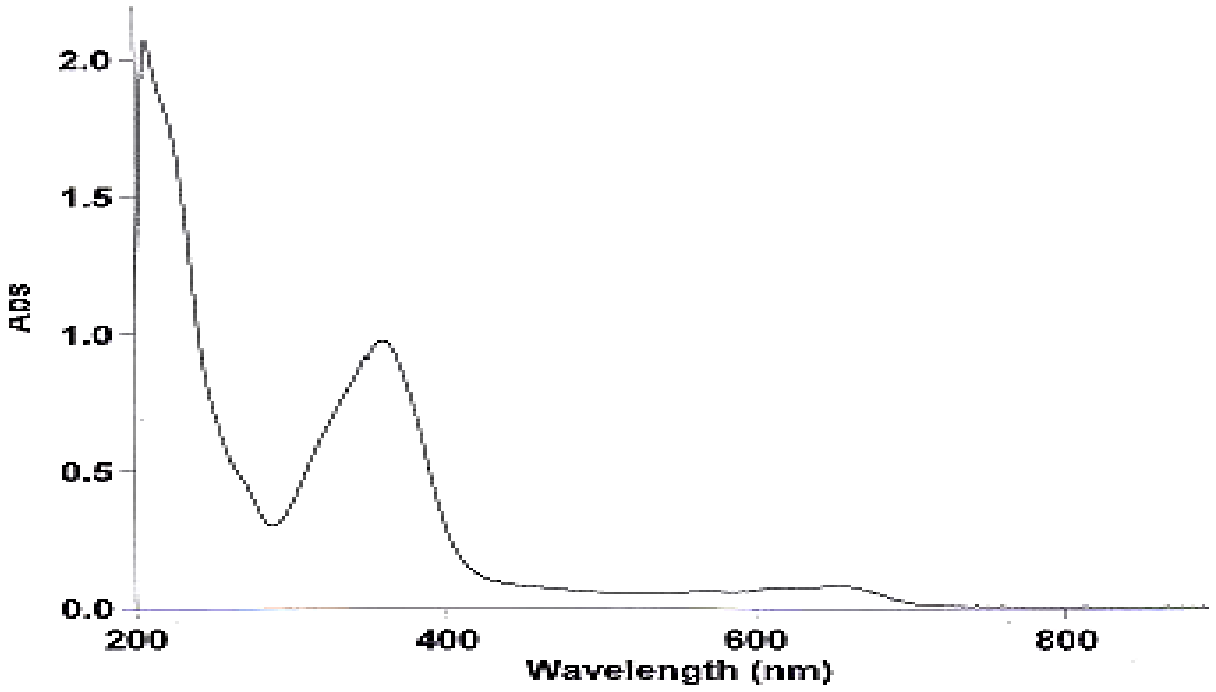


***Fig.(5) UV –VIS absorption spectrum of Co- SSZ - Hydroxamate complex.***

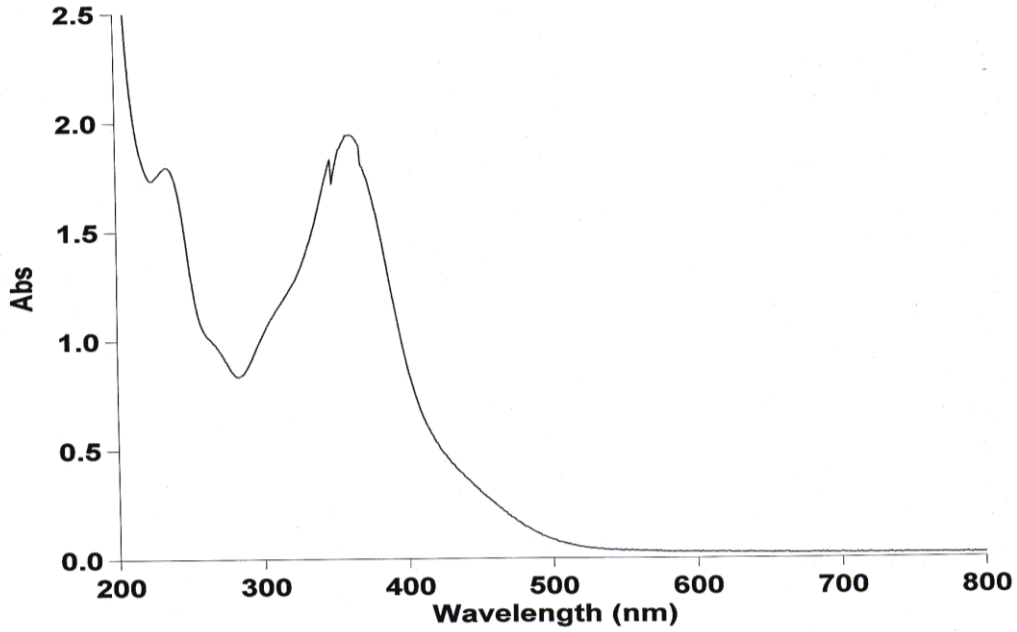


*Fig. (6) UV – VIS absorption spectrum of SSZ.*

**Equations of Reaction**

The various stages in the preparation of hydroxamic acid and the complex as represented by the reactions below:

In the first step the carboxylic group of SSZ was converted into an anhydride by reacted with ethylchloroformate and N-methylmorpholine in THF. In a second step, the anhydride was reacted immediately with hydroxylamine hydrochloride to produce the corresponding hydroxamic acid derivative. The third step was, reacted the hydroxamic acid with cobalt ion to form chelting complex compound with six membered ring via OH of phenolic group and N–H group of hydroxamic acid. This kind of bonding (N bonding model) suggesting deprotonation of nitrogen of the hydroxamic group[23].

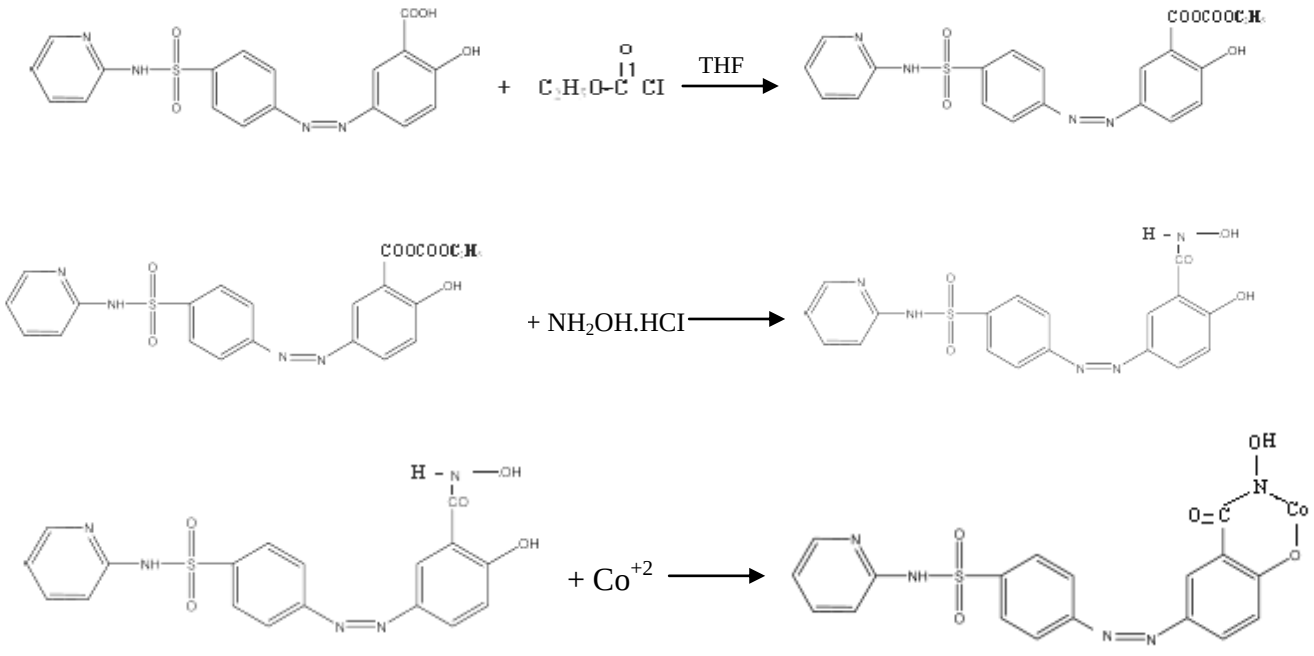


*Fig.(6) Equations of reaction.*

**Job's continuous variation method**

A Job's continuous variation method plot of spectrophotometric data from varying the hydroxamic acid and Co(III) concentrations at PH4, showed the existence of a 1:3 complex M:L.

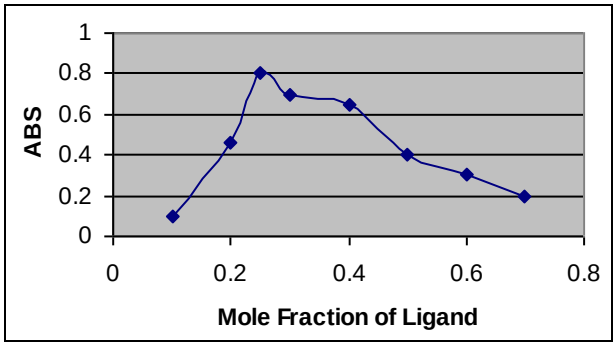


***Fig.(7) Determination of Stoichiometry of complex.***

**Suggested Structural formula of Complex**

The structural formula of the complex is given in Fig.(8), it is concluded that from spectroscopic methods analysis, the hydroxamic acid behaves as a bidentate ligand coordinated to the metal ions through the phenolic OH group and NH group of the hydroxamic acid. It is known that cobalt metal values in solution commonly exists as either Co(II) or Co(III) and that these two oxidation states have widely different chemistries when complexed by a ligand. Under oxidizing conditions, presence of air, normally Co(II)converts to Co(III) when complexed with a bidentate ligand or chelting agent[24]. One that bonds to metal ion through at least two atoms thereby, forming a chelate ring complex and that Co(III) chelate formed is usually irreversible and not readily strippable.

**Magnetic Moment**

The room temperature magnetic moment value for cobalt complex lies within the range for their observed octahedral geometries[25]. The magnetic moment ($\mu_{eff}$) for cobalt complex (0.3 B.M) is expected to contain an even number of electrons.

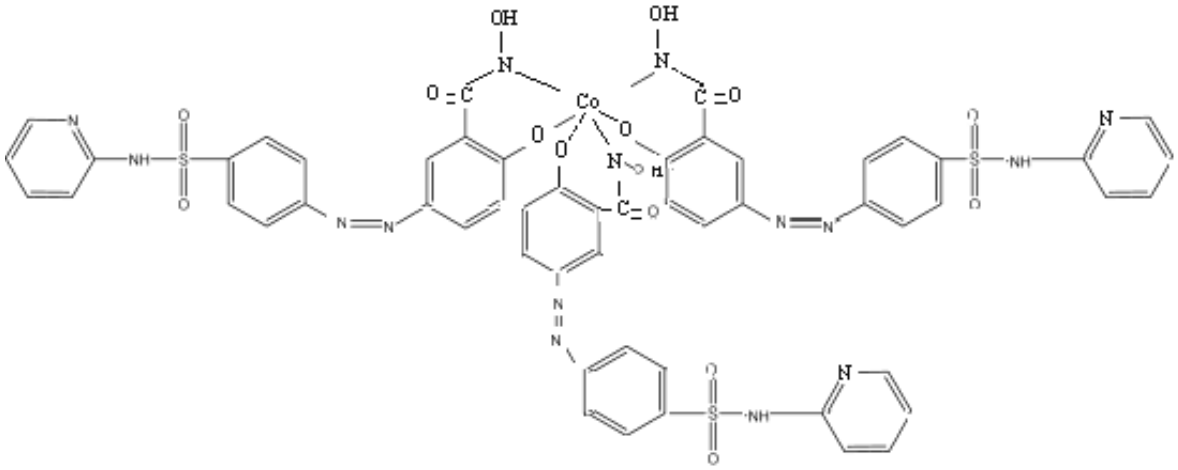

***Fig.(8) Structure of complex.***

**Antimicrobial activity test**

The microbial sensitivity test carried out on Cobalt (III) complex showed activity on the microorganism under investigation. Table (3), shows the microbial sensitivity test against five microorganisms as indicated in the table.

***Table (3)***
***Microbial sensititivity test for Co – SSZ Hydroxamate.***

| *Compound* | *Staph. aureus* | *S.typhylium* | *E.cola* | *Pseudonomias* | *Streptococcus* |
|---|---|---|---|---|---|
| Co-SSZ Hydroxamate complex | +ve | +ve | +ve | +ve | +ve |

**Effect of Interference**

A number of transition metal ions were studied for their possible interference in the determination of Co (III). $Zn^{+2}$ was found to interfere rather severely while $Cd^{+2}$ was medum. $Ni+^{2}$ and $AI^{+3}$ dose not interfere Table (4). RE% is the Relative Error, which is commonly defined as the absolute error divided by the true value. Absolute error, defined as the difference between the observed and the true value. The true value term refers to the absorbance value before addition, which was 0.9.

***Table (4)***
***Effect of interference some metal ions on the abs. of Co-Hydroxamate complex.***

| *Metal ions solution* | *Abs. after addition* | *RE%* |
|---|---|---|
| $Ni+^{2}$ | 0.98 | –8.88 |
| $Cd^{2}$ | 0.77 | 14.44 |
| $AI+^{3}$ | 1.2 | –33 |
| $Zn+^{2}$ | 0.64 | 28 |

**Conclusion**

In this study, anew complex of Co(III) Sulfasalazine hydroxamate was synthesized. Sulfaslazine was reacted with ethylchloro-formate and N-methylmorpholinein in THF to convert the carboxylic group of SSZ to an anhydride.The anhydride was reacted immediately with hydroxylamine hydro-chloride to produce the corresponding hydroxamic acid derivatives. Hydroxamic acid was reacted with Co(III) ions to form chelting complex compound. The microbial sensitivity test shows activity.

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**الخلاصة**

تم تحضير مركب جديد للعقار الطبي Sulfasalazine (SSZ) عن طريق تحضير معقد فلزي مع ايون Co(II) للحصول على معقد Co(III)–SSZ Hydroxamate. شخص المعقد طيفيا باستخدام مطيافية الاشعة فوق البنفسجية و المرئية ومطيافية الاشعة تحت الحمراء. اظهرت الاختبارات الطيفية لتعيين تكافؤية المعقد ان نسبة الفلز الى الليكاند هي ١:٣. لوحظ ان تناسق المعقد الثماني السطوح الذي تم تحضيره يكون من خلال ذرة الـ (N) و مجموعة OH الفينولية لحامض hydroxamic acid. تم دراسة تاثير تداخلات بعض العناصر الفلزية الانتقالية على امتصاصية المعقد، فضلا عن اختبار الحساسية البايولوجية على خمسة انواع من الاحياء المجهرية.