

Synthesis And Spectroscopic Study Of Hydroxamic Acid Derivative: Cobalt (III) Sulfasalazine Hydroxamate

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Introduction

Recent advances in the field of drug discovery have shed light on the capacity of hydroxamic acids to form complexes with a variety of metal ions, in particular iron, zinc, magnesium, and calcium. As a result, hydroxamic acids possess a range of distinct biological and pharmacological properties as a result of this ability (Raj et al., 2016). Strong metal ion bidentate chelators, hydroxamic acids highly chelate with Fe(III); matrix metalloproteases (MMPs), carbonic anhydrase, and tumour necrosis factor- converting enzyme (TACE), Ni(II); urease, and Cu(II) (Nef et al., 2012). Under physiological circumstances, hydroxamic acids may be quickly hydrolyzed into carboxylic acids and hydroxylamine, which is a mutagen. This property of hydroxamic acids is the primary factor that prevents these molecules from being used in therapeutic applications (Flipo et al., 2009). Recent research has demonstrated that hydroxamic acids are donors of nitric oxide,⁴ and that acetylated hydroxamate derivatives have the potential to operate as effective aspirin mimics through the suppression of prostaglandin H₂ synthase.

Because of their powerful ability to chelate metal ions, their NO-releasing properties, their capability, when ionised, to form salt linkages in their complexes with proteins, or when unionised, to engage in key hydrogen bonding interactions, and to provide sites for acylation, hydroxamates have a wide range of applications in biological research (Marmion et al., 2000). There have been reports of several substituted analogues of hydroxamic acid with features such as anti-inflammatory, anti-tubercular, anti-leukemic, anti-microbial, and tumour inhibitory. They are also employed in industry as insecticides, antioxidants, as corrosion inhibitors, and for the extraction of hazardous materials, and they play an essential part in the production of a wide variety of physiologically active pharmaceuticals such as antidepressants, antitumor agents, anti-HIV agents, and antimalarial agents (Gaon et al., 2018). In addition, various physicochemical aspects were researched and were incorporated into this study. Our objective was to design a novel technique for coupling new hydroxamic acid derivatives with cobalt.

Apparatus and Chemicals used

Double beam UV-Visible spectrophotometer, IR spectrophotometer, pH meter, Melting point type. Ethylchlorofotmate, ethanol, tetrahydrofuran, hydroxylamine hydrochloride, N –methylmorpholine, dimethylformamide, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and Azulfidine tablets.

Procedure

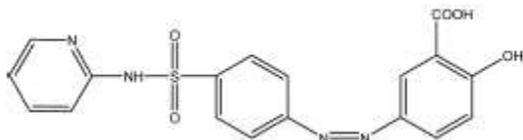


Fig. 1: Chemical structure of Sulfasalazine (SSZ) 5-([P-(2-Pyridylsulfamoyl) phenyl] azo) salicylic acid.

Synthesis of Sulfasalazine Hydroxamic acid (Reddy and Kumar, 2000) - Drop by drop, 0.423 g of ethylchloroformate was added to a solution of 1 g of SSZ and 0.4 g of N- methyl morpholine in 10 ml of tetrahydrofuran at 0 Co, and the mixture was agitated for 2 hours. After removing the solid using a filter, the filtrate was added to a solution that contained 0.229 grammes of hydroxylamine hydrochloride and 0.394 grammes of triethylamine dissolved in 10 millilitres of dimethylformamide. At a temperature of 25 Co, the reaction mixture was agitated for three hours. Evaporation of dimethylformamide took place. The residue was dried after being washed with water and extracted using fifty millilitres of ethyl acetate. This eliminates the need for hydrolysis by allowing all of these reactions to be carried out at room temperature or below.

Preparation of Cobalt-Sulfasalazine Hydroxamate complex

At a temperature of 80 °C, 0.53 gm of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ was added while stirring to 0.5 g of SSZ-hydroxamic acid that was dissolved in 20 ml of EtOH. After adding 1 millilitre of 1N hydrochloric acid to the solution and agitating it for one hour, a precipitate was finally formed. After allowing the precipitate to sit for one more night, it was extracted using filtering, then washed with EtOH, and finally dried.

Determination of stoichiometry of complex

In order to discover the characteristics of the complicated system, Job's approach of continuous variations was applied. In this procedure, a series of mixtures are generated in which the concentrations of the two constituents in the mixture vary, but the total concentration of those constituents remains the same. 0.01M solutions of SSZ – hydroxamic acid and Co (II) ions were prepared both in 0.1N HCl.

After transferring 1, 3, 5, 7, and 9 ml of SSZ- hydroxamic acid to five 50 ml volumetric flasks, the following amounts of Co(II) ions were added: 9, 7, 5, 3, and 1 ml, respectively. The mixture was then diluted with 0.1 N HCl.

In accordance with the specifications of the methodology, the total concentration of SSZ-hydroxamic acid and Co(II) ions in each flask was maintained at a constant value. At least thirty minutes were allowed for the reaction to continue until it reached equilibrium. At a wavelength of 652 nm, the absorbances of the mixes were evaluated. In most cases, the curve will exhibit a maximum when the amole fraction corresponding to the complex that forms is reached.

Interference study of metal ions solutions

Under optimal conditions, the influence of a variety of transition metal ions on the detection of cobalt was investigated. The goal was to identify any potential for interference. After performing the necessary dilutions from 0.1 M metal solutions in distilled water, 0.01 M Standard solutions for nickel (II), cadmium (II), aluminium (III), and zinc (II) were created.

Qualitative Analysis

It is possible to determine the structure of a compound based solely on its spectra; but, in most cases, the spectra must be supplemented with additional information about the unknown, such as its physical state, its solubility, and a confirmatory test for functional groups.

Hydroxamic acid test

In 1 millilitre of ethanol, dissolved approximately 30 milligrammes of the unknown substance. To this, added 1 millilitre of 1 N hydrochloric acid, followed by 1 drop of an aqueous solution containing 10% iron chloride. The colour red blue indicated a positive test. When combined with iron (III), hydroxamic acid produces a complex that is reddish-blue in colour.

Result and Discussion

Infrared spectrum Analysis

The characteristically large hydrogen-bonded stretching bands at 2820 cm^{-1} can be seen in the infrared spectrum of SSZ Fig.(2), which demonstrates that acids exist as hydrogen-bonded structures. This broad band, which is overlaid on the C-H stretching, in addition to a prominent carbonyl band at 1676 cm^{-1} , shows that it is COOH (Morrison and Durham, 1978). The SO_2 group can be recognised by the presence of the prominent band in the regions between 1200 and 1120 cm^{-1} , which is caused by the symmetric vibration (Fig. 2, 3 and 4). Fig. 3 depicts the spectrum of hydroxamic acid, which may be identified by the vibration that is caused by the N-H group. The N-H group can be identified by the doublet N-H stretching near 3063 cm^{-1} and the N-H bending band in the plane near 1587 cm^{-1} . The phenolic group is responsible for the presence of a band in the infrared spectrum of both SSZ and hydroxamic acid at 3134 cm^{-1} and 3136 cm^{-1} , respectively. The technique of following the stretching vibration band of CO in the complex is to be done in order to determine whether or not OH from the phenolic group of SSZ is involved in the coordination. In the case of the complex, examination revealed that the CO is shifted to a lower wavenumber, moving from 1280 cm^{-1} in the case of SSZ and hydroxamic acid to 1084 cm^{-1} in the case of the complex (Brown and Roche, 1982).

Based on this data, it appears that the phenolic group is involved in the complexation, and that SSZ functions as a bidentate. Another essential validating that confirms the involvement of OH phenolic group in the coordination process is the lower shift of (OH), which goes from 1394 cm^{-1} in the free hydroxamic acid to 1359 cm^{-1} in the complex. This shift occurs at the carbon atom.

Because the CO vibration is positioned at 1676 cm^{-1} , 1618 cm^{-1} , and 1622 cm^{-1} in the SSZ, hydroxamic acid, and complex, respectively, it is difficult to explain it to the involvement of CO group in coordination because these three frequencies are so close together.

The drop in the NH band from 3063 cm^{-1} in the hydroxamic acid to 2360 cm^{-1} in the complex reveals N-bonding of mode, which suggests deprotonation of the nitrogen atom in the hydroxamic acid group²⁰. This can be seen as a decrease from 3063 cm^{-1} to 2360 cm^{-1} .

The appearance of bands at 533 cm^{-1} as a consequence of the M-O band offers conclusive evidence regarding the bonding of oxygen to the metal ions. Suggesting deprotonation of phenolic group of the hydroxamic acid. 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 respectively²¹. This spectrum indicate octahedral cobalt (II) complex²².

Electronic Absorption Spectra

In a solution of EtOH, the electronic absorption spectra of both the complex and the reagent were recorded (Fig. 5,6). According to the electronic spectra, it was discovered that the UV spectrum of the Co (III) complex exhibited a band with a moderate amount of intensity at 652 nm. This band was determined to correspond 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 cannot be observed even with concentrated solution. It's possible that it was eliminated out in the low-energy tail of the charge transfer transition. The UV spectra of the reagent SSZ show three bands at 362 nm, 348 nm, and 235 nm, with the first two bands being attributed to transitions and the third band being related to n transitions.

The UV spectra of the reagent SSZ show three bands at 362 nm, 348 nm, and 235 nm, with the first two bands being attributed to $\pi \rightarrow \pi^*$ transitions and the third band being related to $n \rightarrow \pi^*$ transitions.

In comparison to those of the reagent, the spectra of the complex exhibit distinct differences. In addition to this, the electronic absorption spectra of the complex show that there is a band at 652 nm that can be seen very clearly. This helps to improve the development of a new complex of Co(II) Sulfasalazine hydroxamate.

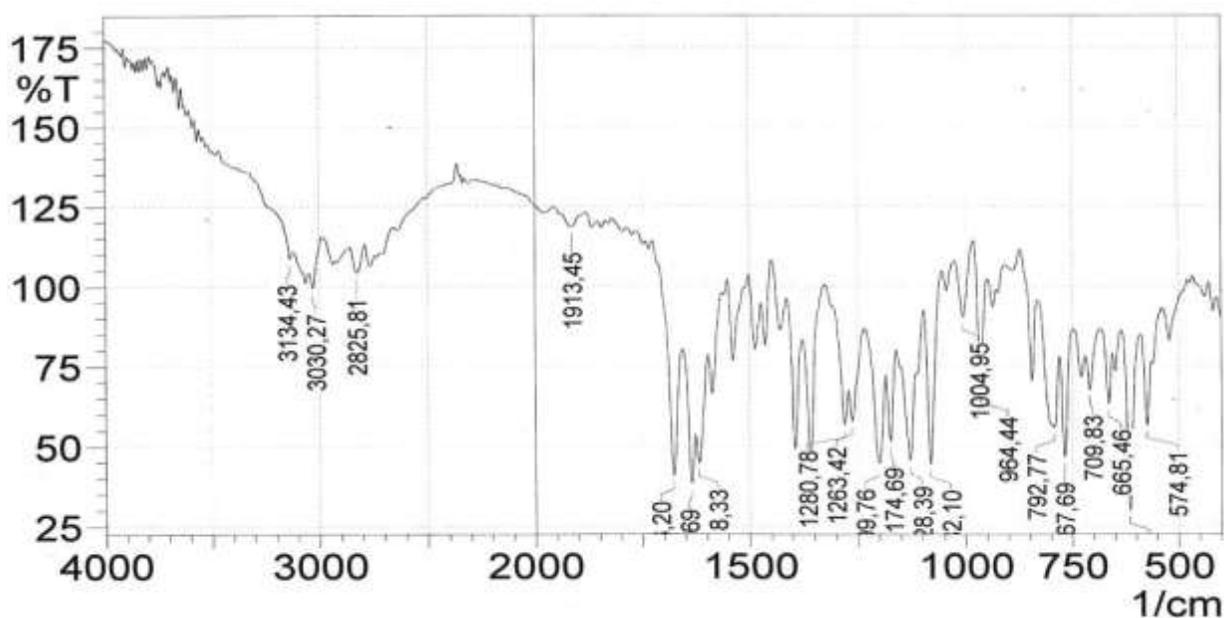


Fig.2: IR Spectrum of Sulfasalazine (SSZ)

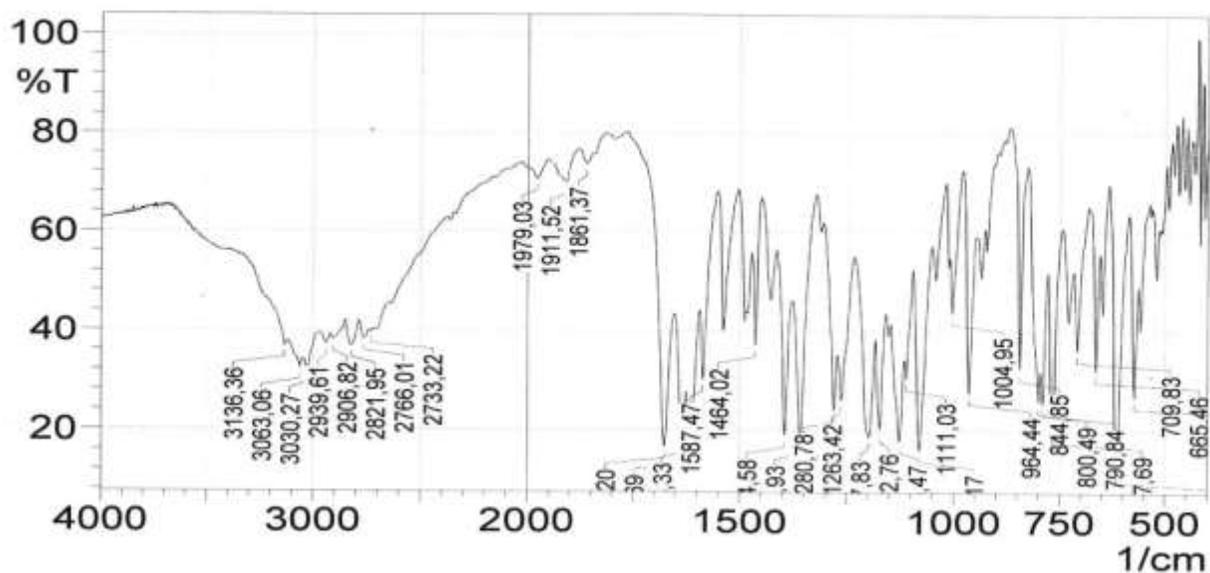


Fig.(3) IR Spectrum of SSZ - Hydroxamic acid

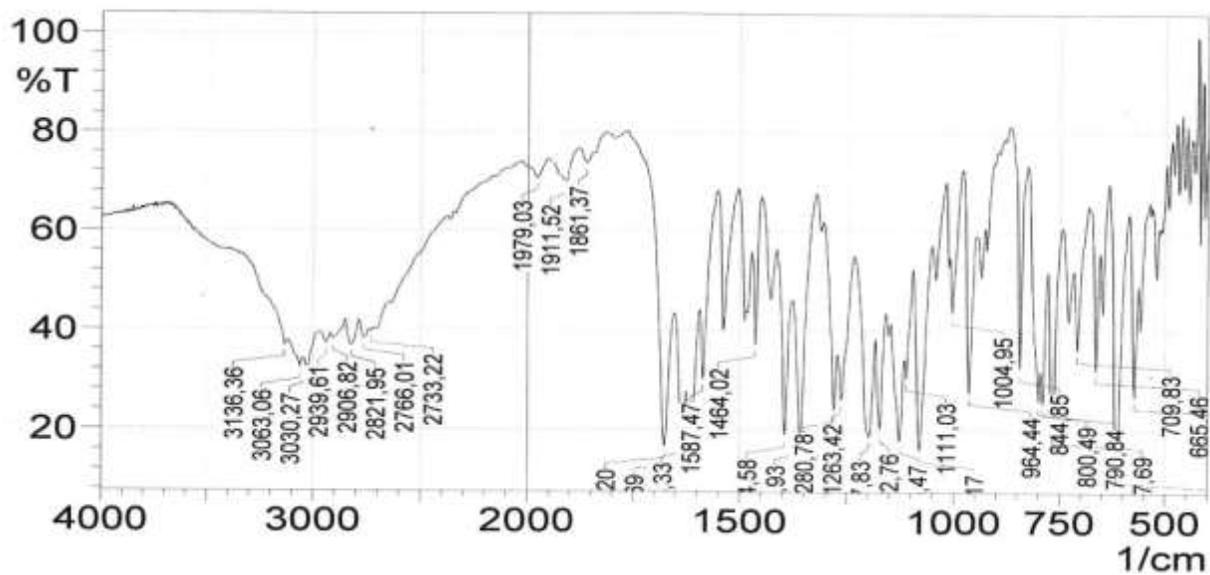


Fig. 4: IR Spectrum of Co \square SSZ - Hydroxamate complex

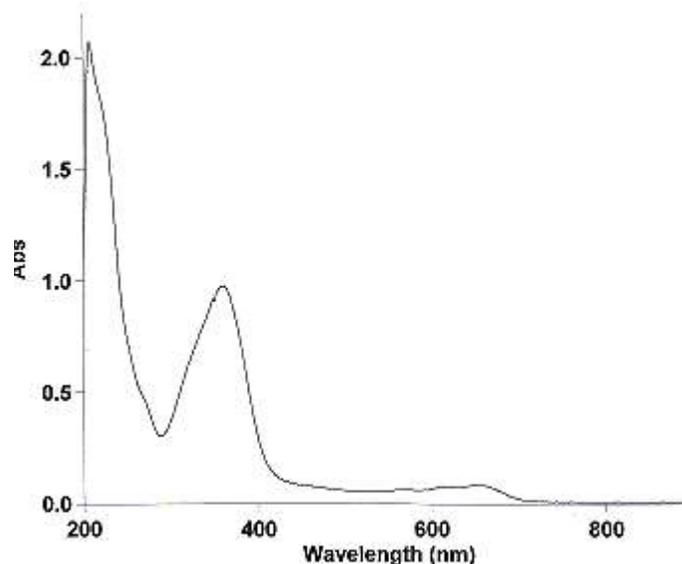


Fig.(5) UV –VIS absorption spectrum of Co- SSZ - Hydroxamate complex.

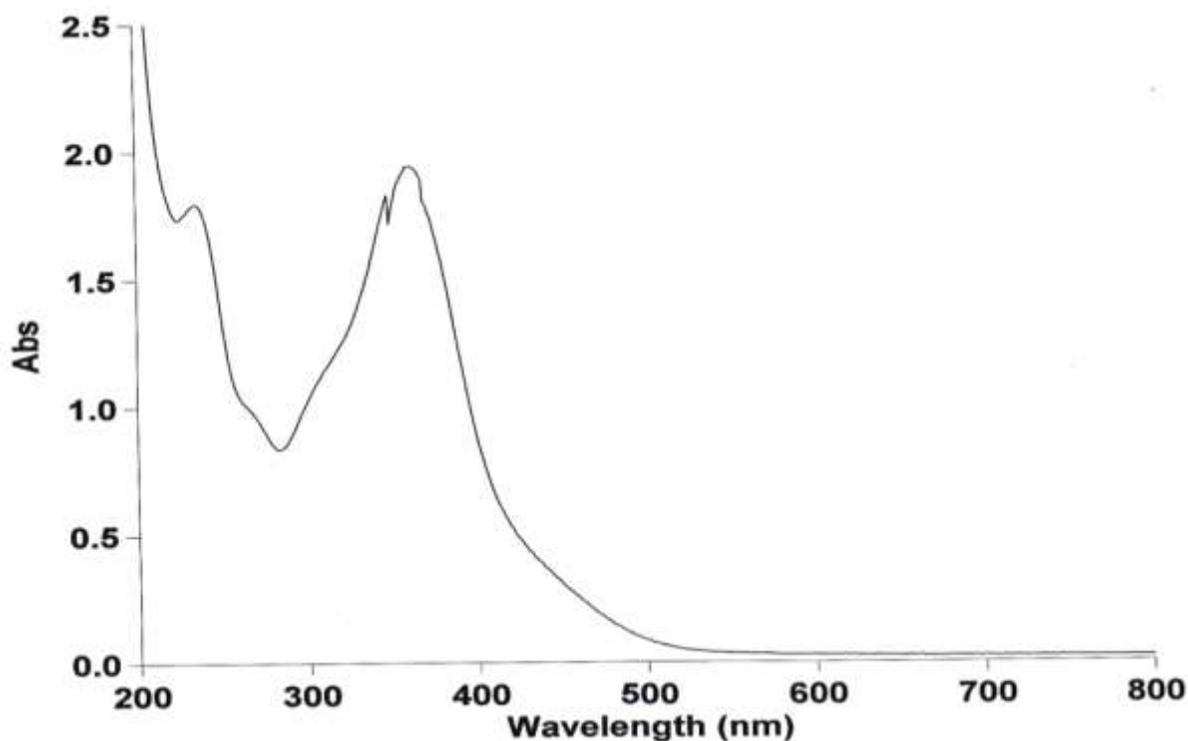


Fig. (6) UV – VIS absorption spectrum of SSZ

Reactions

In the first step of the process, the carboxylic group of SSZ reacted with ethylchloroformate and N-methylmorpholine in THF, which resulted in the formation of an anhydride. The anhydride was then

promptly reacted with hydroxylamine hydrochloride in a second stage to obtain the equivalent hydroxamic acid derivative. In the third stage, the hydroxamic acid was reacted with cobalt ion to generate a chelating complex chemical. This product had a six-membered ring and was formed through the OH group of the phenolic group and the N-H group of the hydroxamic acid. This type of interaction, known as the N bonding model, indicates that the nitrogen in the hydroxamic group has lost its proton.

Job's continuous variation method

A Job's continuous variation technique plot of spectrophotometric data obtained by altering the amounts of hydroxamic acid and Co(III) at PH4 revealed the presence of a 1:3 complex M:L.

Effect of Interference

The potential for influence from a variety of transition metal ions was investigated during the process of determining the value of cobalt (Co). It was discovered that Zn²⁺ caused a significant interference, while Cd²⁺ had a moderate impact. Table (4) shows that Ni²⁺ and Al³⁺ do not interfere with one another. The relative error, abbreviated as RE%, is calculated by taking the absolute error and dividing it by the value that is actually being measured. The difference between the value that was observed and the value that actually existed is what statisticians refer to as the absolute error. The absorbance value before to the addition was 0.9, which is what is meant when the term "true value" is used.

Conclusion

In the course of this research, the synthesis of a novel complex of Co(II) Sulfasalazine hydroxamate was carried out. In the presence of THF, sulfasalazine was reacted with ethylchloro-formate and N-methylmorpholine so that the carboxylic group of SSZ could be converted into an anhydride. In order to obtain the relevant hydroxamic acid derivatives, the anhydride was promptly reacted with hydroxylamine hydrochloride. In order to produce a chelating complex molecule, hydroxamic acid was treated with cations of cobalt. The results of the test for microbial sensitivity suggest activity.

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