

**PRELIMINARY STEPS TOWARDS ESTABLISHMENT OF A
UV-SPECTROPHOTOMETRIC METHOD FOR THE
ANALYSIS AND ESTIMATION OF B-LACTAM ANTIBIOTICS
VIA CONVERSION INTO FERRIC HYDROXAMATE
COMPLEX AS AN INDEX OF DETERMINATION
(AMOXICILLIN MODEL)**

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ABSTRACT

The main aim of the present investigation is to perform preliminary steps for the establishment and development of a new analytical technique for the analysis of β -lactam antibiotics, in general, executed and illustrated by amoxicillin as a model. The method requires a stepwise synthesis of amoxicillin hydroxamate ferric ion complex as an index of determination of the drug in capsule dosage form. Amoxicillin hydroxamic acid was prepared via treatment of amoxicillin with hydroxylamine, after establishment of the appropriate reaction conditions that allowed the nucleophilic attack of hydroxylamine to take place, stereoselectively, at the carbonyl group of the β -lactam ring leading to the formation of amoxicillin hydroxamic acid. The latter acid was treated with an aqueous solution of ferric chloride, the purple colored amoxicillin hydroxamate ferric ion complex. The complex was obtained, qualitatively, in good yield and moderate stability. The stability of the complex was improved when the reaction was conducted in the aprotic solvent dimethylsulphoxide, instead of water. The specificity of the attack at the β -lactam ring was verified through conducting a control experiment involving reaction of hydroxylamine with acetamide, which formed an unstable colored complex, when treated with ferric chloride aqueous solution. The noticeable low yield and extreme instability of the latter complex was evident from the faint purple, transient color. Further evidence for this stereoselective nucleophilic attack at the carbonyl of the β -lactam ring was also evident and being confirmed by the disappearance of the carbonyl absorption (1776 cm^{-1}) frequency in the IR-spectrum of the amoxicillin ferric ion complex. Moreover, a number of experiments have been carried out, which involved monitoring the stability of the complex towards solvent polarity, pH variations, resistance to temperature changes and time. A quantitative UV-spectrophotometric analysis was used for the determination of the stoichiometric ratio of the two reactants by adopting Job's method of continuous variation and the mole ratio method, whereupon, the ratio of amoxicillin hydroxamic acid to that of ferric ion is calculated to be 1:6, based on the absorption of the complex at λ_{max} 520 nm. Accordingly, the following chemical formula was suggested: $\text{Fe}_6\text{C}_{16}\text{H}_{17}\text{N}_4\text{SO}_6$ and proposed chemical structures for amoxicillin hydroxamate ferric ion complex were reached.

Keywords: Amoxicillin, hydroxamic acid, hydroxamate ferric ion complex, Job's, method.

INTRODUCTION

β -lactam antibiotics are used in health and medical circles due to their high efficiency in eradication of many infectious diseases. One notable member of this class of antibiotics is amoxicillin [(2S,5R,6R)-6-[[[(2R)-2-Amino-2-(4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid trihydrate (Figure 1)¹. Amoxicillin is classified as a moderate-spectrum, 3- or β -lactam antibiotic. It is widely used to treat bacterial infections caused by susceptible microorganisms. It is active against a wide range of Gram-positive, and a limited range of Gram-negative organisms. It acts by inhibiting the synthesis of bacterial cell walls. It also inhibits cross-linkage between the linear peptidoglycan polymer chains that make up a major component of the cell wall of Gram-positive bacteria. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β -lactam antibiotics. Amoxicillin is susceptible to degradation by (β -lactamase-producing bacteria), and so may be given with clavulanic acid to decrease its susceptibility². A number of analytical methods have been developed and are widely being used for the analysis of β -lactam antibiotics. Most of these methods have employed amoxicillin as a marker of β -lactam moiety. Literature search revealed that previous studies attempted for the determination of amoxicillin in pharmaceutical preparations containing only amoxicillin included spectrophotometry³⁻¹⁰ spectrofluorimetry¹¹ HPLC¹²⁻¹⁴, flow-injection analysis^{7,15-20}, voltammetry and polarography^{21,22} and titrimetry²³. Determination of amoxicillin in the presence of sulbactam sodium, clavulanic acid, fluxacilline and metronidazole was accomplished by using HPLC²⁴⁻³⁰ TLC³¹, CE³² and chemometry³³. However, the literature search revealed that there were no attempts in using derivative UV spectrophotometric methods for the analysis of β -lactam antibiotics, in general or even amoxicillin as a representative.

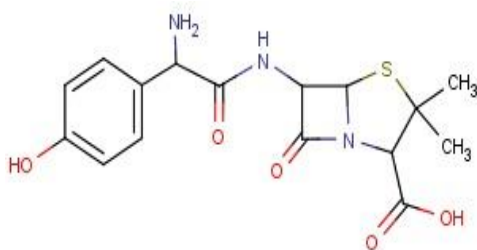


Fig. 1: Amoxicillin

The amino group in hydroxylamines (HONR₂) is more nucleophilic than simple amines. Solvents such as dimethyl formamide DMF and dimethyl sulphoxide DMSO are commonly being used in reactions involving nucleophilic addition of hydroxyl amines to carbonyl compounds. These are aprotic but polar enough to solubilize the nucleophile, leading to high reactivity of the reagent.³⁴

It is basically known that hydroxylamine reacts, nucleophilically, with aldehydes and ketones to give crystalline colored oximes that form also colored complexes with ferric ions. The nucleophilic potency of hydroxyl amine was also being shown by its rapid reaction with penicillins to give the corresponding hydroxamic acid, which forms a colored complex with ferric ion that can be determined colorimetrically³⁵.

Hydroxamic acids are important bioligands.³⁶ They are involved in numerous biological processes including metal-ion transport and inhibition of metalloenzymes^{37, 38}. Enormous amount of information has accumulated about hydroxamic acid residues with respect to their biomedical applications, synthesis and the determination of the structures of their metal complexes.³⁹⁻⁴¹ Complexation of metal ions with hydroxamic acids is the starting point of a number of analytical determinations.⁴¹ All hydroxamic acids, react with ferric chloride to give rust brown complex salts. These colored complexes form the basis for the sensitive qualitative and quantitative determination of carboxylic acids and their derivatives. The ferric hydroxamate formation has also been used as a qualitative test for the presence of aromatic amides⁴².

Job's method, also known as the method of continuous variations, is a versatile spectrophotometric method that has been used, successfully, to determine the composition of many complexes in solution.⁴³ The Job's method could also be used in the semi-quantitative determinations of the mole ratio between a metal ion and the ligand in the complex. The mole ratio method is another spectrophotometric method that has been adopted to determine stoichiometry of complexes⁴⁴.

However, the literature search revealed that there were also no attempts in using derivative UV spectrophotometric methods for the analysis of amoxicillin or its use in the analysis of β -lactam antibiotics. The principle on which the method adopted in the present research work is based on the fact that β -lactam rings are susceptible to nucleophilic attack by nucleophiles such as hydroxylamines⁴⁵. It is expected that

nucleophilic attack by hydroxylamine would lead to ring opening, thus leading to the formation of the corresponding hydroxamic acid, Figure 2. Simultaneous conversion of the hydroxamic acid into the ferric ion complex affords a red color complex. The latter could be used as a derivative β -lactam complex that could, quantitatively, be estimated by UV/Vis spectrophotometric procedure in combination with Job's Method and the Mole Ratio Method. Hence, the main aim of this investigation is to synthesize this ferric ion complex, investigate its formation, examine its stability and estimate its level of concentration as an index of β -lactam moiety in amoxicillin.

MATERIALS AND METHODS

Reaction of Amoxicillin with hydroxylamine hydrochloride

A saturated solution of amoxicillin was made in six test tubes, by dissolving the drug (100 mg) in absolute ethanol (2 ml). 1M Hydroxylamine hydrochloride (2ml) was added to five of the test tubes. The tubes were then heated in a water bath maintained at 100 C for specific duration of time. The tubes were, then, cooled to room temperature and one drop of aqueous ferric chloride (5%) solution was added. Development of any coloration was recorded. The sixth tube was left and kept at room temperature to test the formation of the acid at this temperature. Table 1.

Reaction of hydroxylamine hydrochloride with acetamide (control experiment)

Acetamide (100 mg) was placed in six test tubes and then dissolved in absolute ethanol (1 ml) to form a saturated solution. 1M Hydroxylamine hydrochloride (1 ml) was added to five of the test tubes. The five test tubes were, then, heated in a water bath maintained at 100 C for 1, 2, 3, 4 and 5 minutes for consecutive tubes. The sixth test tube was left at room temperature. Time for heating the test tubes was recorded for each, see Table (2). Test tubes were then removed after the elapse of specific time duration for each one. The test tubes were cooled to room temperature and one drop of aqueous ferric chloride (5%) solution was then added. Development of a red coloration was observed in each of the five heated tubes. A very faint transient color was observed on the sixth tube left at room temperature.

UV-spectral analysis of the reaction of hydroxylamine hydrochloride with amoxicillin in different mole ratios (1:1, 2:1 and 3:1) using ferric chloride as a chromogen

Three accurate separate weights (0.20g) of amoxicillin trihydrate were, separately, placed in three 100 ml volumetric flasks, dissolved in three different volumes (5 ml, 10 ml and 15 ml) of hydroxylamine hydrochloride solution in DMSO (0.6706 %). The three mixtures were shaken and sonicated for 30 minutes and the volumes were made up to the mark by DMSO. Three 10 ml volumes each of these three solutions were transferred to three 50 ml, separate volumetric flasks. Ferric chloride in DMSO (7 ml, 1.614 %) was added to each flask. An instant reddish brown coloration was obtained; the volume was then made up to 50 ml by DMSO. Instantly, each of the three solutions was scanned in a UV/Vis-spectrophotometer set at a wavelength range 300-750 nm. The three solutions were left to stand for 24 hours at room temperature. UV-scanning was again performed on these solutions at the same wavelength range, Table 5.

IR spectral analysis of the reaction products of hydroxylamine hydrochloride with amoxicillin in different molar ratios (1:1, 2:1 and 3:1)

Three accurate separate weights (3 \times 0.2g) of amoxicillin trihydrate were, separately, placed in three 50 ml volumetric flasks. Three volumes of hydroxylamine hydrochloride aqueous solution (0.67%, 5 ml, 10 ml and 15 ml) were added into the flasks and sonicated for 30 minutes. The sonicated mixtures were dried overnight; at room temperature on betray dishes. The crystals, so formed, were collected and an IR-spectral analysis was performed.

Determination of the λ_{max} of amoxicillin ferric hydroxamate complex in aqueous and acidic media

Amoxicillin trihydrate (0.05 g) was placed in 100 ml volumetric flask and dissolved in water (10 ml). Aqueous hydroxylamine hydrochloride (5 ml, 0.19%) was added and the mixture was well shaken and sonicated for 30 minutes. An acidic aqueous solution of ferric chloride (0.1 M) was prepared. The aqueous ferric chloride (5ml, 0.44%) was then added to the mixture. A reddish brown coloration developed and started, gradually, to fade out. The volume was made up to 100 ml by distilled water. The solution was scanned in a UV/Vis-spectrophotometer set up in the range 200-750 nm. Another solution was scanned within the same wavelength range after the elapse of 24 hours. The experiment was repeated using the constant concentrations of hydroxylamine hydrochloride, ferric chloride and amoxicillin mentioned previously. Table 3.

Determination of the λ_{\max} of the ferric hydroxamate amoxicillin complex in DMSO

Amoxicillin trihydrate (0.05 g) was placed in a 100 ml volumetric flask, and dissolved in DMSO (10 ml) followed by addition of hydroxylamine hydrochloride in DMSO (5ml, 0.19%). The mixture was well shaken and sonicated for 30 minutes. Ferric chloride in DMSO (5ml, 0.44%) was added to the sonicated mixture. A stable reddish brown colored complex was obtained. The solution was completed to volume by DMSO. The solution was scanned in a UV/Vis-spectrophotometer set up in the wavelength range 200-600 nm. Another solution was scanned within the same wavelength range after the elapse of 24 hours.

Another two experiments were performed using two dilute solutions of the amoxicillin ferric hydroxamate complex, prepared via taking 15 ml from the stock complex solution, previously obtained and completed to 50 ml by DMSO. One of the resulting solutions was scanned in a UV/Vis-spectrophotometer set up in the wavelength range 200-600 nm. And the other was scanned within the same wavelength range after the elapse of 24 hours, Table 4.

Determination of the empirical formula of the amoxicillin ferric hydroxamate complex by Job's method

Amoxicillin trihydrate (0.05 g) was placed in 100 ml volumetric flask. Hydroxylamine hydrochloride in DMSO (5 ml, 0.17%) was added. The mixture was well shaken and sonicated for 30 minutes. The volume was then completed to volume by DMSO. Eleven varying volumes (0 to 10 ml) of this sonicated solution were treated with successive varying volumes (10 to 0) of ferric chloride in DMSO (0.37%), The increment of each variation is 1.0 for each solution Table 6.

Determination of empirical formula of amoxicillin hydroxamate ferric ion complex by the Mole Ratio method)

Two standard solutions in DMSO one of hydroxyl amine hydrochloride (0.66%), the other of ferric chloride (1.55 %), were prepared. Amoxicillin (0.2 gm) was dissolved in the hydroxylamine hydrochloride (5 ml) in a 100 ml volumetric flask. The solution was sonicated for 30 minutes, and then the volume was made up to 100 ml by DMSO. 10 ml of this stock solution of amoxicillin hydroxamic acid were placed in seven volumetric flasks. To each of these seven volumetric flasks a serial volumes of 1, 2, 3, 4, 5, 6 and 7 ml of ferric chloride standard solution were added

(solutions of group I), where the ferric chloride concentration was fixed and the hydroxyl amine concentration was varied. An instant reddish brown colouration appeared, after each addition. Each of the seven solutions were scanned, individually, in a UV-spectrophotometer set at the visible region (300-750 nm). For each a single broad band was obtained at a maximum wavelength of absorbance λ_{\max} 520, Table 7.

Another experiment was then performed using the same stock standard solutions and utilizing the same procedure, but the volume of the ferric chloride solution was kept constant (3 ml) and that of amoxicillin hydroxamic acid was varied 4, 6, 8, 10, 12, 14, 16 that is, a concentration range $3.80 \times 10^{-5} \text{M}$ to $1.50 \times 10^{-3} \text{M}$ (group II solutions), Table 8.

RESULTS AND DISCUSSION

Previous methods adopted in the analysis of β -lactam antibiotics require expensive high grade solvents, sophisticated instruments and numerous steps of analysis that are time consuming. The analytical procedure adopted in the present study involves the synthesis and analysis of amoxicillin ferric ion complex that overcome some drawbacks of previous methods of analysis. Furthermore, these ferric ion complexes could be used, generally, as a quantitative estimation of the β lactam rings in any suspected drug adopting a UV/Vis spectrophotometric analysis in combination with Job's Method and the Mole Ratio Method. Moreover, the synthesis of amoxicillin hydroxamate ferric ion complex may itself be of considerable importance as a source of ferric ions.



Fig. 2: Amoxicillin hydroxamic acid

The present research study involves a number of phases, each of vital synthetic and analytical importance. The first being the reaction of the β -lactam ring of amoxicillin with the hydroxylamine, whose aim is the determination of the stoichiometric ratio of the amoxicillin hydroxamic acid and ferric ion.

The reaction of hydroxylamine with amoxicillin was attempted in duplicates: one trial at room

temperature and the other in which the reaction mixture is warmed to 100 C. After an elapse of appropriate time, few mls of an aqueous solution of ferric chloride were added, instantly a reddish brown color developed in both reaction mixtures, which indicated the formation of amoxicillin hydroxamate ferric ion complex. It was observed that the color started to fade out gradually with time indicating the instability of the complex. Alternatively, it was found that when the reaction was carried in a non-aqueous system, aprotic solvent, DMSO, and in excess of ferric chloride, more intense and persistent reddish brown color was produced. This was, qualitatively, indicative of more yield and increasing stability of the complex in this aprotic solvent. In an attempt to verify that nucleophilic attack takes place, preferably, at the carbonyl group of the β -lactam ring and does not occur at the amide carbonyl of the side chain, another experiment was conducted. Acetamide was used as a reactant (control) instead of amoxicillin, but establishing the same reaction conditions. A very faint and transient purple coloration was produced. This control reaction demonstrated that nucleophilic attack of hydroxylamine is predominantly, targeting the carbonyl group of the β -lactam ring, rather than that of the amide side chain, leading to synchronous ring opening.

Examination of the stability of amoxicillin hydroxamate ferric ion complex towards changes of pH, time and dilution of the reaction mixture with water have been monitored via the measurement of absorbance at λ_{\max} 487.5 nm of the complex using UV-Vis-spectrophotometric analysis. The results are summarized in Table 1 and Table 2. It could be noticed from Table 1 that there is slight decrease in absorbance and slight blue shift of the wavelength to 484.0 nm after few minutes, which is indicative that an initial and progressive gradual instability of the complex has started to occur. On the elapse of 24 hrs, the wavelength suffered remarkable blue shift to 353 nm, which indicates a complete destruction of the complex. On the other hand, an increase of the acidity of the medium of the complex to pH 3.0 resulted in a recognized red shift to 494.0 nm and a decreased absorbance to 0.388, which indicates that the initial increment of instability may occur on decrease of pH. The maximum wavelength of absorption λ_{\max} of amoxicillin hydroxamate ferric ion complex has not undergone any structural

changes after an elapse of few minutes from the time of preparation as the initial λ_{\max} 487.5 nm suffers a trivial blue shift change to λ_{\max} 484.0 nm. It was then concluded that analytical studies for the complex should be carried out promptly after its preparation in a medium of appropriate pH, Table 2, It could be noticed from Table 4 that when the reactants are made in DMSO a very slight and negligible change of wavelength of 2.0 nm has occurred, which indicates that the complex is stable in non-aqueous aprotic solvent. Alternatively, the stability even prevails after 24 hours compared to that when the reaction is conducted in an aqueous medium. It has, also, been observed that dilution with this solvent has no effect on stability.

IR-spectral analysis of the reaction products of hydroxylamine hydrochloride with amoxicillin in different molar ratios 1:1, 2:1 and 3:1 reveals that the spectrum of the amoxicillin hydroxamate is void of the band at 1776 cm^{-1} which, was very intense band in the spectrum of amoxicillin standard. The disappearance of this band of the carbonyl group of the β -lactam ring is, clearly, indicative that nucleophilic attack has occurred, solely, on the carbonyl of the β -lactam ring.

Furthermore, UV-spectrophotometric analysis were attempted to confirm that there is no reaction in the amide side chain of amoxicillin molecule with even excess hydroxylamine hydrochloride. This was carried out by the reaction of hydroxylamine hydrochloride with amoxicillin in different mole ratios (1:1, 2:1 and 3:1), with FeCl_3 concentration being kept constant. For all of these three mole ratios a reddish brown colored complex was obtained after addition of ferric chloride in DMSO to amoxicillin hydroxamic acid in DMSO. When the number of moles of hydroxyl amine hydrochloride added was increased, the concentration of amoxicillin hydroxamate ferric ion complex was decreased by 15.6 %, Graph 1. UV-scanning has shown a very broad single band with maximum absorbance at approximately 520 nm Table 3. This confirmed that the ferric ion complexes with only one kind of hydroxamic acid, which, presumably, results from the opening of the β -lactam ring. It could also be observed that there is an extremely insignificant small red shift ranging from 2-6 nm in the UV-absorbance for the three solutions after being kept for 24 hours in DMSO, which indicated the stability of the complex over time, Table 3.

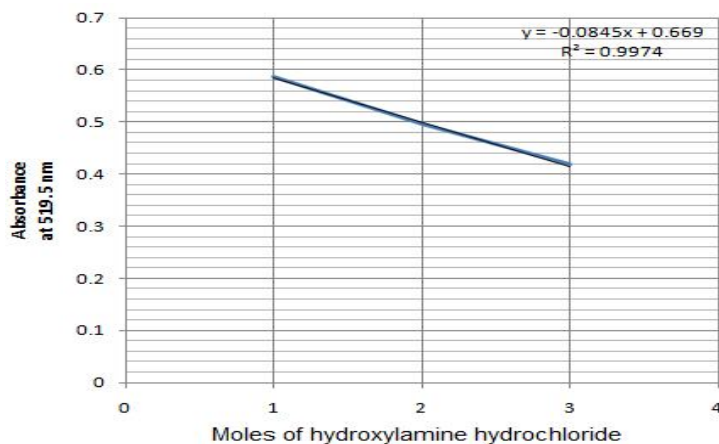


Fig. 3: Graph 1: Absorbance of solutions of amoxicillin and hydroxylamine hydrochloride within mole ratios of 1:1, 1:2 and 1:3

The empirical formula of the complex was obtained by the use of Job's method via the determination of the mole ratio of amoxicillin hydroxamic acid and the ferric ion. As amoxicillin trihydrate contains 12.49% moisture, then it follows that its moisture content is 0.04376 g. And that 100 g of anhydrous amoxicillin contains 99.72 g pure amoxicillin. The application of Job's method starts with the preparation of two standard solutions of amoxicillin hydroxamic acid (1.94×10^{-3} M) and ferric chloride solution (2.288×10^{-3} M). The solutions were mixed together in a two varying reversed volumes to get a final volume of 10 ml in each case. The reddish brown amoxicillin hydroxamate ferric ion complex so

formed was scanned in a UV-spectrophotometer set up at 529 nm. These two experiments were represented by a plot of absorbance against volume of ferric chloride Graph 2, which is based on Job's method. By The linear regression of ascending and descending lines, as represented by the blue lines in the graph, where the cross of the lines represents the stoichiometric point (7.5 ml amoxicillin hydroxamic acid and 2.5 ml of ferric chloride). Using simple mathematical equations, calculations have been made to determine the mole ratio which was found to be 1:6. The molecular formula of the complex was proposed to be: $\text{Fe}_6\text{C}_{16}\text{H}_{17}\text{N}_4\text{S O}_6$.

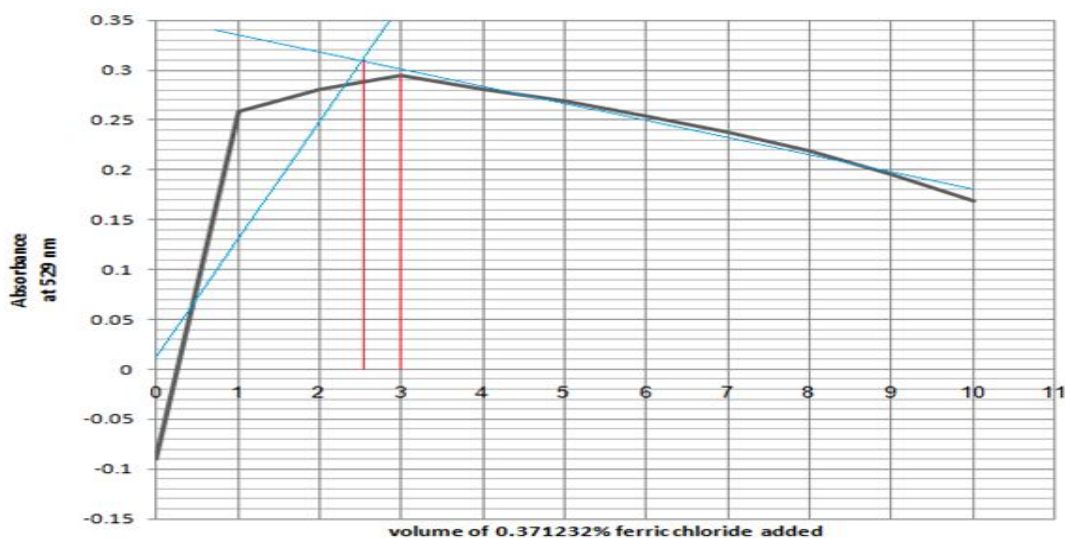


Fig. 4: Graph 2: Determination of the Mole Ratio of Amoxicillin hydroxamic acid and Ferric ion via Job's Method

The Mole Ratio method, another method for the determination of the molecular formula of

amoxicillin hydroxamate ferric ion complex has been attempted, which consists of mixing the

two solutions of amoxicillin hydroxamic acid and ferric chloride solutions, both in DMSO, in such a manner that one solution volume is kept constant, while the other is varied. The experiment was repeated by reversing the trend of the addition. In both cases, the resulting complex was scanned by UV-spectrophotometer set up at λ_{\max} 520 nm. Moreover, there is no remarkable differences in the λ_{\max} values between the two sets of solutions. It could also be noticed from Graph

3 and Graph 4 that the point of inflection at which the slope is changed, is the stoichiometric point between amoxicillin hydroxamic acid and ferric ion at which the absorbance is 0.45714 for Group 1 solutions and 0.418 for Group II solutions. Via the use of mathematical formulae and conducting simple calculations the mole ratio of amoxicillin hydroxamic acid and ferric ions was found to be 1:6, a verification of what had been determined by the Job's method.

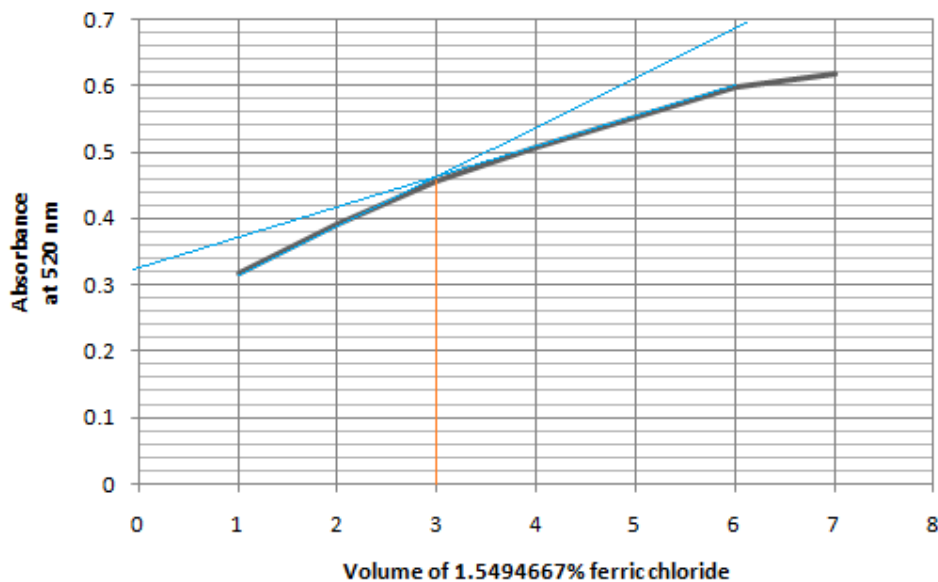


Fig. 5: Graph 3: Mole Ratio Method solutions of Group (I): Amoxicillin hydroxamic acid is kept constant (10 ml) while that of ferric chloride varied

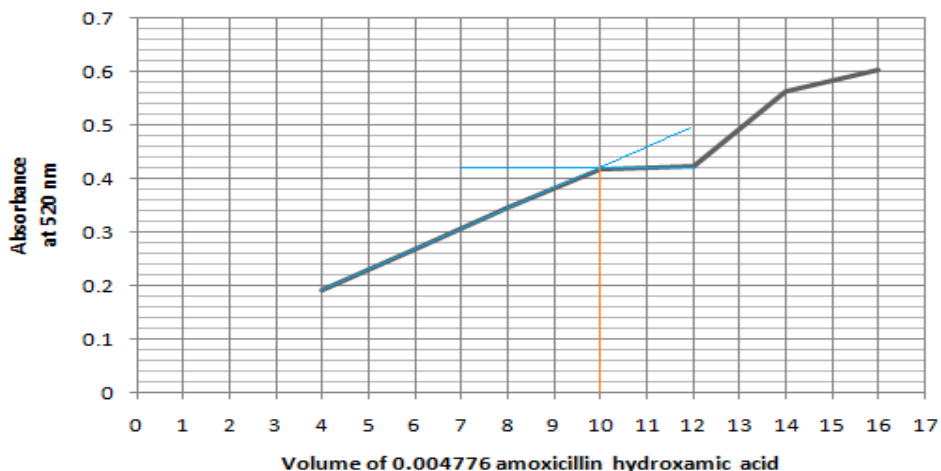


Fig. 6: Graph 4: Mole Ratio method solutions of Group (II): Ferric chloride is kept constant (10ml) while that of amoxiciliin hydroxamic acid varied

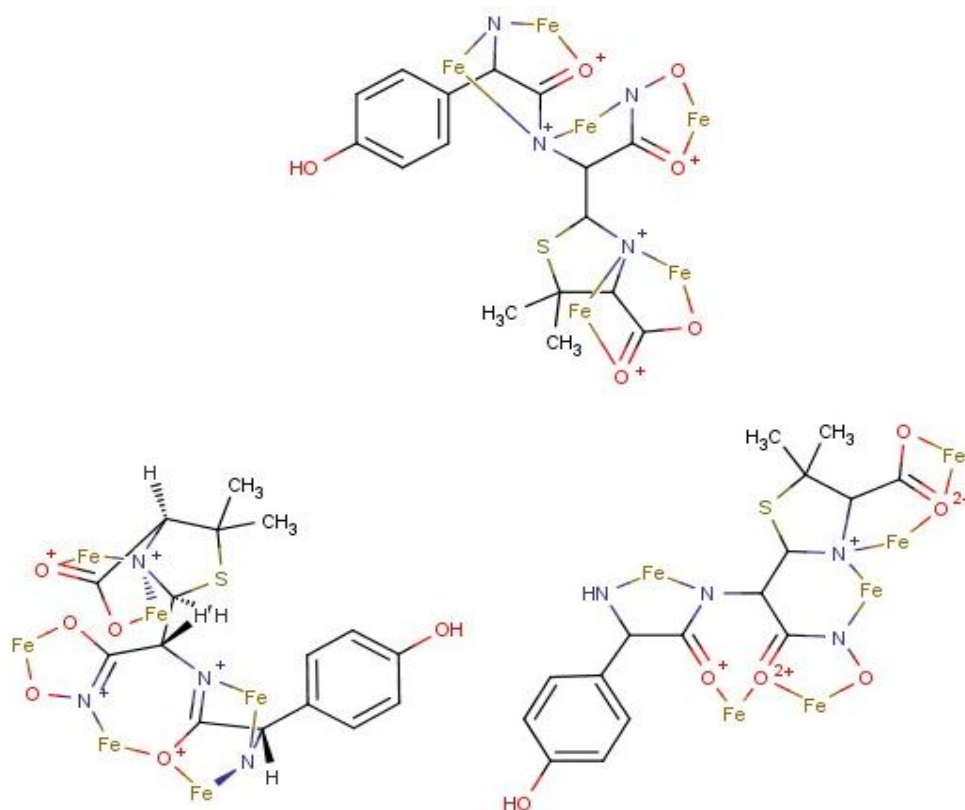


Fig. 7: The proposed chemical structures of amoxicillin hydroxamate ferric ion complex

It could be concluded that the reaction between amoxicillin and hydroxylamine hydrochloride takes place readily due to formation of amoxicillin hydroxamic acid which produces a reddish brown complex with ferric chloride solution. By conducting a control experiment it was confirmed that the nucleophilic attack of hydroxylamine hydrochloride took place, stereo-specifically, on the carbonyl group of the β -lactam ring with simultaneous ring opening to form amoxicillin hydroxamic acid. The latter is so stable that it withstands heating to a temperature near 100 C beyond which it is only stable for 5 minutes. While the ferric hydroxamate complex is unstable in the aqueous medium it is very stable in aprotic solvents such as DMSO. It is advisable to maintain the reaction system at or near pH of 3. UV-spectrophotometric analysis set at λ_{\max} 520 nm utilizing Job's method of contentions variation and mole ratio method, have revealed that the ratio of amoxicillin hydroxamic acid to ferric chloride is 1:6.3 and 1:6, respectively. Accordingly, the following proposed chemical formula: $C_{16}H_{17}Fe_6N_4O_6S$

and chemical structure of the complex were proposed.

Based on the fact that this research investigation is just a preliminary step towards estimation of β -lactam antibiotics in general, then it should be extended to apply this method to other β -lactam antibiotics. It is also recommended that a suitable chromatographic procedure should be used to isolate the amoxicillin hydroxamate ferric ion complex. The present work has opened a door for further research investigations that should be undertaken to study the complexation of amoxicillin hydroxamic acid with other ions of the following elements such as Al, Zn, Pb and Hg.

ACKNOWLEDGEMENT

The authors would like to convey their thanks and gratitude to UMST University for funding this research project and providing the necessary laboratory facilities. Thanks are due to Prof. Sulfab of the Chemistry Department, Faculty of Science, U of K for his beneficial comments.

Table 1: λ_{\max} of amoxicillin hydroxamate ferric ion complex in aqueous, neutral and acidic media

Solution No.	Solutions	Time of scanning	λ Scanning range (nm)	λ_{\max} (nm)	Absorbance
1	0.05% amoxicillin hydroxamate as ferric ion complex	Freshly prepared solution	200-750	487.5	0.465
2	0.05% amoxicillin hydroxamate as ferric ion complex	After few minuets	200-750	484.5	0.342
3	0.05% amoxicillin hydroxamate as ferric ion complex in acidic medium pH 3	Freshly prepared	200-750	494	0.388
4	0.05% amoxicillin hydroxamate as ferric ion complex	After 24 hours	200-600	353	2.055

Table 2: Determination of λ_{\max} of amoxicillin hydroxamate ferric ion complex in DMSO

Solution No.	Solutions	The time of scanning after preparation	Wavelength scanning range	λ_{\max} nm	Absorbance
1	0.05% amoxicillin hydroxamate ferric ion complex	Freshly prepared	200-750 nm	516	1.55
2	0.05% amoxicillin hydroxamate ferric ion complex	After 24 hrs.	200-600 nm	518	1.15
3	0.0075% amoxicillin hydroxamate ferric ion complex	Freshly prepared	200-750 nm	522	0.54
4	0.0075% amoxicillin hydroxamate ferric ion complex	After 24 hours.	200-600 nm	523	0.36

Table 3: UV-Spectrophotometric Scanning of the amoxicillin ferric ion hydroxamate complex produced via different mole ratio of hydroxylamine hydrochloride

sample no	weight of amoxicillin trihydrate	volume of hydroxylamine added	amoxicillin to hydroxylamine mole ratio	λ_{\max} in nm	Total volume in ml	Absorbance	λ_{\max} after 24 hrs	absorbance after 24 hrs
1	0.2 g	5 ml	1:01	520	50	0.587	522	0.502
2	0.2 g	10 ml	1:02	520	50	0.495	525	0.317
3	0.2 g	15 ml	1:03	520	50	0.418	526	0.228

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