Determination of Stability Constants and Thermodynamic parameters of Cefotaxime –Fe(III) Complex at Different Temperatures

O. V. Ikpeazu¹, I. E. Otuokere^{2*} and K. K. Igwe³

¹ Department of Biochemistry, Abia State University, Uturu

² Department of Chemistry, Michael Okpara University of Agriculture, Umudike, Nigeria

³ Department of Vet Biochemistry and Animal Production, Michael Okpara University of Agriculture, Umudike, Nigeria

*Corresponding author: ifeanyiotuokere@gmail.com +2347065297631

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ABSTRACT

Cefotaxime, a β -lactam antibiotic, has a structure which enables it to act as a chelating agent. The formation of Fe(III) complex with cefotaxime has been studied colorimetrically at an absorption maximum of 480 nm at different temperatures. The data showed that Fe(III) and cefotaxime combine in the molar ratio of 1:1 at pH 7.4 with ionic strength maintained using 0.1M KNO₃. The stability constants of the complex were calculated to be $1.56 - 1.90 \times 10^4$ by continuous variation method and $1.34 - 1.71 \times 10^4$ by mole ratio method at 25 and 40 °C respectively. ΔH^θ values for the complex were calculated to be -1.02×10^4 and -1.05×10^4 J by continuous variation method and mole ratio method respectively. ΔG^θ of the complex were calculated to be $-2.44 - (-2.51) \times 10^4$ J by continuous variation method and $-2.41 - (-2.48) \times 10^4$ J by mole ratio method at 25 and 40 °C. ΔS^θ of the complex were calculated to be $2.44 - 2.51 \times 10^4$ J/K by continuous variation method and $-2.41 - 2.48) \times 10^4$ J/K by mole ratio method at 25 and 40 °C respectively. Cefotaxime is a good chelating agent and can be an efficient antidote in the therapy of copper overload or poisoning.

Keywords: Cefotaxime, stability constant, enthalpy, entropy, free energy, complex

1 INTRODUCTION

Cefotaxime is an antibiotic used in the treatment of bacterial infections [1]. Specifically it is used in the treatment of joint infections, pelvic inflammatory disease, meningitis, pneumonia, urinary tract infections, sepsis, gonorrhea, and cellulitis [1]. It is administered either by injection into a vein or muscle [1]. Common side effects are nausea, allergic reactions and inflammation at the site of injection [1]. Other side effects are *Clostridium difficile* diarrhea [1]. It is not recommended in patients who have had previous anaphylaxis to a penicillin [1]. Relatively, it is safe for use during pregnancy and breastfeeding [1, 2]. It is among the third-generation cephalosporin family of medications and works by interfering with the bacteria's cell wall [1]. Cefotaxime was discovered in 1976, and was commercially available for use in 1980 [3,4]. It is on the list of World Health Organization's Essential Medicines, the safest and most effective medicines needed in a health

system [5]. It is available as a generic medication [1]. Cefotaxime is a β -lactam antibiotic that inhibits the synthesis of bacterial cell wall by binding to one or more of the penicillin-binding proteins (PBPs). This inhibits the final transpeptidation step of peptidoglycan synthesis in bacterial cell walls, thus inhibiting cell wall biosynthesis. Bacteria eventually lyse due to ongoing activity of cell wall autolytic enzymes (autolysins and murein hydrolases) in the absence of cell wall assembly [6]. Due to the mechanism of their attack on bacterial cell wall synthesis, β -lactams are considered to be bactericidal [7].

From ancient times, the special role of iron in health and disease in man has been recognized [8]. Early medicinal uses of iron by Egyptians, Hindus, Greeks, and Romans have been reported [9,10]. During the 17th century, iron was used in the treatment of chlorosis (green disease), a condition that resulted from the iron deficiency [11]. However, it was not until 1932 that the

importance of iron was finally settled by the convincing proof that inorganic iron was needed for hemoglobin synthesis [12]. For many decades, nutritional interest in iron focused on its role in hemoglobin formation and oxygen transport [13]. Recently, low iron intake and/or bioavailability are responsible for most anemia in industrialized world, they account for only about half of the anemia in developing countries [14], where infectious and inflammatory diseases exist.

Cefotaxime chelating ability could result due to the presence of C=O, NH₂, COOH, C OOR, NH and NO electron donating groups (Figure 1). The synthesis and antibacterial activity of cefotaxime complexes of Mn(II), Fe(II), Co(II), Ni(II), Cu(II) and Cd(II) have been reported [15]. Spectroscopic method suggested a tetrahedral geometry for the metal complexes of cefotaxime. (Figure 2). The amino, β -lactamic and carboxylic groups of cefotaxime formed thermodynamically stable saturated rings by interacting with transition metals [15]. Spectrophotometric study of stability constant of cimetidine-Ni(II) complex was reported by Tirmizi and co-workers [16]. Studies of complex formation between curcumin and Hg(II) by spectrophotometric method have been published by Waranyoupalin and co-workers [17]. Determination of protonation constants and stability constants of lanthanides with derivative of

(10-[4-aminobenzyl(hydroxyl)phosphonylmethyl]-1,4,7,10-tetraazacyclo-1,4,7-triacetic acid complexes by UV-Vis spectrophotometry and potentiometry have also been reported [18]. However, to the best of authors knowledge, stability constants of cefotaxime – Fe(III) complex at different temperatures have yet not appeared in the literature. These stability constants are useful to study the effects of cefotaxime on trace elements and mineral metabolism. It is possible that changes in trace element and mineral concentration induced by cefotaxime can be an efficient antidote in the therapy of iron overload or poisoning. In the present study, colorimetric methods were used for the determination of stability constants and

thermodynamic parameters of Fe(III) with cefotaxime at 25 and 40 °C respectively.

Figure 1: Structure of cefotaxime

Figure 2: Tentative structure of cefotaxime metal complexes, M = Mn(II), Co(II), Ni(II), Cu(II) and Cd(II) [15].

2. MATERIALS AND METHODS

2.1. Instrumentation

Colorimetric measurements were performed on auto colorimeter ME-51. Orion Versa Star Pro pH Benchtop meter (VSRAR10 series) calibrated with standard buffer solutions of pH 4 and 10, was used for pH measurements.

2.2. Reagents

All the chemicals used were of analytical grade purity. Cefotaxime was purchased from Nitin Life sciences Limited, Indian FeCl₃.6H₂O was purchased from Merck Germany. Double-distilled water was used throughout the experiment.

2.3. Preparation of 2 x 10⁻² M FeCl₃.6H₂O

FeCl₃.6H₂O (5.406 g, 20 m mol, M. Wt. = 270.30 g/mol) was dissolved in freshly distilled water in a beaker and was made up to the mark in a 1000 cm³ volumetric flask.

2.4. Preparation of 2 x 10⁻² M cefotaxime
Cefotaxime (9.1094 g, 20 m mol, M. Wt. = 455.47 g/mol) was dissolved in freshly distilled water in a beaker and was made up to the mark in a 1000 cm³ volumetric flask.

2.5. Procedure for continuous variation method
FeCl₃.6H₂O (2 x 10⁻² M) (0, 1, 2, 3, 4, 5, 6 cm³) was pipetted out and transferred into seven 50 cm³ volumetric flasks. Cefotaxime (2 x 10⁻² M) (6, 5, 4, 3, 2, 1, 0 cm³) was added, respectively to the Fe(III) solution so that the mole fraction remained constant. The pH adjusted to 7.4 and ionic strength maintained constant by using 0.1 M KNO₃. Their absorbance were measured at 480 nm (maximum absorbance of the complex) and at a temperature of 25 and 40 °C, respectively.

2.6. Procedure for mole ratio method
FeCl₃.6H₂O (2 x 10⁻² M) (2 cm³) was pipetted out and transferred into each of the seven 50 cm³ volumetric flasks. Cefotaxime (2 x 10⁻² M) (1, 2, 3, 4, 5, 6, 7 cm³) was added to each of the Fe(III) solution respectively. Wavelength of maximum absorbance of the complex (480 nm) was noted against blank reagent FeCl₃.6H₂O. Their absorbances were measured at 480 nm and at a temperature of 25 and 40 °C, respectively.

2.7. Calculation of stability constant
Equation 1 [19] was applied in the calculation of stability constant.

$$K_{cef} = \frac{1 - \alpha}{m^m \cdot n^n (\alpha)^{m+n} (C)^{m+n-1}} \quad \text{Equation 1}$$

Where C is the concentration of the complex at stoichiometry point, α is the degree of dissociation, m and n are the corresponding stoichiometric coefficients of metal and ligand respectively.

The degree of dissociation (α) was calculated using equations 2, 3 and 4 [19].

$$A_{\alpha} = A_o - A_{max} \quad \text{---Equation 2}$$

$$A_{\alpha} = \epsilon b C \quad \text{---Equation 3}$$

$$\alpha = \frac{A_{\alpha}}{\epsilon b C} \quad \text{---Equation 4}$$

Where A_{max} is absorbance value of the maximum at experimental curve that represents the maximum quantity of the complex that is formed. A_o is absorbance value corresponding to the intersect point of the theoretical straight lines. A_{α} is the absorbance value of the part of dissociated concentration of complex. ϵ is molar absorptivity, b is cell thickness, C is a concentration of complex at stoichiometry point. ΔH , ΔG and ΔS were calculated using equations 5, 6 and 7 respectively.

$$\ln \frac{K_2}{K_1} = \frac{-H^{\theta}}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \quad \text{---Equation 5}$$

$$\Delta G^{\theta} = -RT \ln K_{cef} \quad \text{---Equation 6}$$

$$\Delta G^{\theta} = \Delta H^{\theta} - T \Delta S^{\theta} \quad \text{---Equation 7}$$

3. RESULTS AND DISCUSSION

3.1. The properties of complex

The absorption spectra of cefotaxime-Fe(III) complex and FeCl₃.6H₂O are shown in Figure 3. The absorption spectra were recorded at wavelength range of 400 – 670 nm. The reaction of cefotaxime with FeCl₃.6H₂O was investigated at two different temperatures i.e. 25 and 40 °C. It was observed that cefotaxime with FeCl₃.6H₂O formed a milky colour, water soluble complex. The absorption maximum of the complex was 480 nm. Cefotaxime and FeCl₃.6H₂O does not absorb significantly at this wavelength hence 480 nm was used for the analytical measurements. In aqueous solution, iron exist as [Fe(H₂O)₆]³⁺, and λ_{max} was observed at 430 nm. Ironaquo complex is a labile complex because water behaved as a weak ligand. Cefotaxime displaced water from [Fe(H₂O)₆]³⁺ to form a stable cefotaxime - Fe(III) complex.

Figure 3: Absorption spectra of cefotaxime-Fe(III) complex (2×10^{-2} M) (Series 1) and $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (2×10^{-2} M)(Series 2)

Figure 5: Job's curves for stability constants of equimolar solutions at 40°C

Figure 4 Job's curves for stability constants of equimolar solutions at 25°C

Figure 6: Mole ratio method curves for stability constant at 25°C

Figure 7: Mole ratio method curves for stability constant at 40°C

Table 1: Experimental data of cefotaxime-Fe(III) complex at 480 nm by continuous variation method

S/N	FeCl ₃ .6HO (2 x 10 ⁻² M)	Cefotaxim e (2x10 ⁻² M)	Mole fraction of Fe(III)	Absorbance at 480 nm	
				25°C	40°C
1	0.00	6.00	0.00	0.61	0.64
2	1.00	5.00	0.17	0.80	0.78
3	2.00	4.00	0.33	1.30	1.33
4	3.00	3.00	0.50	2.00	2.00
5	4.00	2.00	0.66	1.30	1.34
6	5.00	1.00	0.83	0.60	0.61
7	6.00	0.00	1.00	0.10	0.11

Table 2: Experimental data of cefotaxime-Fe(III) complex at 480 nm by mole ratio method

S/N	FeCl ₃ .6HO (2x10 ⁻² M)	Cefotaxim e (2x 10 ⁻² M)	Vol of Cefotaxime/ vol of Fe(III)	Absorbance at 480 nm	
				25 °C	40 °C
1	2.00	1.00	0.5	1.69	1.70
2	2.00	2.00	1.0	1.90	1.89
3	2.00	3.00	1.5	1.95	1.95
4	2.00	4.00	2.0	1.96	1.96
5	2.00	5.00	2.5	1.97	1.97
6	2.00	6.00	3.0	1.99	1.99
7	2.00	7.00	3.5	2.00	2.00

Table 3: Calculated stability constants and Gibbs free energies for cefotaxime-Fe(III) complex

S/N	Method	Metal : ligand ratio	Stability constant		ΔG (J)	
			25 °C	40 °C	25 °C	40 °C
1	Continuous variation	1:1	1.90 x 10 ⁴	1.56 x 10 ⁴	-2.44 x 10 ⁴	-2.51 x 10 ⁴
2	Mole ratio	1:1	1.71 x 10 ⁴	1.40 x 10 ⁴	-2.42 x 10 ⁴	-2.48 x 10 ⁴

Table 4: Calculated enthalpy and entropy change for cefotaxime-Fe(III) complex

S/N	Method	$\Delta H^\ominus$ (J)	ΔS (J/K)	
			25 °C	40 °C
1	Continuous variation	-1.02 x 10 ⁴	2.44 x 10 ⁴	2.51x 10 ⁴
2	Mole ratio	-1.05 x 10 ⁴	2.41 x 10 ⁴	2.48x 10 ⁴

The experimental data of cefotaxime-Fe(III) complex at 480 nm by continuous variation method is shown in Table 1 while the Job's plot at 25 and 40 °C are presented in Figures 4 and 5 respectively. The extrapolated value at the point of cross-section on continuous variation plot (Figures 4 and 5) corresponds to the total absorbance of the complex, indicating that the complex formation process has been completed. The mole fraction of Fe(III) at the point of intersection are 0.50 and 0.50 at 25 and 40 °C respectively. This corresponded to metal:ligand ratio of 1:1. This was in agreement with the proposed structure reported in literature [15].

The experimental data of cefotaxime-Fe(III) complex at 480 nm by mole ratio method is shown in Table 2 while the mole ratio plot at 25 and 40 °C are presented in Figures 6 and 7 respectively. The extrapolated value at the point of cross-section on mole ratio plot (Figures 6 and 7) corresponds to the total absorbance of the complex, indicating that the complex formation process has been completed. The vol. of cefotaxime/vol. of Fe(III) at the point of intersection are 1.00 and 1.00 at 25 and 40 °C respectively. This corresponded to metal:ligand ratio of 1:1. This was in agreement with the proposed structure reported in literature [15].

Continuous variation method and mole ratio suggested 1:1 metal:ligand ratio (Table 3). The positive values of the stability constants implied that the complex was stable (Table 3). Similar positive values of stability constant have also been reported in literature [20]. The results of stability constant suggested that cefotaxime could be effective against Fe(III) toxicity. It is efficient as Fe(III) chelating agent in the therapy of iron overload. The negative values of the free energies (Table 3) showed that the complexes were formed spontaneously. The values of the stability constants decrease with increase in temperature because the reactions were exothermic. The positive value of ΔS indicates increase in entropy (Table 4). Cefotaxime behaved as a tetradentate ligand at pH=7.4 this is in agreement with the finding in literature [15]. Both Job method and mole ratio methods supported the metal to ligand ratio of

1:1 as evidenced from the results of elemental analysis of Fe(II) - cefotaxime complex reported in literature [15]. Complexation occurred at pH=7.4 at 25 and 40 °C. This showed that the complex was stable both at room temperature and higher temperature. The stability constant values obtained from continuous variation and were compared with that of mole ratio method. It can be seen from the Tables 3 and 4 that the values obtained by both methods are in fair agreement.

CONCLUSION

Cefotaxime, a β -lactam antibiotic forms a reasonably stable complex with Fe(III). Job's method of analysis corresponded well with the analogous values obtained using mole ratio method of analysis. Owing to a high formation constant at body temperature, cefotaxime intake can remove iron from the body and this may disturb the functions of enzymes and can cause anaemia and weight loss. The Job's continuous variation and mole ratio methods data showed that Fe(III) and cefotaxime combine in the molar ratio of 1:1. The stability constant results suggest that cefotaxime used in the study is a good chelator agent and can be an efficient antidote in the therapy of Fe(III) overload or poisoning.

REFERENCES

1. The American Society of Health-System Pharmacists (2016), Cefotaxime Sodium, Retrieved 8 December 2016.
2. H. Richart (2015). Tarascon Pocket Pharmacopoeia 2015 Deluxe Lab-Coat Edition. Jones & Bartlett Learning. 87.
3. S.R. Walker (2012). Trends and Changes in Drug Research and Development. Springer Science & Business Media. 109.
4. F. Janos, G.C. Robin (2006). Analogue-based Drug Discovery. John Wiley & Sons. 494.

5. World Health Organization (2019). World Health Organization model list of essential medicines: 21st list 2019.
6. Cefotaxime drug information Archived (2007) Wayback Machine
7. U.S. Food and Drug Administration (2016). U.S. Department of Health and Human Services. Claforan Sterile (cefotaxime for injection, USP) and Injection (cefotaxime injection, USP).
8. J.L. Beard and H.D. Dawson (1997). Iron. In: B.L. O'Dell, R.A. Sunde , editors. Handbook of Nutritionally Essential Mineral Elements. New York: CRC Press; 275-334.
9. R.J. Wood and A Ronnenberg (2005). Iron. In: M.E. Shils, M. Shike, A.C. Ross, B. Caballero, R.J. Cousins, editors. Modern Nutrition in Health and Disease. 10th ed. Baltimore: Lippinco Williams & Wilkins, 248-70.
10. L.R. McDowell (1995). Minerals in Animal and Human Nutrition. 2nd ed. Amsterdam: Elsevier Science, p. 660.
11. K.Y. Guggenheim (1995) Chlorosis: The rise and disappearance of a nutritional disease. *J Nutr*, **125**, 1822 - 1825.
12. R. Yip and P.R. Dallman (1996). Iron. In: E.E. Ziegler, L.J. Filer, editors. Present knowledge in nutrition. 7th ed. Washington DC: ILSI Press, 278-92.
13. E.J. Underwood and N.F. Suttle (1999).. The mineral nutrition of livestock. 3rd ed. Wallingford: CABI International Publishing, 614.
14. WHO (2006). Guidelines on food fortification with micronutrients. In: Allen L, de Benoist B, Dary O, Hurrell R, editors. Geneva: WHO and FAO, 236.
15. J.R. Anaconda and G.D. Silva (2005). Synthesis and antibacterial activity of cefotaxime metal complexes, *Journal of Chilean Chemical Society*, **50**(2), 447 – 450.
16. S. A. Tirmizi, F.H. Wattoo, M. H. S. Wattoo, S. Sarwar , A.N. Memon and A.B. Ghangro (2012). Spectrophotometric study of stability constants of cimetidine–Ni(II) complex at different temperatures, *Arabian Journal of Chemistry*, **5**, 309–314
17. R. Waranyoupalin, S. Wongnawa, M. Wongnawa, C. Pakawatchai, P. Panichayupakaranant, P. Sherdschoopongse (2009). Studies of complex formation between curcumin and Hg(II) by spectrophotometric method: A new approach to overcome peak overlap, *Cent. Eur. J. Chem.* **7**(3), 388-394
18. M. Reková, P. Vanura and V. Jedináková-Krízová (2009) Determination of Protonation Constants and Stability Constants of Lanthanides with Derivative of H₄DOTA Complexes by the UV-VIS Spectrophotometry and Potentiometry, *The Open Inorganic Chemistry Journal*, **3**, 26 – 32
19. R.F. Abbas (2017). Spectrophotometric determination of stability constant by classical and Modified Varagas equations for procaine penicillin G using diazotization reaction depending on stoichiometric curves, *International Journal of ChemTech Research*, **10**(2), 485 – 496.
20. A.C. Tella, J.A. Obaleye and G.K. Obiyenwa (2011), Spectrophotometric Study of Stability Constants of Dapsone- Cu(II) Complex at Different Temperatures, *Journal of Pharmacy Research*, **4**(1), 241-244