

DIRECT AND DERIVATIVE SPECTROPHOTOMETRIC ESTIMATION OF GEMIFLOXACINMESYLATE BY CHELATION WITH Cr (III) ION

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ABSTRACT

A simple, sensitive and accurate spectrophotometric method was described for the determination of Gemifloxacin mesylate (GFX) a broad spectrum flouroquinolone anti bacterial either in pure form or in the tablet. The method is based on chelate formation between GFX and Chromium(Cr III) in aqueous media. The complex showed an absorption maximum at 545nm for zero order, 1st derivative at 620nm and Second derivative at 660nm respectively with apparent molar absorptivities of $1.07 \times 10^4 \text{ L-M}^{-1}\text{Cm}^{-1}$; $7.01 \times 10^3 \text{ L-M}^{-1}\text{Cm}^{-1}$ for 1st order derivative, $1.04 \times 10^4 \text{ LM}^{-1}\text{Cm}^{-1}$ for 2nd order derivative respectively. The solution of the complex obeyed beer's law in the concentration range of 2 to 20 µg/ml for zero order, 1 to 15 µg/ml for 1st order and 1 to 25 µg/ml for 2nd order respectively. The limit of detection and Limit of quantification were calculated and RSD were less than 0.2866. The chelate composition between GFX and Cr(III) ion was found to be 1:1 ratio determined by Job's continuous method and by Molar ratio method. The proposed method was applied for the determination of GFX in tablets without interference from common excipients. The results obtained by the application of this procedure showed percentage recoveries were 99.9 ± 0.1463 for zero order, 100.2 ± 0.1065 for 1st order and 100.6 ± 0.1589 for 2nd order respectively.

Key words: Flouroquinolone, Gemifloxacin mesylate, Chelate, Aqueous media, Spectrophotometric Pharmaceutical formulation.

INTRODUCTION

Gemifloxacin (GMF) chemically R,S-7-(3 amino methyl 4- syn methoxyimino-1pyrrolidiny)-1cyclopropyl-6-flouro-1,4,dihydro 4- oxo-1,8 naphthyridine-3-carboxylic acid methane- sulphonate¹⁻³ is a new flouroquinolone antibacterial compound with enhanced affinity for bacterial topoisomerase-IV and is being used for the treatment of respiratory and urinary tract infections.⁴⁻⁶ Literature review revealed few analytical methods for the determination of GMF include HPLC, electrophoresis, uv-spectrophotometry concerning visible spectrophotometry very few methods have been reported and no derivative spectrophotometry has been reported, hence sensitive and accurate, direct, derivative spectrophotometric method has been viewed⁷⁻¹⁰. The purpose for this present study was to develop direct and derivative spectrophotometric, stability indicating procedure for the selective determination of GFX mesylate by chelation with Cr(III) ion, to develop procedure capable of quantitation and describe and validate the structural ability of GFX to chelate with Cr(III), which is used in the preparation of wide range of drugs, and methods based on chelation of drug with iron, a cobalt and chromium have been studied and prospective work will be the study using proposed chelation procedures by direct and derivative spectroscopy, which not have been previously studied.

EXPERIMENTAL

Apparatus

All absorption Spectra were made using SCHIMADZU-160 A U.V-VIS Spectrophotometer equipped with 10mm matched Quartz cells.

Materials and Reagents

Chromium chloride ($\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$)¹¹⁻¹⁶ $1 \times 10^{-2}\text{M}$ solution in distilled water was prepared and standardized Iodometrically after oxidation to Dichromate¹⁷.

GFX $1 \times 10^{-3}\text{M}$ solution was prepared by dissolving 38.9mg in 100ml of distilled water and GFX 0.1 mg/ml in distilled water was prepared. GFX solutions were freshly prepared and stable for 24 hours at room temperature.

Gemez (Majesta, a division of Glenmark Pharmaceuticals Ltd., Mumbai.) Labelled to contain 320 mg of Gemifloxacin mesylate. G-cin (Hetero Drugs Ltd., Hyderabad) Labelled to contain 320 mg of Gemifloxacin mesylate were used.

Chelation of GFX with Cr(III)

To a 10 ml volumetric flask, transferred 1ml of $1 \times 10^{-3}\text{M}$ GFX stock solution and 2ml of $1 \times 10^{-2}\text{M}$ Cr(III)Cl_3 is added water shaken vigorously for 5 minutes and heat on a waterbath at $60 \pm 5^\circ\text{C}$ for 5, 10, 15, 20, 30 minutes respectively, cool and complete volume with distilled water. The resulted purple colour chelate was scanned in between 200-800nm against a blank. The λ -max for the Chelate at Zero order is 545nm, first order derivative is obtained at 620 nm and second order derivative at 660 nm. Fig 1- Fig 3.

Procedure for dosage form

An accurately weighed amount of finely powdered tablet equivalent to 100mg of drug was dissolved in about 10ml of distilled water and transferred in to 100ml of calibrated volumetric flask and after 15minutes mechanical shaking was filtered into a 100ml of calibrated volumetric flask through Whatmann no:41 filter paper and was diluted to 100ml with distilled water and the same procedure was followed as described above

Optimum conditions

Effect of pH: At acidic PH there was disappearance of the purple colour chelate and at basic PH there was formation of turbidity.

Effect of reagent concentration: To 1ml of $1 \times 10^{-3}\text{M}$ GFX stock solution, aliquots of 0.2 to 4ml of $1 \times 10^{-2}\text{M}$ reagent solution was added into 10 ml Volumetric flask and make up to the volume to 10ml with distilled water and the absorbance values at 545nm, 1st order derivative at 620nm and 2nd order derivative at 660nm were taken. Investigation of metal ion concentration revealed that 2ml of $1 \times 10^{-2}\text{M}$ Cr(III) solution was sufficient for optimum and maximum colour intensity of the chelate of GFX using $40\mu\text{g/ml}$ concentration Fig .4.

Effect of heating: There was increase in the intensity of absorption after heating up to 60°C for 10 minutes in a thermostated water bath Fig. 5.

Determination of chelate stability and composition

The composition of the chelate¹⁸⁻²³ of GFX with Cr(III) ion used was studied by Job's continuous method and Molar ratio method. The chelate of 1:1 ratio was obtained between GFX and Cr(III) . The stability constants of formed chelate were calculated and the values of $\log \beta$ was 8.794×10^5 . The results were tabulated in Table-1.

Linearity range and quantification procedure

Beer's law was found to be obeyed in the concentration range of 2 to $20\mu\text{g/ml}$ for Zero order, 1 to $15\mu\text{g/ml}$ for 1st order derivative and 1 to $25\mu\text{g/ml}$ for 2nd order derivative. $A(1\%, 1\text{cm})$ was calculated. The results were tabulated in Table-2.

Assay of dosage form²⁴⁻³²

An accurately weighed amount of finely powdered tablet equivalent to 100mg of drug was dissolved in about 10ml of distilled water and transferred in to 100ml of calibrated volumetric flask and after

15minutes of mechanical shaking was filtered into a 100ml of calibrated volumetric flask through Whatmann no:41 filter paper and was diluted to 100ml with distilled water and the same procedure was followed as described above. The results were tabulated in Table-3.

Interference study

Potential interference by the excipients in the dosage form was also studied , samples were prepared by mixing fixed amounts of common excipients such as lactose, Micro crystalline cellulose ,Talc, Magnesium stearate and Starch. The good percentage recoveries were obtained indicating no interference was observed .The results were tabulated in Table-4.

RESULTS AND DISCUSSIONS

The linearity range of GFX and Cr(III) chelate covered over a range of 2to 20 μ g/ml to 1to25 μ g/ml of GFX and A(1%,1Cm) were 1.07×10^{-4} for Zero order, 7.01×10^{-3} for 1st order and $1.04 \times 10^{-4} \text{ LM}^{-1} \text{ cm}^{-1}$ for 2nd order respectively. The drug chelate absorbances were plotted against the corresponding concentrations. The data fitted to the equation $Y=a+bx$, where Y is absorbance at relevant maxima, X is the drug concentration in μ g/ml , b is the slope and a is the Intercept of the calibration curve. The regression parameters were shown in Table no:2. The correlation Coefficient ranged from 0.997 to 0.999 indicating exact linearity. The accuracy of the proposed procedure were 99.9 to 100.6%. Repeatability and reproducibility were evaluated and RSD % ranged from 0.1082 to 0.2057. The limit of detection does not exceed 1.4 and whereas limit of Quantification was between 1.46 to 4.23. Proposed procedure for GFX is a stability indicating one which can be used for the determination without interference with the dosage form. The drug being water soluble and considered more selective in determining the structure ability of the drug to chelate with Cr(III) ion, in addition, the derivative spectra normally contain more apparent spectral details than the normal spectra, more selective and sensitive in eliminating the background interference of complex matrix in resolving individual drug ,drug additives and drug decomposition both interfered.

REFERENCES

1. The Merck index, 13th edn., Merck and Co Inc., White house station, N.J., USA ,779.
2. M.Mathew, V.Das Gupta, R.E.Bailey . *Drug Dev. Ind. Pharm.*, **8**,1995).
3. N. Ekpe and T.Jacobsen, *Drug Dev. Ind. Pharm.*, **25(9)**,1057, (1999).
4. J.I. Oh, M.J. Pack, M.Y. Ahn, C.Y. Kim, C.Y. Hong, I.C. Kim and J.H. Kwak, *Antimicrob Agents Chemother.*, **40**, 1564(1996).
5. M.G. Cormican and R.N. Jones, *Antimicrob Agents Chemother.*, **41**, 204(1997).
6. A.F. Hohl, R. Frei, V. Ponter, A. Von graevenitz , C. Knapp, J. Washington , Johnson Dand, R.Jones, *N. Clin. Microbiol Infect.*, **4**, 280(1998).
7. E. Doyle, S.E. Fowles, D.F. Mc Donnell, Mc Carthy and S.A. White, *J. Chromatogr. B*, **746**, 191(2000).
8. J.V. Ramji, N.E. Austin, G.W. Boyle, M.H. Chalker , G. Duncan, A.J. Fairless , F.J. Hollis, D.F. Mc Donnell, T.J. Musick and P.C. Shardlow , *Drug Metabolism and Disposition.*, **29**, 435(2001).
9. Seung I Cho, Jiyeon Shim, Min-su kim, Yong-kweon kim and Doo Soo Chung, *J. Chromatogr. A.*, **1055**, 241(2004).
10. Won Lee and Chang Yang Hong, *J. Chromatogr. A.*, **879**, 113(2000)
11. F. Salama et.al, *I.J.Pharm. Biomed. Anal.*, **33**, 411-421(2003).
12. Lijiljana Zivanovic, et.al., *Mikrochim. Acta(Wien)*, **1**, 49-54 (1990)
13. J.H. Yoe , A.L. Jones, *Ind. Eng. Chem., Anal.* , **6**, 111(1944).
14. X.Y.Xu, J.H.Lu, M.L.Wang, C.Xu, R.L.Wang, L.Y.He, *Yaowuu-Fenxi-Zazhi*, **17(3)**, 169(1997).
15. G.W.Sluggett, J.D.Stong, J.H.Adams, Z.Zhao, *J.Pharm.Biomed. Anal.*, **25 (34)** ,357(2001).
16. J. Inezedy, *Analytical Applications of Complex Equilibria*, College House Westergate, UK, (1976).
17. Vogel's textbook of Quantitative Inorganic Analysis 4TH. Ed.(1978)
18. R. Foster, *Organic charge transfer complexes*, Academic press, London, 51, 387(1969).

19. P. Job , *Anal. Chim* , **16**, 97(1936).
20. L.R. Melby , The Chemistry of the Cyano Group Ed. S. Patai, Interscience, NewYork., 639-670(1970).
21. A.N. Martin, J. Swarbrick , A. Cammarata , “Physical Pharmacy,” 3rd Ed., Lee & Febiger, Philadelphia, PA, p. 344(1969).
22. A. Ringbom , *Z. Anal. Chem.*, **115**, 332-343 (1939)
23. C.N.R. Rao , S.N. Bhat, P.C. Dwivedi, *Applied SpectroscopyReviews*, E.G. Brame , Ed. Dekker , New York , **5**, 1-170(1972).
24. I.B. Lories, E.L.K. Naglaa , K.S. Jihan, S. Mohamed, *J. Pharma. Biomed, Analysis.*, **21**, 133(1991).
25. M.A. Magda, B. Saied, M.E.L. Adi Sobhi and A.K. Afaf, *Analyst*, **109**, 1417(1984).
26. F.A.A. Hassan, S. Gamal and M. O. Nabil, *Analyst*, **116**, 387(1991).
27. (a.)M.V. Krishna and D.G. Sankar, *E-Journal of Chemistry*, **5(3)**,515-520 (2008), (b.) M.V. Krishna and D.G. Sankar , *E-Journal of Chemistry*, **5(3)**,493-498(2008)
28. H.A. Benesi, J.H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703-2707(1949).
29. A.N. Martin , J. Swarbrick , A. Cammarata, “Physical Pharmacy,” 3rd Ed., Lee and Febiger, Philadelphia, PA, 344(1969).
30. A. Ringbom , *Z. Anal. Chem.*, **115**, 332-343 (1939)
31. IUPAC Compendium of Analytical Nomenclature, Definitive Rules,ed. by Irving H. M. N. H., Freiser H., West T. S., Pergamon Press, Ox-ford,1981.
32. J. C. Miller, J.N. Miller, Significance tests “Statistics in AnalyticalChemistry,” Chapt. 3, 3rd ed., Ellis Horwood , Chichester, (1993).

Table-1: Stability constants of Gemifloxacin mesylate chelate with Chromium ion in distilled water by Job’s method

Parameters	GFX-Cr(III) λ_{max} at 545nm
Total molar conc	$1 \times 10^{-5} \text{M}$
N	2.303
A/A _{ex}	0.1021
β^*	8.794×10^5
Log β	5.9441

Table-2: Results of validation

Parameter	GFX-Cr(III) 545nm	GFX- Cr(III) 1st derivative 620nm	GFX- Cr(III) 2 nd derivative 660nm
Linearity range($\mu\text{g/ml}$)	2-20	1-15	1-25
LOD($\mu\text{g/ml}$)	1.4	0.9	0.4
LOQ($\mu\text{g/ml}$)	4.23	2.7	1.46
Slope	0.010	0.007	0.010
Intercept	0.001	0.001	0.003
Correlation coefficient	0.999	0.999	0.997
Accuracy	99.9	100.2	100.6
%RSD	0.1463	0.01065	0.01589

Table-3: Results of the determination of GF X by the proposed method in their dosage form compared with reference methods

Recovery $\pm$ %S.D					
	GFX- Cr(III) At 545nm	GFX- Cr(III) At 620nm 1 st derivative	GFX- Cr(III) At 660nm 2 nd derivative	Reference methods ^{a,b}	
GEMEZ	100.05 $\pm$ 0.1706 N=6	100.4 $\pm$ 0.1668 N=6	100.5 $\pm$ 0.1624 N=6	99.89 $\pm$ 0.48 N=6	99.99 $\pm$ 0.39 N=6
G-CIN	100.01 $\pm$ 0.1572 N=6	100.3 $\pm$ 0.1628 N=6	100.6 $\pm$ 0.1283 N=6		

a, b indicates utility of σ and π acceptors for the spectrophotometric determination of Gemifloxacin Mesylate in pharmaceutical formulation

Table-4: Determination of Gemifloxacin in presence of common excipients by the proposed method

Excipient	Recovery ^a (% $\pm$ S.D)		
	GFX-Cr(III) Zero order At 545nm	GFX-Cr(III) 1 st derivative At 620nm	GFX-Cr(III) 2 nd derivative At 660nm
Lactose(10mg)	100.013 $\pm$ 0.1950	100.3 $\pm$ 0.162	100.5 $\pm$ 0.1690
Talc(10mg)	100.03 $\pm$ 0.1246	100.3 $\pm$ 0.1701	100.5 $\pm$ 0.1519
Magnesium stearate(10mg)	99.95 $\pm$ 0.1980	100.4 $\pm$ 0.1369	100.5 $\pm$ 0.2103
Starch(10mg)			
Microcrystalline cellulose	100.03 $\pm$ 0.2866 100 $\pm$ 0.2	100.4 $\pm$ 0.21 100.2 $\pm$ 0.1695	100.6 $\pm$ 0.1939 100.6 $\pm$ 0.1961

(a)Values are mean of six determinations.

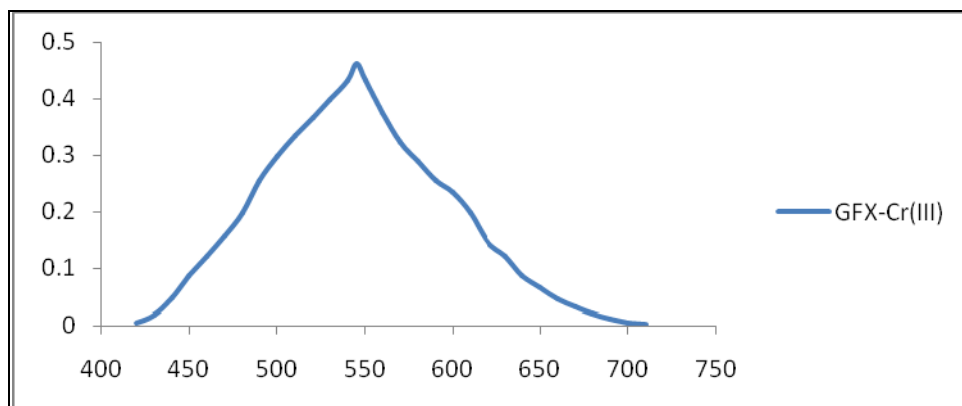


Fig.-1: Absorption spectra of 40 μ g/ml GFX complex with 1×10^{-2} M Cr(III)

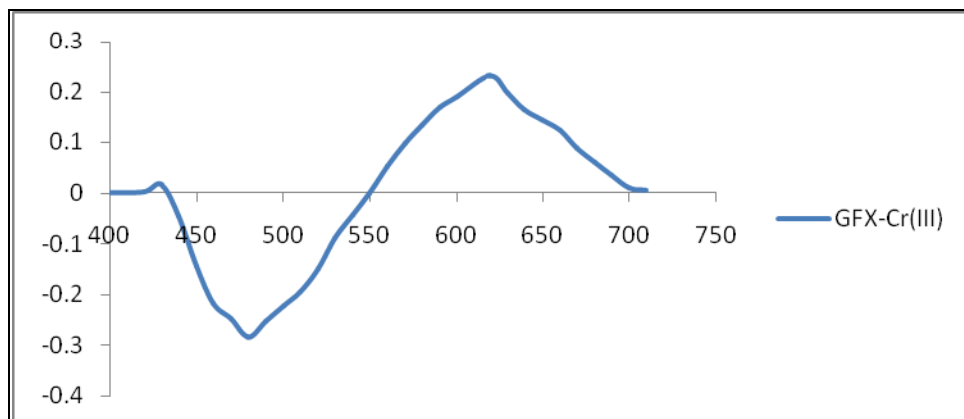


Fig.-2: First order derivative spectra of 40 μg/ml GFX complex with 1×10^{-2} M Cr(III) .

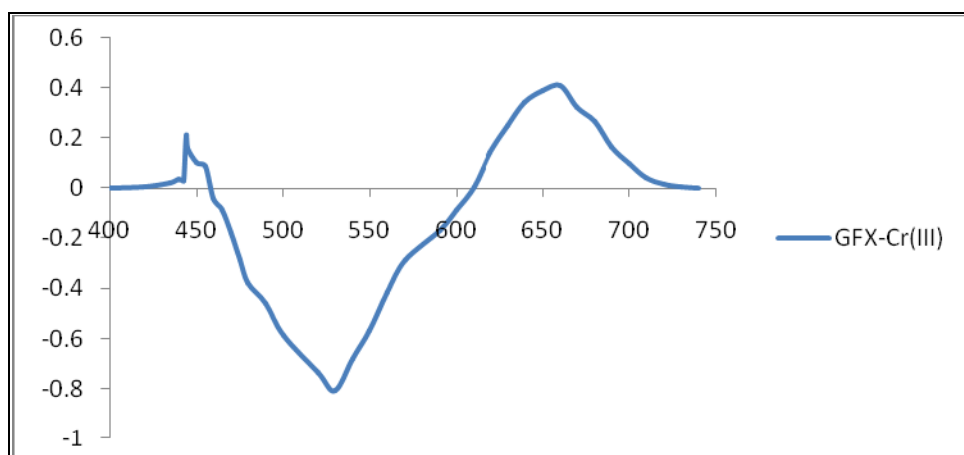


Fig.-3: Second order derivative spectra of 40 μg/ml GFX complex with 1×10^{-2} M Cr(III) .

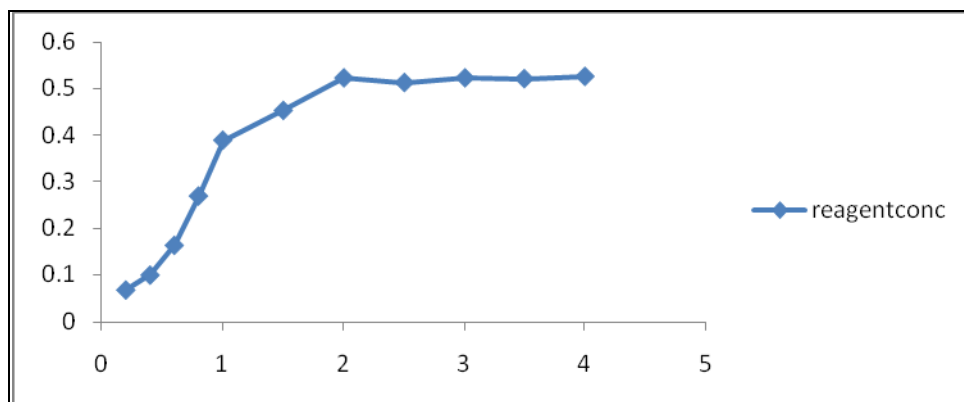


Fig.-4: Effect of reagent concentration on the formation of GFX complex with Cr(III)io

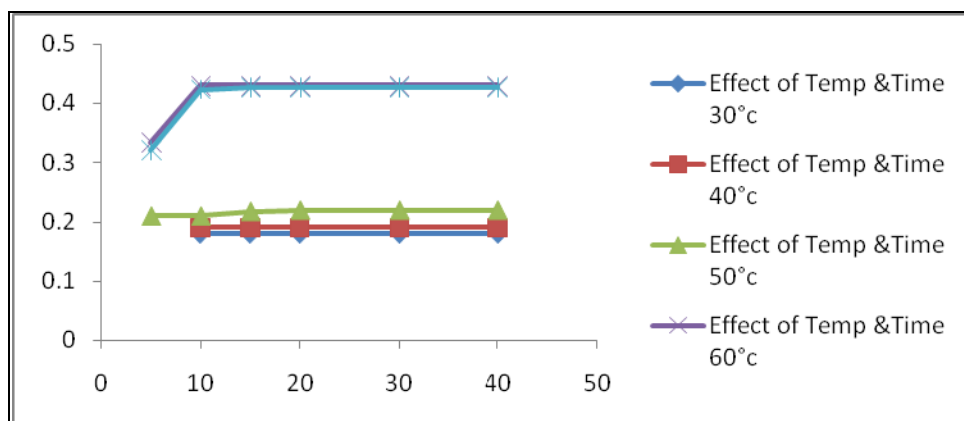


Fig.-5: Effect of Temperature and Time of heating on GFX-Cr(III) complex

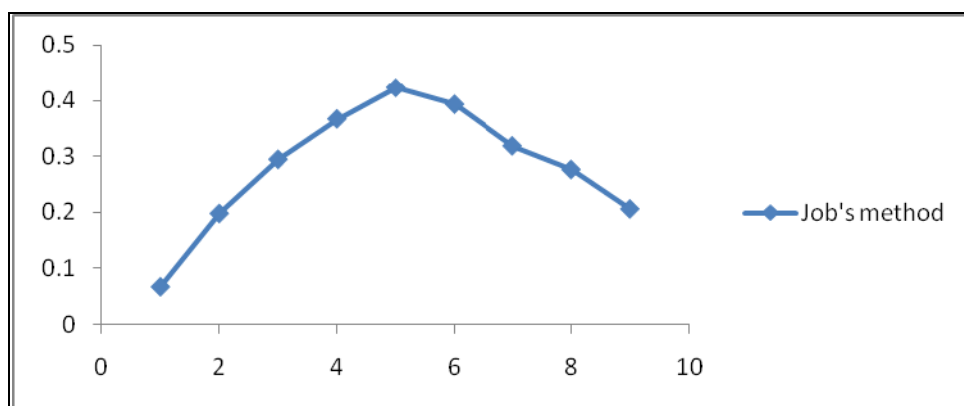


Fig.-6: Job's method for GFX complex with Cr(III) ion at 545nm.

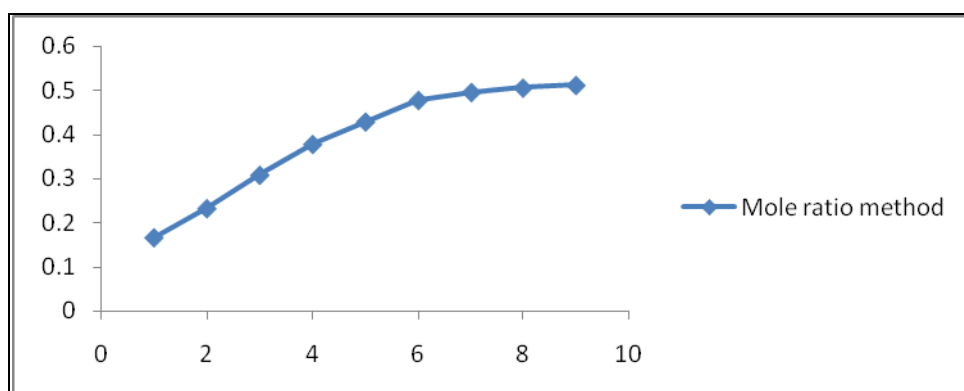


Fig.-7: Mole ratio method for GFX complex with Cr(III) ion at 545nm.

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