



Spectrophotometric Determination Studies of Cefotaxime (CFX) and their CFX- Cd (II) and CFX- Cu (II) Complexes

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ABSTRACT

In this work Cefotaxime (CFX) and its Cd (II) and Cu (II) metal complexes determination were carried out by spectrophotometric method. The following experimental procedures like effect of pH, effect of metal ion concentration, effect of drug concentration, effect of time, effect of organic solvents and the composition of the complex by mole ratio and job's continuous variation method and interference studies were carried out for the determination of metal ions and drug. In this research work, it is clear that Cd forms a stable 1:1 colored complex with CFX in basic medium and Cu forms stable 1:1 colored complex with CFX in acidic medium. Effect of pH was studied for CFX- Cd (II) and CFX- Cu (II) complexes; it is noticed from that at pH 11 CFX- Cd (II) and at pH 5.5 CFX- Cu (II) colored complexes were formed. In these reactions can be found the metal ions in the range 1.12 to 6.74 and 3.812 to 8.895 $\mu\text{g/ml}$ and the drug in the range 0.02 to 0.14 mg/ml respectively for CFX- Cu (II) and CFX- Cd (II) complexes. Effect of time clearly suggest, that the maximum absorbance is obtained only after heating the mixture to 40° & 60°C respectively for CFX- Cd (II) and CFX- Cu (II) complexes. It has been observed that the absorbance values remain constant thereafter; it indicates the formation of 1:1 stable metal complexes.

Keywords: Spectrophotometric Determination, Cefotaxime, Cadmium, Copper.

INTRODUCTION

The cephalosporins are β -lactam antibiotics isolated from cephalosporium species and/or prepared semi-synthetically. This comes under the class of 7-amino cephalosporonic acid (7-ACA) derivatives and are much more acid-stable

than the corresponding 6-APA compounds. The cephalosporins have a mechanism of action similar to that of penicillin, mainly; they inhibit the cross-linking of the peptidoglycan units in the bacterial cell wall by inhibiting the transpeptidase enzyme. Cefotaxime is a third generation cephalosporin, it was the first to become available and made a

landmark in cephalosporin history as it proved to be the answer to the microbes resistant to 1st and 2nd generation agents. It is less active than first generation drugs against gram-positive but have a much expanded spectrum of activity¹ against gram – negative organisms. Cefotaxime chemically Sodium (6*R*, 7*R*)-3-[(acet-yloxy) methyl]-7-[(*Z*)-2-(2-aminothiazol-4-yl)-2-(methoxyimino) acetyl]-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate. It is less active than first generation drugs against gram-positive but have a much expanded spectrum of activity against gram – negative organisms. The structure of the cefotaxime (Fig.1) is shown below.

It inhibits bacterial wall synthesis of actively dividing cells by binding to one or more penicillin binding proteins. These proteins serve as enzymes that catalyse the final stages of bacterial cell wall synthesis. As a result a defective cell wall is formed that is osmotically unstable. It has good activity against²⁻³ *S. Aurens*, *S. Fecails*, *Bacteroids* and many enterobacteriaceae. Cefotaxime has wide clinical applications including treatment of infection in the respiratory tract, skin, bone and joint gynecologic, septicemia, urinary tract, and documented or suspected meningitis.

Most of the analytical chemists having greater attention these compounds (cephalosporins) for their determination in pharmaceutical formulations⁴ by using metal ions. Cephalosporins very important today because serious ailments are controlled by these drugs. Many researchers have also dedicated their efforts to develop a simple, rapid and sensitive analytical method for the determination⁵⁻⁶ of cephalosporins in pure, dosage form and biological samples. Generally, the assay of cephalosporins previously been achieved by several analytical techniques such⁷⁻⁸ as, HPLC voltametry, ionometry, flow injection analysis, fluorimetry⁹⁻¹⁰ etc.,

In this research article carried out a spectrophotometric method for determination of Cefotaxime sodium in pure and pharmaceutical formulation dosage form by using cadmium and copper. In this method reaction of drug with cadmium (II) in basic and with copper (II) in acidic condition respectively to form stoichiometrically a yellow CFX-Cd (II) and light yellow color CFX-Cu(II) complexes

at optimum conditions. This method is convenient, simple, rapid, economically viable and selective method for the determination of drug as well as metal ions in pharmaceutical formulations.

EXPERIMENTAL

All the molecular absorption measurements were made using UV-Vis spectrophotometer type Shimadzu model UV-160 equipped with 10mm matched quartz cell. For pH measurement it is used a pH Meter (Elico) with combined electrode. Well calibrated glassware was used to carry out experiments. Hydrochloric acid, sodium acetate, acetic acid, ammonia, methanol, ammonium chloride, cadmium chloride and copper chloride were purchased from Merck. Cefotaxime tablets were purchased from Ranbaxy laboratories Ltd. All the used chemicals were of analytical reagent grade; deionized water was used for diluting the reagents and samples.

Preparation of Buffer Solution

The buffer solutions are prepared by mixing appropriate quantities of the following solutions. Exact pH of the solutions was measured using pH meter.

Preparation of Cefotaxime solution

20 tablets of cefotaxime is ground into a fine powder and 100 mg of the powder is weighed into a 100ml standard flask. Solution of 5M HCL is added and the solution is heated on boiling water bath for 30 minutes. The solution is heated on boiling water bath for 30 minutes. The solution is cooled; 5ml of DMF is added and made up to the mark with double distilled water. 10ml of this solution is neutralized with 10ml of 2.5M NaOH and the solution is made to 100ml to obtain a final concentration of 0.1 mg/ml. This solution is used for further studies after appropriate dilutions.

Preparation of Cu (II) and Cd (II) metal solutions

Fresh stock solutions of 1×10^{-4} M of metal salts (Cu (II), 2.5mg /100 ml & Cd (II), 3.5 mg /100 ml), were prepared by dissolving accurately weighed amounts of the metal salts in appropriate volumes of de-ionized water.

Preparation of CFX-Cd (II) complex

CFX-Cd (II) solution was prepared, 1 ml of 1×10^{-3} M copper sulphate solution, 8 ml of pH 5.5 buffer, 1 ml of drug solution of 0.1mg/ml concentration are taken in a 10ml volumetric flask. The contents are mixed well to get a solution of

uniform concentration. Similarly, a blank solution without the drug is prepared.

Preparation of CFX- Cu (II) complex

CFX-Cu (II) solution was prepared, 1 ml of 1×10^{-3} M copper sulphate solution, 8 ml of

Buffer components of the solution	pH
0.1M Hydrochloric acid	1.0
0.2M sodium acetate solution + 0.1M Hydrochloric acid	2.0 – 3.0
0.2M Acetic acid + 0.2M sodium acetate	4.0 – 7.0
Ammonia + Ammonium chloride solution	8.0 – 11.0

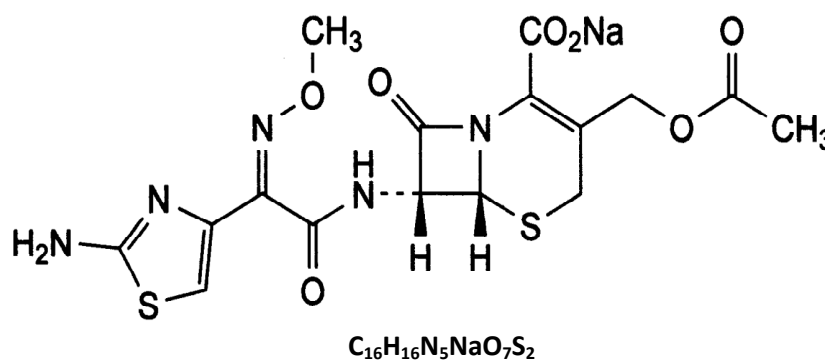
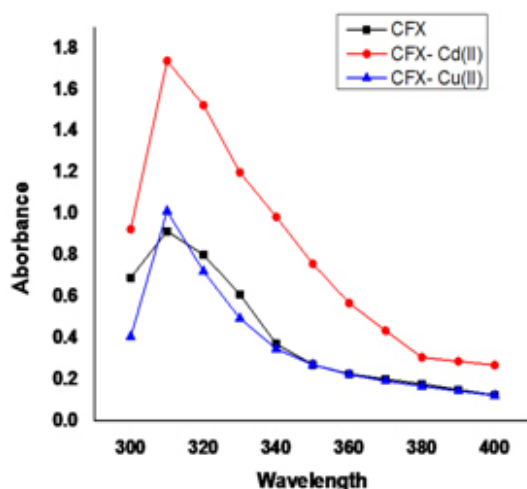


Fig. 1: The chemical structure of cefotaxime



[CFX = 0.1 mg/ml; Cd (II)] = 1×10^{-4} M;
Cu (II) = 1×10^{-4} M; λ range = 300 – 400 nm.]

Fig. 2: The absorbance spectral data of cefotaxime (CFX) and its metal complexes

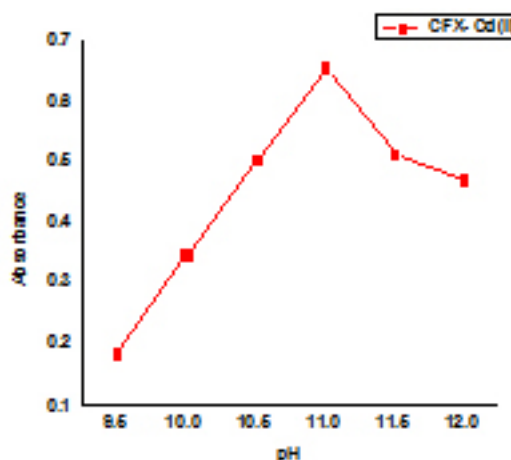


Fig. 3: Effect of pH on absorbance in CFX- Cd (II)

pH 5.5 buffer, 1ml of drug solution of 0.1mg/ml concentration are taken in a 10ml volumetric flask. The contents are mixed well to get a solution of uniform concentration. Similarly, a blank solution without the drug is prepared.

RESULTS AND DISCUSSION

The absorbance spectral data of cefotaxime (CFX) and their CFX-Cd (II) and CFX- Cu (II) complexes.

The spectrum of the CFX, CFX- Cd (II) and CFX- Cu (II) solutions were recorded in the wavelength range 300 to 400 nm against a blank solution. The spectrums of reaction mixtures are recorded against their respective blank solutions were shown in (Fig.2) Table.1.

Effect of pH

The author has carried out the effect of pH for the development of color between cadmium, copper and CFX. The data revealed that cadmium

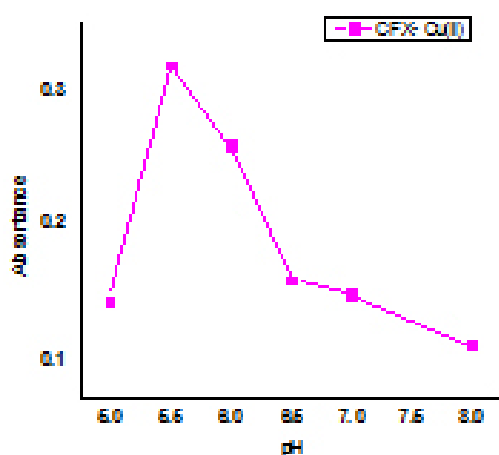


Fig. 4: Effect of pH on absorbance in CFX- Cu (II) complex

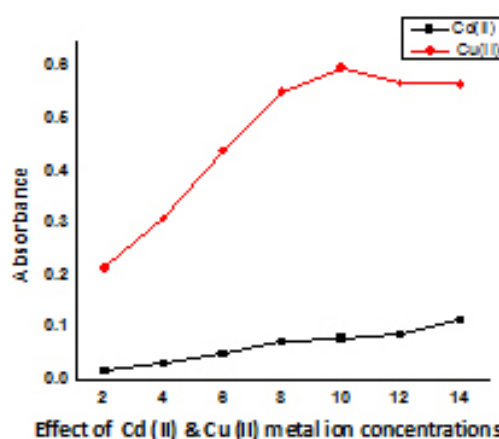


Fig. 5: The Effect of metal ion concentrations in CFX- Cd (II) and CFX- Cu(II) Complexes (Beer's law)

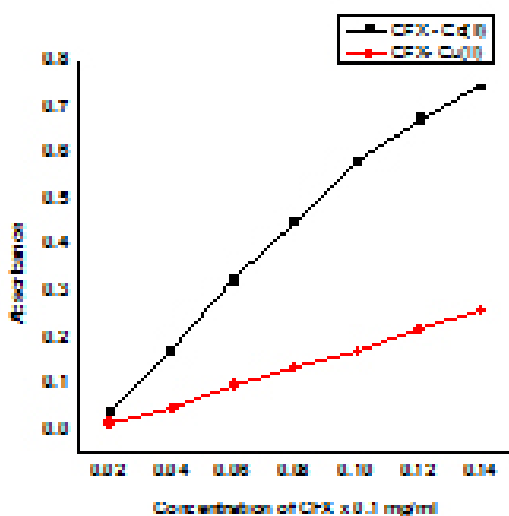


Fig. 6: Effect of Drug concentrations in CFX- Cd(II) and CFX- Cu(II) Complexes (Beer's law)

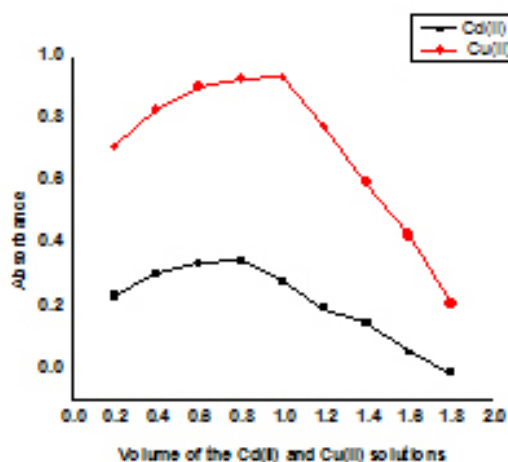
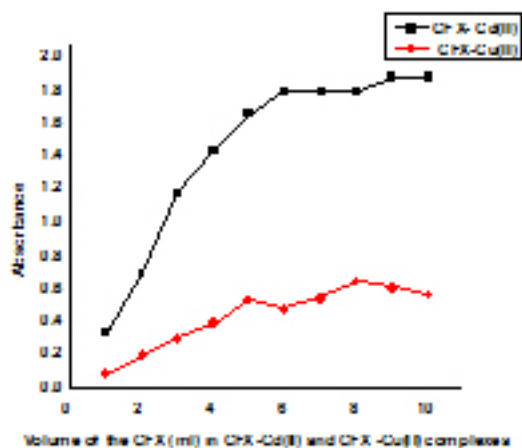


Fig.7: Job's continues variation Method

forms a complex in the basic and copper forms the complex¹¹⁻¹² in acidic medium. Hence, effect of pH is studied in the range 9.5 to 11.5 for CFX- Cd (II) and 5.0 to 8.0 for CFX- Cu (II) complexes. Absorbance of metal complexes increased compared with the drug solution; it indicates the formation of metal complexes. It is noticed from the graph that pH of 11



[Cd(II)] = 1×10^{-4} M
[Cu(II)] = 1×10^{-4} M
[CFX] = 0.1 mg/ml
[CFX] = 0.1 mg/ml
pH = 11
pH = 5.5
Absorbance at 350nm
Absorbance at 350nm

Fig. 8: Mole ratio Method

and 5.5 were suitable for carrying out further studies. The absorbance was maximized and the solutions were remains clear for quite a long time after the development of color. Graph is plotted (Figures 3 & 4) between pH and absorbance values are given in Table.2. The intensity of the color increases with time, but the maximum color develop is possible¹³ only after heating the system for half an hour at 50 to 60°C. In explaining the results on CFX- Cd (II) and CFX- Cu (II) complexes¹⁴⁻¹⁵, the author has proved that hydrolysis of the drug are not required for complex formation. The unhydrolysed drug is sufficient and responsible for the color development. Only to achieve completion of the reaction, the system is to be heated to 50 to 60°C.

Effect of metal ion concentration

The effect of cadmium and copper concentrations on the absorbance the concentration of the metal ions vary in the range 1.1 to 6×10^{-5} M and 6 to 14×10^{-5} M respectively, for CFX-Cd (II) and CFX- Cu (II) keeping the CFX concentration is constant. The absorbance values are measured at 350nm against a blank solution containing no reagent. The absorbance values increase progressively and a linear graph is obtained between cadmium concentration [16] and absorbance and copper concentration and absorbance. The data are shown in Table 3 and Fig 5. These were fitted

Table 1: Spectral data of CFX, CFX-Cd (II) and CFX- Cu (II) complexes

[CFX = 0.1 mg/ml; Cd (II) = 1×10^{-4} M; Cu (II) = 1×10^{-4} M; λ range =300 – 400 nm.]				
S. No.	λ	Absorbance of CFX	Absorbance of CFX – Cd(II)	Absorbance of CFX – Cu(II)
1	300	0.690	0.925	0.406
2	310	0.915	1.738	1.012
3	320	0.801	1.523	0.721
4	330	0.610	1.199	0.494
5	340	0.370	0.984	0.346
4	350	0.270	0.757	0.270
7	360	0.225	0.568	0.223
8	370	0.200	0.434	0.191
9	380	0.177	0.305	0.166
10	390	0.149	0.286	0.143
11	400	0.123	0.268	0.121

into the equations $A_{350} = 0.0405x + 0.4141$ and $A_{350} = 0.0073x - 0.0012$ respectively for CFX-Cd (II) and CFX- Cu (II).

Effect of drug concentration

The concentration of the drug is varied from 0.02 mg/ml to 0.14mg/ml keeping the metal ion concentration constant. The values of absorbance are measured at 350nm against the blank solution containing no reagent. The results are tabulated and shown in Table 4 (Fig.6) is also drawn between the concentration of the drug and absorbance values.

Table 2: Effect of pH on absorbance in CFX- Cd (II) and CFX- Cu (II) complexes

[Cd (II)] = 1×10^{-4} M; CFX = 0.1 mg/ml; Cu (II)] = 1×10^{-4} M CFX= 0.1 mg/ml; Absorbance at 350nm)

S. No	CFX-Cd(II) complex pH	Absorbance	CFX-Cu (II) complex pH	Absorbance
1	9.5	0.187	5.0	0.145
2	10.0	0.349	5.5	0.318
3	10.5	0.502	6.0	0.259
4	11.0	0.654	6.5	0.160
5	11.5	0.513	7.0	0.149
6	12.0	0.470	8.0	0.112

It is clearly seen¹⁷⁻¹⁸ from the graph that a linear relationship exists between the drug concentration and absorbance.

Effect of time

To best the reproducibility of the results and stability of the complex formed, the author measured the absorbance values for the same solutions¹⁹⁻²⁰ containing CFX- Cd (II) and CFX- Cu (II) complexes for one hour at an interval of 10 minutes. These values are shown in Table 5. From the data, it is clear that the absorbance values increase with time but maximum color intensity is obtained only after heating the system for 40 minutes. In CFX- Cd (II) complex the absorbance values²¹ were started from 0.065 to 0.751, where as in CFX- Cu (II) complex absorbance values noticed at 0.035 to 0.261. Based on these observations, the absorbance values are measured in each case only after heating the system and achieving maximum color development. From the table, it is clear that the maximum absorbance is obtained only after heating the mixture to 40° & 60°C respectively for CFX- Cd (II) and CFX- Cu (II) complexes. It has been observed that the absorbance values remain constant thereafter.

Effect of organic solvents

In these experiments relating to the effect of solvents. Therefore, carried out the effect of acetone, dioxane, DMF and methanol. In these studies, the

Table 3: Effect of Metal Ions Concentrations (Beer's law) in CFX-Cd (II) and CFX -Cu (II) complexes

S. No	Effect of Metal ion concentration in CFX- Cd (II) complex		Effect of Metal ion Concentration in CFX-Cu (II) complex	
	[Cd(II)] x 10 ⁵ M	Absorbance	[Cu(II)] x 10 ⁵ M	Absorbance
1.	2	0.019	2	0.216
2	4	0.032	4	0.311
3	6	0.052	6	0.441
4	8	0.075	8	0.552
5	10	0.082	10	0.598
6	12	0.089	12	0.570
7	14	0.115	14	0.569

[Cd(II)] = 1×10^{-4} M

[CFX] = 0.1 mg/ml

pH = 11

Absorbance at 350nm

[Cu(II)] = 1×10^{-4} M

[CFX] = 0.1 mg/ml

pH = 5.5

Absorbance at 350nm

and drug are continuously varied. The absorbance values of each mixture are measured at 350nm against the respective reagent blank. The values are presented in Table 7. When volume of the reagent is plotted against the absorbance values an 'inverted U-shaped curves' were obtained as shown in the Figure 7. It can be deduced from the graphs that Cd and Cu forms a stable 1: 1 complex²⁴⁻²⁵ with CFX under these experimental conditions. The stability constant values of these complexes are 6.7×10^6 and

17.6×10^6 respectively for CFX- Cd (II) and CFX- Cu (II).

Mole ratio method

In this method, the volume of the metal ions was maintained constant and that of CFX is continuously varied. The absorbance was measured at 350nm against the reagent blank. A graph is drawn between the volume of CFX and absorbance²⁶. The data are shown in Table 8 and Fig 8. The point of

Table 6 : Effect of organic solvents on absorbance

S. No	Effect of organic solvents on absorbance in CFX- Cd (II) complex		Effect of organic solvents on absorbance in CFX-Cu (II) complex	
	Solvent (50% V/V)	Absorbance	Solvent (50% V/V)	Absorbance
1.	None	0.487	None	0.109
2	Acetone	0.729	Acetone	0.429
3	Dioxane	0.107	Dioxane	0.113
4	DMF	0.409	DMF	0.221
5	Methanol	0.450	Methanol	0.194

Table 7: Job's Continuous Variation Method

S.No	Job's Continuous Variation Method of CFX- Cd (II) complex		Job's Continuous Variation Method of CFX-Cu (II) complex	
	Volume of Cd in ml	Absorbance	Volume of Cu(II) in ml	Absorbance
1.	0.2	0.234	0.2	0.707
2	0.4	0.302	0.4	0.829
3	0.6	0.339	0.6	0.901
4	0.8	0.343	0.8	0.928
5	1.0	0.277	1.0	0.930
6	1.2	0.195	1.2	0.773
7	1.4	0.149	1.4	0.595
8	1.6	0.052	1.6	0.427
9	1.8	-0.012	1.8	0.212

the intersections in graph gives the composition of these complexes²⁷⁻²⁸. It is seen from the figure that Cd and Cu forms 1:1 complex with CFX under these experimental conditions.

Interference Studies

In a complexation reaction between the metal ion and the ligand, there is a possibility of other metal ions to interfere. Anions might influence the color formation reaction. Hence, the author has

Table 8: Mole – Ratio Method for the determination of CFX- Cd (II) and CFX- Cu (II) complexes

S.No	Mole – Ratio Method of CFX- Cd (II) complex		Mole – Ratio Method of CFX-Cu (II) complex	
	Volume of CFX in ml	Absorbance	Volume of CFX in ml	Absorbance
		[Cd(II)] = 1 x 10 ⁻⁴ M [CFX] = 0.1 mg/ml pH = 11 Absorbance at 350nm		[Cu(II)] = 1 x 10 ⁻⁴ M [CFX] = 0.1 mg/ml pH = 5.5 Absorbance at 350nm
1.	1	0.346	1	0.091
2	2	0.705	2	0.210
3	3	1.186	3	0.316
4	4	1.451	4	0.402
5	5	1.670	5	0.540
6	6	1.804	6	0.489
7	7	1.802	7	0.557
8	8	1.802	8	0.652
9	9	1.893	9	0.620
10	10	1.890	10	0.570

Table 9: Interference by Cations and Anions in CFX- Cd (II) and Cu (II) complexes

S. No	Interference by Cations and Anions in CFX- Cd (II) complex				Interference by Cations and Anions in CFX- Cu (II) complex			
	Added Cations (1 x 10 ⁻³ M)	Absorbance	Added Cations (1 x 10 ⁻³ M)	Absorbance	Added Cations (1 x 10 ⁻³ M)	Absorbance	Added Cations (1 x 10 ⁻³ M)	Absorbance
1	None	0.852	None	0.846	None	0.128	None	0.167
2	Nickel	0.504	Chloride	0.755	Nickel	0.115	Chloride	0.163
3	Cobalt	0.742	Nitrate	0.769	Cobalt	0.116	Nitrate	0.176
4	Copper	0.404	Sulphate	0.796	Cadmium	0.120	Sulphate	0.176
5	Molybdenum	0.774	Oxalate	0.819	Molybdenum	0.100	Oxalate	0.191
6	Manganese	0.882	Phosphate	0.769	Manganese	0.117	Phosphate	0.173
7	—	—	Tartrate	0.780	—	—	Tartrate	0.133

chosen certain bivalent cations and some common anions carry out the interference studies²⁸⁻²⁹ on CFX- Cd (II) and CFX – Cu (II) complexes. To know the effect of various cations and anions on the determination of Cd and Cu, We have studied the effect of various metal ions which are usually associated with Cd and Cu. The data are presented in Table 9. From the data, it is seen in the CFX- Cd (II) complex, most of the anions can be tolerated³⁰⁻³³ where as cations studied seriously interfere with the determination of cadmium. Where as in CFX- Cu (II) complex the absorbance values are revealed that neither the cations nor the anions seriously interfere with this color formation reaction.

CONCLUSION

Thus, in this research work, it is clear that Cd form a stable 1:1 colored complex with CFX in basic medium and Cu forms stable 1:1 colored complex with CFX in acidic medium. Effect of pH is studied for CFX- Cd (II) and CFX- Cu (II) complexes; it is noticed from that at pH 11 CFX- Cd (II) and 5.5 CFX- Cu (II) colored complexes were formed. From

mole ratio method and Jobs continuous variation methods, it clearly showed that Cd and Cu forms a stable 1: 1 complexes with CFX and the stability constant values of these complexes were 6.7×10^6 and 17.6×10^6 respectively for CFX- Cd (II) and CFX- Cu (II). In these reactions can be spectrophotometric determination of metal ions in the range 1.12 to 6.74, 3.812 to 8.895 $\mu\text{g/ml}$, and the drug in the range 0.02 to 0.14 mg/ml respectively for CFX- Cu(II) and CFX- Cu(II) complexes. Effect of time clearly suggest, that the maximum absorbance is obtained only after heating the mixture to 40° & 60°C respectively for CFX- Cd (II) and CFX- Cu (II) complexes. It has been observed that the absorbance values remain constant thereafter. This color reaction can be used advantageously for the determination of metal ion as well as the drug. As this method is based on the measurement of absorbance at 350nm, this can be readily adopted by any pharmaceutical laboratory involved in the preparation of cephalosporins. This method is can be used for the determination of metal ions as well as drug in pharmaceutical formulations because it is a simple, convenient and rapid technique.

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