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# 속 이온과 CFP 상호작용: 흡수 형광 분광법에 의한 금속 이온과 CFP의 착물 형성

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# Interaction of CFP with Metal ions: Complex Formation of CFP with Metal ion by Absorption and Fluorescence Spectrophotometery

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**요 약.** 산성 용액에서 $Ca^{2-}$, $Mg^{2+}$, $Mn^{2+}$, $Fe^{3+}$, $Co^{2-}$, $Ni^{2-}$, $Cu^{2-}$ 및 $Zn^{2-}$와 Cefpodoxime proxetil (CFP)의 상호작용을 분광학적으로 조사한 결과 1:1 착물이 형성됨을 알 수 있다. 순수한 약품의 흡수스펙트럼은 270과 345 nm에서 두 개의 현저한 봉오리를 보였다. 여러 pH에서 스펙트럼은 두 개의 isosbestic 점(305과 330 nm)을 나타내었다. 이는 용액상에서 약품의 쯔비터 이온이 존재함을 의미한다. 다른 농도의 금속이온에서 CFP의 형광방출 스펙트럼은 chelating enhancement fluorescence(CHEF)효과에 의해 형광강도가 증가함을 알 수 있었다. 착체의 화학량론은 Job's와 Benesi-Hildebrand 방법에 의해 결정되었다. 착체의 안정도는 다음 순서와 같다. $Ca^{2-} < Mg^{2-} < Co^{2+} < Ni^{2-} < Zn^{2+} < Mn^{2+} < Cu^{2-} < Fe^{3-}$.

**주제어:** CFP; 형강증강; 안정도 상수; 착체형성; CHEF

**ABSTRACT.** Spectrophotometric investigation of the interaction of Cefpodoxime proxetil (CFP) with $Ca^{2+}$, $Mg^{2+}$, $Mn^{2+}$, $Fe^{3-}$, $Co^{2-}$, $Ni^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ in acidic medium showed the formation of 1:1 complex. The absorption spectrum of pure drug exhibits two prominent peaks at 270 and 345 nm. Its spectra scanned at several pH exhibited two isosbestic points (305 and 330 nm) indicating the presence of zwitterionic condition of drug in solution phase. The fluorescence emission spectra of CFP in presence of different concentrations of metal ions showed enhancement in fluorescence intensity which is ascribed to chelating enhancement fluorescence effect (CHEF). The stoichiometry of the complexes was determined by Job's and Benesi-Hildebrand method. The stability of the complexes follow the order $Ca^{2+} < Mg^{2+} < Co^{2-} < Ni^{2+} < Zn^{2-} < Mn^{2-} < Cu^{2-} < Fe^{3-}$.

**Keywords:** CFP, Fluorescence enhancement, Stability constant, Complex formation, CHEF

## INTRODUCTION

Cefpodoxime proxetil (CFP) is a semisynthetic β-lactum antibiotic known as (RS)-1(isopropoxycarbonyloxy) ethyl (+)-(6R, 7R)-7-[2-(2-amino-4-thiazolyl)-2-{(Z) methoxyimino} acetamido]-3-methoxymethyl-8-oxo-5-thia-1-azabicyclo [4.2.0] oct-2-ene-2-carboxylate. It is an ester prodrug of cefpodoxime acid where a proxetil radical is attached to cefpodoxime acid (*Fig.* 1). It is the third generation cephalosporin ester. used in the treatment of upper respiratory tract and urinary tract infection. In biological system cefpodoxime undergoes ester hydrolysis and converted into cefpodoxime acid to exhibit its antibiotic activity.[1,2] It has an asymmetric carbon at position 4 and is supplied as racemic mixture of R and S-enantiomers. Only a few methods are reported to quantify

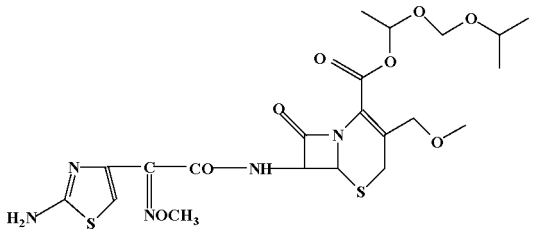

*Fig.* 1. Structure Cefpodoxime Proxetil (CFP).

CFP,[3,4] nevertheless the analytical methods of detection of CFP are handful, and they employ RP-HPLC. These methods are based on separation of the R- and S-isomers.[5] However, both the isomers are reported to exhibit similar biological activity,[1] the use of highly sophisticated and time-consuming methods is not always required for routine analysis of CFP from the different dosage forms.

The discovery of cephalosporin from *C.acremonium* culture by Brotzu[6] and demonstration of its remarkable stability towards aqueous solution even at pH 2 as well as its excellent *in vitro* activity against penicillin-resistant organisms by Abraham and Newton,[7] were major breakthroughs in the history of β-lactam antibiotics. The realization that fungi might be a good source for novel antibiotics spurred microbiologists to develop novel soil-screening programmes for the investigation of microbial culture leading to the discovery of several non-classical β-lactams like carbapenem[8] and oxacephems.[9] The reactivity of β-lactam antibiotics is fundamentally linked to antimicrobial activity, it led Woodward[10] to design and synthesize carbapenem group of compounds for evaluation of their antibacterial activity through systematic screening of the soil microorganism. Numerous of these compounds show broad-spectrum antimicrobial activity as predicted earlier and it was further substantiated by the subsequent discovery of thienamycin. It is unstable in its pure form and hence its derivatives are used which also led to the introduction of imipenem, which is regarded as one of the most effective drugs among the β-lactam antibiotics. Although several methods have been developed to determine the drug in biological fluids and pharmaceutical preparations[11] no effort seems to have been made to investigate the interaction of the drug with metal ions.

In the present work an earnest effort has been made to study the interaction of the CFP with metal ions by fluorescence emission and absorption spectrophotometric measurements. Since quenching or enhancement in fluorescence intensity of the drug in presence of metal ions occurs, the spectra of the drug in presence of different concentrations of metal ions such as $Ca^{2-}$, $Mg^{2+}$, $Mn^{2-}$, $Fe^{3-}$, $Co^{2-}$, $Ni^{2-}$, $Cu^{2-}$, and $Zn^{2+}$ were scanned. The ratio of the drug to metal ions was determined by Job's method and Benesi-Hildebrand methods.[12,13] The absorption spectra of the drug in the pH range 2.32-11.50 were run to see the zwitterionic condition, apparent ionization constant and the isosbestic point which indicate the presence of different species in solution. The stability constant of the complexes formed between the drug and metal

*Table* 1. Stability constant and other thermodynamic parameters of CFP complexes (Job's method)

| Metal | logK | | Gibbs energy change | Enthalpy change | Entropy change |
|---|---|---|---|---|---|
| | 25 °C | 35 °C | (-ΔG)(kJ.mol$^{-1}$) | (ΔH) (J.mol$^{-1}$) | (ΔS) (J.mol$^{-1}$ K$^{-1}$) |
| $Ca^{2-}$ | 5.600 | 5.846 | 31.95 | 270.22 | 108.13 |
| $Mg^{2+}$ | 5.915 | 6.044 | 33.75 | 164.93 | 113.82 |
| $Mn^{2+}$ | 6.200 | 6.289 | 35.38 | 49.97 | 118.91 |
| $Fe^{3-}$ | 6.287 | 6.356 | 35.87 | 260.34 | 121.22 |
| $Co^{3+}$ | 5.983 | 6.122 | 34.13 | 244.95 | 115.38 |
| $Ni^{2-}$ | 6.130 | 6.276 | 34.97 | 178.43 | 117.97 |
| $Cu^{2+}$ | 6.211 | 6.315 | 35.44 | 240.25 | 119.93 |
| $Zn^{2-}$ | 6.184 | 6.297 | 35.28 | 190.29 | 119.04 |

*Table* 2. Stability constant, Gibbs energy change of CFP complexes (Benesi-Hildebrand method) at 25 °C

| Metal | logK | Gibbs energy change ($-\Delta G$) ($kJ.mol^{-1}$) | $R^2$ |
|---|---|---|---|
| $Ca^{2+}$ | 6.854 | 39.11 | 0.9843 |
| $Mg^{2-}$ | 6.869 | 39.19 | 0.9784 |
| $Mn^{2-}$ | 6.886 | 39.29 | 0.9769 |
| $Fe^{3+}$ | 6.919 | 39.48 | 0.9914 |
| $Co^{2-}$ | 6.883 | 39.27 | 0.9807 |
| $Ni^{2+}$ | 6.884 | 39.28 | 0.9845 |
| $Cu^{2-}$ | 6.896 | 39.34 | 0.9668 |
| $Zn^{2+}$ | 6.889 | 39.30 | 0.9706 |

ion was also evaluated.

## EXPERIMENTAL

### Instruments

The absorption spectra were obtained with Elico-SL-169 double beam UV-visible spectrophotometer. Fluorescence emission spectra were scanned with Hitachi-F-2500FL-spetrophotometer. All potentiometric measurements were done with Elico-LI-120 pH meter.

### Methods and materials

Double distilled water was used throughout Cefpodoxime proxetil (Lupin pharmaceutical Ltd. India), sodium hydroxide and metal chloride (Merck Ltd. Mumbai, India) and HCl (Ranbaxy fine chem. Ltd. India) were used as received.

### Preparation of solution

Stock solution of Cefpodoxime proxetil and metal salts of $1\times10^{-3}$ M were prepared in $1\times10^{-2}$ M HCl. Stock solution of drug was stored at 4 °C.

### Spectrophotometric method

Solutions of equimolar concentration ($1\times10^{-4}$ M) of CFP and metal ions were prepared. The pH of the drug was adjusted between 2.32 to 11.50 by adding sodium hydroxide and hydrochloric acid ($1\times10^{-1}$ M and $1\times10^{-2}$ M respectively). The absorption spectra were recorded in the range 250-380 nm. The ratio of metal to CFP was determined by Job's method. The linearity of CFP was found in the range $2\times10^{-5}$ to $6\times10^{-4}$ mg/ml and the correlation factor ($R^2$) 0.9236.

### Fluorescence study

Solution of the CFP ($7\times10^{-6}$ M) and those of metal ions ($1\times10^{-6}$ to $7\times10^{-6}$ M) were prepared. To prepare dilute solutions, an aliquot of stock solution was placed in a 10 ml volumetric flask and made up to the mark with distilled water. Spectra were recorded immediately after sample preparation in the optimum wavelength range 370-430 nm at optimum excitation wavelength of 335 nm. For calibration curve an aliquot of stock solution ($1\times10^{-6}$ to $3\times10^{-5}$ mg/ml) was prepared which showed linearity with correlation factor ($R^2$) 0.9652

## RESULTS AND DISCUSSION

### Spectrophotometric study

The absorption spectrum of CFP ($1\times10^{-4}$ M) was run in the region 250-380 nm. It exhibited two peaks at 270 and 345 nm (*Fig.* 2). Since the first peak is very strong it was selected for further absorption studies.

When the spectra of the drug were run at varying pH in the region 250-380 nm two isosbestic points, one at 305 nm and another at 330 nm were observed which indicated the presence of zwitter ionic condition in solution (*Fig.* 3).[14] The appa-

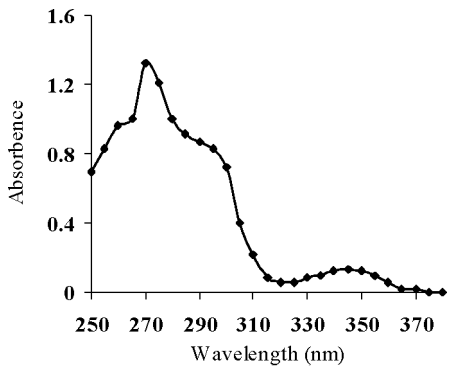


*Fig.* 2. Absorption spectrum of CFP ($1\times10^{-4}$ M) at pH 5.15.

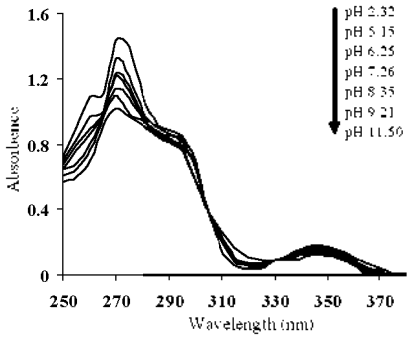


*Fig.* 3. Absorption Spectra of CFP ($1\times10^{-4}$ M) in (2.32-11.50) pH range.

rent ionization constant (pKa') of the drug was calculated (8.92) by the following equation.

$$pKa' = pH + \log\{(A_I - A_M)/(A - A_M)\} \quad (1)$$

where. $A_I$ = absorbance of drug in basic medium. $A_M$ = absorbance of drug in acidic medium. A= absorbance of drug in aqueous medium.

The absorption spectra corresponding to the metal. drug and complexes were obtained in acidic medium. The concentration of cations and drug (CFP) were $1\times10^{-4}$ M. The stoichiometry of the complexes was obtained by Job's methods[12,15] a sample of resulting plots is shown in *Fig.* 4. The stability constant of chelate formed is calculated by following equation

$$K = \frac{A/A_{ex}\,C_X}{(C_M - A/A_{ex}\,C_X)(C_L - A/A_{ex}\,C_X)} \quad (2)$$

where. K is the stability constant of the metal chelate formed in the solution, M = metal. L = ligand. X = mole fraction of the ligand at maximum absorption. $A/A_{ex}$ is the ratio of the absorbance to that indicated by the tangent for the same wavelength. $C_x$, $C_M$ and $C_L$ are the limiting concentration of complex, metal ion and the drug respectively.

The stability constant is related to the standard enthalpy changes ΔH and other thermodynamic function by the equation:

$$\Delta G = -2.303RT\log K = \Delta H - T\Delta S \quad (3)$$

where R is the gas constant T is the experimental

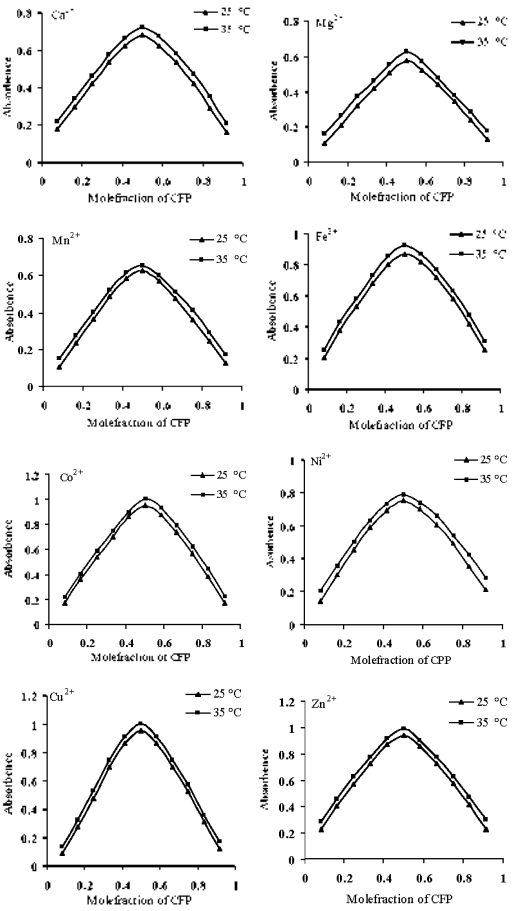


*Fig.* 4. Continuous variation curves of CFP and metal complexes.

temperature, K is the binding constant at the corresponding temperature.

From the value of stability constant at different temperature the enthalpy changes can be calculated by using the equation:

$$\log K_2/K_1 = [1/T_1 - 1/T2]\Delta H/2.303R \quad (4)$$

The negative value of ΔG for the complexation process suggests spontaneous nature of such process.[16,17] The positive value of ΔH suggests that these processes are endothermic and are favourable at higher temperature. Also it is entropically favourable. The positive value of H and S indicates that hydrophobic force may play a major

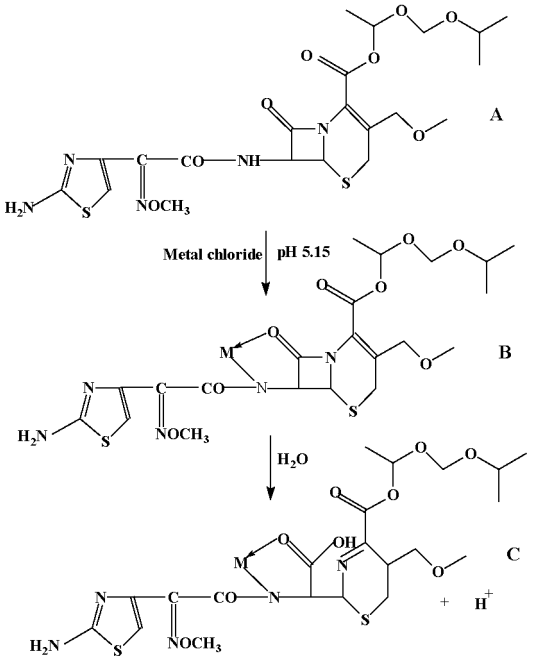


*Fig.* 5. Mechanism of binding of CFP with metal ions: A. CFP, B. CFP-metal complex, C hydrolyzed form of complex.

role in the interaction.[18] It is reasonable to think, on the basis of these results that the metal ions form a five membered chelate ring with CFP.

**Fluorescence study**

The fluorescence emission spectrum of the pure drug is markedly different from its absorption spectrum in UV region. The emission spectrum run between 350-450 nm at excitation wavelength of 335 nm showed a peak at 400 nm. The addition of metal ions to the drug causes enhancement or quenching in the fluorescence spectrum. We have noted an enhancement in fluorescence intensity at 400 nm in each case ($Ca^{2+}$, $Mg^{2+}$, $Mn^{2-}$, $Fe^{3-}$, $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$ and $Zn^{2+}$) (*Fig.* 6) although the shape and position of emission spectrum remains unchanged.

The stoichiometry and stability constant of the complexes was determined by Benesi-Hildbrand method.[13] The Benesi-Hildebrand plots were examine to further characterize the stoichiometry of

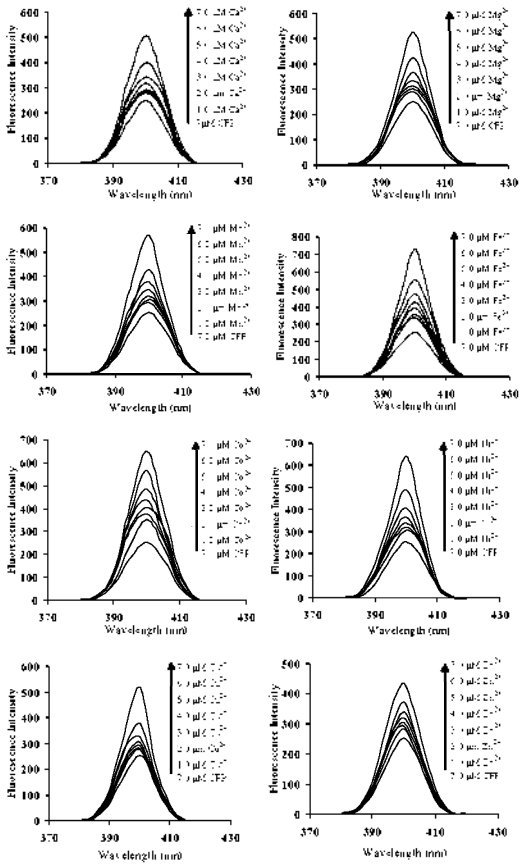


*Fig.* 6. Fluorescence enhancement (at $\lambda_{ex}$ 335nm) of CFP in presence of metal ions.

the CFP-metal complexes. In case of a 1:1 complex, the following equation is applicable:

$$1/F\text{-}F_o = 1/(F_\infty\text{-}F_o)K\,[Me]_o + 1/(F_\infty\text{-}F_o) \quad (6)$$

In this approach, a graph of $1/(F - F_o)$ versus $1/[Me]_o$, was made where F is the observed fluorescence at each concentration tested, $F_o$ is the fluorescence intensity of analyte in the absence of metal ion, $F_\infty$ is the maximum fluorescence intensity in presence of metal ions and $[Me]_o$ is the concentration of metal ion. A linear plot is required for this double reciprocal plot in order to conclude 1:1 stoichiometry. In such cases, a linear relationship has to be obtained when $1/(F - F_o)$ versus $1/[Me]_o$ is plotted. The stability constant is determined by dividing the intercept by the slope

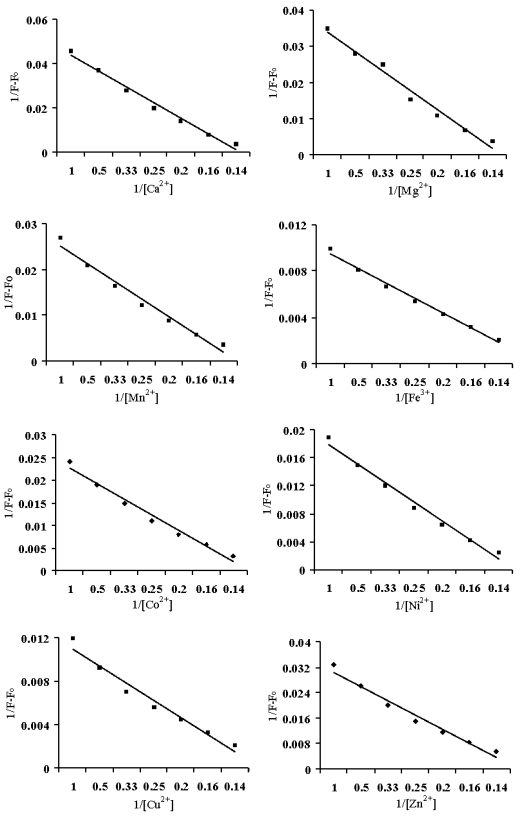


*Fig.* 7. Benesi-Hildebrand's plots for 1:1 (CFP: metal complexes)

of the straight line obtained in the double-reciprocal plot. (*Fig.* 7)

**Influence of metal ion concentrations**

The influence of metal ions concentration was studied in the range of $1\times10^{-6}$-$10\times10^{-6}$ M. The fluorescence study of interaction CFP with metal ions in acidic aqueous medium showed 1:1 stoichiometry. Both these study also support the formation of 1:1 CFP:Metal complex, with the possibility of metal ions chelating with CFP by β-actum carbonyl carbon and acidamide nitrogen.

In general, the phenomenon of enhancement is observed because the complexation by cations causes increase in the redox potential of the donor so that the relevant HOMO energy decreases to a level lower than that of fluorophore. Consequently the excited state energy of fluorophore is dumped as a visible emission.[19]

The reason of this enhancement lies in strong perturbation of the excited state upon coordination of the metal ion. A low lying internal charge transfer state due to the presence of electron donor and acceptor group in the CFP is the lowest excited state, this state is however, a less emitting state and in equilibrium with the $\pi$-$\pi^*$ excited state of the molecule. upon coordination with metal cation the PCT interaction becomes weaker since the electron withdrawing group is now electron rich moiety due to the deprotonation of –NH group necessary for coordination of metal cation.

In the present work an attempt has been made to study the interaction of the CFP with metal ions by fluorescence emission, absorption spectrophotometric and measurements. Since enhancement in fluorescence intensity of the CFP in presence of the metal ions occurs, the spectra of the drug in presence of different concentrations of several metal ions were scanned. The ratio of the drug to metal ions was determined by Job's and Benesi-Hildbrand method. The absorption spectra of the CFP was run at different pH to see the zwitterionic condition, apparent ionization constant and the isosbestic point indicating the presence of different species in solution. The stability constant of the complex formed between the drug and metal ion was also evaluated.

## CONCLUSION

In this paper the nature and magnitude of the interaction of CFP with biologically important metal ion was investigated by fluorescence spectra and UV spectra. The experimental result indicated the formation of 1:1 complex of CFP with metal ions in acidic medium by Job's and Benesi-Hildbrand method. The absorption spectra at different pH showed the presence of two isosbestic point indicating the existence of zwitterionic condition. The thermodynamic parameters showed that the interaction between CFP and metal ion was spontaneous, and that the hydrophobic force was a major factor in the interaction.

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