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Effect of Metal Ions on the Thermodynamics of Water: 1-Butanol Partitioning of Ciprofloxacin

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Abstract
The purpose of this study is to determine the effect of metal cations on the partitioning of ciprofloxacin. The thermodynamics of partitioning of ciprofloxacin between aqueous buffer (pH 7.4) and 1-butanol was studied alone, and in the presence of compounds containing metal cations which included Fe^{2+}, Al^{3+}, Mg^{2+}, Fe^{3+}, K^{+}, Ca^{2+} and Na^{+}. The determinations were carried out at 20°C, 25°C, 30°C, 37°C and 45°C and the absorbance values of the solutions were determined using UV-visible spectrophotometry at appropriate wavelengths of maximum absorption. The results showed that partition coefficient of ciprofloxacin between aqueous buffer:1-butanol medium was 0.176 at 25°C, demonstrating that ciprofloxacin is not predominantly lipophilic. The van't Hoff thermodynamic analyses of the partitioning revealed that the transfer of ciprofloxacin from the aqueous to the organic phase was exothermic. The presence of metal cations caused a decrease in the partition coefficient of ciprofloxacin and increased the standard change in Gibbs free energy (ΔG^{o}_{p}) because of complexation leading to the formation of a more polar product with concomitant increase in aqueous solubility and hence a reduction in the transfer of ciprofloxacin from the aqueous phase. When the amount of metal cation was doubled, there was a further though insignificant increase in ΔG^{o}_{p} values showing that the transfer of ciprofloxacin from the aqueous phase was less favourable and will therefore require more energy before partitioning can occur. The presence of metal cations decreased the partitioning of ciprofloxacin from the aqueous phase to 1-butanol. This may explain the reported reduction in absorption, and consequently reduced bioavailability and antibacterial activity observed when ciprofloxacin was co-administered with metal cations.
Key words: Ciprofloxacin, cations, thermodynamics, partitioning, buffer, 1-butanol

Introduction
Ciprofloxacin, 1-cyclopropyl-6-fluoro-1,4-dihydro-7-(piperazin-1-yl)-4-oxo-quinoline-3-carboxylic acid is a second generation fluoroquinolone which has broader antibacterial spectrum and better pharmacokinetic profile compared to earlier quinolones and first generation fluoroquinolones[1-3]. Generally, the fluoroquinolones have become a major group of synthetic antibiotics with a broad spectrum of activity. Despite of the numerous advantages associated with the increasing uses of the fluoroquinolones in chemotherapy, great attention has been drawn to the need to examine their possible interactions with other substances and the effect of such interactions.[4] More recently, possible interactions between the 4-quinolones and metal cations which are normally co-administered (for the purpose of supplementation; phosphate binding or acid neutralizations) have been reported. In fact, the interaction of metal cations with the 4-oxo-3-carboxylic acid moiety of the quinolones has been proven without ambiguity.
The interaction with these metal cations has affected the pharmacokinetic profile of the drug leading to reduced absorption, bioavailability and hence antibacterial activity. These *in vitro* and *in vivo* studies showed that the activity of the fluoroquinolones and bioavailability when co-administered orally were reduced to varying extents in the presence of the metal cations[5 — 9]. Although, many proposals have been made concerning the mechanism and effect of these interactions leading to the reduction in absorption, bioavailability and antibacterial activity, it became necessary to examine the physicochemical properties of the complexes resulting from the interactions. Therefore, this study was carried out in order to determine the effect of the complexation of metal cations with ciprofloxacin on the thermodynamics of its partitioning across an aqueous buffer: 1-butanol system.

Materials and Methods
***Preparation of aqueous buffer*:** Phosphate buffer of pH 7.4 solution was prepared by mixing 50 ml of 0.2M potassium dihydrogen phosphate (KH_2PO_4) with 39.50 ml of O.2M sodium hydroxide and diluting to 200 ml with de-ionized water.
Preparation of stock solution of ciprofloxacin: The buffer solution was used to prepare stock solutions of ciprofloxacin (10 mg/l00ml, which is 100 µg/ml or 1.30 x 10^4M) and solutions of the drugs containing the metal cations were prepared

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as follows: ferric ammonium citrate (25 mg/100ml or 5.1 x 10^{-4}M), ferrous sulphate (15 mg/100ml or 5.4 x 10^{-4}M), calcium carbonate (25 mg/100ml or 2.5 x 10^{-3}M), aluminium hydroxide (25 mg/100ml or 3.2 x 10^{-3}M), magnesium sulphate heptahydrate (25 mg/100ml or 1.0 x 10^{-3}M), potassium chloride (25 mg/100ml or 3.35 x 10^{-3}M) and sodium bicarbonate (67.20 mg/100ml or 7.4 x 10^{-3}M). The amount of metal cations used (in their salt form) was proportionate to their oral single doses compared with the equivalent oral 400 mg of ciprofloxacin.

***Partitioning studies*:** Exactly 20 m1 of 100 µg/ml of ciprofloxacin in buffer (pH 7.4) was mixed with 20 ml of 1-butanol and agitated for 5 h in a temperature-regulated water bath set at 20°, 25°, 30°, 37° and 45°C, (±0.1°C), respectively. After equilibrium, the two phases were separated by means of a separating funnel and a 1 ml clear solution was removed from the buffer fraction and diluted serially.

The concentrations of ciprofloxacin in the buffer were determined by measuring absorbance using UV/Visible spectrophotometer at 272nm. From the absorbance values, the solubility of ciprofloxacin in buffer (pH 7.4) was calculated by interpolating from the previously constructed calibration plots. The concentration of ciprofloxacin in the 1-butanol fraction is the difference between the initial and final concentration of ciprofloxacin in the buffer phase. The partition coefficient (k_p) at different temperatures was calculated as the concentration of ciprofloxacin in the 1-butanol phase against the concentration in the aqueous buffer phase.

Based on the calculated partition coefficient (k_p) values at the different temperatures, van't Hoff plot of ln k_p versus l/T (reciprocal of temperature in Kelvin) was prepared and the thermodynamic parameters of the partitioning of ciprofloxacin between 1-butanol and the aqueous buffer (pH 7.4) were calculated.

The procedure was repeated in the presence of varying amounts of different metal cations, taking absorbances at 364nm and determining the partition coefficient (at different temperatures) in the presence of compounds containing metal actions, and the thermodynamic parameters were also derived. The amount of compounds containing the different metal cations used in this study was also doubled so as to study the effect of increased concentration of the metal cations on the partitioning of the fluoroquinolones.

Results and Discussion

The application of the principles of thermodynamics has long been recognized as the most fundamental approach to the study of physical and chemical changes such as solubility and partitioning. Biological processes are essentially physical and chemical by nature and are therefore controlled by the exchange of energy; so that thermodynamic concepts are also applicable to biological systems.[10] The considerations of these physicochemical parameters and related thermodynamic parameters are fundamental to discussing several important aspects of drug action.

Lipophilicity is a measure of the distribution of a drug between the aqueous and lipid phases i.e. oil-water partition coefficient. If the partition coefficient is determined over the temperature range 20 - 45°C, the thermodynamics of transfer can be calculated. Although the partition coefficient is most commonly sought, the thermodynamic parameters are important in explaining the molecular extent of distribution of the drug between the aqueous and the lipid phases.

Therefore, the knowledge of the partitioning of ciprofloxacin alone, and in the presence of metal cations and the thermodynamic considerations in the interpretation of partitioning behaviour is a matter of interest because it provides information on the hydrophobic nature of compounds and their potential for interactions.

The partition coefficient of a compound has been shown over the years to be the single most important property controlling the transport of substances in living organisms because partition coefficients provide information on the hydrophobic nature of the compounds and their potential for interactions with various regions of bilayer membranes.[11]

The organic medium used for the solubility and partitioning studies was 1-butanol, instead of 1-octanol, which has been regarded as the best organic solvent that simulate the hydrophilic-hydrophobic nature of the biological membrane for extra-thermodynamic studies.[12 – 13]

Although the 1-octanol-water system is generally used for the measurement of partition coefficient, many other solvent pairs have been used such as the cyclohexane-water system, alcohol-water system. In fact, semipolar solvents have been found to yield better correlations with the partitioning of solutes obtained in model membranes compared to non-polar solvents like cyclohexane. [10, 14-15]

In this study, 1-butanol was used because it is more polar than 1-octanol and it was easier for spectrophotometric determination. Moreover, the solubility of the fluoroquinolones was very low in 1-octanol.

The partition coefficients of ciprofloxacin were studied at different temperatures in order to evaluate the temperature dependence of partitioning and also evaluate the thermodynamic functions of the partitioning of ciprofloxacin.

The temperature dependence of the partition coefficients of pure ciprofloxacin between aqueous phosphate buffer (pH 7.4) and 1-butanol is shown in Table 1. From the results, the partition coefficient of the ciprofloxacin at the thermodynamic temperature of 25°C is 0.176. The partition coefficients of ciprofloxacin decreased as temperature increased because it tended to remain in the aqueous medium with increasing temperature rather than partition into the

more non-polar 1-butanol medium. The thermodynamic parameters (standard changes in free energy, enthalpy and entropy) at 25°C that are usually related to the transfer of ciprofloxacin from the aqueous medium to the organic medium showed that the standard change in free energy of partitioning (ΔG^{o}_{p}) at 25°C has positive values of +4.30 KJ/mol, standard changes in enthalpy of partitioning (ΔH^{o}_{p}) has a negative value of -2.04 KJ/mol and the standard changes in entropy of partitioning (ΔS^{o}_{p}) has a negative value of -21.27 J/mol.K. The entropy values reported as J/mol.K are low compared to the free energy and enthalpy values that are in KJ/mol.

From the free energy values, the transfer of ciprofloxacin from the aqueous medium to the organic medium is not favourable since ciprofloxacin tended to remain more in the aqueous phase; however, the spontaneity of the transfer from aqueous phase to 1-butanol phase is dependent on temperature and the relative magnitude of the standard change in enthalpy and standard change in entropy values. The negative sign of the standard change in enthalpy (ΔH^{o}_{p}) indicates that the transfer of the fluoroquinolones is enthalpy-driven. But the negative sign of the entropy value for ciprofloxacin shows that the partitioning is not entropically favoured because the standard change in free energy (ΔG^{o}_{p}) becomes more positive when the standard change in entropy (ΔS^{o}_{p}) is negative. Hence, the partitioning can only be due to adsorption phenomenon.[16]

The transfer of ciprofloxacin from aqueous medium to organic medium required more energy to bring out ciprofloxacin from the aqueous phase after breaking the solute-aqueous phase and solvent-solvent interactions and depositing it in 1-butanol phase, creating solute:1-butanol phase and 1-butanol phase : 1-butanol phase interactions. These processes require work and energy which can be measured thermodynamically.

The configuration of the solute, the kind of arrangement in the crystal lattice, increased polarity of solvent, reduced surface tension of solvent and factors that influenced the solubility of the solute, will affect the process of solubilization and hence partitioning of solutes. The relative magnitude and sign of the standard change in enthalpy (ΔH^{o}_{p}) and then the standard change in entropy (ΔS^{o}_{p}) which relates to the degree of disorderliness, determines the extent of solubility and hence the transfer of the solute from the buffered aqueous medium to the 1-butanol medium.

Table1: Results for the Partitioning of Ciprofloxacin between 1-Butanol and Aqueous Buffers (pH 7.4) as a Function of Temperature

Temperature (^{o}C)	Temperature (K)	1/T (K^{-1})	Solubility in Butanol (S_o) (mol/L x 10^{-3})	Solubility in Buffer (S_w) (mol/L x 10^{-3})	$K_p = S_o/S_w$	ln K_p
20	293	0.00341	0.0393	0.2199	0.179	-1.720
25	298	0.00336	0.0388	0.2204	0.176	-1.737
30	303	0.00330	0.0387	0.2207	0.175	-1.743
37	310	0.00323	0.0381	0.2211	0.172	-1.760
45	313	0.00314	0.0371	0.2221	0.167	-1.790

With the addition of the compounds containing the metal cations, ciprofloxacin tended to remain more in the aqueous phase rather than partition into the organic phase. This is due to increase in aqueous solubility of the ciprofloxacin in the presence of the metal cations. Ferrous (Fe^{2+}) and aluminium (Al^{3+}) ions have been mostly implicated from previous studies. This is because the ciprofloxacin-metal complex is more polar that ciprofloxacin and required more polar aqueous buffer to stabilize it.[8, 17, 18]

All the metal cations used in this study caused varying decrease in the partitioning of the ciprofloxacin at any given temperature and depending of the type of metal cation, as shown in Fig. 1 and the thermodynamic parameters are presented in Table 2 below.

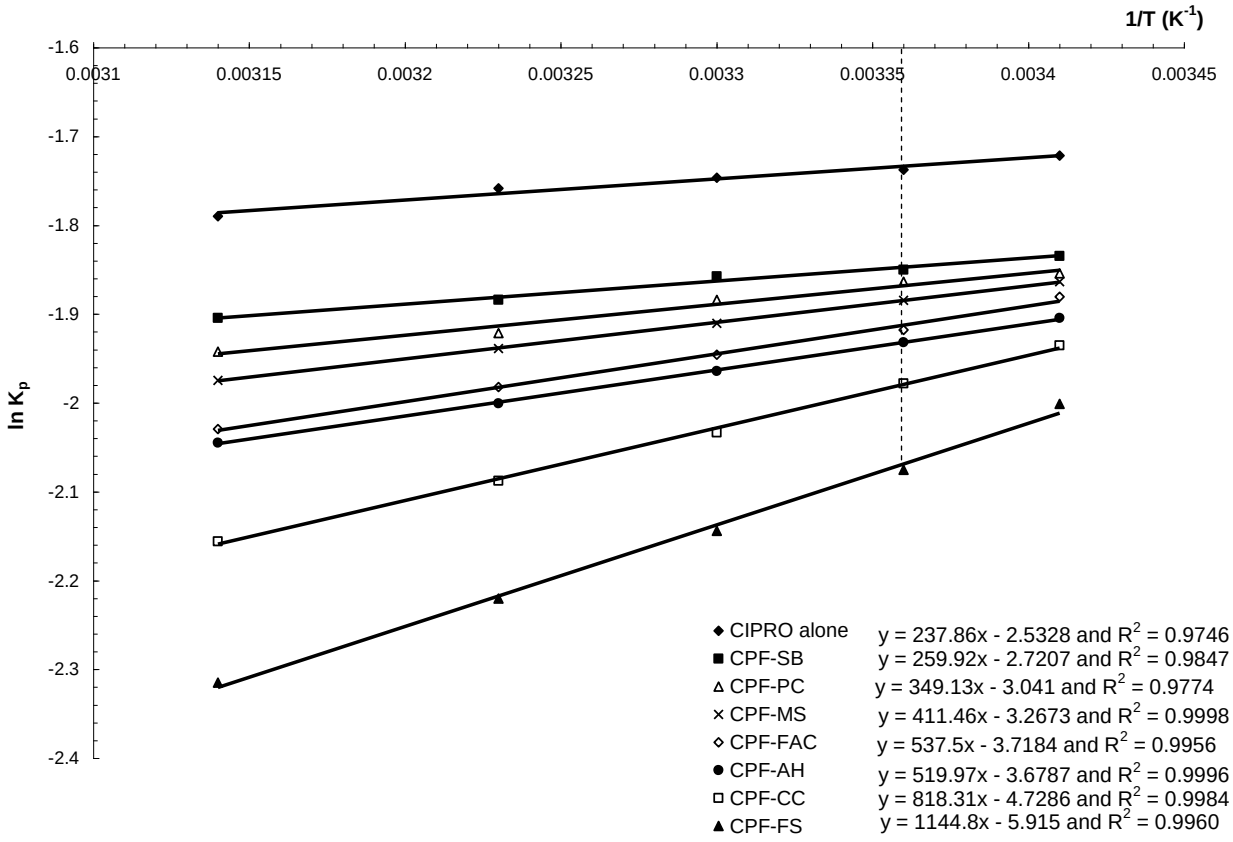

Fig. 1: van't Hoff Plots of ln K_p versus 1/T for the partitioning of ciprofloxacin between 1-butanol and aqueous buffer (pH 7.4) in the presence of metal cations

KEY**:** CPF = Ciprofloxacin; SB = Sodium bicarbonate; PC = Potassium chloride;
MS = Magnesium sulphate; AH = Aluminium hydroxide; FAC = Ferric Ammonium Citrate;
CC = Calcium carbonate; FS = Ferrous Sulphate

Table 2: Results of the partitioning of ciprofloxacin between 1-butanol and aqueous buffer in the presence of metal cations and their thermodynamic parameters at 25^{o}C

Ciprofloxacin + Metal Cation	Slope of Plot (K^{-1})	ΔG^{o}_{p} (KJ/mol)	ΔH^{o}_{p} (KJ/mol)	ΔS^{o}_{p} (J/mol.K)
CPF	+237.86	+4.39	-1.98	-21.37
CPF + SB	+259.92	+4.58	-2.16	-22.63
CPF + PC	+349.13	+4.62	-2.90	-25.24
CPF + MS	+411.46	+4.67	-3.42	-27.15
CPF + AH	+519.97	+4.79	-4.32	-30.57
CPF + FAC	+537.50	+4.75	-4.47	-30.94
CPF + CC	+818.31	+4.90	-6.80	-39.28
CPF + FS	+1144.80	+5.14	-9.52	-49.19

KEY**:** CPF = Ciprofloxacin; SB = Sodium bicarbonate; PC = Potassium chloride;
MS = Magnesium sulphate; AH = Aluminium hydroxide; FAC = Ferric Ammonium Citrate;
CC = Calcium carbonate; FS = Ferrous Sulphate

Using the thermodynamic temperature of 25°C, there was a 1.12-fold decrease in the partition coefficient of ciprofloxacin when co-administered with (Na^+), a 1.13-fold decrease with potassium ion (K^+), a 1.16-fold decrease with magnesium ion (Mg^{2+}), a 1.20-fold decrease with ferric ion (Fe^{3+}), a 1.21-fold decrease with aluminium ion (Al^{3+}), a 1.27-fold decrease with calcium ion (Ca^{2+}), and then a 1.40 fold decrease with ferrous ion (Fe^{2+}). The decreasing effect of the metal cations on the partition coefficient of ciprofloxacin is as follows:

$Fe^{2+} > Ca^{2+} > Al^{3+} > Fe^{3+} > Mg^{2+} > K^+ > Na^+$

The results of this study followed the trend observed by Eboka and Okeri[8] on the effect of metal cations on the aqueous solubility of ciprofloxacin, except for magnesium ion (Mg^{2+}). It was reported that the magnesium ions caused an initial increase in aqueous solubility but that as the concentration of the Mg^{2+} increased, aqueous solubility then decreased. The effect of the magnesium ion (Mg^{2+}) on partitioning of ciprofloxacin in this study therefore, may be due to the fact the concentrations of the magnesium cation was low.

The thermodynamics of the transfer of ciprofloxacin from aqueous buffer to 1-butanol in the presence of metal cations showed that the standard change in free energy of partitioning (ΔG°_{p}) at 25°C gave more positive values. The ΔG°_{p} value for pure ciprofloxacin was +4.39 KJ/mol, the value increased to +4.58 KJ/mol for Na^+, +4.62 KJ/mol for K^+, +4.67 KJ/mol for Mg^{2+}, +4.75 KJ/mol for Fe^{3+}, +4.79 KJ/mol for Al^{3+}, +4.90 KJ/mol for Ca^{2+} and 5.14 KJ/mol for Fe^{2+}.

Comparing the standard change in enthalpy of partitioning (ΔH°_{p}) for pure ciprofloxacin which was -1.98 KJ/mol, it increased to -2.16 KJ/mol for Na^+, -2.90 KJ/mol for K^+, -3.42 KJ/mol for Mg^{2+}, -4.32 KJ/mol for Al^{3+}, - 4.47 KJ/mol for Fe^{3+}, -6.80 KJ/mol for Ca^{2+} and -9.52 KJ/mol for Fe^{2+}. It meant that the transfer of ciprofloxacin from the aqueous medium to the organic medium in the presence of all the metal cations studied showed that the partitioning was exothermic.

The standard changes in entropy of partitioning (ΔS°_{p}) for pure ciprofloxacin gave a negative value of -21.37 J/mol.K and also negative values in the presence of Na^+ (-22.63 J/mol.K), K^+ (-25.24 J/mol.K), Mg^{2+} (-27.1 J/mol.K), Al^{3+} (-30.57 J/mol.K), Fe^{3+} (-30.94 J/mol.K), Ca^{2+} (-39.27 J/mol.K) and Fe^{2+} (-49.19 J/mol.K). This showed that the partitioning of ciprofloxacin in the presence of all the metal cations gave more negative entropy values showing that the partitioning was not entropically favoured. In this case where standard changes in enthalpy and entropy have negative values the partitioning is enthalpy favoured but not favoured entropically; thus, the spontaneity of partitioning is dependent on the relative size or magnitude of the enthalpy and entropy values.

When the amount of metal cations was doubled, there was only a little further decrease in the partitioning of ciprofloxacin in the presence of ferrous, calcium and aluminium ions, while there was little or no significant decrease for the other metals.

The partition coefficients of the ciprofloxacin decreased in the presence of the metal cations because aqueous solubility increased in each case. This is because the metal cations increased the polarity of water, reduced the interfacial surface tension of the water molecules and enhanced the activity of the solute in the aqueous medium as a result of complexation. Also, the thermodynamic parameters of the partitioning for ciprofloxacin were affected to varying degrees depending on the metal cation. Divalent and trivalent cations reduced the partition coefficient of ciprofloxacin more than the monovalent cations.

The general implication of the reduction in partitioning in the presence of metal cations is that there would be decreased absorption of the fluoroquinolones from the gastrointestinal tract and hence reduced bioavailability. The reduced partitioning in the presence of metal cations would also cause reduced penetration through bacterial cell wall and hence the observed decreased antibacterial activity. Hoffken et al[6], Nix et al[19] and Brouwers et al[20] reported that the absorption of ciprofloxacin was reduced in the presence of antacids and compounds containing iron. Also, Smith[5] reported that the presence of metal cations increased the minimum inhibitory concentration (MIC) of ciprofloxacin by many folds, and hence reduced antibacterial activity of ciprofloxacin.

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