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Thermodynamics of the Aqueous Solubility of some Fluoroquinolones

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ABSTRACT
The fluoroquinolones have been reported to form complexes with metal cations when co-administered with compounds containing metallic cations such as the antacids and haematinics. This complex formation leads to reduction in bioavailability and antibacterial activity. Although different reasons have been adduced for the observed effects, however, no significant complexation as observed at some concentrations of the metal cations at which significant reduction in bioavailability of the fluoquinolones was reported. This showed that other mechanisms besides complexation and chelation interactions must contribute to the observed effects. Thus, some physicochemical properties of the fluoroquinolones need to be considered. This work is a study of the energetic of the thermodynamics of the aqueous solubility of three fluoroquinolones namely ciprofloxacin, ofloxacin and norfloxacin, so as to establish their correlation with absorption processes and penetration across bacterial cell wall. The experimental conditions were simulated to mimic normal physiological conditions and the thermodynamics of the solubility of the fluoroquinolones in aqueous buffer (pH 7.4) was studied. The determinations were carried out at 20, 25, 30, 37 and 45 ^{o}C, and the absorbance values of the saturated solutions of the fluoquinolones were determined spectrophotometrically. The results showed that increased solubility of the fluoroquinolones in aqueous buffer with increasing temperature.

INTRODUCTION

The synthesis of fluorinated quinolones known as fluoroquinolones has really opened up the field of quinolone antibacterial chemotherapy. The fluoroquinolones have enhanced activity against a wide range of Gram-negative as well as Gram-positive cocci and Enterobacteriaceae. The increased potency of the fluoroquinolones due to enhanced activity, a broader antibacterial spectrum or improved pharmacokinetic properties has greatly expanded their potential usefulness in clinical practice. The fluoroquinolones are widely distributed in the body tissue and achieve excellent urinary concentration hence; they are useful for the treatment of urinary, respiratory, gastrointestinal, skin, soft tissue, bone and sexually transmitted infections due to intracellular pathogens [1,2].

The differences in the activity of the quinolones are as a result of the differences in substituents [3] which may be responsible for differences in absorption and tissue penetration and these may potentially be predicted by their physicochemical properties [4].

But despite 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. [5 – 7]

Generally, the quinolones are poorly soluble in water in the pH range of 6 and 8; hence they form needle-shaped crystals in acid urine. In other words, the low water solubility of the 4-quinolones makes them to be prone to precipitation even under more acidic conditions than urine. It is apparently due to this property that crystalluria has been observed in man and animals. However, the salt forms of the fluoroquinolones are freely soluble and are generally stable in an aqueous solution. The water solubility at physiological pH varies across these compounds, depending on the substitutions on the quinolone carboxylic acid nucleus. Oral or parenteral liquid formulations of quinolones usually contain soluble salts (e.g. hydrochloride salt) in stable aqueous solutions.[8 – 10]

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The study on aqueous solubility and the thermodynamics of the aqueous solubility of the fluoroquinolones may be necessary to understanding transport of the fluoroquinolones across biologic membranes, whether bacterial or gastrointestinal membranes. This is because the bioavailability of a drug, minimum inhibitory concentration (MIC) and hence its antimicrobial activity are dependent on the entry of the drug molecule into the bacterial cell and its interaction with its target within the cell. Therefore, for the quinolone drug to be active, it has to be present in appropriate concentration at the site of action and must necessarily be absorbed through various membranes. Solubility characteristics of a drug have profound influence on the ease with which it crosses a biologic membrane and thus determine the rate and extent of absorption. Also, the rate of penetration of the quinolone drug across membranes depends on various physicochemical properties of the drug such as lipophilicity [11, 12].

Thermodynamics is a branch of science that deals with the transformations of energy from one form to another and it helps us to understand why some processes are feasible while others are not. It does this by exploring the energy changes accompanying the process or reaction and thus determines whether the process or reaction will take place spontaneously or not, as well as determine the extent of the reaction or process. The spontaineity of solubility of a drug and its transport across the bacterial cell wall depends on the relative size of the changes in the enthalpy and entropy values. The interplay of the changes in enthalpy (ΔH_s^o) and entropy (ΔS_s^o) determines the free energy change (ΔG_s^o) and hence, whether the dissolution of a drug will occur spontaneously. If in the solution process, the change in enthalpy is negative and the change in entropy is positive, dissolution is favoured because the change in free energy will be negative.

METHODOLOGY

The three fluoroquinolones used for this work were supplied by reputable Pharmaceutical Companies in Nigeria. Norfloxacin and ciprofloxacin were obtained from Sam Pharmaceuticals (Ilorin, Nigeria) and ofloxacin (manufactured by Hoechst, Germany) was obtained from Nigeria-German Company, Lagos, Nigeria.

Ultra-violet/Visible spectrophotometer (UV/Vis Spectrometer, model T70, PG Instruments Ltd, United Kingdom), thermostated shaker bath (Gallenkamp, United Kingdom), water deioniser (Model 6C Houseman [Burnham] Ltd, United Kingdom), sensitive analytical balance (b15, Mettler, Toledo, Switzerland), pH meter (Model 3020, serial no. 4519, Jenway, United Kingdom) with a ThermoOrion combination glass electrode (Orion Research, Boston, MA) were used.

(a) Preparation of Buffer (pH 7.4): Solutions of phosphate buffer of pH 7.4 were prepared by mixing 50 ml of 0.2M potassium dihydrogen phosphate (KH_2PO_4) with 39.50 ml of 0.2M sodium hydroxide (NaOH) and diluting to 200 ml with de-ionized water.

(b) Preparation of Stock Solutions of Fluoroquinolones in Buffer (pH 7.4): Stock solutions of ciprofloxacin (5 mg/100ml, which is 50 μg/ml or 1.30 x 10^{-4}M), ofloxacin (10 mg/100ml, which is 100 μg/ml or 2.8 x 10^{-4}M) and norfloxacin (10 mg/100ml, which is 100 μg/ml or 3.0 x 10^{-4}M) were prepared in phosphate buffer (pH 7.4) to simulate physiological pH. All stock solutions of fluoroquinolones were wrapped in aluminium foil and stored in amber coloured bottles to prevent or minimize photo-degradation.

(c) Preparation of Calibration Plot for the Fluoroquinolones in Buffer (pH 7.4): Standard solutions of the fluoroquinolones in buffer (pH 7.4) were prepared by transferring aliquots of 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 ml of stock solution of fluoroquinolones into separate 25 ml volumetric flasks and made up to the 25 ml mark with buffer (pH 7.4). The standard solutions were then diluted with the phosphate buffer (pH 7.4) and the absorbances of the final solutions were recorded at the wavelength of maximum absorption between 260 and 300nm using the UV/Visible spectrophotometer.

The Beer-Lambert's (calibration) plots for the three fluoroquinolones were then prepared by plotting absorbance values against the concentration of fluoroquinolone.

(d) Determination of the Aqueous Solubility of Fluoroquinolone in Buffer (pH 7.4) at Different Temperature: Known weights (20 mg) of finely divided pure fluoroquinolone powder were placed in dried and clean volumetric flasks and 5 ml of the phosphate buffer (pH 7.4) was added to simulate physiological pH. The solutions were protected from light by wrapping the flasks in aluminium foil and agitated for 5 h in a temperature-regulated water bath set at 20, 25, 30, 37 and 45 ^oC, (±0.1^oC), respectively.

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After equilibrium was achieved, a 1 ml clear solution was removed from the saturated solution in the flask and serially diluted with buffer (pH 7.4) and the absorbances of the final solutions were measured using the UV/Visible spectrophotometer at wavelengths of maximum absorption already determined with the pure fluoroquinolones above. From the absorbance values, the solubility of the fluoroquinolones in buffer (pH 7.4) was calculated after interpolation of the corresponding concentration from the already prepared calibration plots.

Based on the aqueous solubility at different temperatures, van't Hoff plot of ln S versus 1/T (reciprocal of temperature) was prepared and the thermodynamic parameters of the aqueous solubility of the fluoroquinolones in buffer (pH 7.4) were calculated.

RESULTS AND DISCUSSION

Solutions of known concentration of the fluoroquinolones were prepared in aqueous phosphate buffer adjusted to pH 7.4 (physiologic pH similar to that of the lower part of the gastrointestinal tract and blood) and at this pH, most drugs have their highest distribution coefficient values because the undissociated molecules dominate.[13,14]

The solubilities of the fluoroquinolones were studied at different temperatures in order to evaluate the change of solubility with temperature and also evaluate the thermodynamic functions of the solubility of the fluoroquinolones.

The solubilities of the three fluoroquinolones used in this study were determined spectrophotometrically at their appropriate wavelengths of maximum absorption. The values of the thermodynamic parameters of transfer can be rationalized in terms of the solvation of the fluoroquinolones in the aqueous medium.

The solubility of the three fluoroquinolones (in aqueous phosphate buffer of pH 7.4) at thermodynamic temperature of 25^{o}C were independently determined to be 120 µg/ml (i.e. 3.11 x 10^{-4} M) for Ciprofloxacin, 360 µg/ml (i.e. 1.13 x 10^{-3} M) for Norfloxacin and 2,050 µg/ml (i.e. 5.67 x 10^{-3} M) for Ofloxacin. Thus, ofloxacin was the most soluble of the three fluoroquinolones followed by norfloxacin and the least was ciprofloxacin although it was in the hydrochloride form.

The solubility of norfloxacin was reported by Swanson et al [14] to be 0.45 mg/ml (450 µg/ml) at pH 7.5 and 25^{o}C, while Ross and Riley [15] reported 0.38 mg/ml (380 µg/ml) for norfloxacin at pH of 7. On the other hand, Ross and Riley [15] reported the solubility of ofloxacin to be 3.23 mg/ml (3,230 µg/ml) and the solubility of ciprofloxacin to be 0.09 mg/ml (90 µg/ml), at the pH of 7. [15]

Thus, the solubilities of norfloxacin and ofloxacin as obtained in this study were slightly lower than the values obtained in earlier studies at about the same pH condition [14, 15]. On the other hand, the solubility of ciprofloxacin as obtained in this study at the pH of 7 was higher than the reported value [15]. The results for the solubility of norfloxacin and ciprofloxacin compared well with the results of Ross and Riley obtained at the pH of 7 (the difference observed may be due to the small change in pH from 7 to 7.4. However, the solubility is much more reduced in the case of ofloxacin and this could also be due to the small pH variations.

The difference in solubility of the fluoroquinolones can be attributed to the nature of the fluoroquinolone and certain properties of the crystals. For example, ofloxacin has an additional ring structure and a chiral centre that may affect the crystal lattice packing and hence the solubility.

Some fluoroquinolones have been found to be more soluble in acidic conditions [16 – 20], but it has been reported that the zwitterionic form of the fluoroquinolones, which is predominant at the isoelectric point of the molecule at pH close to 7, has the lowest solubility [21].

In order to improve solubility and bioavailability of poorly water soluble drugs, many methods such as preparation of solid dispersions [22] and cyclodextrin inclusion complexes [23, 24] have been used.

From the results, it is clear that the aqueous solubility increased with increasing temperature. The temperature dependence of solubility for all the three fluoroquinolones as presented in the van't Hoff plots (Figure 1) of natural logarithm of solubility (ln S) against the reciprocal of temperature (T^{-1} in ^{o}K) shows that increase in solubility with increasing temperature. In all the van't plot for the three fluoroquinolones, straight lines with negative slopes and correlation coefficients (r^2) of greater than 0.98 were obtained for the solubility of the fluoroquinolones in aqueous buffer; therefore, the van't Hoff method was useful for the respective thermodynamic analyses [25 – 27].

From the van't Hoff plots, the thermodynamic functions (standard free energy change, standard enthalpy change and standard entropy change) of the solubility of the three fluoroquinolones were

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determined and the values are summarized in Table 1. The sign and magnitude of the respective thermodynamic functions have been successfully used in making relevant observations as to the molecular basis of solubility of these fluoroquinolones.

The thermodynamics of the solubilities of the three fluoroquinolones in aqueous buffer showed that the standard free energy change of solubilization (ΔG_s^o) at 25°C has positive values in all the cases; +20.09 KJ/mol for ciprofloxacin, +16.82 KJ/mol for norfloxacin and +12.82 KJ/mol for ofloxacin. The lesser the standard free energy (ΔG_s^o) values, the greater the solubility of a compound since it will take lesser energy to break the crystal lattice structure. The change in standard free energy value was lowest for ofloxacin and hence, it is the most soluble of the three fluoroquinolones used in this study.

Although the change in standard free energies of the solubilization of the three fluoroquinolones in aqueous buffer were not favourable, the spontaneity of the solubilization of the three fluoroquinolones in aqueous buffer (pH 7.4) was dependent on temperature and the relative size of the change in standard enthalpies (ΔH_s^o) and the change in standard entropies (ΔS_s^o). Work was done on the system by agitation (shaking) so as to enhance the dissolution of the fluoroquinolones and dissolution was also enhanced when heat was supplied by raising the temperature.

The standard changes in enthalpy of solubilization (ΔH_s^o) of the three fluoroquinolones are as follows: +14.83 KJ/mol for ciprofloxacin, +9.68 KJ/mol for norfloxacin and +6.91 KJ/mol for ofloxacin. The positive signs of the standard change in enthalpy will ordinarily indicate that the solubilization of the fluoroquinolones in aqueous buffer (pH 7.4) was not enthalpy-driven since the transfer of energy was markedly endothermic, hence the solubilization of the three fluoroquinolones increased with increasing temperature which indicated that the process was energetically-driven.

Generally, if the change in enthalpy of solution was sufficiently positive, the change in entropy term should be large enough to overcome the change in enthalpy factor, else the compound will be insoluble.

On the other hand, the standard changes in entropy of solubilization (ΔS_s^o) of the three fluoroquinolones gave negative values for all the three fluoroquinolones as follows: –17.65 J/mol.K for ciprofloxacin, –19.82 J/mol.K for ofloxacin, and –23.96 J/mol.K for norfloxacin. The change in entropy values reported as J/mol.K are low compared to the change in enthalpy values that are in KJ/mol.

For the three fluoroquinolones, the standard change in entropy value for ciprofloxacin was the highest (because it has least negative value relatively and hence more positive) in terms of its magnitude and the negative sign, which led to a positive value of change in standard free energy change considering the equation $\Delta G_s^o = \Delta H_s^o - T\Delta S_s^o$.

The three fluoroquinolones used in this study had varying disruptive effects on the structure of water and hence varying entropy values and the general trend showed that, the solubilization process for these fluoroquinolones was not entropy-driven because the free energy change increased as ΔS_s^o was negative. The more positive the entropy change, the greater the degree of randomness or disorder of the reaction system and the more favourably disposed would be the process.

Dissolution generally occurs starting at a relatively ordered (low entropy) state and progressed to a disorderly (high entropy) state due to the disruption of solute-solute and solvent-solvent interactions. The negative entropy values may be due to the loss of freedom of movement of water molecules and solute molecules as a result of hydration.

It has been reported that when the change in standard entropy gives negative values, solubilization mechanism is only based on adsorption rather than micelle formation and penetration. Also the structuring of solvent molecules based on their dielectric constant (e.g. water has a high dielectric constant leading to the creation of dipoles) plays significant roles in the dissolution of solutes [27].

Therefore, the positive values of change in standard free energy and change in standard enthalpy values, and negative change in entropy values also showed that the fluoroquinolones are ionic and solubilization was due to interaction of the solute with the solvent through adsorption phenomenon [27].

That the solubilization of the three fluoroquinolones in aqueous buffer (pH 7.4) increased with temperature is based on the interplay between enthalpy and entropy. Ofloxacin was the most soluble of the three fluoroquinolones, because it had the least change in free energy values and the least positive change in enthalpy value.

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CONCLUSION

For these three fluoroquinolones, increase in temperature increased solubility because the heat of solution is positive (i.e. heat is absorbed) thereby providing more thermal energy. This energy barrier for solubilization was overcome by increasing temperature and by work on the system through agitation which increased disorderliness and hence entropy.

The van't Hoff plots showed that the enthalpy of solubilization (ΔH_s^o) in the aqueous buffer was positive for all the fluoroquinolones, thus the solubilization process was endothermic. The standard free energy change (ΔG_s^o) for solubilization of all the fluoroquinolones in the aqueous buffer was also positive showing that the solubilization process was not spontaneous. The standard change in entropy for the solubilization (ΔS_s^o) of these fluoroquinolones in the aqueous buffer was negative. Thus, solubilization was by adsorption rather than by micelle formation and penetration. The $T\Delta S_s^o$ term contribution to standard free energy change (ΔG_s^o) was more predominant than the ΔH_s^o term, thus solubilization of the fluoroquinolones was entropically controlled.

Fig. 1: Van't Hoff Plot of In S versus 1/T for the Solubility of the Fluoroquinilones in Aqueous Buffer (pH 7.4)

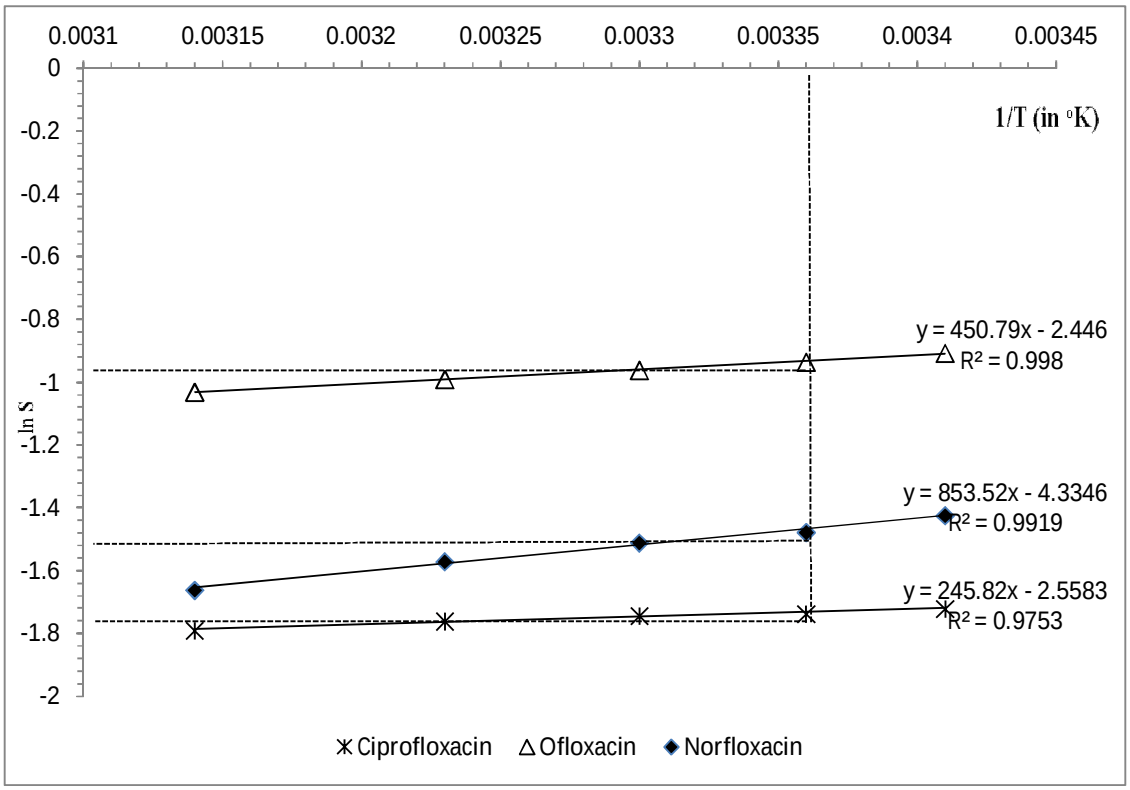

Table 1: Result of the Solubility of the Fluoroquinilones in Aqueous Buffer (pH 7.4) and Thermodynamic Parameters at 25°C

Fluoroquinolone	Slope of Plot (K^{-1})	ΔG_s^o (KJ/mol)	ΔH_s^o (KJ/mol)	ΔS_s^o (J/mol.K)
Norfloxacin	-1164.50	+16.82	+9.68	-23.96
Ofloxacin	-831.57	+12.82	+6.91	-19.82
Ciprofloxacin	-1784.10	+20.09	+14.83	-17.65

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