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The Physicochemical And Antibacterial Properties Of Ciprofloxacin-Mg^{2+} Complex.

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ABSTRACT

Purpose: Co-administration of quinolone antibiotics with cation-containing medicaments such as, antacids has been reported to influence the overall bioavailability leading to subtherapeutic plasma concentrations of these antibiotics in humans.

Objectives of the study: The present work was designed to evaluate the binding constant, binding molar ratio, influence of temperature on the binding constant of ciprofloxacin-Mg^{2+} and to determine the antimicrobial activity of ciprofloxacin and ciprofloxacin-Mg^{2+}.

Methods: Job's method of continuous variation and Benesi-Hildebrand equation were adopted to determine the molar ratio and stability constant respectively. The antibacterial activity was determined by the Agar diffusion method.

Results: A complexation molar ratio of 1:1 was obtained for ciprofloxacin-Mg^{2+} complex. The stability constants were 3.59 and 3.50 at 25°C and 60°C respectively. There was a significant difference between the zones of inhibition of ciprofloxacin-Mg^{2+} complex and that of ciprofloxacin alone against *E. coli, P. aeruginosa, and S. aureus* ($p < 0.05$). This difference showed that the complex formed was not as active as ciprofloxacin.

Conclusion: The present studies have shown that ciprofloxacin readily complex with Mg^{2+} and that the stability constant was temperature dependent. The antibacterial activity of ciprofloxacin was markedly reduced in the presence of Mg^{2+}. Concomitant administration of ciprofloxacin with Mg^{2+} containing medicaments should be avoided to prevent resistance.

Key words: Ciprofloxacin-Mg^{2+} complex, stability constant, antibacterial activity.

INTRODUCTION

Ciprofloxacin is a **fluoroquinolone** antibiotic that is widely prescribed for a number of bacterial infections[1,2,3].

It is the most commonly used fluoroquinolone, a broad spectrum antibiotic, effective against both gram positive and gram negative organisms, especially active against the latter. It has an excellent activity against the enterobacteriaceae, and is also effective against Herpes influenza, penicillinase producing *Neisseria gonorrhoea*, staphylococcal infection, intracellular pathogens such as mycobacterium tuberculosis, and mycoplasma [1,2,3].

The complex absorption profile and pharmacokinetics of the fluoroquinolones tend to predispose them to various types of drug interactions [1,2,3]. These drug interactions ultimately influence the blood concentrations of the fluoroquinolones. Liver biotransformation enzymes are very important for drug metabolism and disposition when fluoroquinolones are co-administered with enzyme suppressing drugs like tolbutamide and cimetidine. This often results in toxic levels in the blood[4].

Studies have shown marked reduction in blood levels of fluoroquinolone antibiotics when co-administered together with other medicaments such as antacids containing cations. These drug-drug interactions result in poor absorption and sub-therapeutic blood levels. The patient fails to respond clinically. Furthermore, reduced blood concentrations may generate resistance to such antibiotics by the microbes.

Other drugs showing incompatibility with the fluoroquinolones include cyclosporine, warfarin and many non-steroidal anti-inflammatory drugs (NSAIDs) [5,6,7]. Clinical and laboratory studies have documented an interaction between fluoroquinolones and multivalent metal cations, such as aluminium, magnesium calcium, iron, and zinc[8]. Extraneous factors that might interfere with drug bioavailability may reduce tissue levels and alter the effectiveness of the drug. Medicaments such as **antacids** containing aluminium can significantly impair the absorption of **ciprofloxacin** from the gastro intestinal tract when co-administered to humans. The proposed mechanism for this impairment is the formation of a poorly absorbed chelation complex. The co-administration of sucralfate, which contains 16 aluminium ions per molecule, reduces the bioavailability of ciprofloxacin by at least 30% [8].

Patients who receive antimicrobial treatment may require nutritional supplements. A significant reduction in maximum serum concentration-time curve (AUC) of ciprofloxacin when administered concurrently with Osmolite or Pulmonary (Ross Laboratories, Columbus) has been reported[8,9].

Since the various interactions are not of equal intensity for all fluoroquinolones, it is best to avoid these combinations whenever possible. If the combination of a multivalent metal cation and a fluoroquinolone can not be avoided, their doses should be staggered to minimize this

potential drug interaction. It has been suggested that the lower serum levels of the fluoroquinolones when co-administered with Mg^{2+} and Al^{3+} containing antacids were due to antibacterial, inactive chelate complexes between the metallic ions and the drug[7,10,11].

Generally, metals have a definite affinity for organic ligands. Donor–acceptor complex are formed from election – donating molecules or ions known as ligands and acceptor ion with an incomplete valence shell like metal ions. Molecules like ethylene diamine forming complexes involving bi-, tri–or poly-dentate ligands are called chelating agents. Chelating agents that confer water solubility by complexation with insoluble entities are known as sequestering agents. Inorganic cations contained in some medicaments are known to sequester organic moieties by chelation reactions and rendering these organic molecules more water-soluble[12].

Metal ions are generally hard acids forming bonds with hard bases (ligand) mentioned above. Metals in the system bind to hard bases like water in the system, playing an important rôle in fluid retention, excretion and transport in vivo, and out of the animal[12,13].

There are so many inorganic elements in the body incorporated or fixed in enzymes and protein[13].

This research was carried out to determine the molar ratio, stability constant, influence of temperature on the stability constant and the antimicrobial activity of ciprofloxacin-Mg^{2+} complex.

METHOD

Materials and equipments

Spectrophotometric measurements were performed using an ultraviolet/visible spectrophotometer- Pye Unicam SP4 800 model (courtesy May & Baker Nigeria PLC, Ikeja, Lagos).

Ciprofloxacin (potency=99%, Courtesy Sam Pharmaceutical, Ilorin, Nigeria).

All the reagents used were of analytical grade.

Microbial strains of bacteria used include Gram positive bacteria: *Staphylococcus aureus* (American Typed Culture Commission, ATCC 25923) and Gram negative bacteria:

Escherichia coli (ATCC 25922) and *Psuedomonas aeruginosa* (ATCC 27853) all obtained from the Pharmaceutical Microbiology Laboratory of the Faculty of Pharmacy, University of Lagos. All the microbial strains were identified and characterized in the laboratory. The test microorganisms were grown on Mueller–Hinton (MH) agar plates (Hi-Media India) and suspended in MH broth prior to use for antimicrobial susceptibility testing.

The physicochemical method

The ligand, 1.0×10^{-4}M of $MgSO_4.7H_2O$ was placed in each of eight test tubes and to these were added 1.0 ml to 8.0 ml of 10^{-4}M ciprofloxacin and finally made up to 10.0 ml with 0.1 M HCl. The absorbances of the substrate ligand complexes were determined at 25°C and 60°C with the wavelength fixed at 330 nm. The complexation constant was derived using the Benesi-Hildebrand method[14].

Also, 1.0 ml of 1×10^{-4}M of ciprofloxacin was placed in each of eight test tubes and to each was added 1.0 ml to 8.0 ml of 10^{-4}M $MgSO_4.7H_2O$. The volume was made up to 10.0 ml with 0.1 M HCl. The absorbances of the substrate-ligand complexes were determined at 330nm. The molar ratio was determined using the Job's method of continuous variation[14].

Effect of magnesium ion on antibacterial activity of quinolones.

The Agar well-diffusion method of Hugo and Russell (2002) was adopted[15]. A stock solution of 160µg/ml of various quinolones was prepared from which various concentrations of 80.0, 40.0, 20.0, 10.0, 5.0, 2.0 and 1.0 µg/ml were prepared.

The respective complex solution were prepared with a fixed metallic ion concentration of 20 µg/ml and allowed to stand for two hours to attain equilibrium.

MH – agar plates were seeded with eight hours old – broth culture of respective bacteria (turbidity equivalent to 0.5 Mc Farland turbidity standards).

Two wells (6mm diameter) were made in each of these plates using a sterile cork borer. A 0.1ml of each concentration of ciprofloxacin and ciprofloxacin-Mg^{2+} complex solution were added respectively into the wells using sterilized pipettes and allowed for diffusion at room temperature for two hours. The plates were incubated at 37°C for 24 hours. Mg^{2+} solution was used as control. The experiment was repeated three times and the mean values of the zone diameter were recorded for antimicrobial activity.

RESULTS

Figures 1-3 showed the physicochemical result obtained for ciprofloxacin-Mg^{2+} complex. Using the Benesi-Hildebrand method, the stability constant (expressed as log K) was 3.59 at 25°C and 3.50 at 60°C (Fig. 1 and 2). The molar ratio obtained was 1:1 (Fig.3).

Fig. 4-6 gave a comparation between the zones of inhibition of ciprofloxacin and ciprofloxacin-Mg^{2+} complex against *E. coli, P. aeruginosa and S. aureus.*

The regression equations obtained from the antibacterial data showed that there was a good correlation between the zones of inhibition and concentration of ciprofloxacin.

The data obtained were subjected to the SPSS software for statistical analysis. It showed that there was a significant difference between the activity of ciprofloxacin and ciprofloxacin-Mg^{2+} complex ($p < 0.05$).

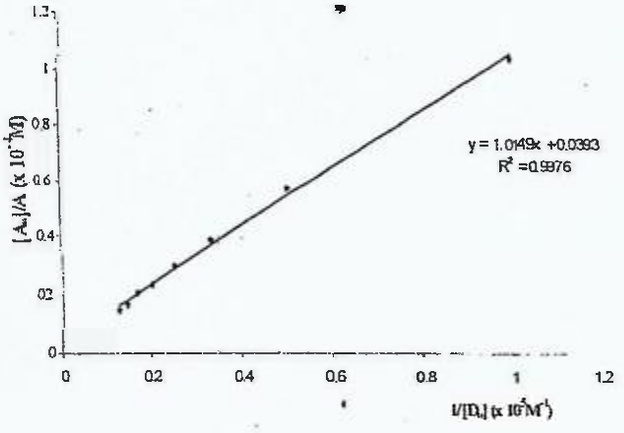

Fig.1. A Benesi-Hildebrand plot using Mg^{2+} as electron acceptor, ciprofloxacin as donor and 0.1M HCl as solvent at 25°C.

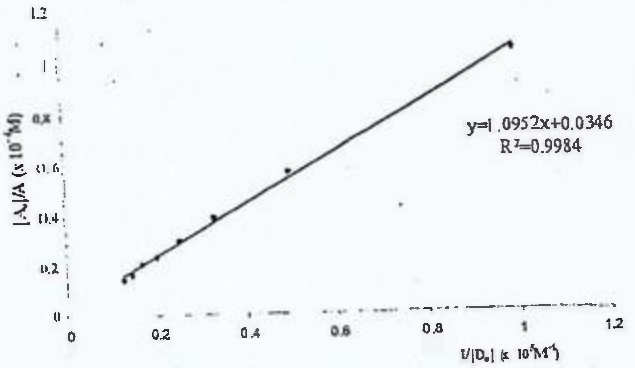

Fig.2. A Benesi-Hildebrand plot, using Mg^{2+} as electron acceptor, ciprofloxacin as donor and 0.1M HCl as solvent at 60°C.

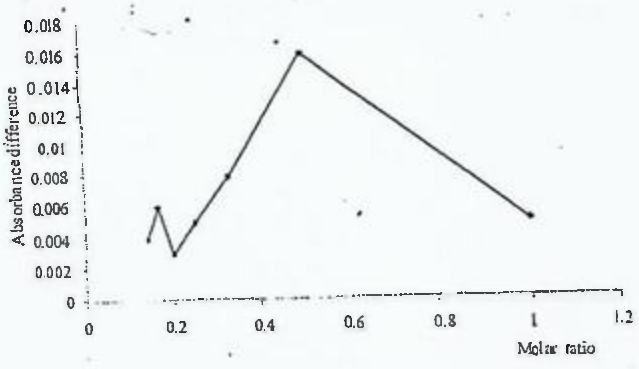

Fig.3. Absorbance difference against the molar ratio of ciprofloxacin-Mg^{2+}.

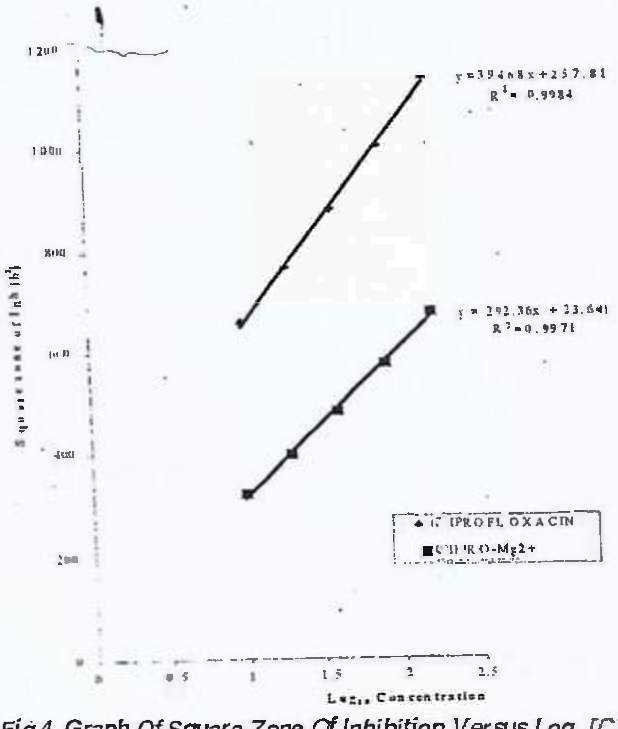

Fig.4. Graph Of Square Zone Of Inhibition Versus $Log_{10}[C]$ Showing The Effect Of Mg^{2+} On Antibacterial Activity Of Ciprofloxacin Against E. Coli.

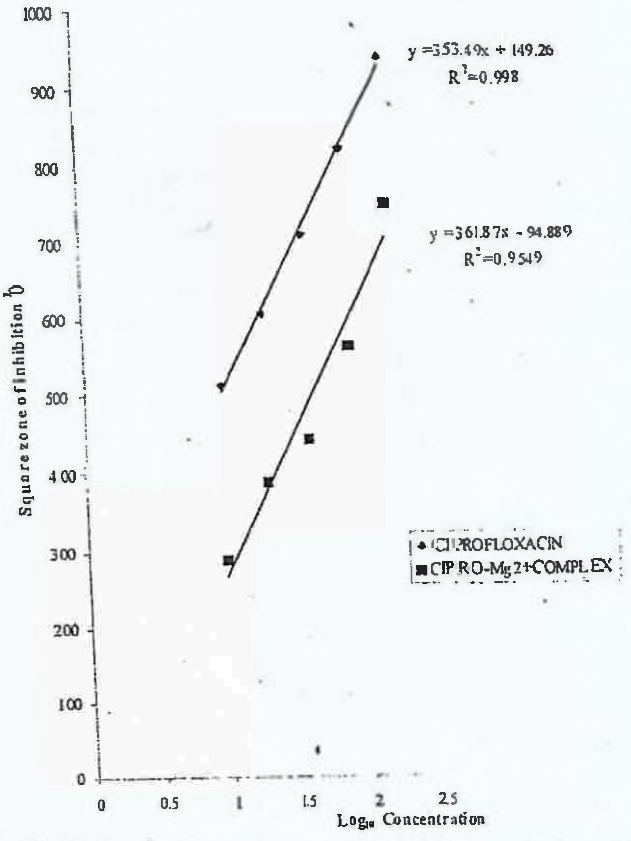

Fig.6. Graph of square zone of inhibition versus log $_{10}$[C] showing the effect of Mg^{2+} on the antibacterial activity of Ciprofloxacin against S. aureus

DISCUSSION

Physicochemical study

Employing the Benesi – Hildebrand equation, appropriate plots of $[A_o]/A$ vs. $1/[D_o]$ revealed that change in temperature of reaction or medium had effect on the complexation constant, K. The stability constant, (expressed as logK), obtained for ciprofloxacin: Mg^{2+} at 25°C and 60°C are 3.59 and 3.5 respectively. It was observed that ciprofloxacin-Mg^{2+}complex was more stable at room temperature than at higher temperature.

The result obtained using Job's method of continuous variation showed that the molar ratio of ciprofloxacin-Mg^{2+} complex using 0.1 M HCl as solvent was 1:1.

The complexation constants obtained showed that the ciprofloxacin-Mg^{2+} complex was stable.

This showed that concomitant administration of quinolones with drug formulations or food supplements containing metal ions possibly results into complexes.

Invitro antimicrobial assay.

The invitro antimicrobial study showed that the metal ions reduced the antibacterial activity of ciprofloxacin. There was a significant difference between the antibacterial activity of ciprofloxacin and ciprofloxacin–Mg^{2+} complex ($p < 0.05$). This showed that the pure ciprofloxacin was relatively more potent than ciprofloxacin-Mg^{2+} complex (Fig.4-6).

CONCLUSION

The stability constant obtained showed that ciprofloxacin-Mg^{2+} complex was stable with a molar ratio 1:1. The complex was stable at room temperature than at higher temperature.

The invitro antimicrobial study showed that the presence of Mg^{2+} reduced the antibacterial activity of ciprofloxacin.

In conclusion, ciprofloxacin formed a complex with Mg^{2+} which was less potent compared to ciprofloxacin as an antibiotic agent. Concomitant administration of ciprofloxacin with haematinics, food supplements, antacids and formulations containing Mg^{2+} ions should be avoided to reduce or prevent resistance to ciprofloxacin.

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