An Interaction Study of Metal Ions Complexation with Cephradine on Its Antibacterial Activity

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Abstract—This research work comprises of interaction studies of cephradine with essential metals and investigation of antimicrobial activity of cephradine. Cephradine is included among the cephalosporin drug class which is active against a wide range of gram positive and negative bacteria. Cephradine has been interacted with Zn, Fe metal salts in vitro. All the reaction conditions were simulated to natural environments. The antimicrobial activity of the drug and the complexes also were determined. The studies were carried out in buffer at PH 7.4 in variable ratios of drug and metal both at room temperature. It has been observed that almost major proportion of drug is complexed with metals. There is a effect of PH on drug metal complexation. It has been observed that cephradine interacts with metals a PH 7.4. The stability constant of these complexes were determined in order to evaluate their possible in vivo implications. In order to investigate the number of metal ions involved in the complexation with cephradine complexes were elucidated by exploiting various spectrophotometric methods. The ultraviolet studies of these complexes were carried out and compared. The test samples were tested against Staphylococcus aureus. The standard cephradine disk also tested against Staphylococcus aureus. We observed that the antimicrobial activity of cephradine decreases when it forms complexes with ZnSO₄.7H₂O and FeSO₄.7H₂O by antimicrobial studies.

Keywords—Antimicrobial activity, cephradine, spectrophotometric methods, complexation.

I. INTRODUCTION

The principal aim of the present study was to probe the in vitro interactions of Cephradine with different essential metal salts and to determine the bioavailability in terms of antimicrobial activity of Cephradine after drug metal interactions at pH 7.4. The interaction of transition metal ions with biological molecules provides one of the most fascinating areas of co-ordination chemistry. Some transitional metals are essential to human biochemical process. For example, zinc is an important co factor for several enzymatic reactions in the human body, vitaminine B12 has a cobalt atom and its core, and hemoglobin contains iron. Likewise Cu, Mn, Se, Cr, Mo are all trace elements, which are important in the human diet. Another subset of metals includes those used therapeutically in medicine, Al, Bi, Au, Ga, Li and Ag are all part of the medical armamentarium[1]. These transition metals also interact with different drugs. The lipophilisity of the drug is increased through the formation of chelates and drug action is significantly increased due to effective permeability of the drug into the site of action. Interaction of various metal ions with antibiotics may enhance their antimicrobial activity as compared to that of free ligands[2]. A drug interaction is a situation in which a substance affects the activity of a drug, i.e. the effects are increased or decreased, or they produce a new effect that neither produces on its own. However, interactions may also exist between drugs & foods (drug-food interactions), as well as drugs & herbs (drug-herb interactions). People taking antidepressant drugs such as Monoamine oxidase inhibitors should not take food containing tyramine. Hypertensive crisis may occur (An example of drug-food interactions). These may occur out of accidental misuse or due to lack of knowledge about the active ingredients involved in the relevant substances[3]. Consequently, the metal complexes can be utilized for the transport of selected organic chemotherapeutic drugs to target organs, or for the decorration of those toxic organic compounds which are able, before or after metabolic activation of reacting with metals or 1:1 complexes. It is emphasized that degree to which metal ions interact in vivo should employ the conditional constants which take into account competition from other ions specially Ca²⁺, H⁺ and OH⁻ the genotoxic consequences of the virus chemical factors involved in chelation, along with examples: kinetics, stabilization or oxidation state, lipophilicity, the mixed ligand formation, are discussed. Metal ion bond with ligands in some process, and to oxidized and reduced in biological systems[4]. The important metal present in the body is iron which plays a central role in all living cells. Generally iron complexes are used in the transport of oxygen in the blood and tissues[5]. An adult at rest consumes 250ml of pure oxygen per minute, this oxygen carried by the metal complex transport system known heame, alloying the oxygen to leave the blood when it reaches the tissue. The heame group is metal complex, with iron as central metal atom, which bind or released molecular oxygen.[6] Cephradine is a semisynthetic cephalosporin antibiotic developed at the Squibb Institute for Medical Research, chemically designed as 7-[D-2-amino-(1,4-cyclohexadiene-1-yl)acetamido]-3-methyl-8-oxo-5-thia-1-azayabicyclo-octa-2-ene-2-carboxylic Acid or 7-[2-amino-2-(1,4-cyclohexadienyl)acetamido]-desacyetyl-cephalosporanic acid.[7] It is defined as a hydrated form containing 3-6% of water, which is not a stoichiometric hydrate since the water moves freely in the crystal lattice.[8] Cephradine dihydrate, which crystallizes from aqueous solution under controlled conditions, is very stable and resistant to oxidation. However, on dehydration the dehydrate becomes very unstable.[9] The structure of this was determined by a single crystal x-ray diffraction. The IR spectra of cephradine monohydrate & dihydrate, NMR and mass spectrum are reported.[10]
II. MATERIALS & METHODS

Preparation of Stock Solution:

Cephradine solution 250 ml of 1×10^{-2} stock solution of cephradine was prepared by dissolving 0.8735 gm of cephradine (Gifted sample from Medicon Laboratories Ltd.) in 250 ml of demineralised water in a 250 ml volumetric flask. The stock solution was diluted to desired strength by buffer solution.

Preparation of Metal Solutions:

For the preparation of 0.1M metal solution, zinc sulfate heptahydrate (0.0287gm), ferrous sulfate heptahydrate (0.0151 gm) (source: Merck Specialties Private Ltd. Mumbai, India) were weighted accurately and introduced with the help of funnel in 100 ml volumetric flask separately, dissolved in deminerolised water and make up to the mark with the same solvent. These primary solutions were further diluted ten folds in the same solvent and the final solutions were 0.01 M concentration.

Preparation of Buffer Solution:

To prepare buffer solution 1.76 gm of disodium hydrogen phosphate was dissolved in demineralised water with 2.43 gm of sodium di hydrogen phosphate (source: Merck Specialties Private Ltd. Mumbai, India) and pH was adjusted to 7.4 and the volume was made to 1000 ml with the same solution.

Preparation of standard curve of cephradine:

Cephradine stock solution at pH 7.4 and concentration of 1×10^{-5} M was added in different concentrations to ten test tubes, to have the following concentrations: 9×10^{-5} M, 8×10^{-5} M, 7×10^{-5} M, 6×10^{-5} M, 5×10^{-5} M, 4×10^{-5} M, 3×10^{-5} M, 2×10^{-5} M, 1×10^{-5} M. The solutions were then properly mixed. The absorbance values of the solutions were determined at 262 nm by UV spectrophotometer (PG Instrument Ltd, Leicestershire, United Kingdom). As a control of reference sample, phosphate buffer solution of pH 7.4 was used.

Disc Diffusion Method:

Solution of known concentration (3µg/ml) of the test samples are made by dissolving measured amount of the samples in calculated volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganism. Standard antibiotic discs and blank discs (impregnated with solvent) are used as positive and negative control. These plates are then kept at low temperature (4°C) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials dissolve and diffuse out of the sample disc. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel. As a result there is a gradual change of test materials concentrations in the media surrounding the disc.

Placement of Disc, Diffusion and Incubation:

The sample disks impregnated separately with the test material and standard antibiotic disk were placed gently on the solidified agar plates freshly seeded with test organism with the help of a sterile forceps to assure complete contact with medium surface. The spatial arrangement of the disks was such that the disks were no closer than 15 mm to the edge of the plate and for enough apart to prevent overlapping the zones of inhibition. The plates were then inverted and kept in a refrigerator for about 24 hrs at 4°C. This is sufficient time for the material to diffuse to considerable area of the medium. Finally the plates were incubated at 37°C for 24 hrs.

Determination of Zone of Inhibition:

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms surrounding the discs which gives clear zone of inhibition. After incubation, the antimicrobial activities of the test materials were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent millimeter scale.

III. RESULTS AND DISCUSSION

The standard curve was obtained by plotting the absorbance values against the corresponding concentrations.

From the above figure we can observe that the absorbance of cephradine increases with increasing concentration.

Spectral Analysis of Cephradine with ZnSO₄·7H₂O and FeSO₄·7H₂O:

This figure shows that absorbance of cephradine is quite different from absorbance of cephradine and metal complexes. Interaction between drug and metal may lead to form complexes which have different light absorption capacity and spectrum pattern is altered.

**Effect of Metals on Cephradine by Job’s Method of Continuous Variation:**

The molar ratios of the complexes of cephradine with metal salts were estimated by job’s method of continuous variation. The observed absorbance values were measured in pH 7.4 at various concentration (1 × 10⁻⁵ to 9 × 10⁻⁵ M) of cephradine and metal salts at 262 nm. The Job’s plots at pH 7.4 were obtained by plotting absorbance difference against the mole fraction of the drug (cephradine) which are presented in the following table.

**Antimicrobial Investigation**

The test samples Cephradine + ZnSO₄.7H₂O and Cephradine + FeSO₄.7H₂O were tested against *Staphylococcus aureus*. The standard cephradine disk also tested against *Staphylococcus aureus*. The results of the antimicrobial activity, measured in terms of diameter of zone of inhibition in mm are showed in table.

Coments:

So from the above table we can say that the antimicrobial activity of cephradine decreases when it forms complexes with ZnSO₄.7H₂O and FeSO₄.7H₂O.

**IV. Conclusion**

In the present work, the interaction of an important antimicrobial drug, Cephradine with Zinc Sulfate and Ferrous Sulfate has been studied in the aqueous system at 7.4 by a variety of physical method like inspection of spectral behavior, Job’s method of continuous variation by spectrophotometry. From spectral study, it has been seen that cephradine gives a sharp peak at 262 nm. When Zinc Sulfate and Ferrous Sulfate salt mixed with Cephradine at 1:1 ratio, the intensity of the peak changes remarkably, i.e. absorption characteristics are altered due to interaction but the position of the compound do not shift. The antimicrobial screening of an agent is essential to ascertain its spectrum against various types of pathogenic organisms. The susceptibility of organism (bacteria in this case) to antimicrobial agents can be measured *in vitro* by a number of techniques among which the disk diffusion method using different concentration of the agents absorbed on material filter paper disks, is widely acceptable for the preliminary evaluation of antimicrobial activity. Job’s plot has given the molar ratio of complexes of Cephradine and Zinc Sulfate, and Ferrous Sulfate. At pH 7.4 Cephradine forms strong 1:1 complexes with Zinc Sulfate and Ferrous Sulfate indicated as ‘^-’ shaped curves. These curves may indicate strong kinetics of complexation between cephradine with Zinc Sulfate and Ferrous Sulfate. The test samples were tested against *Staphylococcus aureus*. The standard cephradine disk also tested against *Staphylococcus aureus*. We observed that the antimicrobial activity of cephradine decreases when it forms complexes with ZnSO₄.7H₂O and FeSO₄.7H₂O.

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