

# Ceftriaxone with Antacid and Metal Complexation and Investigation of Antimicrobial Activity, *In-Vitro* Demonstration

Pinkey Ghose<sup>1</sup>, Md. Shahidul Islam<sup>\*1</sup>

<sup>1</sup>Department of Pharmacy, University of Science and Technology Chittagong (USTC), Chattogram, Bangladesh  
E- mail: \*<sup>1</sup>s\_i\_liton@yahoo.com

**Abstract**— The research work comprises of interaction studies of ceftriaxone with essential metal, antacid and investigation of antimicrobial activity of ceftriaxone. Ceftriaxone is included among the cephalosporins drug class which is active against a wide range of gram positive and negative bacteria. Since the presence of compelling ligand may affect the bioavailability of a metal in the blood or tissues, therefore in order to study the portable interaction of ceftriaxone with essential and trace elements present in the body. Ceftriaxone has been interacted with Zn (metal), Ca (antacid) as an in-vitro analysis. All the reaction conditions were simulated to natural environment. Also the anti-microbial activity of the drug and the complexes were determined. There is an effect of pH on drug metal complexation. It has observed that ceftriaxone interacts with metal on a pH 7.4, the stability constant of these complexes were determined in order to evaluate their possible in vivo implications. This research work confirms that there was a possible interaction between the ceftriaxone and metal Zn and antacid Ca which was confirms by jobs plot method and by antimicrobial investigation it was confirmed that the zone of inhibition of ceftriaxone with Metal Zn and antacid Ca reduced from 16 mm to 14mm & 11mm respectfully. The standard ceftriaxone disk also tested against *Staphylococcus aureus*. In order to investigate the number of metal ions involved in the complexation with ceftriaxone complexes were elucidated by plotting various UV spectrophotometric methods. The ultraviolet studies of these complexes were carried out and compared.

**Keywords**— Ceftriaxone, Interaction, Complexation, job's Plot, Antimicrobial activity.

## I. INTRODUCTION

A drug interaction can simply be defined as an interaction between a drug and any other substance that prevents the drug from performing as expected. A drug interaction is a situation in which a substance affects the activity of a drug when both are administered together. This action can be synergistic or antagonistic or a new effect can be produced that neither produces on its own. Typically, interactions between drugs come to mind. However, interactions may also exist between drugs and foods, as well as drugs and medicinal plants or herbs. People taking antidepressant drugs such as monoamine oxidase inhibitors should not take food containing tyramine as hypertensive crisis may occur. These interactions may occur out of accidental misuse or due to lack of knowledge about the active ingredients involved in the relevant substances.[1] Two drugs are antagonistic when their interaction causes a decrease in the effects of one or both of the drugs[2],[3]. The different responses of a receptor to the action of a drug has resulted in a number of classifications, which use terms such as "partial agonist", "competitive agonist" etc. These concepts have fundamental applications in the pharmacodynamics of these interactions. The proliferation of existing classifications at this level, along with the fact that the exact reaction mechanisms for many drugs are not well understood means that it is almost impossible to offer a clear classification for these concepts. It is even likely that many authors would misapply any given classification [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 as hemoglobin, allowing the oxygen to leave the blood when it reaches the tissue. The hemoglobin group is metal complex, with iron as central metal atom, which binds or releases molecular oxygen.[6] Cephadrine 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-azabicyclo-octa-2-ene-2-carboxylic Acid or 7-[2-amino-2-(1,4-cyclohexadienyl)acetamido]-desacetyl-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]. Cephadrine 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 cephadrine monohydrate & dihydrate, NMR and mass spectrum are reported. Ceftriaxone is a synthetic broad spectrum antimicrobial agent for parenteral administration. Ceftriaxone is an antibiotic which falls under the group of drugs called cephalosporins. It is used to treat different types of bacteria in the lower respiratory tract and skin and skin structure infections. Ceftriaxone for injection USP is a sterile, semisynthetic, broad-spectrum cephalosporin antibiotic for intravenous or intramuscular administration [11]

## II. MATERIALS & METHODS

All the chemicals used here were analytical grade and were stored under sorted under optimum storage conditions. The experimental mixtures & solutions were prepared in standard volumetric flasks about one hour prior to recording the data.

Ceftriaxone solution 250 ml of  $1 \times 10^{-2}$  M was prepared by dissolving 1.386 gm of ceftriaxone in 250 ml of demineralized 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 0.01 M metal solution, zinc sulfate hepta hydrate (0.28754 gm) was weighed accurately and introduced with the help of funnel in 100 ml volumetric flask separately, dissolved in demineralized 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 0.0001 M concentration.

### *Preparation of antacid solutions:*

For the preparation of 0.01 M antacid solution, calcium hydroxide (0.0740gm) was weighed accurately and introduced with the help of funnel in 100 ml volumetric flask separately, dissolved in demineralized 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 0.0001 M concentration.

### *Preparation of buffer solutions:*

To prepare buffer solution 1.76 gm of disodium hydrogen phosphate was dissolved in demineralized water with 2.43 gm of solution dihydrogen phosphate and pH was adjusted to 7.4 and the volume was made to 1000 ml with the same solution.

### *Preparation of standard curve of Ceftriaxone:*

Ceftriaxone stock solution at pH 7.4 and concentration of  $1 \times 10^{-5}$  M was added in different concentrations to ten test tubes, to have the following concentrations:  $9 \times 10^{-5}$  M,  $8 \times 10^{-5}$  M,  $7 \times 10^{-5}$  M,  $6 \times 10^{-5}$  M,  $5 \times 10^{-5}$  M,  $4 \times 10^{-5}$  M,  $3 \times 10^{-5}$  M,  $2 \times 10^{-5}$  M,  $1 \times 10^{-5}$  M. The solutions were then properly mixed. The absorbance values of the solutions were determined at 262 nm by uv spectrometer. As a control of reference sample, phosphate buffer solution of pH 7.4 was used. The standard curve was obtained by plotting the absorbance of values against the corresponding concentrations.

### *Disc Diffusion Method:*

Solution of known concentration ( $3 \mu\text{g/ml}$ ) 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 [11]. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganism, Standard antibiotic discs and blank discs are used as positive and negative control. These plates are then kept at low temperature ( $4^\circ\text{C}$ ) 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 [12].

### *Placement of disc, diffusion and incubation:*

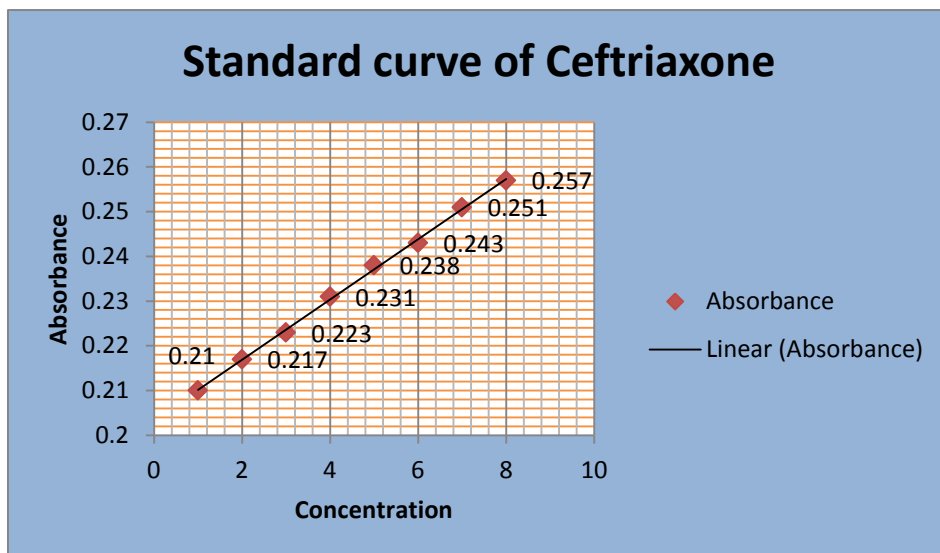
The sample disks impregnated separately with the test material and standard antibiotic disk were placed gently on the solidified agar plates freshly seeded with test organism with the help of a sterile forceps to assure complete contact with medium surface. The spatial arrangement of the disks was such that the disks were no closer than 15 mm to the edge of the plate and enough apart to prevent overlapping the zones of inhibition. The plates were then inverted and kept in a refrigerator for about 24 hrs at  $4^\circ\text{C}$  [13]. This is sufficient time for the material to diffuse to considerable area of the medium. Finally the plates were incubated at  $37^\circ\text{C}$  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 DISCUSSIONS

From the following figure we can observe that the absorbance of ceftriaxone increases with the increasing concentration according to Beer Lambert's Law.



Spectral studies of interaction of ceftriaxone with metals: Spectral analysis of ceftriaxone with  $ZnSO_4 \cdot 7H_2O$ .

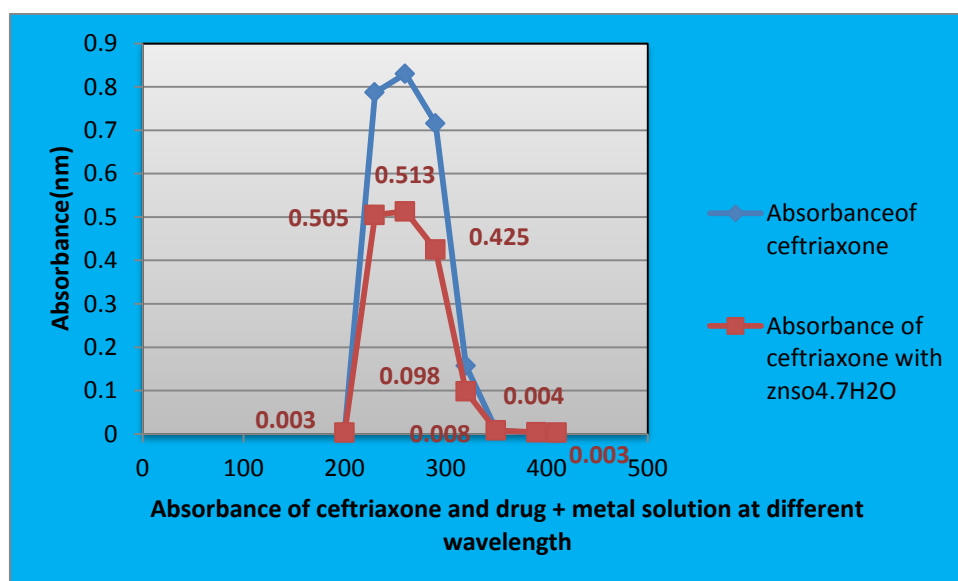


Figure: Absorbance of ceftriaxone with  $ZnSO_4 \cdot 7H_2O$

From the figure we can observe that the absorbance of ceftriaxone is different when it interacts with zinc sulfate.

Spectral analysis of ceftriaxone with  $Ca(OH)_2$ :

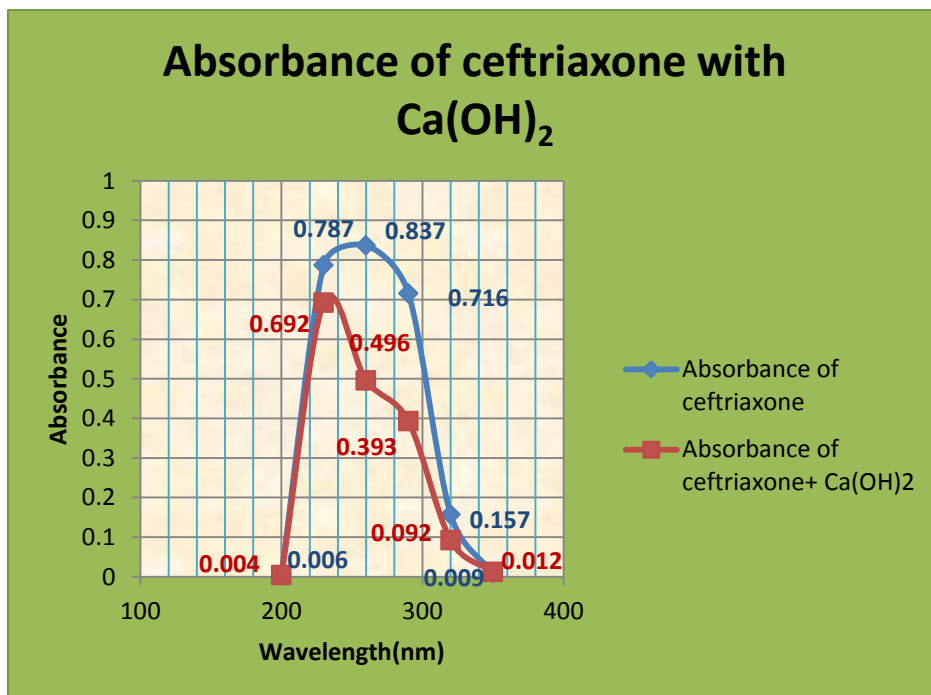
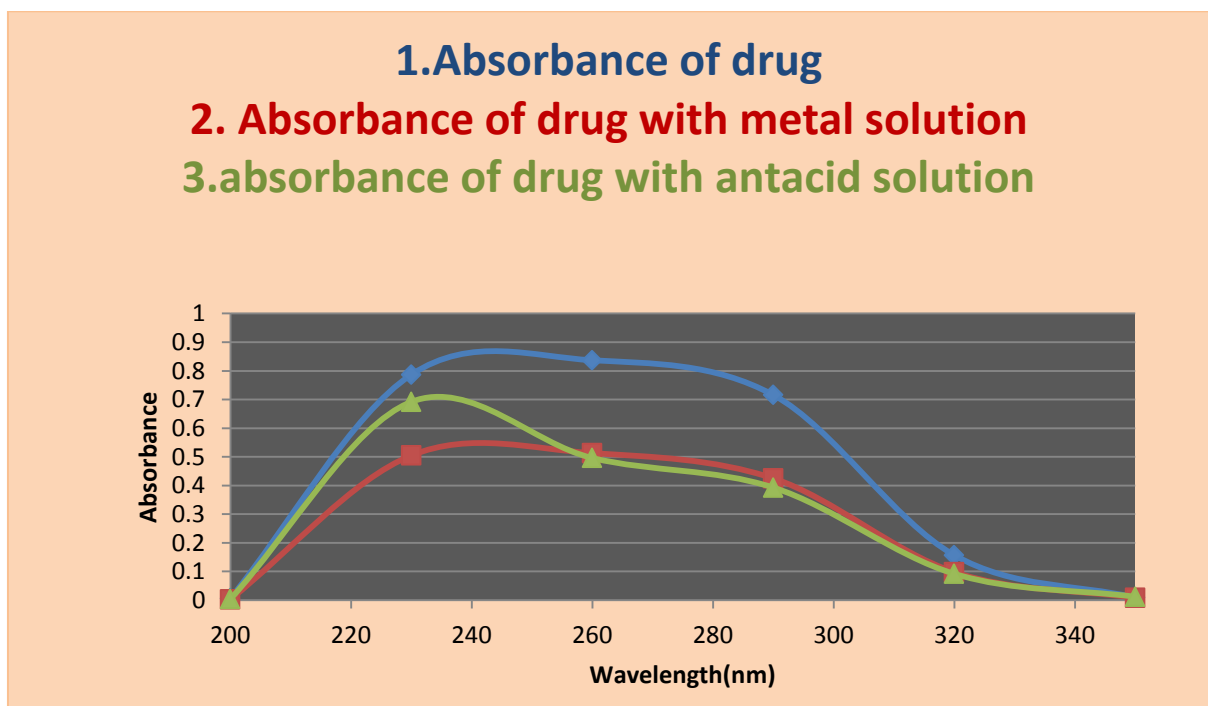


Figure: Spectral plot of drug and drug with Ca(OH)<sub>2</sub>

From the figure we can observe that the absorbance of ceftriaxone is different when it interacts with Ca(OH)<sub>2</sub>.

*Spectral analysis of Ceftriaxone with ZnSO<sub>4</sub>. 7H<sub>2</sub>O and Ca(OH)<sub>2</sub>:*

Graph: This graph shows that absorbance of ceftriaxone is quite different from absorbance of ceftriaxone and metal complexes. The intensity of the peak of ceftriaxone changes remarkably i.e; absorption characteristics are altered due to interaction but the position of the compound does not shift. Interaction between drug and metal may lead to form complexes which have different light absorption capacity and spectrum pattern is altered. So any alteration and spectrum behavior is regarded as a tool for primary interaction from the spectral studies.



*Effect of metals on ceftriaxone by Job's method of continuous variation:*

The molar ratios of the complexes of ceftriaxone with metal salts were estimated by Job's method. The observed absorbance values were measured in pH 7.4 at various concentration ( $1 \times 10^{-5}$  to  $9 \times 10^{-5}$  M) of ceftriaxone and metal salts at 280 nm. The Job's plots at pH were obtained by plotting absorbance difference against the mole fraction of the drug (Ceftriaxone) which are presented in the following table.

Values of job's plot of ceftriaxone and  $ZnSO_4 \cdot 7H_2O$

Concentration of ceftriaxone $M \times 10^{-5}$	Absorbance of Ceftriaxone A	Concentration of $ZnSO_4 \cdot 7H_2O$ $M \times 10^{-5}$	Absorbance of $ZnSO_4 \cdot 7H_2O$ B	Absorbance of mixture C	Absorbance difference $D=(A+B)-C$
1	0.210	9	0.181	0.106	0.285
2	0.217	8	0.180	0.105	0.292
3	0.223	7	0.181	0.105	0.299
4	0.231	6	0.181	0.108	0.304
5	0.238	5	0.181	0.108	0.325
6	0.243	4	0.188	0.110	0.321
7	0.251	3	0.179	0.115	0.315
8	0.257	2	0.177	0.114	0.314
9	0.261	1	0.176	0.123	0.314

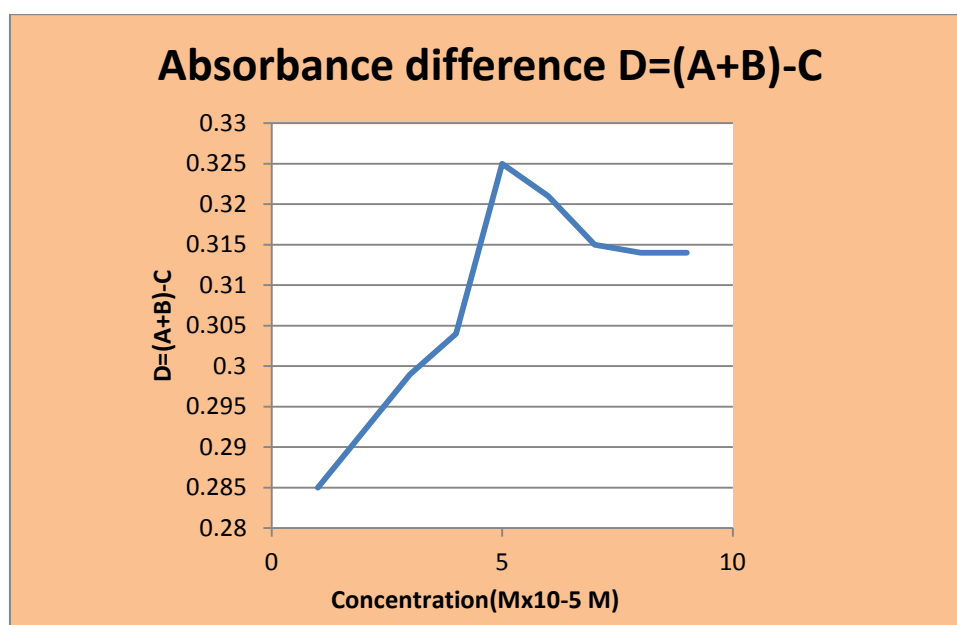


Figure: Absorbance difference of ceftriaxone and  $ZnSO_4 \cdot 7H_2O$

From the above figure we can observe that ceftriaxone forms strong 1:1 complexes with zinc sulfate hepta hydrate which is indicated as inverted 'V' shaped curve.

Values of Job plot of ceftriaxone and  $Ca(OH)_2$

Concentration of ceftriaxone $M \times 10^{-5}$	Absorbance of Ceftriaxone A	Concentration of $Ca(OH)_2$ $M \times 10^{-5}$	Absorbance of $Ca(OH)_2$ B	Absorbance of mixture C	Absorbance difference $D=(A+B)-C$
1	0.210	9	0.086	0.108	0.188
2	0.217	8	0.082	0.106	0.193
3	0.223	7	0.089	0.112	0.2
4	0.231	6	0.092	0.112	0.211
5	0.238	5	0.099	0.115	0.222
6	0.243	4	0.093	0.117	0.219
7	0.251	3	0.084	0.117	0.218
8	0.257	2	0.081	0.122	0.216
9	0.261	1	0.079	0.126	0.214

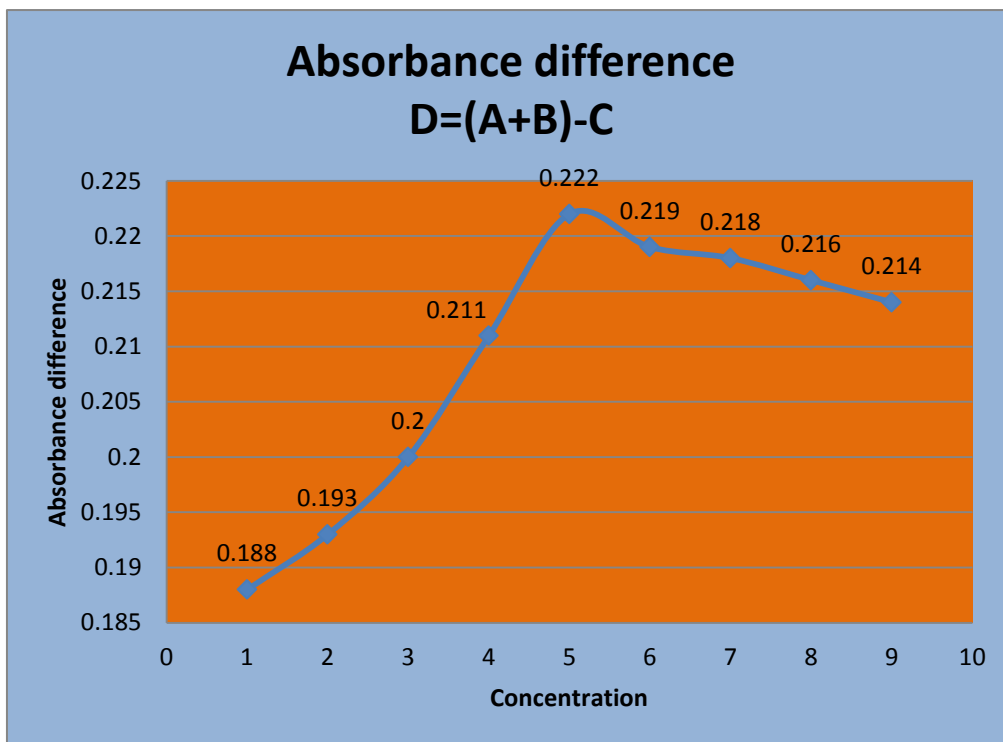


Figure: Absorbance difference of ceftriaxone and Ca(OH)<sub>2</sub>

From the figure we can observe that ceftriaxone forms strong 1:1 complexes with calcium hydroxide which is indicated as inverted 'v' shaped curve.

The test samples were tested against *Staphylococcus aureus*. The standard ceftriaxone 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.

Bacteria used	Standard disk (zone of inhibition/mm)	Sample disk (zone of inhibition)
<i>Staphylococcus aureus</i>	16 mm	Ceftriaxone+ ZnSO <sub>4</sub> .7H <sub>2</sub> O
		14mm
<i>Staphylococcus aureus</i>	16 mm	Ceftriaxone+ Ca(OH) <sub>2</sub>
		11mm

Figure: Antimicrobial sensitivity testing of ceftriaxone against *Staphylococcus aureus* after interacting with ZnSO<sub>4</sub>.7H<sub>2</sub>O and Ca(OH)<sub>2</sub> solution respectively.

Here, D= drug solution(antibiotic)

A=Antacid solution.....Ca(OH)<sub>2</sub>

M= Metal solution.....ZnSO<sub>4</sub>.7H<sub>2</sub>O



## IV. CONCLUSION

In this present work, the interaction of an important antimicrobial drug, ceftriaxone with Zinc Sulfate and antacid calcium hydroxide 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 this spectral study, it has been seen that ceftriaxone gives a sharp peak at 262 nm. When Zinc Sulfate and antacid solution, calcium hydroxide mixed with ceftriaxone 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 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 ceftriaxone and Zinc Sulfate, and antacid solution, calcium hydroxide. At pH 7.4 ceftriaxone forms strong 1:1 complexes with Zinc Sulfate and antacid solution, calcium hydroxide indicated as '∧' shaped curves. These curves may indicate strong kinetics of complexation between ceftriaxone with Zinc Sulfate and antacid solution, calcium hydroxide. The test samples were tested against *Staphylococcus aureus*. The standard ceftriaxone disk also tested against *Staphylococcus aureus*. It was observed that the antimicrobial activity of ceftriaxone decreases when it forms complexes with  $ZnSO_4 \cdot 7H_2O$  and antacid solution, calcium hydroxide. So, by antimicrobial investigation it was confirmed that the zone of inhibition of ceftriaxone with Metals Zn, Ca reduced from 16 mm to 14mm & 11mm respectively.

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