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Gemifloxacin with Different Essential Metals, In Vitro Interaction and Investigation of Antimicrobial Activity

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***Abstract*—** *The research work comprises of interaction studies of Gemifloxacin with essential metals and investigation of antimicrobial activity of Gemifloxacin. Gemifloxacin is included among the fluoroquinolones 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 probable interaction of Gemifloxacin with essential and trace elements present in the body. Gemifloxacin has been interacted with Fe – (Ferrous), Zn- (Zinc) metal salts in vitro. All the reaction conditions were simulated to natural environment. Also the antimicrobial activity of the drug and the complexes were determined. There is a effect of PH on drug metal complexation. It has observed that Gemifloxacin 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. In order to investigate the number of metal ions involved in the complexion with Gemifloxacin complexes were elucidated by exploiting various spectrophotometric methods. The ultraviolet studies of these complexes were carried out and compared*.

***Keywords*—** *Gemifloxacin, Complexation, Interaction, job's Plot, Antimicrobial Study*.

I. INTRODUCTION

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. Typically, interaction between drugs come to mind (1). 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 (2). These may occur out of accidental misuse or due to lack of knowledge about the active ingredients involved in the relevant substances. (3) Drug interactions may also occur outside the body i.e.; in-vitro. Some classic examples include that thiopentone and suxamethonium should not be placed in the same syringe and same for benzylpenicillin and heparin. (4) Gemifloxacin is a fourth generation synthetic broad-spectrum fluorinated quinolone antibacterial agent for oral administration (http://en.wikipe-dia.org/wiki/Gemifloxacin_mesylate). It is discovered (5) that is present in two forms; either as free gemifloxacin base or as gemifloxacin mesylate salt. Gemifloxacin has a broad-spectrum activity against both Gram-negative and Gram-positive microorganisms (6). The new oxime-pyrrolidine derivative moiety of gemifloxacin is the responsible moiety for its new unique activity as compared to the reported fluoroquinolnes (7).

II. MATERIAL AND METHODS

Chemical and Reagents:

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

TABLE 1. List of chemicals and reagents

SL No	Name	Source
1	Gemifloxacin	Gift samples from medicon laboratories ltd
2	Ferrous sulphate	Merck ltd, Mumbai, India
3	Zinc sulfate	Merck ltd, Mumbai, India
4	Sodium di-hydrogen phosphate	USTC, Foys lake, Chittagong, dept of pharmacy
5	Disodium hydrogen phosphate	USTC, Foys lake, Chittagong, dept of pharmacy
6	Demineralised water	Taj scientific , Anderkilla , Chittagong

Preperations of Stock Solution

Gemifloxacin solution 100 ml of $1^{x}10^{-2}$ was prepared by dissolving 485.49 gm of Gemifloxaciline in 100 ml of demineralised water in a 100 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, ferrous sulfate hepta hydrate (0.0151gm), Zinc sulfate (0.0287 gm) were weighted accurately and introduced with the help of funnel in 100 ml volumetric flask separately, dissolved in demineralised 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.

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Preparation of Buffer Solution

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

Principle of Disc Diffusion Method

Solution of known concentration (314/m1) 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 (8).The plate are then incubated at 37 °C for 24 hours to allow maximum growth of the organism. If the test materils have any antimicrobial activity, it will inhibit the growth of the micoorganisms and a cler, distict zone of inhibition will be visualized surrounding the medium. The antimicrobial activity of the test agent is determined by measuring the diameter of zone of inhibition expressed in millimeter. The experiment is carried out more than once and the mean of the readings is required.

III. Results and Discussion

Gemifloxacin stock solution at pH 7.4 and concentration of 1x 10^3 M was added in different concentrations to ten test tubes, to have the following concentrations: 9x 10^{-5} M, 8x 10^{-5} M ,7x1() $^{-5}$M ,6x 10 $^{-5}$M ,5x10 $^{-5}$M ,4x10 $^{-5}$M ,3x 10 $^{-5}$M ,2x10 $^{-5}$M ,1x10^{-5}M.

The solutions were then properly mixed. The absorbance values of the solutions were determined at 279 nm by UV spectrophotometer. As a control of reference sample, phosphate buffer solution of Ph 7.4 was used. The standard curve was obtained by plotting the absorbance values against the corresponding concentrations

TABLE 2. Spectral analysis of Gemifloxacin with FeSo4. 7 H2O

Wavelength	Absorbance of Gemifloxacin	Absorbance of Gemifloxacin & FeSO4.7 H20
200	0.013	0.010
205	0.010	0.026
210	0.012	0.029
215	0.001	0.018

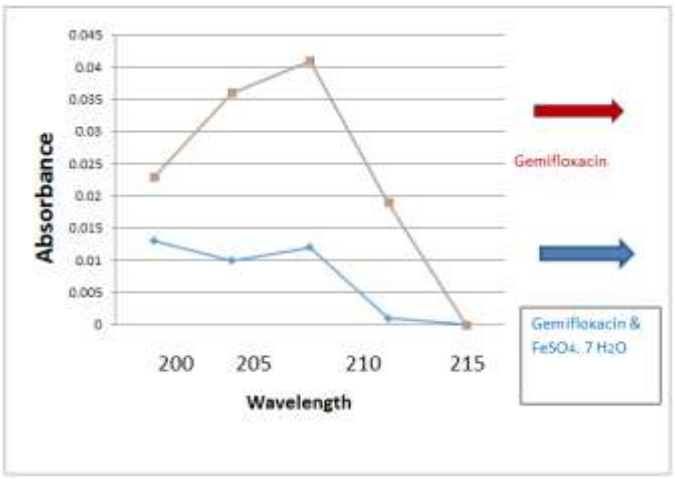

Fig. 1. Spectral plot of Drug with FeSO4. 7 H2O

In x- axis: wavelength
In y- axis: absorbance

From the above figure we can observe that the absorbance of Gemifloxacin is different when it interacts with FeSO4.7 H2O

TABLE 3. Spectral analysis of Gemifloxacin with ZnSO4 .7 H2O

Wavelength	Absorbance of Gemifloxacin	Absorbance of Gemifloxacin + ZnSO4. 7 H2O
200	0.013	0.009
205	0.010	0.007
210	0.012	0.004
215	0.001	0.003

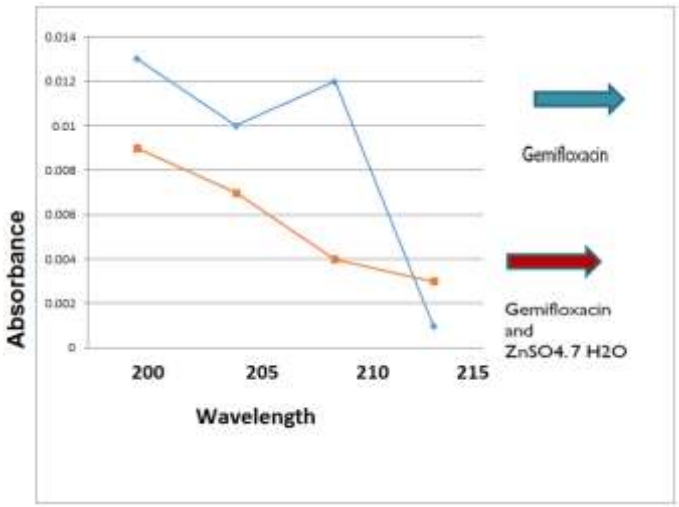

Fig. 2. Spectral plot of Drug with ZnSO4. 7 H2O

In x- axis: wavelength
In y- axis: absorbance

From the above figure we can observe that the absorbance of Gemifloxacin is different when it interacts with ZnSO4.7 H2O

Spectral analysis of Gemifloxacin with FeSO4. 7H2O and ZnSO4.7 H2O:

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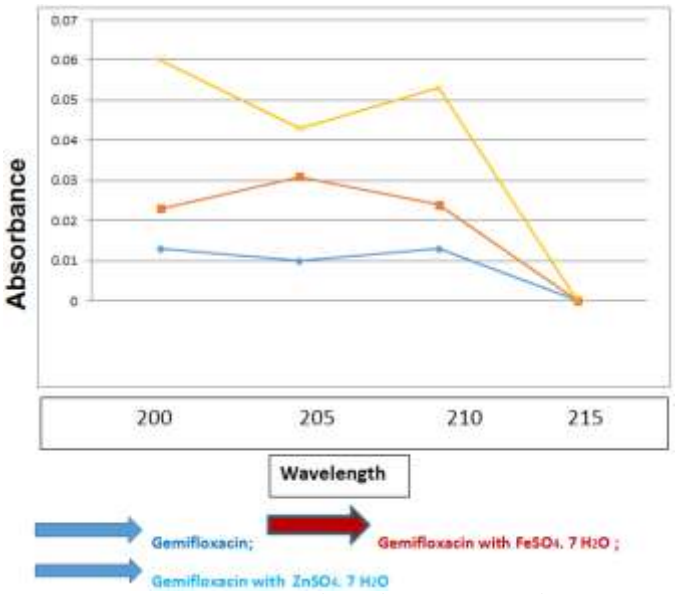

Fig. 3. Combined spectral curve of Gemifloxacin and Gemifloxacin with metal.

This figure shows that absorbance of Gemifloxacin is quite different from absobance of Gemifloxacin and metal complexes.

The intensity of the peak of ciprofloxacin 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 n spectral behavior is regarded as a tool for primary interaction from the spectral studies.

Effect of metals on ciprofloxacin by Job's method of continuous variation

The molar ratios of the complexes of Gemifloxacin 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 ($1x10^{-5}$ to 9 x 10^{-5} M) of Gemifloxacin and metal salts at 279 nm. The Job's plots at pH 7.4 were obtained by plotting absorbance difference against the mole fraction of the drug (Gemifloxacin) which are presented in the following table.

TABLE 4. Values of job plot of Gemifloxacin and FeSO4. 7 H2O

Conc. of Gemifloxacin M x 10^{-5}	Absorbance of Gemifloxacin A	Conc. of $FeSO_4.7H_2O$	Absorbance of $FeSO_4.7\ H_2O$ B	Absorbance of Mixture C	Absorbance difference D= (A+B) - C
1	0.137	9	0.078	0.061	0.154
2	0.278	8	0.075	0.105	0.248
3	0.423	7	0.070	0.170	0.323
4	0.546	6	0.054	0.233	0.367
5	0.646	5	0.050	0.290	0.406
6	0.765	4	0.031	0.412	0.384
7	0.854	3	0.030	0.554	0.330
8	0.949	2	0.024	0.677	0.296
9	0.998	1	0.021	0.829	0.191

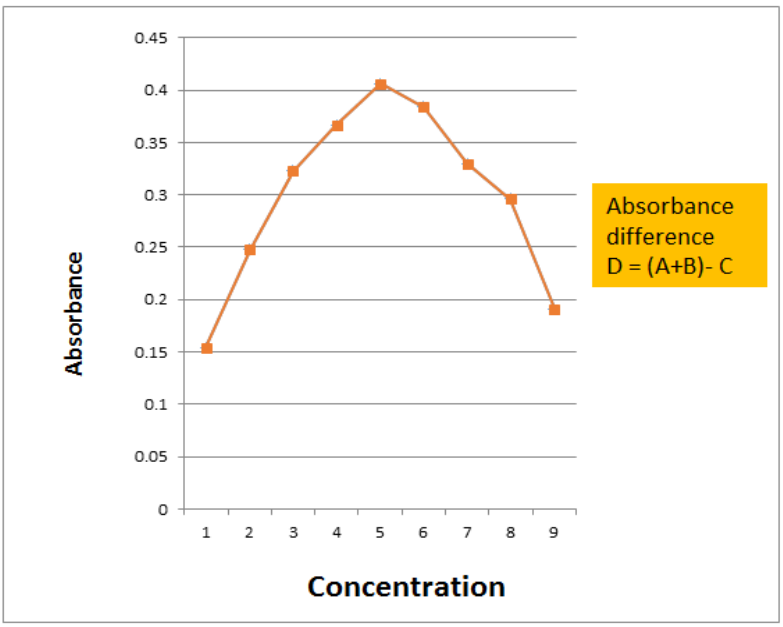

Fig. 4. Absorbance difference of Gemifloxacin and FeSO4. 7 H2O

From the above figure we can observe that Gemifloxacin forms strong 1:1 complexes with Ferrous sulfate hepta hydrate which is indicated as '^' shaped curve.

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TABLE 5. Values of job plot of Gemifloxacin and ZnSO4. 7 H2O

Conc. of Gemifloxacin M x 10^{-5}	Absorbance of Gemifloxacin A	Conc. of $ZnSO_4.7H_2O$	Absorbance of $ZnSO_4.7H_2O$ B	Absorbance of Mixture C	Absorbance difference D= (A+B) - C
1	0.115	9	0.054	0.018	0.151
2	0.204	8	0.051	0.027	0.228
3	0.319	7	0.032	0.078	0.273
4	0.415	6	0.022	0.105	0.332
5	0.511	5	0.019	0.145	0.385
6	0.645	4	0.012	0.305	0.352
7	0.737	3	0.009	0.417	0.329
8	0.834	2	0.009	0.539	0.304
9	0.956	1	0.006	0.718	0.244

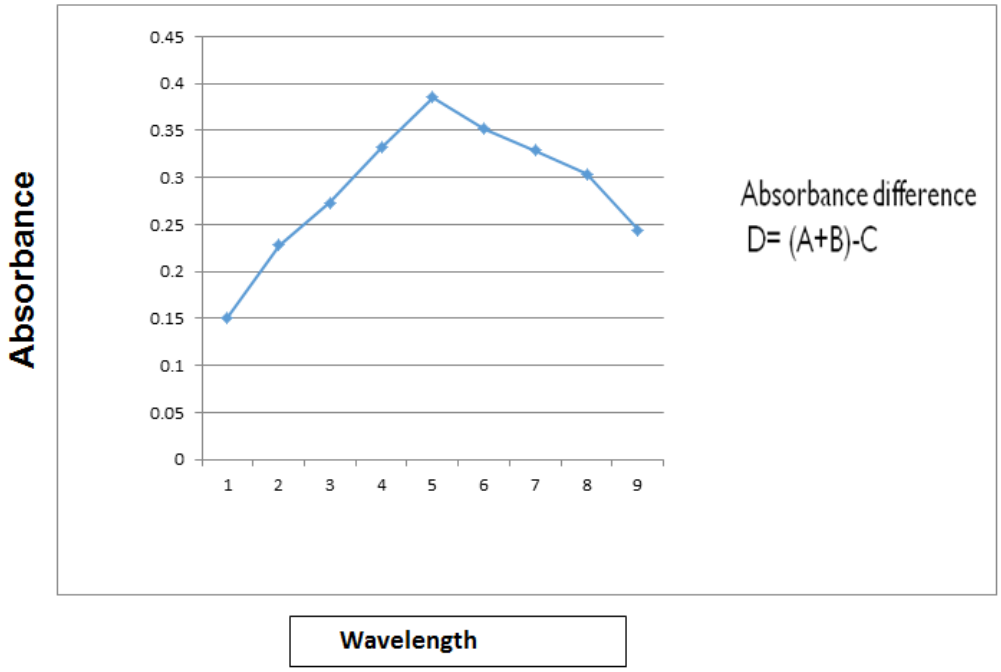

Fig. 5. Absorbance difference of Gemifloxacin and ZnSO4. 7 H2O

From the above figure we can observe that Gemifloxacin forms strong 1:1 complexes with Zinc sulfate hepta hydrate which is indicated a '^' shaped curve.

Antimicrobial Study:

The test samples were tested Staphylococcus aureus. The standard Gemifloxacin 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:

TABLE 6. Results of the antimicrobial activity

Bacteria Used	Standard disc (Zone of Inhibition)	Sample disc (Zone of Inhibition)
Staphylococcus aureus	16 mm	Gemifloxacin
		16mm
Staphylococcus aureus	16 mm	Gemifloxacin +F ($FeSO_4.7H_2O$)
		11mm
Staphylococcus aureus	16 mm	Gemifloxacin + M ($ZnSO_4.7H_2O$)
		13mm

Fig. 6. Evaluation of microbial study of Gemifloxacin with essential metals

G= Gemifloxacin
F = Metal (FeSO4.7 H2O)
M= another metal (ZnSO4. 7 H2O)

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IV. CONCLUSION

In the present work, the interaction of an important antimicrobial drug, Gemifloxacin with Ferrous Sulfate and Zinc Sulfate at 7.4 by a variety of physical method like inspection of spectral behavior, Job's method of continuous variation. From spectral study, it has been seen that Gemifloxacin gives a sharp peak at 279 nm. When Ferrous Sulfate and Zinc Sulfate salt mixed with Gemifloxacin at 1:1 ratio, the intensity of the peak changes remarkably (absorbance decreases) 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 Gemifloxacin and Ferrous Sulfate, and Zinc Sulfate. At pH 7.4 Gemifloxacin forms strong 1:1 complexes with Ferrous Sulfate and Zinc Sulfate indicated as `^' shaped curves. These curves may indicate strong kinetics of complexation between Gemifloxacin and Ferrous Sulfate and Zinc Sulfate. When drug individually act with Ferrous Sulfate and Zinc Sulfate curve of their absorbance are verify. It is known to us; the availability of the drug represents the amount (quantity or concentration) of drug It helps in the study of selection the best dosage form for treatment. And obviously very important in adjusting the effective dose and dose ranges.

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