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RESEARCH ARTICLE

UV SPECTROPHOTOMETRIC STUDY OF AMOXICILLIN–ANTACIDS INTERACTION AND ESTIMATION OF ANTIMICROBIAL ACTIVITY

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Abstract

Background and aims: Interaction between a drug and another element which causes a drug interaction when it prevents the medication from functioning as intended. Usually, an interaction can change how the drug is absorbed, distributed, metabolized and eliminated by the body. The purpose of this study is to assess the type and strength of *in-vitro* complexation that may be caused by an interaction between Amoxicillin with Antacids and investigation of antimicrobial study of Amoxicillin.
Methodology: This work ensures as a possible interaction between Amoxicillin with $Mg(OH)_2$ (Antacid-1) and $CaCO_3$ (Antacid-2) which was determined by Job's plot approach. Investigating the number of $Mg(OH)_2$ (Antacid-1) and $CaCO_3$ (Antacid-2) connected in the complexation with Amoxicillin complexes was demonstrated by exploiting several spectrophotometric methods. Ultraviolet studies of these were carried out and balanced. The microbial sensitivity assessment is important to identify whether Amoxicillin's efficacy has changed as a result of its interaction with antacids.
Findings: The study supported that there is a probable interaction between Amoxicillin with antacids which is confirmed by job's plot method. The Amoxicillin absorbance is verily different from absorbance of Amoxicillin – $Mg(OH)_2$ (Antacid-1) and Amoxicillin- $CaCO_3$ (Antacid-2). And the intensity of the peak of Amoxicillin changes particularly when Amoxicillin forms strong 1:1 complex with $Mg(OH)_2$ (Antacid-1) and $CaCO_3$ (Antacid-2). These curves were able to indicate strong kinetics of complexation of Amoxicillin with antacids. By Antimicrobial investigation against gram positive bacteria, it was certain that the zone of inhibition of Amoxicillin with $Mg(OH)_2$ (Antacid-1) and $CaCO_3$ (Antacid-2) reduced from 15 mm, 11 mm and 9 mm respectively against gram positive bacteria and Amoxicillin with $Mg(OH)_2$ and $CaCO_3$ did not exhibit any detectable antibacterial action when tested against gram negative bacteria.
Conclusion: It has been demonstrated that the standard absorbance of Amoxicillin differs significantly from the absorbance of drug-antacid and the antimicrobial activity is also reduced due to interaction.
Keywords: Amoxicillin, antacid, antimicrobial activity, interaction, Job's plot.

INTRODUCTION

Any disease or situation brought on by bacterial growth or toxins is referred to as a bacterial infection. Anywhere in our body, including epidermis, gut, lungs, heart, blood and brain, can become ill from harmful bacteria. Infections can be brought on by bacteria that are usually not harmful but find their way into an area of our body[1,2]. Bacterial infections are brought on by living single celled and they are capable of self-replication. Viral illnesses are caused by non-cellular organisms. To multiply, viruses always need to spread to people or living things[3].

Infectious organisms like bacteria and protozoa are killed (bactericidal) or have their growth inhibited (bacteriostatic) by chemical substances known as antibiotics. The beta lactams, which are divided into penicillins, cephalosporins, carbapenems, and monobactams are the oldest class of antibiotics still used in medicine[4,5]. All but one of these substances have outstanding safety profiles. Most bacterial illnesses can be treated with antibiotics but there aren't many viral infections that can be treated with medication[6].

Antacids and multivitamin formulations are the most frequently co-administered medications with anti-

biotics because it is well known that extended antibiotic therapy produces anemia, stomach discomfort, or acidity[7]. Amoxicillin kills bacteria by block-ing the development of their cell walls as a beta-lactam antibiotic that is a member of the popular aminopenicillin antibiotic class[8-10]. All beta lactam antibiotics possess the same molecular structure, the beta-lactam ring, which accounts for their shared characteristics. Penicillin-like drugs have a long history in medicine and are still widely used today[11].

Antimicrobial resistance exerts a leading challenge to the health of human. Since 1940s, antibiotics have disclosed and resolved healthcare and turned the mortality and morbidity of different infectious diseases. Nevertheless, many microbial species which are adapted at speedily developing strength to existing novel medicines, giving up few effective treatment choice[12,13]. Researches are occurred in the division on pathogenesis and antimicrobial resistance on gram positive and gram negative organisms both. In this research, both gram positive and gram negative are provided their antimicrobial activity[14-16].

Moreover, several *in vitro* studies have exhibited the interaction between antibiotics and antacids[17-20]. Though Amoxicillin resistance rises against gram positive as well as gram negative bacteria along with antacids which have determined in this work. The aim of this present study is to evaluate *in-vitro* complexation nature of Amoxicillin with antacids and investigate antimicrobial activity of Amoxicillin.

MATERIALS AND METHODS

All of the chemicals utilized in this project were of the analytical grade and were organized for optimal storage. About an hour before collecting the data, standard volumetric flasks were used to create the experimental solutions. Amoxicillin was obtained from Alvion laboratories Ltd as the gift sample. Magnesium hydroxide (Antacid-1) and Calcium carbonate (Antacid-2) from Merck ltd, Mumbai, India. Disodium hydrogen Phosphate and Sodium di-hydrogen phosphate were obtained from Department of Pharmacy, USTC, Foy's lake, Chittagong.

Preparation of stock solution

Amoxicillin, 0.36541 gram within 100 ml of demineralized water in a 100 ml volumetric flask was used for preparing 100 ml. By using buffer solution, stock solution which was diluted to the necessary capacity.

Preparation of antacid solutions

Antacid-1, Magnesium hydroxide, $Mg(OH)_2$ (0.05853 gm) and Antacid-2, Calcium carbonate, $CaCO_3$ (0.10009 gm) were precisely weighed and proposed with use of funnels in 100 ml volumetric flasks separately, dissolved in demineralized water and made up to the required concentration using the same solvent. The final solutions had a concentration of 0.01M after being further ten times diluted in the same solvent as the initial solutions.

Preparation of standard curve of Amoxicillin

The concentration of 1×10^{-5} M Amoxicillin stock solution was measured and applied in various amounts for producing 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.

These solutions were applicably combined after that. Using a UV spectrophotometer, absorbance values of the solutions were calculated at 256 nm. The reference sample's control was a phosphate buffer solution. Plotting the absorbance readings against the corresponding concentrations led to the creation of the standard curve.

Disc diffusion method

Dissolving measured amounts of test samples in predicted solvents volumes, a solution of the test samples with a defined concentration (3 µg/ml) is created. The test compounds are then impregnated into dried, sterile, 6mm diameter filter paper discs using a micropipette. Standard discs of antibiotics and blank discs (saturated with solvent) which are used as positive and negative controls. Discs containing test material that placed on nutrient agar medium uniformly with test microorganism[21]. For maximum diffusion, these are then held at low temperature (4°C) for about 24 hours. Dried discs absorb the surrounding media's moisture during this time, after which test components diffuse and dissolve out of sample disc. Physical law that governs diffusion of molecules through agar gel that causes the diffusion to happen. There is consequently a progressive alteration in the test concentrations in the medium around the disc. Then the plates are kept at 37°C for 24 hours to promote the organism's growth. And a clear, definite zone of inhibition (ZOI) will be visible encircling the medium if the test materials which have antibacterial activity since hinder the growth of microorganisms. The diameter of the inhibition zone, reported in millimeters, is used to calculate the test agent's antimicrobial activity. The experiment is run multiple times because average readings is needed[22].

Discernment of antimicrobial activity by Zone of inhibition

Ability of test agents to suppress the development of microbes around the discs, which results in a distinct zone of inhibition, serves as an indicator of their antimicrobial efficacy. Using a clear millimeter scale, diameter of the zone of inhibition was measured after incubation to determine the test materials' antibacterial properties.

RESULTS AND DISCUSSION

Penicillin-type medications were a significant advance in medicine at the time and are still in use today. Although Amoxicillin is a reliable and inexpensive antibiotic, not all infections respond well to it. It is a potent broad-spectrum antibiotic that works well for treating bacterial infections in general and pediatric bacterial pneumonia in particular. And it is combined with Clavulanic acid, which is beta-lactamase inhibitor, to prolong the spectrum of activity against penicillinase occurring bacteria. Antacids are simple compounds that, when consumed, react with stomach acid and reduce the acidity of the stomach's contents. They are mostly used to treat hyperchlorhydria and peptic ulcer.

Antacids that contain calcium or magnesium are more widely used than H2-antagonists or proton pump inhibitors. $Mg(OH)_2$ is used to treat indigestion, heartburn, and other symptoms brought on by having too much stomach acid. It is an antacid that lessens the stomach's acid production. Many commercial antacid formulas contain calcium, either as carbonate or chloride. Patients with osteoporosis or other bone-related illnesses frequently take calcium carbonate tablets or supplements as part of their treatment regimens. Present work describes Amoxicillin, a significant antibiotic medication, interacts with Magnesium hydroxide as well as Calcium carbonate using a range of physical techniques, including spectrum behavior analysis and Job's method of continuous variation. According to spectral analysis, amoxicillin exhibits a distinct peak at 256 nm. When Amoxicillinis treated in a 1:1 ratio with Magnesium hydroxide and Calcium carbonate, the strength of the peak varies noticeably (absorbance decreases), altering the absorption properties due to the interaction but not altering the compound's position. An agent must undergo antimicrobial screening to determine its range of activity against different kinds of pathogenic organisms. Numerous methods exist for determining an organism's sensitivity to antimicrobial compounds *in vitro*, however the disk diffusion method, which uses various concentrations of the agents absorbed on filter paper in the disks, is generally accepted for initial assessment of antimicrobial activity[10]. At first, standard curve of Amoxicillin was obtained by plotting absorbance value against respective concentrations. It follows the Beer-Lamberts law because we know that concentration and absorbance are directly proportional to each other and from Lambert's law it shows path length and absorbance are directly proportional.

Depending on Beer-Lambert's law, we can see from the preceding figure that Amoxicillin's absorbance rises as concentration increases (Figure 1). Figure 4 shows that when Amoxicillin interacts with Magnesium hydroxide (Antacid-1) its absorbance changes. As seen in this Figure 5, Amoxicillin absorbs differently when it interacts with Calcium Carbonate (Antacid-2).

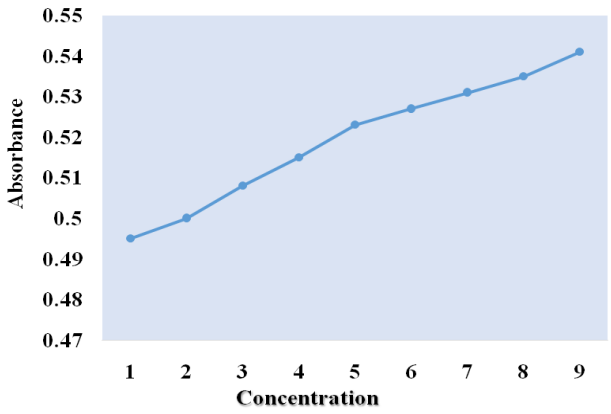

Figure 1: Standard curve of Amoxicillin at 257nm.

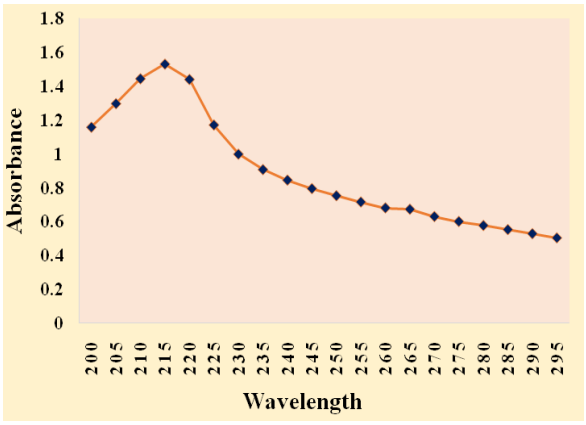

Figure 2: Spectral Curve of $Mg(OH)_2$ at different wavelength.

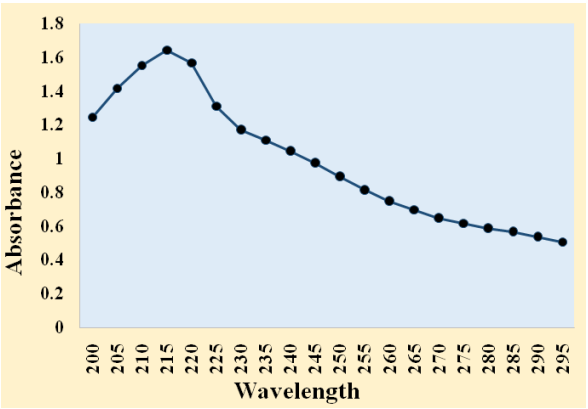

Figure 3: At different wavelength spectral analysis of $CaCO_3$

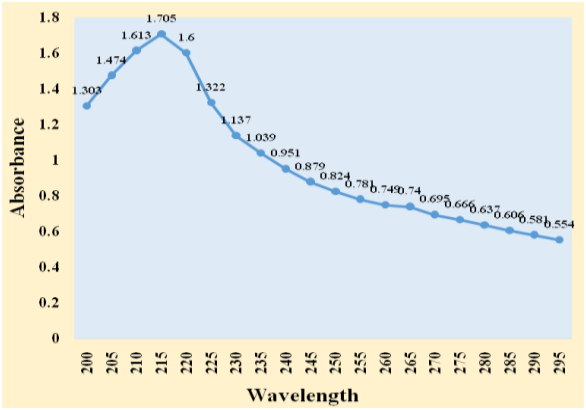

Figure 4: Spectral curve of Amoxicillin with $Mg(OH)_2$.

From Figure 6, it is easy to say that spectral analysis of Amoxicillin with Magnesium hydroxide is quite different from Amoxicillin with Calcium carbonate.

By using the method of Job's of continuous variation, molar ratios of the complexes of amoxicillin with antacids were calculated. The reported absorbance values were measured at 256 nm with a range of Amoxicillin concentrations (1×10^{-5} to 9×10^{-5}M) and antacids. The absorbance difference against the drug's mole fraction was plotted to produce Job's plots (Figure

7). The given figure demonstrates how Amoxicillin and Magnesium hydroxide mix to form potent 1:1 complexes, which are represented by the'^' shaped curve (Figure 7).

Figure 8, indicate that Amoxicillin and calcium carbonate combine to produce powerful 1:1 complexes, which are shown as '^'shaped curve. Figure 9 displays how absorbance differs, or Amoxicillin and $Mg(OH)_2$ and Amoxicillin and $CaCO_3$ are dissimilar from one another. Figure 10 demonstrates how Amoxicillin absorbance varies from Amoxicillin absorbances when combined with $Mg(OH)_2$ and $CaCO_3$.

Antimicrobial study

S. aureus was used to test the test samples. *S. aureus* was tested against the common amoxicillin disk as well. The following table displays the results of the antibacterial activity, assessed as the diameter of zone of inhibition in mm. Amoxicillin shows significant action against *S. aureus* (gram positive bacteria) because it is superfluous effective for infections which are caused by the gram positive bacteria (Table 1)[23].

It can see that Amoxicillin doesn't show any activity against *E. Coli* (gram negative bacteria) but it shows its activity against both gram positive and gram negative bacteria and this absence of activity is because of variation in organism and Amoxicillin resistance (Table 1).

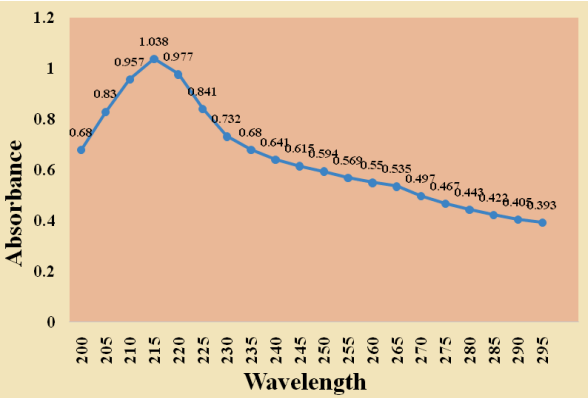

Figure 5: Spectral analysis of Amoxicillin with $CaCO_3$.

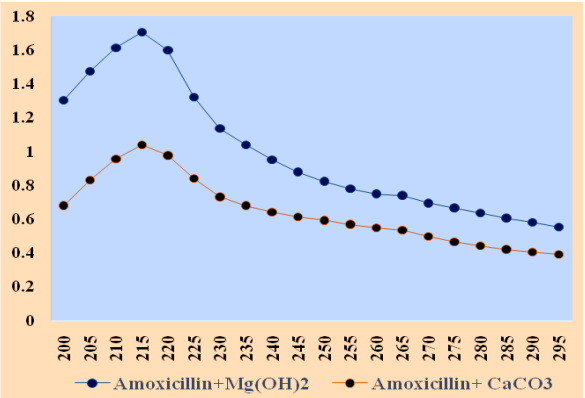

Figure 6: Combined spectral analysis of Amoxicillin with different antacids.

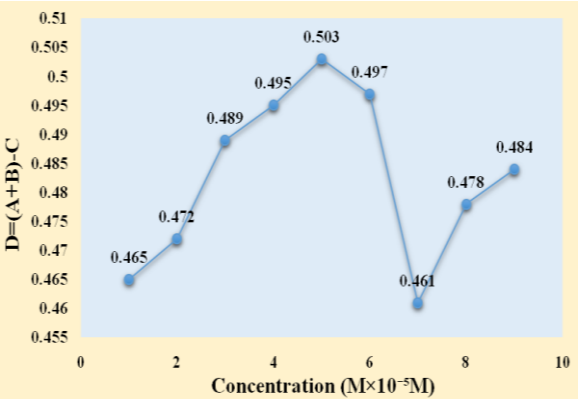

Figure 7: Absorbance difference of Amoxicillin with Magnesium hydroxide, $Mg(OH)_2$.

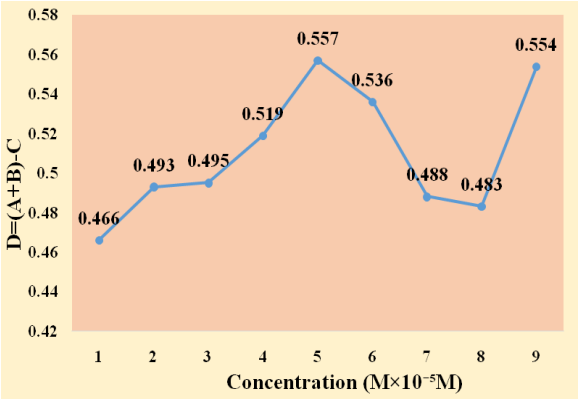

Figure 8: Absorbance difference of Amoxicillin with Calcium carbonate, $CaCO_3$.

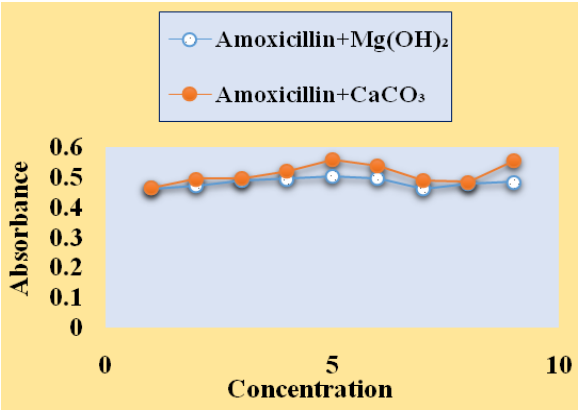

Figure 9: Combined absorbance difference of drug with different antacid.

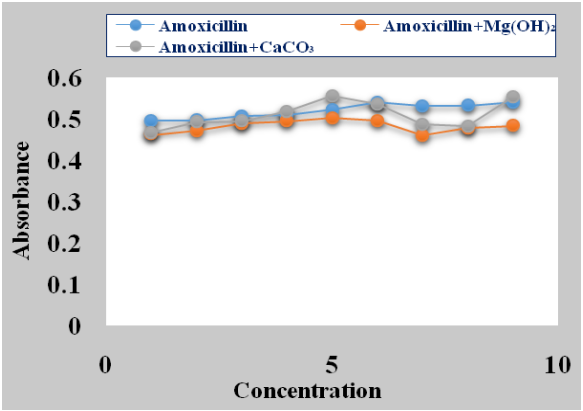

Figure 10: Combined absorbance of drug with different antacid.

Figure 11: Antimicrobial sensitivity of Amoxicillin against *S. aureus.*

Figure 12: Antimicrobial sensitivity of Amoxicillin against *E. coli.*

D=Antibiotic solution (Amoxicillin);
D_1=Amoxicillin solution + Antacid solution-1; $Mg(OH)_2$
D_2=Amoxicillin solution + Antacid solution-2, $CaCO_3$.

Table 1: Activity against *S. aureus* and *E. coli.*

Bacteria used	Standard disk (zone of inhibition/mm)	Sample disk (zone of inhibition)
S. aureus	16 mm	Amoxicillin 15 mm
S. aureus	16 mm	Amoxicillin+$Mg(OH)_2$ 11 mm
S. aureus	16mm	Amoxicillin+$CaCO_3$ 9mm
E. coli	6 mm	Amoxicillin 6 mm
E. coli	6 mm	Amoxicillin+ $Mg(OH)_2$ 6 mm
E. coli	6 mm	Amoxicillin+$CaCO_3$ 6mm

CONCLUSIONS

Amoxicillin produces potent 1:1 complexes with magnesium hydroxide and calcium carbonate, which are represented as'^'shaped curves. Antimicrobial testing against gram positive bacteria revealed that it was verified that zone of inhibition of Amoxicillin with $Mg(OH)_2$ (Antacid-1) and $CaCO_3$ (Antacid-2) lowered from 15 mm, 11 mm and 9 mm respectively. And also the activity against gram negative bacteria, Amoxicillin with $Mg(OH)_2$ and $CaCO_3$ don't show any recordable antimicrobial effect. We are aware that the drug's availability corresponds to its concentration or quantity. It aids in research of choosing the most best and effective dosage type for treatment. Furthermore, it is crucial to modify the ranges of dosage and effective dose. Study concludes that the standard absorbance of Amoxicillin differs significantly from the absorbance of drug-antacid and the antimicrobial activity is also reduced due to interaction.

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DATA AVAILABILITY

The datasets generated during this study are available from the corresponding author upon reasonable request.

AUTHOR'S CONTRIBUTION

Islam MS: writing original draft, methodology. **Mojumder TJ:** research design, data collection. Final manuscript was read and approved by all authors.

CONFLICT OF INTEREST

No conflict of interest associated with this work.

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