In vitro interaction of Amoxicillin with Calcium Chloride (Fused) at pH 2.4 and pH 7.4

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ABSTRACT
This study was aimed to evaluate the in vitro complexation nature and strength of complex which may be formed due to interaction between Amoxicillin and Calcium chloride (CaCl₂). The interaction of Amoxicillin and Calcium chloride (fused) has been studied in aqueous systems at a fixed temperature (37 ± 0.5) °C and under different pH (pH 2.4 and pH 7.4) by using some physical methods as spectral observation, Job’s method of continuous variation, Ardon’s method. From spectrophotometric study, Amoxicillin gives a sharp peak at 272 nm when Calcium chloride mixed with Amoxicillin in 1:1 ratio the intensity of the peak of Amoxicillin changes remarkably due to interaction. The jobs plot was obtained by plotting absorbance difference against the mole fraction of the each drug at pH 2.4 and pH 7.4. Amoxicillin forms strong 1:1 complex with Calcium chloride and reverse V-Shaped curves indicate the formation of 1:1 complexes of Amoxicillin with Calcium chloride. These may indicate strong kinetics of complexation between Amoxicillin with Calcium chloride. The value of stability constant for the complexation of Amoxicillin with calcium chloride at pH 2.4 and pH 7.4 were obtained from the spectral data using Ardon’s plot. The value of stability constant for the drug-metal system at pH 2.4 and pH 7.4 are 5.54 and 6.67 respectively. At pH 2.4 it is found that Amoxicillin form relatively stable complex with Calcium chloride (stability constant 6.67) is high in comparison to pH 7.4. It can therefore be concluded that a careful consideration is needed during concurrent administration of Amoxicillin with Calcium chloride.

INTRODUCTION
According to biopharmaceutical classification system, Amoxicillin is a beta-lactam antibiotic categorized as a class III drug with low permeability (Wu and Benet, 2005). The drug is orthodoxy administered as capsule or as oral suspension. The drug shows absorption through two mechanisms energy dependent active mechanism and electro-chemical gradient dependent passive mechanism but has poor oral absorption due to complete ionization under gastrointestinal pH conditions and exhibits very low lipid solubility (Tsuji et al., 1981). In rat plasma profile, the drug has exposed poor bioavailability (Chesa-Jimenez et al., 1994). It had been also confirmed from the reports extracted from a regional perfusion technique in vivo in humans (Lennernas et al., 2002). On the list of causes of poor oral bioavailability of medication, the permeability of intestinal epithelium plays an important role in passive transport. Several factors apparently influence the permeability of intestine and thereby affect the absorption of substances with the gut, for instance, herbs, chemicals, stress, inflammation, toxins and probiotics (Rao, 2009). On the list of distinctive forms of salt, calcium chloride is really a solid that is certainly competent at absorbing quite a lot of liquid. Calcium chloride injected to help remedy internal hydrofluoric acid burns, quite a few to treat magnesium intoxication (Leoci et al., 2014). Calcium chloride injection may antagonize cardiac toxicity as measured by electrocardiogram. It can help to shield the myocardium from dangerously high amounts of serum potassium in hyperkalemia (Hack et al., 2004). Calcium chloride can often quickly treat calcium channel blocker toxicity (Graudins and Wong, 2010), in the uncomfortable side effects of drugs for instance diltiazem (Cardizem)-helping avoid potential cardiac arrest (Isbister, 2002).
Calcium chloride which exists to be a metal complex present in types of food additives such as, milk, egg, bones, cereals, marine fishes etc. So our study is aimed to investigate the in vitro complexation properties and strength of complex which may be formed due to interaction between Amoxicillin and Calcium chloride (CaCl₂) at different pH range.

MATERIALS AND METHOD

Materials
Amoxicillin and Calcium chloride (fused) were kind gifts from department of Pharmacy, BGC Trust University Bangladesh.

Spectral studies
Initial detection of complexation of Amoxicillin and Calcium chloride (fused) has done from the nature of spectra of pure compounds as well as their 1:1 mixtures in chloride buffer 2.4 and phosphate buffer solution of 7.4 at the following concentration: 0.9×10⁻⁵ M, 0.8×10⁻⁵ M, 0.7×10⁻⁵ M, 0.6×10⁻⁵ M, 0.5×10⁻⁵ M, 0.4×10⁻⁵ M, 0.3×10⁻⁵ M, 0.2×10⁻⁵ M, 0.1×10⁻⁵ M.

Job’s spectrophotometric method of continuous variation
In this method, absorbance of series of Amoxicillin and Calcium chloride (fused) mixtures with molar ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 at pH 2.4 and pH 7.4 were measured by keeping the total moles constant. The absorbance of Amoxicillin and Calcium chloride (fused) solutions was measured at 272 nm which is the absorption maxima of Amoxicillin. The observed absorbance of the mixtures at various mole fractions was subtracted from the sum of the values for free Amoxicillin and free Calcium chloride (fused) (Vogel, 1978).

The absorbance differences (D) were then plotted against the mole fractions of drugs in the mixtures. A curve, thus, obtained showed a maximum at a point, which indicated the molar ratios of Amoxicillin to Calcium chloride (fused) in the complex.

The Ardon’s spectrophotometric methods
In this method concentrations of Amoxicillin varied while keeping the concentrations of Calcium chloride (fused) fixed 2×10⁻⁴ M. All the experiments were performed in buffer at pH 2.4 and pH 7.4. The absorbance of free Amoxicillin solutions was measured at 272 nm using UV-VISIBLE spectrophotometer. For calculation, the Ardon’s equation was used. This equation is given below (Ardon, 1957):

\[
\frac{1}{D - \epsilon_A C} = \frac{1}{KC (\epsilon_{com} - \epsilon_A) B} + \frac{1}{C (\epsilon_{com} - \epsilon_A)}
\]

Where,
- \(D = \) Absorbance of the mixture.
- \(B = \) Molar concentration of the Amoxicillin.
- \(C = \) Molar concentration of the Calcium chloride (fused).
- \(\epsilon_{com} = \) Molar extinction co-efficient of the complex.
- \(\epsilon_A = \) Molar extinction co-efficient of the Calcium chloride (fused)

The value was chosen as 1, which is an essential condition for validation of the method. The value for \(1/(D - \epsilon_A C)\) was plotted versus \(1/[C]\) to get the straight lines. The concentration of Calcium chloride was kept constant 2×10⁻⁴ M (denoted by C in the equation) & the concentration of interacting species Amoxicillin was varied (denoted by B in the equation). The 1:1 complex gave a straight line in the plots with an intercept and a slope. The stability constant of the complex was given by the relation, \(K = \text{intercept} / \text{slope}\). It is to be mentioned that this method is only valid for the systems where 1:1 complexes are found.

RESULTS

Spectral study
In spectral studies, it was seen that Amoxicillin gives a sharp peak at 272 nm when Calcium chloride salt mixed Amoxicillin with in 1:1 ratio. The intensity of the peak of Amoxicillin changes remarkably i.e. absorption characteristics are altered due to interaction but the position of the compound do not shift (Fig. 1, 2 and 3).

![Fig. 1: Standard Curve](image1)

![Fig. 2: Spectral studies of Amoxicillin (alone) at pH 7.4 with Amoxicillin & Calcium Chloride (Fused) at pH 7.4](image2)
Study of Job’s method

The molar ratios of the complexes of Amoxicillin with Calcium chloride (fused) were estimated by Job’s method of continuous variation. The observed absorbance values were measured in pH 2.4 and 7.4 at various concentrations (0.1 x 10^{-5} to 0.9 x 10^{-5} M) Amoxicillin with Calcium chloride (fused) at 272 nm. The Job’s plots at pH 2.4 and 7.4 were obtained by plotting absorbance differences against the mole fraction of the drug (Amoxicillin) which are presented in Fig. 4.

Effect of Amoxicillin on Calcium chloride using Ardon’s method

Ardon’s plot confirmed the formation of 1:1 complex of Amoxicillin and Calcium chloride at pH 7.4 and 2.4, since the method is valid only for 1:1 complexes. The values of 1/ |drug| by using the Ardon’s equation:

\[
\frac{1}{D} = \frac{kC}{e_{\text{com}} - e_{A}} [B] + \frac{1}{C} (e_{\text{com}} - e_{A})
\]

This experiment was performed in buffer systems pH 7.4 and 2.4. The data for Ardon’s gave straight lines with intercept which are presented in figure 5 indicate the formation of 1:1 complexes for the system at both pH.

Estimation of Stability Constant

The value of stability constant for the complexation of Amoxicillin with Calcium chloride at pH 7.4 and 2.4 were obtained from the spectral data using Ardon’s plot. The values for stability constant were calculated from the slopes and intercepts of the straight lines from these plots. It was seen from the Ardon’s equation that the values of stability constant was given as [(intercept) / (slope)] of straight line so obtained. i. e. \(k = \frac{\text{intercept}}{\text{slope}}\). The value of intercept and slope were calculated by Least Squares Method using the following equation:

\[ y = mx + C \]

The values of stability constants for the drug-metal system at pH 2.4 and pH 7.4 presented in the table given below:

<table>
<thead>
<tr>
<th>System</th>
<th>Stability Constant, K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin + Calcium chloride</td>
<td></td>
</tr>
<tr>
<td>At pH 2.4</td>
<td>6.67</td>
</tr>
<tr>
<td>At pH 7.4</td>
<td>5.54</td>
</tr>
</tbody>
</table>

At pH 2.4 it is found that Amoxicillin formed stable complexes with Calcium chloride (stability constant 6.67) is high in comparison to pH 7.4.

DISCUSSION

In the present work, the interaction of an important antibiotic, Amoxicillin with Calcium chloride (fused) has been studied in the aqueous system at pH 2.4 and 7.4 by a variety of physical method like inspection of spectral behavior, Job’s method of continuous variation and Ardon’s straight line plots by spectrophotometry. From spectral study, it has been seen that Amoxicillin gives a sharp peak at 272 nm. When Calcium chloride (fused) mixed with Amoxicillin at 1:1 ratio, the intensity of the peak of Amoxicillin changes remarkably (absorbance decreases) i.e., absorption characteristics are altered due to interaction but the position of the compound do not shift. Job’s plot has given the molar ratio of complexes of Amoxicillin and Calcium chloride (fused) At pH 2.4 and 7.4 Amoxicillin forms strong 1:1 complexes with Calcium chloride (fused) indicated as ‘^’ shaped curves. These curves may indicate strong kinetics of complexation between Amoxicillin and Calcium chloride (fused) The Ardon’s spectrophotometric plots also confirm the phenomenon of 1:1 complexation which is indicated by straight lines. The stability
CONFLICT OF INTEREST

The authors have declared that there no conflict of competing interest.

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