Exploring Montelukast Sodium and Calcium Chloride Interactions: A Comparative Study at Physiological and Gastric pH Levels

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Abstract:
Montelukast, a leukotriene receptor antagonist (LTRA) is used to prevent an asthmatic attack, shortness of breath and wheezing. As intravenous therapy, Calcium chloride (fused) is used to treat hypocalcemia. Using spectrophotometry, an in vitro study of the interaction between Montelukast sodium and Calcium chloride (fused) was conducted at pH 7.4 and pH 2.4 in aqueous systems at 37 ± 0.5 °C. A reverse V-shaped curve was found from the Job’s plot indicating a strong kinetics between Montelukast sodium and Calcium chloride. The stability constant was obtained from Ardon’s plot for the complexation at both pH values (7.4 and 2.4), which indicates that Montelukast sodium and Calcium chloride relatively form a stable complex at pH 7.4. Therefore, concomitant administration of Montelukast sodium and Calcium chloride (fused) needed careful consideration since there is a possibility of forming a complex which in turn reduces the therapeutic activity.

Keywords: Montelukast sodium, Calcium chloride (fused), Job’s plot, Ardon’s method, Stability constant.

Introduction
According to the biopharmaceutical classification system, Montelukast sodium is a leukotriene receptor antagonist which inhibits the cysteinyl-leukotriene (CysLT1) receptor. Cysteinyl-leukotrienes, lipid mediators released from inflammatory cells, produce airway edema, mucus secretion, and eosinophil migration; reactions associated with the major findings of airway inflammation in asthma (Gupta et al., 2014) (Cheng et al., 1996) and are widely used as prophylactic and seasonal allergies in children and adults (Reiss et al., 1996) (Knorr et al., 1998). In asthma, the ventilation capacity of the airways reduces due to hyperresponsiveness (AHR) to external stimulus and bronchoconstriction by subepithelial fibrosis and hypertrophy and hyperplasia of smooth muscle (Lemanske & Busse, 2003) (Adcock et al., 2008) (Kudo et al., 2013) (Reddel & Levy, 2015).

Montelukast selectively antagonizes leukotriene D4 (LTD4) in the human airway and inhibits the actions of LTD4 at the CysLT1 receptor (Krishna et al., 2015) (Bouchelouche et al., 2001). Cysteinyl leukotriene receptor 1 (CysTr1), a receptor for CysLT, was found to be
responsible for various diseases in human and animal models. In adenoid hypertrophy, Cysltr1 expression was found upregulated (Gao et al., 2018). JNK phosphorylation is also stimulated by the activation of Cysltr (Lei et al., 2019), which in turn triggers inflammation (Kondeti et al., 2016). Reports showed evidence of Montelukast’s antagonistic activity against NF-κB signalling (Maeba et al., 2005).

Montelukast sodium is administered orally as a tablet chewable or oral granules (Mahesh et al., 2012). The drug shows a short half-life, poor solubility in water, and bioavailability was approximately 64% (Jones et al., 2011) (Mougey et al., 2009). The bioavailability of Montelukast sodium can be improved by increasing its solubility. The liver cannot uptake the drug from the delivery system and it is not metabolized by the liver. The drug undergoes first-pass metabolism, but it can be prevented by changing its formulation to SLN (solid nanoparticles) form (Ekambaram et al., 2012). About 99% of plasma proteins binding occurs to Montelukast sodium. Radiolabeled montelukast distributed at a minimum amount through the blood-brain barrier was found in studies done by rats (Zamek-Gliszczynski et al., 2011). Since Montelukast sodium has sensitive moieties in the structure, it may go for oxidation reactions which are also called light-sensitive nature (Tiwari et al., 2018). The dissolution of MS (Montelukast sodium) from ODTs (Orally Disintegrating Tablets) in an acidic medium usually depends on manufacturing methods. The direct compression method is used to prepare all MS ODTs for rapid disintegration in an acidic medium (Chen et al., 2017).

Calcium chloride is a common salt which can be injected to aid remedy internal hydrofluoric acid burns and a few to treat magnesium intoxication (Leoci et al., 2014). The food sources of calcium are milk, eggs, bones, cereals, marine fish etc. (Sultana et al., 2015). An electrocardiogram can measure the antagonizing effect of Calcium chloride on cardiac toxicity. Calcium chloride can protect the myocardium from dangerously high amounts of serum potassium in hyperkalemia by combating them (Hack et al., 2004). Calcium channel blocker toxicity can be treated by Calcium chloride at a rapid rate (Graudins & Wong, 2010), helping to avoid potential cardiac arrest by side effects of drugs for instance diltiazem (Cardizem) (Isbister, 2002). It is also indicated in hypocalcemic tetany where abnormally low levels of calcium in the body cause muscle spasms in paediatrics.

When calcium is taken with bisphosphonates used for osteoporosis, the absorption rate of bisphosphonates decreases, and the same case happens in fluoroquinolone and tetracycline groups. Calcium also can decrease the absorption of phenytoin which is an anticonvulsant and tiludronate disodium which is available to treat Paget’s disease (Peters et al., 2001). The risk of hypercalcemia and hypercalciuria can increase through the interaction of calcium carbonate and vitamin D supplements with thiazide type diuretics. Calcium absorption can be decreased by mineral oil and stimulant laxatives and aluminium-magnesium containing antacids can rise the excretion of urinary calcium. Calcium depletion can occur with long-term use of prednisone.

There are many factors which influence the rate and extent of oral drug absorption such as complex interactions between the physicochemical properties of a drug, Gastrointestinal (GI) physiologic factors and the nature of the formulation. GI pH is an important phenomenon that can be remarkably affected by oral drug absorption and bioavailability. Different parts of the GI tract have different drug-absorptive properties (Abuhelwa et al., 2017).

Almost all of the medicines have such ingredients that can interact with the human body in many ways. Lifestyle and diet have a significant role in the effect of the drug. When a drug interacts with other drugs, foods or metals, this may impair the effect of the primary drug. The effect might rise or decline from the previous effect of the first drug, or the interaction might produce a whole new effect of its own. The interactions between drugs known as drug-drug interactions and interactions between drugs and foods are called drug-food interactions. Besides, drugs and herbs
interaction is designated as drug-herb interactions (Bushra et al., 2011). Patients who are treated with multifarious drugs at the same time are prone to an increased risk of drug-drug interactions (DDIs). The entire research mechanism about DDIs is the root of predicting the occurrence of adverse drug reactions caused by drug interactions (Koenen et al., 2011).

The formulation of new drug candidates with proper dosing and timing can be evaluated by food-drug interaction studies. These interactions often are related to food intake which can affect the rate or extent of systemic drug absorption. The physiologic and physicochemical characteristics and mechanisms of food are well-characterized which affect the drug disposition (Won et al., 2012). It was observed that the use of some conventional herbs, fruits or alcohol often results in a severe adjustment in the way a drug reacts in the patient’s body. In most cases, food affects drug bioavailability due to the food-drug interactions. The interaction can decrease the bioavailability of the primary drug which leads to a high risk of treatment failure. The chelate formation with food components is responsible for these interactions (Bushra et al., 2011).

So, our study aims to investigate the in vitro complexation of Montelukast sodium and Calcium chloride (fused) which may be formed due to interaction and determine the properties and strength of the complex of Montelukast sodium with Calcium chloride (fused) as a contributing factor for determining drug safety and efficacy at different pH range.

Materials and Methods

Materials

Calcium chloride (fused) was a kind gift from the Department of Pharmacy, BGC Trust University, Bangladesh and Montelukast sodium has been collected from Incepta Pharmaceuticals Ltd. Disodium hydrogen phosphate (Na₂HPO₄) (Analytical grade), Potassium dihydrogen phosphate (KH₂PO₄) (Analytical grade), Hydrochloric acid (Analytical grade), and Potassium chloride (Analytical grade) have been procured from Glaxo, U.K.

Characterization

UV/VIS Spectrophotometer (Thermoelectron company, England), pH meter (Hanna, Portugal), Electronic balance (Model AL2504: Mettler Toledo, Switzerland), Metabolic Shaking Incubator (Nickel Electro Ltd., England), Micro syringe (Fisher brand) have been utilized in this study.

Preparation of Buffer Solutions

Chloride Buffer of pH 2.4

250 ml of 0.1M Potassium chloride (Mol. Wt. 74.5) solution was prepared in a volumetric flask (solution A). In another 500 ml volumetric flask, solution B was prepared by dissolving 8.35 ml of 37% concentration of 0.2 M Hydrochloric acid. To prepare a solution of pH 2.4, approximately 250 ml of solution A was properly mixed with approximately 24 ml of solution B. Finally, a pH meter was used to measure the pH of 2.4.

Phosphate Buffer of pH 7.4

To prepare a phosphate buffer of pH 7.4, 500 ml of 0.01M Disodium hydrogen phosphate (Mol. Wt. 141.96) was prepared in a volumetric flask called solution A and then 0.02 M Potassium dihydrogen phosphate (Mol. wt. 136.09) was taken in a 250 ml volumetric flask and adjusted which was solution B. Then approximately 500 ml of solution A was properly mixed with approximately 80 ml of solution B and the solution was adjusted at pH 7.4.

Preparation of Standard Solutions (Amran et al., 2006)

1×10⁻² M solution of Montelukast sodium and 1×10⁻² M solution of Calcium chloride (fused) was dissolved in 100 ml of de-ionized water separately. These stock solutions were diluted to desired strengths by buffer solution to get the working standard solution.

Preparation of Standard Curve

0.1×10⁻⁷ M, 0.2×10⁻⁷ M, 0.3×10⁻⁷ M, 0.4×10⁻⁷ M, 0.5×10⁻⁷ M, 0.6×10⁻⁷ M, 0.7×10⁻⁷ M, 0.8×10⁻⁷ M, 0.9×10⁻⁷ M of working standard solution of
Montelukast sodium were prepared. Then, a standard curve was prepared by plotting absorbance (measured at 285 nm) VS concentration of Montelukast sodium (Fig.1).

The *in vitro* interaction study of Montelukast sodium and Calcium chloride (fused) has been performed by observing absorption spectra, Job’s spectrophotometric continuous variation data and Ardon’s spectrophotometric curves.

**Spectral Studies** (Sayeed et al., 2012) (Mohiuddin et al., 2009)

Initial detection of complexation of Montelukast sodium and Calcium chloride (fused) has been done from the nature of spectra of pure compounds as well as their 1:1 mixtures in a phosphate buffer solution of pH 7.4 and chloride buffer of pH 2.4 at the following concentration 0.9×10⁻⁷ M, 0.8×10⁻⁷ M, 0.7×10⁻⁷ M, 0.6×10⁻⁷ M, 0.5×10⁻⁷ M, 0.4×10⁻⁷ M, 0.3×10⁻⁷ M, 0.2×10⁻⁷ M, 0.1×10⁻⁷ M. The concentrations of the specimen were kept at extremely dilute levels for every situation and the estimation was made by utilizing UV-VIS spectrophotometer. The spectra of the working standard arrangements were recorded between 400 - 200 nm. The spectrum of each kind was compared with the pure samples.

**Job’s Spectrophotometric Method of Continuous Variation** (Remmer et al., 1968)

In this method, keeping the total moles constant, the absorbance of molar ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1 of Montelukast sodium and Calcium chloride (fused) were measured at 285 nm which is the absorption maxima of Montelukast sodium at pH 7.4 and pH 2.4. The value of absorbance at different molar concentrations was deducted from the summation of the values for alone Montelukast sodium and free Calcium chloride (fused). The absorbance differences (D) were plotted against the mole fractions of Montelukast sodium. A curve, thus, obtained showed a maximum at a point, which indicated the molar ratios of Montelukast sodium to Calcium chloride (fused) in the complex.

**Ardon’s Spectrophotometric Method:**
(Ardon, 1957)

In this method, concentrations of Montelukast sodium varied while keeping the concentration of Calcium chloride (fused) fixed at 0.2×10⁻⁷ M. All the studies were observed in buffer at pH 7.4 and pH 2.4. The absorbance of free Montelukast sodium solutions was measured at 285 nm using a UV-visible spectrophotometer.

For calculation, the Ardon’s equation was used. This equation is given below:

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

Where,

D = Absorbance of the mixture.

C = Molar concentration of the Calcium chloride (fused)

\(\varepsilon_{com}\) =Molar extinction co-efficient of the complex.

\(\varepsilon_A\) = Molar extinction co-efficient of the Calcium chloride (fused)

The value was chosen as 1, which is an essential condition for the validation of the method. The value for \(1/ (D - C \varepsilon_A)\) was plotted versus \(1/[C]\) to get the straight lines. The concentration of Calcium chloride was kept constant at 0.2×10⁻⁷ M (denoted by C in the equation) and the concentration of interacting species Montelukast sodium was varied (denoted by B in the
equation). The 1:1 complex yielded a straight line in the plots with an intercept and a slope. The stability constant of the Montelukast sodium and Calcium chloride (fused) was given by the relation,

\[ K = \frac{\text{intercept}}{\text{slope}} \]  

(2)

It is to be mentioned that this method is only valid for the systems where 1:1 complex is found.

Results

In spectral observation analysis, Montelukast sodium when mixed with Calcium chloride (fused) showed some changes in absorption characteristics of the drug molecule including some shifts in the absorption maxima. Thus, shifting in the spectral curve may be regarded as an indicator of the primary interaction of drug and salt. It was seen that Montelukast sodium gives a sharp peak at 285 nm when Calcium chloride (fused) is mixed with Montelukast sodium in 1:1 ratio at pH 7.4 and at pH 2.4 (Fig. 2 and 3).

Figure 2. Spectral Studies of Montelukast Sodium (Alone) with Montelukast Sodium and Calcium Chloride (fused) at pH 7.4

Figure 3. Spectral Studies of Montelukast Sodium (Alone) with Montelukast Sodium and Calcium Chloride (fused) at pH 2.4
The molar ratios of the complexes of Montelukast sodium with Calcium chloride (fused) were estimated by Job’s method of continuous variation. The observed absorbance values were measured in pH 7.4 and pH 2.4 at various concentrations (0.1 × 10^{-7} to 0.9 × 10^{-7} M) of Montelukast sodium with Calcium chloride (fused) at 285 nm (Table 1). The Job’s plots at pH 7.4 and pH 2.4 were obtained by plotting absorbance differences against the mole fraction of the drug (Montelukast sodium) which are presented in Fig. 4.

Table 1. Values of Job’s Plot for Complexation of Montelukast Sodium and Calcium Chloride (Fused)

<table>
<thead>
<tr>
<th>Concentration M×10^{-7}</th>
<th>Absorbance at pH 7.4</th>
<th>Absorbance at pH 2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.029</td>
<td>0.059</td>
</tr>
<tr>
<td>0.2</td>
<td>0.02</td>
<td>0.038</td>
</tr>
<tr>
<td>0.3</td>
<td>0.033</td>
<td>0.028</td>
</tr>
<tr>
<td>0.4</td>
<td>0.048</td>
<td>0.051</td>
</tr>
<tr>
<td>0.5</td>
<td>0.05</td>
<td>0.059</td>
</tr>
<tr>
<td>0.6</td>
<td>0.018</td>
<td>0.085</td>
</tr>
<tr>
<td>0.7</td>
<td>0.037</td>
<td>0.085</td>
</tr>
<tr>
<td>0.8</td>
<td>0.043</td>
<td>0.044</td>
</tr>
<tr>
<td>0.9</td>
<td>0.01</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Table 2. Values of Ardon’s Plot for Complexation of Montelukast Sodium and Calcium Chloride (Fused)

<table>
<thead>
<tr>
<th>1/Montelukast sodium×10^{-7}</th>
<th>1/(D-\varepsilon)\times10^{-2} at pH 7.4</th>
<th>1/(D-\varepsilon)\times10^{-2} at pH 2.4</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>31.25</td>
<td>75.24</td>
</tr>
<tr>
<td>5</td>
<td>24.1</td>
<td>59</td>
</tr>
<tr>
<td>3.33</td>
<td>21.36</td>
<td>50.2</td>
</tr>
<tr>
<td>2.5</td>
<td>20.35</td>
<td>54.34</td>
</tr>
<tr>
<td>2</td>
<td>19.5</td>
<td>30.86</td>
</tr>
<tr>
<td>1.67</td>
<td>15.92</td>
<td>45.23</td>
</tr>
<tr>
<td>1.42</td>
<td>13.55</td>
<td>42.73</td>
</tr>
<tr>
<td>1.25</td>
<td>13.55</td>
<td>40.2</td>
</tr>
<tr>
<td>1.11</td>
<td>14.32</td>
<td>40.2</td>
</tr>
</tbody>
</table>

The formation of the Montelukast sodium and Calcium chloride(fused) complex in a 1:1 ratio at pH 7.4 and pH 2.4 was confirmed by Ardon’s plot since the method is valid only for 1:1 complexes. The values of 1/[drug] by using Ardon’s equation. This experiment was performed in buffer systems pH 7.4 and pH 2.4. The data for Ardon’s gave straight lines with intercepts which are presented in Figure 5 indicating the 1:1 complex formation for the Montelukast sodium and Calcium chloride(fused) at both pH values (Table 2).
**Discussion**

**Estimation of Stability Constant**

By using spectral data from Ardon’s plot, the values of stability constant for the complexation of Montelukast sodium with Calcium chloride at pH 7.4 and pH 2.4. The stability constants were measured from the slopes and the intercepts of the straight lines yielded from these plots. It was seen from Ardon’s equation that the values of stability constant were given as [(intercept)/(slope)] of a straight line, k = (intercept)/(slope).

The Least Squares Method was used to calculate the values of intercept and slope using the following equation:

\[ y = mx + c \]  \hspace{1cm} (3)

**Table 3. The Values of Stability Constants at pH 7.4 and pH 2.4**

<table>
<thead>
<tr>
<th>System</th>
<th>pH</th>
<th>Stability constant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Montelukast sodium &amp; Calcium chloride</td>
<td>7.4</td>
<td>9.688</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>8.784</td>
</tr>
</tbody>
</table>

Montelukast sodium when combined with Calcium chloride forms the stability constant 9.688 and 8.784 at pH 7.4 and pH 2.4, respectively.

Assuring safe and effective drug dosing requires information such as gastrointestinal fraction absorbed (fa), clearance (CL) and volume of distribution (V_D). The volume of distribution (V_D) is assayed by the volumes of binding capabilities of blood, tissues and organs and also affected by the penetrating capabilities and dissociation rates (Ashfak et al., 2017). Precise determination of volume of distribution depends on the plasma exposure. There are generally two mechanisms by which drugs are absorbed through the gut wall. One is energy dependent active mechanism, and another one is an electrochemical gradient-dependent passive mechanism. The drug with poor lipid solubility and complete ionization in gastrointestinal pH conditions has poor absorption (Berezhkovskiy, 2004). It is also a well-known fact that the presence of another drug, ion or food sometimes modifies the absorption of a drug.

The spectral study is used to detect the existence of a band at a particular wavelength for the identification of a particular group in a compound or in general to make sure about certain compounds. Here in the spectral studies,
it was found that Montelukast sodium gives a sharp peak at 285 nm. But when Calcium chloride (fused) is mixed with Montelukast sodium at a 1:1 ratio, the intensity of the peak of Montelukast sodium changes remarkably (absorbance decreases) that is absorption characteristics are changed due to the drug-salt interaction but the shifting of the position of the compound does not alter. The curves obtained from Job’s method show breaks for both pH values at different molar ratios of complexes of Montelukast sodium and Calcium chloride (fused). The ‘‘’ shaped curves indicated the strong complexes of Montelukast sodium and Calcium chloride (fused). Ardon’s spectrophotometric plots established the occurrence of 1:1 complexation, as it was indicated by the obtained straight lines. The stability constants of the complexes were estimated from these straight lines calculated from the values (intercept)/ (slope) of the straight line. The values of stability constant indicate a strong complexation between Montelukast sodium and Calcium chloride at pH 7.4 than pH 2.4.

Multi-drug treatment is a quite common practice where different diseases are concerned. So, the information on drug-drug, drug-salt or drug-food interactions is especially very significant for health professionals (Tsuji et al., 1981). Lack of information about a new drug can be a significant cause of drug potentiality, adverse drug effects or sometimes hospital admission (Bergk et al., 2005).

Conclusion

The study of the complexation between drugs and metal is the subject of many experiments with a large number of physical and chemical parameters, which have been investigated. Until now, there has been no good enough experiment to observe the stability of the complex. In the present work, the interaction of an important anti-asthmatic drug Montelukast sodium with Calcium chloride has been conducted in the aqueous system at pH 7.4 and pH 2.4 by a variety of physical methods, to detect and confirm the nature of complexation of this drug with Calcium chloride.

In many investigations, it was found that Montelukast sodium interacts with calcium metal and formed a complex. Calcium is usually used to treat osteopenia. At the administration of Montelukast sodium with calcium-containing food as like as milk, cheese, yogurt, sardines, and kale, careful consideration should be needed to treat an asthmatic patient.

We, therefore, propose that these will be our future plan to give a conclusive idea about the interaction between Montelukast sodium and Calcium chloride:

1. Further in-vivo studies with a great number of samples should go through.
2. A multicentric study may be carried out.
3. Further in-vitro study should be fulfilled with site-specific probes in different pH.
4. This study should be supplemental with animals and also human beings.

Acknowledgement

The authors wish to thank all the faculties and Lab demonstrators, Department of Pharmacy, BGC Trust University Bangladesh, Chittagong-4381, Bangladesh.

Conflict of Interests

The authors declare no conflict of interest.

References


