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# *In vitro* drug-drug interaction study between Montelukast Sodium and Amlodipine Besylate at gastric and intestinal pH

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**ABSTRACT**

Present study was aimed to evaluate the *in vitro* complexation nature and strength of complex which may be formed due to interaction between Montelukast Sodium and Amlodipine Besylate. The interaction of Montelukast Sodium and Amlodipine Besylate has been studied in aqueous systems at a fixed temperature ($37^0$C) at both gastric pH (pH 1.0 and pH 3.0) and intestinal pH (pH 6.5 and pH 6.8) by using some physical methods as spectral observation, Job's method of continuous variation, Ardon's method. From spectrophotometric study, Montelukast Sodium and Amlodipine Besylate give different spectra when Montelukast Sodium mixed with Amlodipine Besylate in 1:1 ratio the intensity of the spectra of Montelukast Sodium change remarkably due to interaction. The jobs plot was obtained by plotting absorbance difference against the mole fraction of the each drug at pH 1.0, pH 3.0, pH 6.5 and pH 6.8. Montelukast Sodium strong 1:1 complex with Amlodipine Besylate and lower spectra indicate the formation of 1:1 complexes of Montelukast Sodium with Amlodipine Besylate. These may indicate strong kinetics of complexation between Montelukast Sodium with Amlodipine Besylate. The value of stability constant for the complexation of Montelukast Sodium with Amlodipine Besylate at pH 1.0, pH 3.0, pH 6.5 and pH 6.8 were obtained from the spectral data using Ardon's plot. The value of stability constant for the drug-drug system at pH pH 1.0, pH 3.0, pH 6.5 and pH 6.8 are 1.9412, 2.0323, 2.0952 and 2.2203 respectively. At pH 6.8 it is found that Montelukast Sodium form relatively stable complex with Amlodipine Besylate (stability constant 2.2203) is high in comparison to pH 1. It can therefore be concluded that a careful consideration is needed during concurrent administration of Montelukast Sodium with Amlodipine Besylate.

**Key words:** Drug interaction, Montelukast Sodium, Amlodipine Besylate, pH, Ardon's method,

## 1. INTRODUCTION

Montelukast Sodium (Molecular Formula $C_{35}H_{35}ClNNaO_3S$) is a leukotriene receptor antagonist, used in the treatment of asthma [1, 2, 3]. It is usually administered orally. It is not official in IP and BP. Various analytical methods, such as liquid chromatography with fluorescence detection [4, 5, 6], stereoselective HPLC for MTK and its S-enantiomer [7], simultaneous HPLC and derivative spectroscopic method with loratadine [8], stability indicating HPLC method for MTK in tablets and human plasma [9] have been already reported. Montelukast sodium is freely soluble in ethanol, methanol and water and practically insoluble in acetonitrile and its bioavailability is 63% [10, 11].

Amlodipine is a dihydropyridine calcium antagonist (calcium ion-channel blocker) that inhibits the transmembrane influx of calcium ions into vascular smooth muscle and cardiac muscle. It binds to both dihydropyridine and non dihydropyridine binding sites. The contractile processes of cardiac muscle and vascular smooth muscle are dependent upon the movement of extra cellular calcium ions into these cells through specific ion channels. Amlodipine inhibits calcium ion influx across cell membranes selectively, with a greater effect on vascular smooth muscle cells than on cardiac muscle cells [12].

Amlodipine is an ionized compound having ionization value 8.6 (pKa = 8.6) [13]. The bioavailability of amlodipine besylate, the functionality of excipients is improved by co-processed method [14].
Amlodipine besylate ($C_{26}H_{31}ClN_2O_8S$) is a drug that is used for treating high blood pressure, certain types of angina, and coronary heart failure. The drug works by slowing down the rate at which calcium moves to heart, blood vessel walls, allowing better blood flow. Amlodipine besylate is a white crystalline powder with a molecular weight of 567.1. It is slightly soluble in water and sparingly soluble in ethanol. It is extensively (about 90%) converted to inactive metabolites via hepatic metabolism with 10% of the parent compound and 60% of the metabolites excreted in urine. Its bioavailability is between 64-90% [15, 16].

The present study was to find out the drug-drug interactions (DDIs) as well as to determine the stability of the complexes, which could be formed after interaction between Montelukast Sodium and Amlodipine besylate at various pH. The values of stability constants were determined by using Job's continuous-variation analysis and Ardon's spectrophotometric measurement methods.

## 2. MATERIALS AND METHODS

**2.1 Drugs and chemicals:** Montelukast Sodium and Amlodipine Besylate were collected from Square Pharmaceuticals Ltd., Dhaka, Bangladesh. Sodium dihydrogen orthophosphate and di-sodium hydrogen orthophosphate, used for the preparation of buffer solutions were purchased from Merck, Germany. Sodium hydroxide and Sodium chloride were purchased from Riedel De Haen Ag, Seelze-Hannover- Germany. All chemicals and reagents were of analytical grade

**2.2 Equipments:** UV-Visible spectrometer (Model No. UV-1600, Shimadzu, Japan), pH meter (Hanna-HI981.7, USA), analytical balance (Model No. LF 224 DR, Shinko Denshi Co Ltd, Japan), and a thermostated water bath (Shimadzu, Japan) were used for the test.

**2.3 Preparation of standard solutions:** Montelukast Sodium ($1 \times 10^{-3}$ M) and Amlodipine Besylate ($1 \times 10^{-3}$ M) were dissolved in distilled water to prepare the stock solutions. These stock solutions were diluted to desired strengths ($1 \times 10^{-4}$ M) by buffer solutions to obtain the working standard solutions.

**2.4 Absorption spectrum analysis:** In observation of the spectra, the absorption characteristics of Montelukast Sodium and Amlodipine Besylate and their 1:1 mixtures in the solutions of buffers [17, 18] pH 1.0, 3.0, 6.5 and 6.8 were compared with those of each interacting species. The concentrations of the sample were kept at very dilute levels in each case and the measurements made using UV-VIS spectrophotometer. The spectra of the working standard solutions ($1 \times 10^{-4}$ M) were recorded between 400 - 200 nm. The spectra were compared with those of the pure samples in each case.

**2.5 Job's Spectrophotometric method:** According to Job's method [19] the absorbance of series of Montelukast Sodium with Amlodipine Besylate in different molar ratios 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1 were measured by keeping the total mole constant. The observed absorbance of the mixtures at various mole fractions was subtracted from sum of the values for free drugs (Montelukast Sodium and Amlodipine Besylate). The absorbance difference (D) was then plotted against the mole fractions of the drug in the mixtures. If the formation constant is reasonably favorable, two straight lines of different slopes that intersect at a mole ratio that corresponds to the combining ratio in the complex are obtained [20].

**2.6 Ardon's spectrometric method:** In the Ardon's Spectrophotometric method, [21] concentrations of Montelukast Sodium was varied while keeping the concentrations of Amlodipine Besylate fixed at 1 X $10^{-4}$ M. All the experiments were performed in buffer at pH 1.0, 3.0, 6.5 and 6.8. The absorbances of solutions were measured at 283 nm using UV-VIS spectrophotometer. The Ardon's equation was used for calculation. This equation is given below-

$1/[D-\varepsilon AC]=1/KC(\varepsilon com -\varepsilon A)[B]+1/C.(\varepsilon com -\varepsilon A)$

Where, D = Absorbance of the mixture, B = Molar concentration of the Montelukast Sodium. C = Molar concentration of the other drug $\in_{com}$= Molar extinction co-efficient of the complex, $\in_A$ = Molar extinction co-efficient of the Montelukast Sodium.

The value of n was chosen as 1, which is an essential condition for validation of the method. The value for $1 / (D - \in_A C)$ was plotted versus 1 / D to get the straight lines.
The stability constant of the complex was given by the relation, K = intercept / slope
It is to be mentioned that this method is only valid for the systems where 1:1 complexes are found.

**2.7 Statistical analysis:** The results were expressed as the mean. Regression analysis was performed to

calculate slope and intercept. P<0.05 and P<0.001 were considered as statistically significant. Student's t test was performed between Stability Constants. Statistical programs used were GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA).

## 3. RESULTS

**3.1 Spectral study:** The drugs studied showed absorption in UV-VIS region. The molecular species of Montelukast Sodium when mixed with Amlodipine Besylate showed some changes in absorption characteristics of this molecule (Amlodipine Besylate) including some shifts in the absorption maxima. Thus alteration in spectral pattern may be regarded as an indicator for the primary interaction among these drugs. The UV absorption values of the drug and drug mixtures were measured at 200-400 nm. 1 ml of $10^{-4}$ M Montelukast Sodium and 1 ml of $10^{-4}$ M Amlodipine Besylate were mixed and absorbances were measured within the range of 200-400 nm. Before that individual absorbance of $10^{-4}$ M Montelukast Sodium and Amlodipine Besylate were measured (Figure 1).

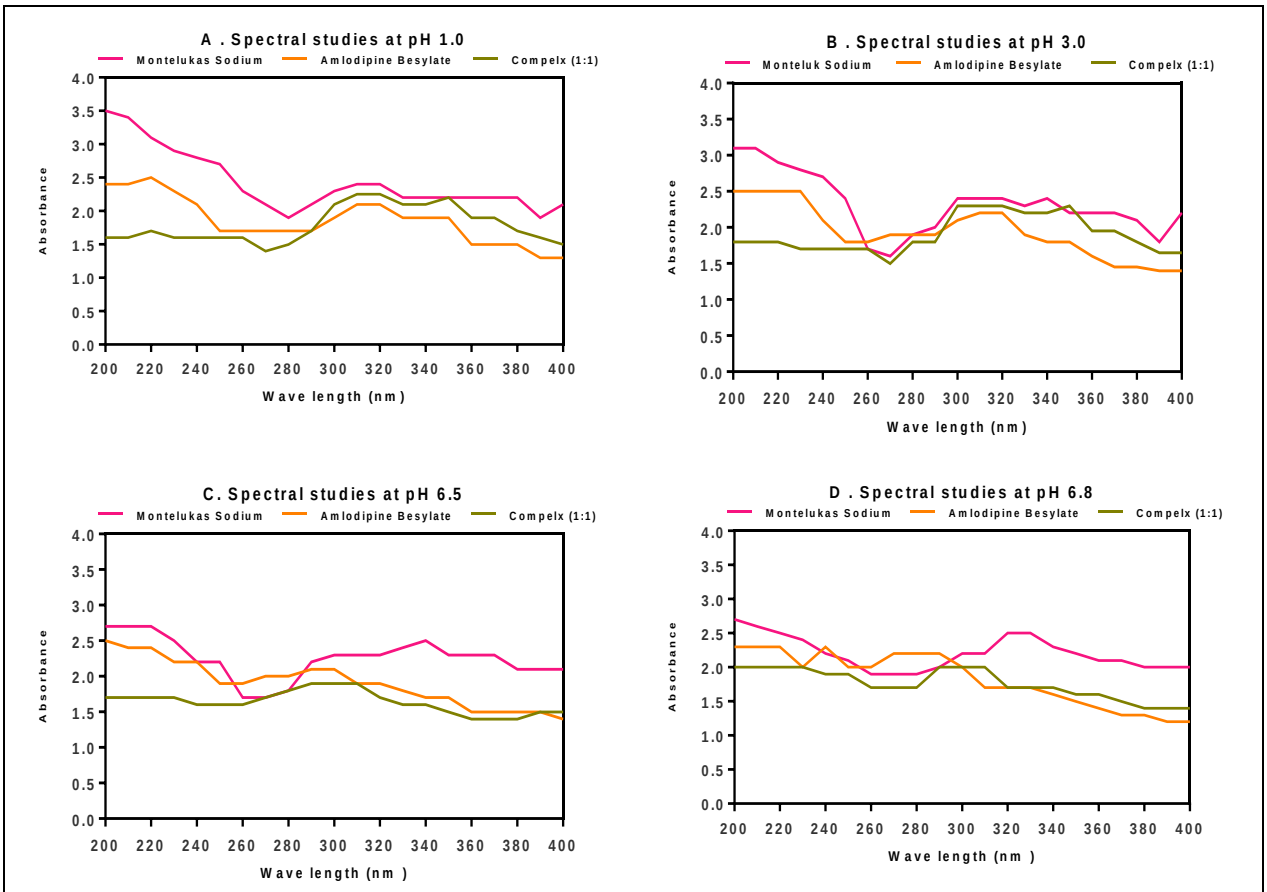


**Figure 1: Spectral studies of Montelukast Sodium, Amlodipine Besylate and their (1:1) complex at different pH.**

### 3.2 Study of Job's method

The molar ratios of the complexes of Montelukast Sodium with Amlodipine Besylate were estimated by Job's method of continuous variation. The observed absorbance values were measured in pH 1.0, 3.0, 6.5 and 6.8 at various concentrations (0.1 x $10^{-4}$ to 0.9 x $10^{-4}$ M) Montelukast Sodium with Amlodipine Besylate of at 283 nm. The Job's plots at pH 1.0, 3.0, 6.5 and 6.8 were obtained by plotting absorbance differences against the mole fraction of the drug (Montelukast Sodium) which are presented in Table 1and Figure 2.

**Table 1: Absorbance of Montelukast Sodium at different pH (using Job's method)**

| Concentration of Montelukast Sodium (1 X $10^{-4}$) | Absorbance(D value) | | | |
|---|---|---|---|---|
| | pH 1.0 | pH 3.0 | pH 6.5 | pH 6.8 |
| 1 | 1.584 | 1.822 | 1.436 | 1.705 |
| 2 | 1.488 | 1.772 | 1.432 | 1.531 |
| 3 | 1.374 | 1.652 | 1.352 | 1.407 |
| 4 | 1.260 | 1.538 | 1.209 | 1.344 |
| 5 | 1.073 | 1.381 | 1.082 | 1.174 |
| 6 | 0.903 | 1.195 | 0.858 | 1.054 |
| 7 | 0.726 | 0.961 | 0.730 | 0.835 |
| 8 | 0.569 | 0.773 | 0.524 | 0.554 |
| 9 | 0.421 | 0.378 | 0.315 | 0.353 |

Values are presented as Mean (n=3).

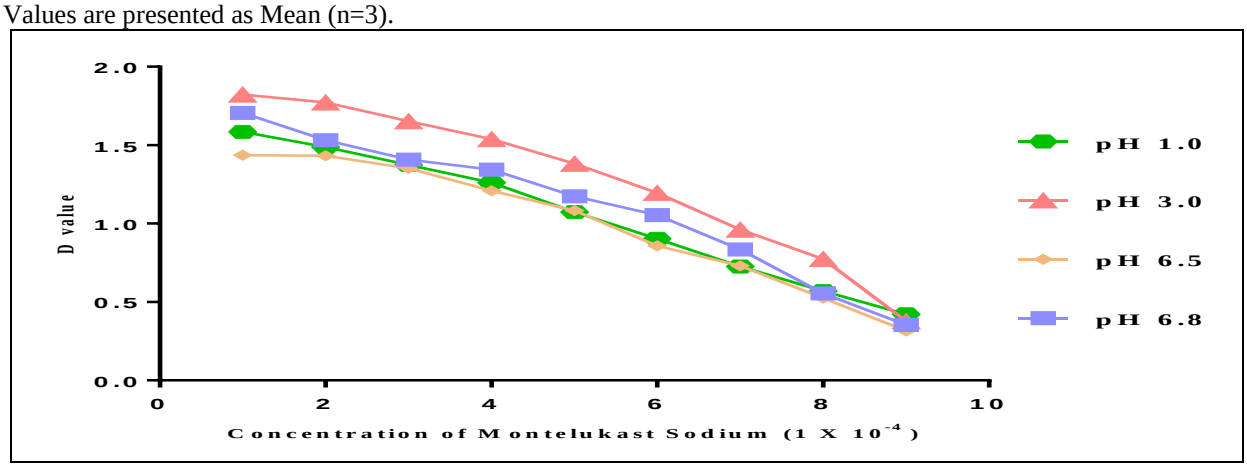


**Figure 2: Job's plot for complexation of Montelukast Sodium with Amlodipine Besylate at 300 nm.**

**3.3 Effect of Montelukast Sodium on Amlodipine Besylate using Ardon's method**

Ardon's plot confirmed the formation of 1:1 complex of Montelukast Sodium and Amlodipine Besylate at pH 1.0, 3.0, 6.5 and 6.8, since the method is valid only for 1:1 complexes. The values of 1/ [drug] by using the Ardon's equation:

1/[D-εAC]=1/KC(εcom -εA)[B]+1/C. (εcom –εA)

This experiment was performed in buffer systems pH 1.0, 3.0, 6.5 and 6.8.The data for Ardon's gave straight lines with intercept which are presented in Table 2 and Figure 3 indicate the formation of 1:1 complexes for the system at both pH.

**Table 2: Absorbance of Montelukast Sodium at different pH (using Ardon's method, when concentration of Amlodipine Besylate is constant)**

| Concentration of Montelukast Sodium (1/D X $10^{-4}$) | Absorbance (D value) | | | |
|---|---|---|---|---|
| | pH 1.0 | pH 3.0 | pH 6.5 | pH 6.8 |
| 1.000 | 27.02703 | 29.41176 | 21.73913 | 14.92537 |
| 0.500 | 12.19512 | 14.70588 | 9.52381 | 5.586592 |
| 0.333 | 6.578947 | 7.8125 | 4.672897 | 2.283105 |
| 0.250 | 5.405405 | 5.434783 | 3.610108 | 1.779359 |
| 0.200 | 4.032258 | 4.149378 | 2.673797 | 1.356852 |
| 0.167 | 3.401361 | 3.636364 | 2.283105 | 1.133787 |
| 0.143 | 2.415459 | 2.785515 | 2.109705 | 1.082251 |

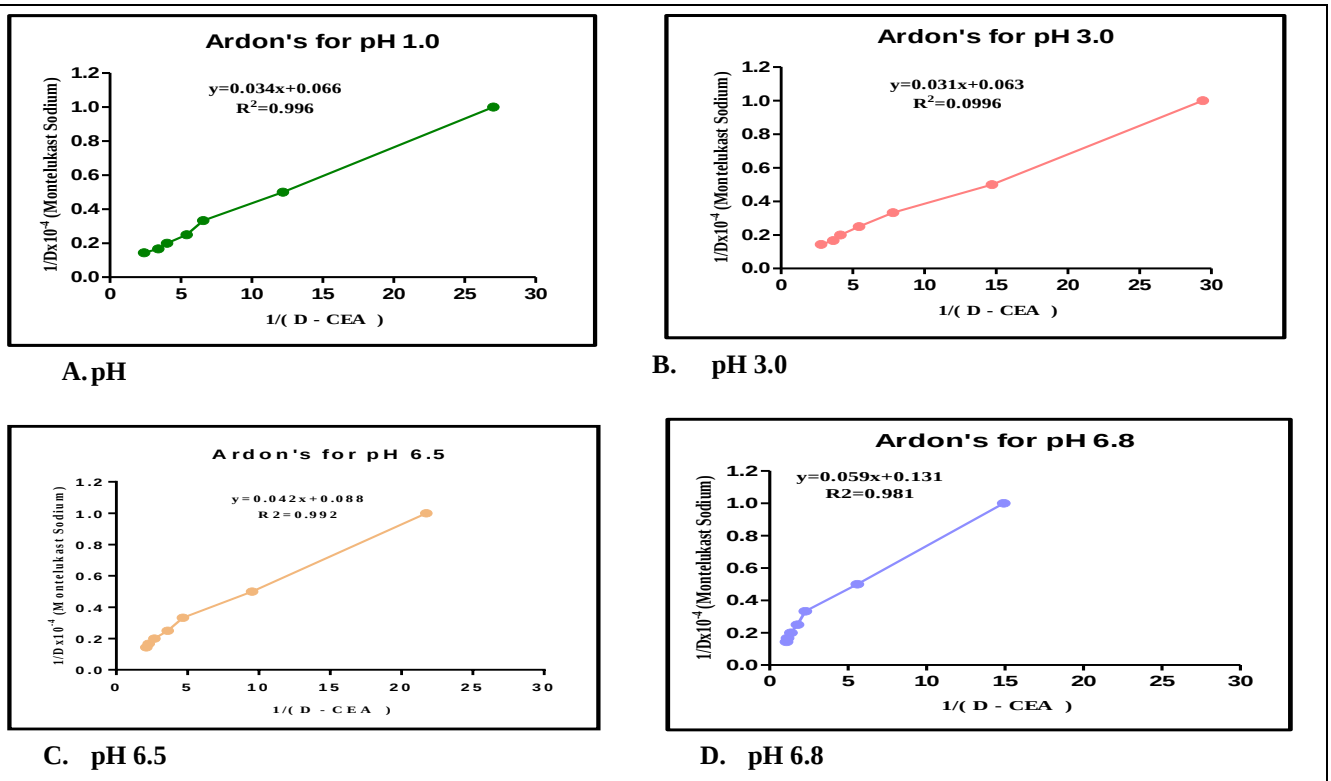


**FIGURE 3: Ardon's plot for complexation of Montelukast Sodium with Amlodipine Besylate at 300 nm for A. pH 1.0, B. pH 3.0, C. pH 6.5 and D. pH 6.8.**

### 3.4 Estimation of Stability Constant

The value of stability constant for the complexation of Montelukast Sodium with Amlodipine Besylate at pH 1.0, 3.0, 6.5 and 6.8 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 = (intercept) / (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-drug system at pH 1.0, 3.0, 6.5 and 6.8 presented in the table given below:

**TABLE 3: Stability constant of Montelukast Sodium with Amlodipine Besylate at different pH.**

| System | pH | Stability Constants |
|---|---|---|
| Interaction of Montelukast Sodium with Amlodipine Besylate | 1.0 | 1.9412** |
| | 3.0 | 2.0323** |
| | 6.5 | 2.0952* |
| | 6.8 | 2.2203** |

Values are the mean of triplicate experiments and represented as mean. Values in the same column with different superscripts (*) are significantly different $^{*}P < 0.05$ and $^{**}P < 0.001$. Student's t test was performed to analyze this data set.

## 4. DISCUSSIONS

Initial evidence for complexation of Montelukast Sodium with Amlodipine Besylate came from differences between the spectra of the drugs and those of their mixtures in buffer solutions. Each compound has its unique molecular structure or electronic configuration which is responsible for absorption of light. It is obvious that each compound has its unique molecular structure or electronic configuration which is responsible for absorption of light in the form of ultraviolet or visible form. When Amlodipine Besylate mixed with Montelukast Sodium in 1:1 ratio the intensity of the peak of Montelukast Sodium change remarkably (absorbance decreases) i.e. absorption characteristics are altered due to interaction but the position of the compound do not shift. The Ardon's plots have been used to evaluate the stability constants and it has been observed that when values of 1 / (D - CεA) are plotted against 1/ Drug (Figure 3), these lines are obtained obeying the Ardon's equation. The values of stability constants at different pH are shown in table 3. Stability constants data showed that Montelukast Sodium-Amlodipine Besylate system formed relatively stronger complexes at all pH conditions.

## 5. CONCLUSIONS

Interaction of Montelukast Sodium with Amlodipine Besylate decreased the free drug concentration of both drugs which can result in decreased availability of the drugs at receptors. Ultimately, one or both drugs may show diminished pharmacologic activity. Furthermore, Montelukast Sodium and Amlodipine Besylate lowered protein binding of Amlodipine Besylate, could increase the volume of distribution of Amlodipine Besylate. The results from all these allowed to conclude that Montelukast Sodium formed stable complex with Amlodipine Besylate.

### Competing interests

The authors declare that they have no competing interests.

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