

Evaluation of *in vitro* Interaction of Metformin with Ibuprofen in Aqueous Medium

Shuvashis Saha¹, Rehana Begum¹, Asma Rahman², Md. Zakir Sultan²,
Md. Shah Amran¹ and Md. Amjad Hossain¹

¹Department of Pharmaceutical Chemistry, faculty of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh

²Centre for Advanced Research in Sciences, University of Dhaka, Dhaka-1000, Bangladesh

Abstract

The purpose of the present study was to investigate the *in vitro* complex formation and to study the nature and strength of complexes which could be formed due to interaction of metformin with ibuprofen. Metformin is an antidiabetic drug and ibuprofen is a non steroidal anti-inflammatory drug (NSAID). The *in vitro* interaction of metformin with ibuprofen was studied at room temperature and at different pH conditions. The studies were performed by UV-Visible spectrophotometry, conductometry and reversed - it was phase high performance liquid chromatography (RP-HPLC). It was found that metformin formed stable 1:1 complex with ibuprofen. The interaction may greatly influence the activity of these molecules.

Keywords: Metformin, ibuprofen, interaction, complexation.

Introduction

Now a days, multiple drug therapy is a common and useful practice for the treatment of diseases where two or more drugs are given at the same time or concurrently. The drugs may exhibit effects independently or may interfere or interact with each other. The interaction may be potentiation or antagonism of one drug by another. Sometimes multiple drug therapy is beneficial to the patients and sometime it causes serious harmful effects. Thus the drug interaction study is very much important in respect to both bio-pharmaceutics and pharmacology (Alan *et al.*, 1996).

Knowledge of drug interaction may allow early recognition and prevention of adverse consequences. The most comprehensive understanding of clinically significant drug interaction can be achieved by combining knowledge of the mechanism of drug interaction with recognition of the high-risk patients and the identification of drug with a narrow therapeutic index. Problem arising from the interaction of drugs may be overcome by partial changes in the molecular pattern, by blocking the reactive site in the molecule, by changing the dosage regimen or by avoiding the combined application of interacting drugs.

However, to take any step to manage the interaction problems, the nature of interaction should be known. We should know the possible interaction of a new drug prior to use clinically. For the drugs which are being used conventionally, interaction studies are also very important to detect the problems yet to be found out (Philip *et al.*, 1989).

The purpose of the present study was to investigate *in vitro* complex formation and to study the nature and strength of complexes which could be formed due to interaction of metformin with ibuprofen. Metformin is an anti-diabetic drug. It is the first-line drug of choice for the treatment of type-2 diabetes, particularly in overweight and obese people and those with normal kidney function. Its mode of action is thought to be multifactorial and includes delayed uptake of glucose from the intestinal tract, increased peripheral glucose utilization mediated by increased insulin sensitivity and inhibition of increased hepatic and renal gluconeogenesis. On the other hand, ibuprofen is a NSAID. It is commonly used for the reduction of pain, fever, inflammation and stiffness caused by conditions such as osteoarthritis, kidney stones, rheumatoid arthritis, psoriatic arthritis, gout, ankylosing

spondylitis, menstrual cramps, tendinitis and the treatment of primary dysmenorrhea. It works by inhibiting both the COX-1 and COX-2 enzymes (Alan *et al.*, 1996).

Materials and Methods

Drugs and chemicals: The working standard of Metformin (potency: 99.27%) and Ibuprofen (potency: 99.25%) were the gifts from Beximco Pharma Ltd, Dhaka, Bangladesh. Hydrochloric acid (37%), potassium chloride, orthophosphoric acid, sodium hydroxide, potassium hydroxide, potassium dihydrogen orthophosphate, disodium hydrogen orthophosphate, methanol, ethanol were of analytical grade and purchased from local suppliers.

Preparation of buffer solutions (Bates, 1973; Perrin, 1979)

pH 1.4: This buffer was prepared by mixing 6.57 g of potassium chloride with 119.0 ml of 0.1M hydrochloric acid and diluted up to 1000 ml with demineralized water. Then pH was adjusted to 1.4 with hydrochloric acid. 250 ml of 0.1M hydrochloric acid was prepared by mixing 2.25 ml of 37% hydrochloric acid with demineralized water.

pH 2.4: It was prepared by mixing 6.7 ml of orthophosphoric acid with 50.0 ml of 4% v/v solution of 2M sodium hydroxide and diluted to 1000 ml with demineralized water. pH was adjusted to 2.4 with sodium hydroxide. 100 ml of 2M sodium hydroxide was prepared by dissolving 8.0 g of sodium hydroxide in demineralized water and standardized with oxalic acid.

pH 7.4: This buffer was prepared by mixing 65.4 ml of 0.02M potassium dihydrogen orthophosphate with 289.7 ml of 0.01M disodium hydrogen orthophosphate and diluted to 1000 ml with demineralized water. 100 ml of 0.02M potassium dihydrogen orthophosphate was prepared by dissolving 0.2722 g of potassium dihydrogen orthophosphate in demineralized water and the final volume up to 100 ml. 500 ml of 0.01M disodium hydrogen orthophosphate was prepared by dissolving 0.710 g of disodium hydrogen orthophosphate in demineralized water.

Preparation of stock solutions

Metformin: 100 ml stock solution of 1×10^{-3} M was prepared by dissolving 0.0129 g of metformin in

demineralized water and added 1-2 drops of concentrated hydrochloric acid to dissolve it and finally made the volume up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solutions.

Ibuprofen: 100 ml stock solution of 1×10^{-3} M was prepared by dissolving 0.0206 g of ibuprofen in methanol and demineralized water. Then the volume was made up to 100 ml with the same solvent. The stock solution was diluted to desired strength by buffer solutions.

Drug-drug interaction analysis by observation of absorption spectra: In this procedure, the ultraviolet absorption characteristics of metformin, ibuprofen and their 1:1 mixture in solution at different pHs were taken for comparison. The concentration of samples was kept at very dilute level in each case and the measurements were made using the UV-visible spectrometer (UV-1601, Shimadzu, Japan) equipped with a computer having appropriate software program. The stock solutions of the sample were diluted to appropriate levels by buffer of the desired pH and the spectra were recorded between 200-400 nm.

Drug-drug interaction analysis by Job's method of continuous variations: Job's spectroscopic method of continuous variation plots was carried out to confirm the formation of 1:1 complex between the drugs studied. In this method, solutions of different concentrations of metformin and ibuprofen were prepared using solvent and continuous plots were made by corrected absorbance against the volume fraction of one reactant.

Drug-drug interaction analysis by conductometric titration method: Conductance is an inherent property of an ionic species. The conductance of one species may change due to the interaction with other species. In this method, conductance was changed due to the interaction of varying molar ratios of the species in a mixture. Conductometric titrations in demineralized water system at pH 7.4 were carried out using a Conductometer (Jenway, Switzerland) to find the molar ratios at which complexation occurred. For this 35 ml of 0.05M solution of metformin was taken in a beaker and was titrated individually with gradual addition of 0.05M solution of ibuprofen from a burette. Two titrations were carried out; one was titrated against the other and vice-versa. The conductance at each addition was recorded. Then the

conductance was plotted against molar ratios of the titrants to obtain the conductivity curves. The titration curve showed break at the points of possible interaction.

Drug-drug interaction study by RP-HPLC method:

High-performance liquid chromatography is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components of the mixture. Retention time and absorbance peak of one species in solution may be changed due to the interaction with other species. In the present study, analyses of metformin and ibuprofen were carried out, using HPLC (Shimadzu, Japan) at pH 7.4 with a concentration of 50 μ g/ml where combination of metformin with ibuprofen was 1:1 molar ratio (50 μ g/ml). The studies were conducted twice.

Results and Discussion

In the present study, various methods of analyses were carried out for the determination of drug-drug interactions of metformin with ibuprofen. These methods were the spectroscopic methods of analysis, spectral characteristics, conductometric titrations, Job's method of continuous variations and RP-HPLC. All of these methods of analyses revealed the formation of complexes among the studied drugs.

The drugs studied showed absorption in UV-VIS region. The molecular species of ibuprofen when mixed with metformin, showed some changes in absorption characteristics of this molecule (ibuprofen), 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. The spectra of metformin alone at different pH conditions showed an absorption maximum at 228 nm.

An aliquot of 1 ml of 0.005M metformin and 1 ml of 0.005M ibuprofen were mixed and absorbances were measured within the range of 200-400 nm at pH 1.4. Before that individual absorbance of 0.005M metformin and ibuprofen were measured (Figure 1).

Then 1 ml of 0.005M metformin and 1 ml of 0.005M ibuprofen were mixed and absorbances were measured within the range of 200-400 nm at pH 7.4. Before that

individual absorbance of 0.005M metformin and ibuprofen were measured (Figure 2).

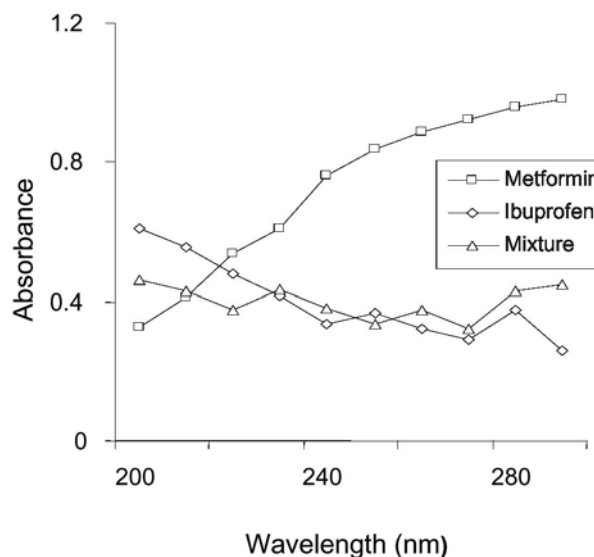


Figure 1. UV spectra of metformin, ibuprofen and their 1:1 mixture at pH 1.4.

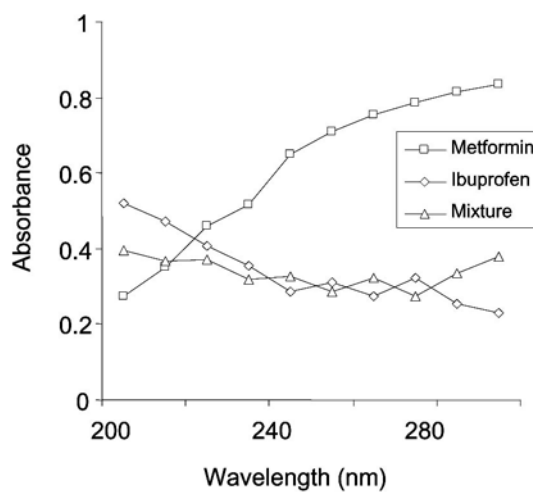


Figure 2. UV spectra of metformin, ibuprofen and their 1:1 mixture at pH 7.4.

From the graphs we found that absorbance of individual metformin and ibuprofen varied with the absorbance of their combination. The graph for combination showed a zigzag line, which was nearest to ibuprofen. It indicated that 1:1 mixture of metformin with ibuprofen showed noticeable changes in the absorption intensities due to interaction. The intensities of absorbance peaks also varied with pH. Change in pH from acidic to basic, shifted the absorbance to higher wavelengths. This

ensured that pH change had significant effects on the interaction between these drugs.

Job's spectroscopic method of continuous variations plots was carried out to confirm the formation of 1:1 complexes between the drugs to be studied. These plots (Figure 3 and 4) gave 'Λ' shaped curve indicating the formation of 1:1 complexes for all systems. When metformin was at a higher concentration compared to ibuprofen in the mixture, the absorbance decreased to the larger extent and the difference became negative indicating a stable interaction.

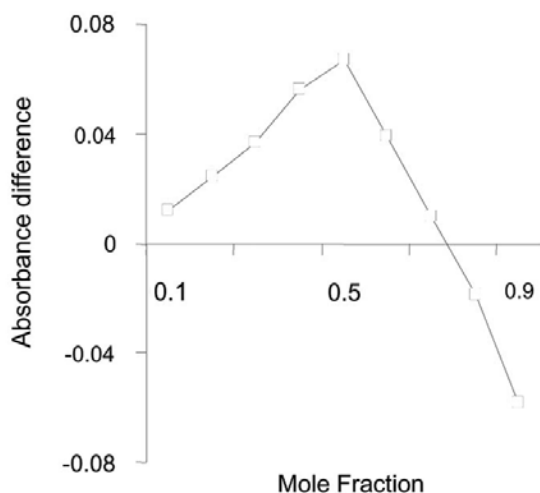


Figure 3. Job's plot for metformin-ibuprofen system at pH 7.4.

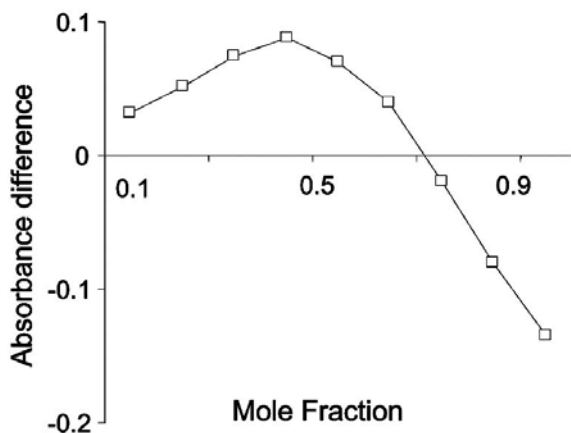


Figure 4. Job's plot for metformin-ibuprofen system at pH 2.4.

The conductance was plotted versus the molar ratios of the titrants for obtaining conductivity curves (Figure 5).

When metformin was titrated with ibuprofen at pH 7.4, the conductivity curve showed distinct break at 1:1 molar ratio. The reverse titration curve also showed a break at 1:1 molar ratio. Thus, the formation of a stable 1:1 complex of metformin with ibuprofen was indicated.

Chromatogram of metformin was taken under following conditions: 10% acetonitrile and 90% phosphate buffer (as mobile phase), C8 column, flow rate 0.7 ml/min, UV 254 nm, injection volume 20 μ l. The obtained chromatogram showed that retention time for metformin was 2.062 min (Figure 6).

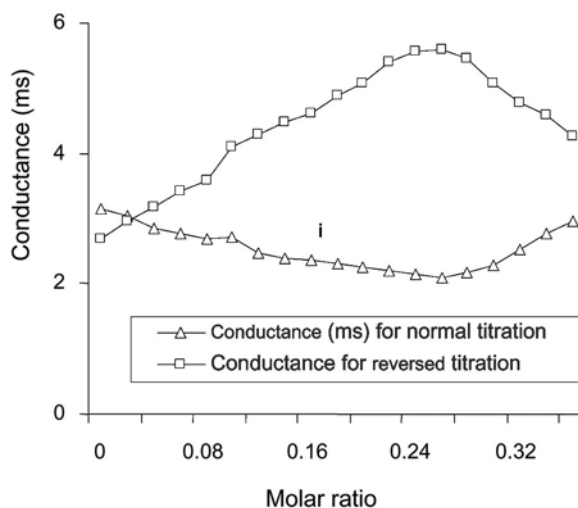


Figure 5. Conductometric titration of metformin with ibuprofen at pH 7.4.

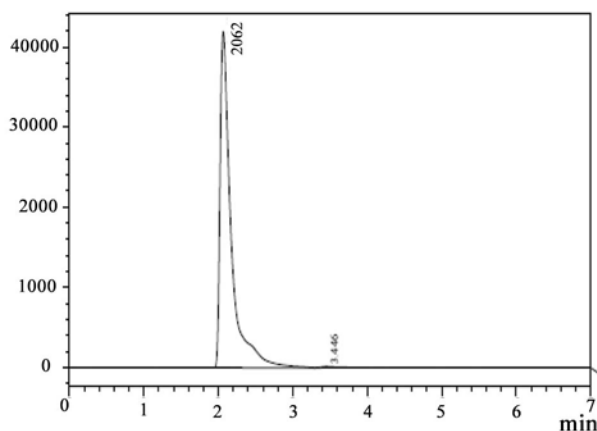


Figure 6. HPLC plot for metformin at pH 7.4.

Chromatogram of ibuprofen was taken under following conditions: 20% acetonitrile and 80% phosphate buffer (as mobile phase), C-8 column, flow rate 0.6

ml/min, UV detection at 215 nm, injection volume 5 μ l. The obtained chromatogram showed that retention time for ibuprofen was 2.46 min. (Figure 7).

Chromatogram of mixture (1:1) of metformin and ibuprofen was taken under following conditions: 10% acetonitrile and 90% phosphate buffer (as mobile phase) 10 min, 10% acetonitrile to 80% acetonitrile for 7 min, 80% acetonitrile for 8 min, C-8 column, flow rate 0.7 ml/min, UV 254 nm, injection volume 20 μ l. The obtained chromatogram showed that retention time for mixture (1:1) was 3.705 min. (Figure 8).

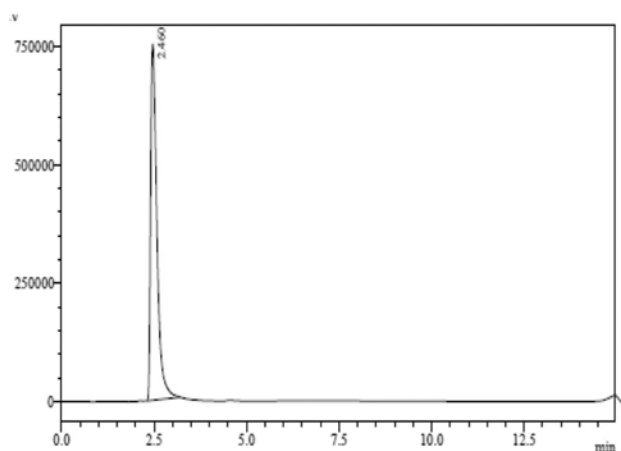


Figure 7. HPLC plot for ibuprofen at pH 7.4.

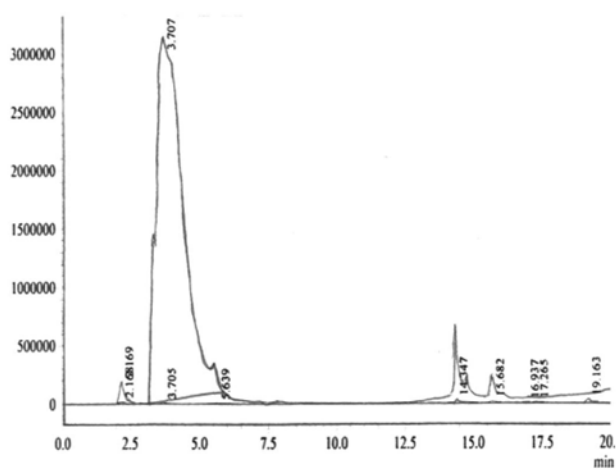


Figure 8. HPLC plot for metformin and ibuprofen at pH 7.4.

In case of the combination of metformin with ibuprofen (1:1) retention time shifted to 3.705 min, which is higher than metformin and ibuprofen alone. It indicated a possible interaction between metformin and ibuprofen.

Moreover, from the graph we also found appearance of more peaks at times 2.168, 5.639, 14.347 and 15.682 min indicating different species in the mixture. Thus, it can be said that a good number of combination products might result due to interaction of metformin with ibuprofen. These results are comparable to the previous studies (Mohiuddin *et al.*, 2009; Ahsan *et al.*, 2011, 2012; Siraji *et al.*, 2012).

Conclusion

From the obtained results and above discussion it can be concluded that metformin formed stable complex with ibuprofen. Therefore, precaution and monitoring must be exercised during concurrent therapy of both drugs.

Acknowledgement

The authors would like to express their gratitude to the authority of Centre for Advanced Research in Sciences (CARS) for their co-operation to carryout some of the experiments in their laboratory.

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