

***In vitro* Interaction of Metronidazole and Mebendazole with Copper (II) and Chromium (III) in Aqueous Media**

F. Siraji¹, A. T. M. Z. Azam¹, M. S. Amran¹, J. N. Islam², F. M. Amjad³, and M. A. Hossain*

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

²Research Center for Mathematical and Physical Sciences, University of Chittagong, Chittagong, Bangladesh

³Department of Surgery, Dhaka Medical College Hospital, Dhaka-1000, Bangladesh

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Abstract

The interaction of metronidazole, an antiprotozoal agent and mebendazole, an important anthelmintic agent with Cu (II) and Cr(III) ions have been studied in aqueous systems at a fixed temperature of $37\pm 0.5^\circ\text{C}$ and at different pH values. It has been found that metronidazole and mebendazole form stable 1:1 complexes with Cu(II), but a less stable complex with Cr(III) ion. The studies were performed by electroanalytical and spectrophotometric methods. Spectral studies helped to detect initial complexation between the species. Conductometric titration indicated the nature of interaction and the stoichiometries of complexation. The conductometric study also indicated the formation of complexes of various molar ratios viz 1:2, 4:1, 3:1, 1:2, 1:3 and 1:4. etc. along with 1:1 complexes at various pH conditions. Similar types of complexation were observed by Job's plots at various pH values. The Ardon's spectroscopic method confirmed the 1:1 complexation and the values of stability constant were calculated using Ardon's plot. Complexation at molar ratios 1:3 and 1:4 can not be ruled out.

Keywords: Metronidazole; Mebendazole; Cu(II); Cr(III); Ardon's plot; Complexation.

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1. Introduction

The study on interaction and complexation of drug molecules with other drugs species and various metal ions is an important field of research in the chemical, biochemical and medicinal point of views [1,2]. Binding and complexation of drug molecules and medicinal substances with metal ions and other species remarkably influence the bioavailabilities and biochemical properties of drugs in our body. Our body possesses a large number of metals to operate its normal physiological activities. We also intake a

*Corresponding author: arshi15du@yahoo.com, zafrulazam@yahoo.com

number of metals in the dosage forms, diet, drinks and other ways and they come in close contact with different drugs and biomolecules present in the dietary and body systems. Drug interaction followed by complexation with metal ions results from donor-acceptor mechanism or Lewis acid-base reaction between two or more different chemical entities or medicinal agents [3-5]. Accordingly, Haider and co-workers [6] investigated the complexation of thiamine (vitamine B₁)-HCl with Co(II), Cu(II), Zn(II), Cd(II) and Mg(II) in the aqueous MeOH media. Meanwhile, we have studied the complexation of barbitone with Cd(II), Ni(II) and Cr(III), of dopamine and diphenyl carbazide with Cu(II) and Zn(II) and complexation of similar other molecules with metal ions in the aqueous and mixed media under varying conditions [7-12]. In our continuing studies to find out the interactions of other drugs molecules with metals ions, here we report the interaction of metronidazole, an antiprotozoal agent [13] and mebendazole, an important anthelmintic agent [13] with Cu(II) and Cr(III) ions.

2. Experimental

2.1. Materials

Metronidazole and mebendazole (product grade) have been obtained from the manufacturer, Drug International, Dhaka, Bangladesh for this study. Copper (II) chloride and chromium (III) chloride were used in this study without further purification. Potassium dihydrogen phosphate, disodium hydrogen phosphate, potassium chloride (analytical grade, BDH, England), hydrochloric acid (AR, China), acetic acid (90%), ethanol ((analytical grade, MERK, Germany) have been used without further purification. Deionized water was used for preparation of working solutions.

2.2. Equipments

UV-Visible Spectrometer (UV – 1601, Shimadzu, Japan), pH meter (Mettler Toledo, Switzerland), conductometer (Mettler Toledo, Switzerland), power sonic (Model No. 510, Seoul, Korea) have been utilized in this study.

2.3. Methods

The *in vitro* interaction study of metronidazole and mebendazole with Cu(II) and Cr(III) ions has been studied by observing absorption spectra, conductometric data, Job's continuous variation [14] and Ardon's spectrophotometric methods [15].

2.4. Spectrophotometric analysis

The initial detection of complex formation of metronidazole and mebendazole with Cu(II) and Cr(III) ions was accomplished by comparing the absorption spectra of pure

compounds and their 1:1 mixtures in buffer solutions at pH 0.8, 1.4, 2.4, 6.4 and 7.4. The concentrations of the samples were kept at very dilute levels in each case and the measurements of the spectra of pure sample as well as their mixtures were made using UV–VIS automatic recording instrument with a constant temperature cell compartment.

Conductometric titration in demineralized system at different pHs were carried out to find out the molar ratios of metronidazole and mebendazole with Cu(II) and Cr(III) at which complexation occurred. Here, 40 ml of 5×10^{-3} M metronidazole or mebendazole solution was taken in a 100 mL beaker and titrated with gradual addition of 5×10^{-2} M solution of Cu(II) or Cr(III) from a burette. Reversely 40 mL each of 5×10^{-3} M solution of Cu(II) or Cr(III) was titrated with gradual addition of 5×10^{-2} M metronidazole or mebendazole solution under similar conditions.

The conductance values (ms or μ s) were plotted against molar ratios of the conducting species in the system. The titration curves showed breaks at the points of possible interaction. All the titrations were performed with solutions adjusted to pH 0.8, 1.4, 2.4, 6.4 and 7.4 using a pH meter.

In Job's spectrophotometric method of continuous-variation, absorbances of series of metro.-Cu(II), metro.-Cr(III) mixtures with various molar ratios at pH 0.8, 1.4, 2.4, 6.4 and 7.4 were measured at 277 nm (for lower pH) and at 328 nm (for higher pH) by keeping the total moles constant in the mixture. Similar experiments were performed for meben.-Cu(II) and meben.-Cr(III) mixtures at pH 0.8, 1.4, 2.4 and the absorbances of these systems were measured at 290 nm. The observed absorbances of the mixtures at various mole fractions were subtracted from the sum of the values for free drug and free ligand under the same conditions. 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 indicates the molar ratios of drug to ligand in the complex.

In the Ardon's Spectrophotometric method, concentrations of metronidazole and mebendazole were varied while the concentrations of the metal was kept fixed (2×10^{-4} M). The absorbance of free metronidazole solutions having pH 0.8, 1.4 and 2.4 were measured at 277 nm and solutions having pH 6.4 and 7.4 were measured at 328 nm using UV–VIS recording spectrophotometer. Similarly, the absorbance of free mebendazole solutions having pH 0.8, 1.4 and 2.4 were measured at 290 nm. For calculations, the Ardon's equation was used. This equation is given below :

$$1/(D - E_A C) = 1/ KC(E_{com} - E_A) [B]^n + 1/ C(E_{com} - E_A)$$

where,

D = absorbance of mixture,

C = molar concentration of the drug,

B = molar concentration of the metal,

E_{com} = molar extinction co-efficient of the complex, and

E_A = molar extinction co-efficient of the drug.

The value of n was chosen as 1, which is an essential condition for validation of the method. The value for $1/(D - E_A C)$ was plotted versus $1/[C]$ to get the straight lines. The concentrations of Cu(II) and Cr(II) were kept constant at 2×10^{-4} M (denoted by B in the equation) and the concentration of interacting species (metro. and meben.) was varied (denoted by C 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-

$$K = \text{intercept /slope}$$

It should be mentioned that this method is valid only for the systems where 1:1 complexes are found.

2.5. Statistical analysis

The results were expressed as mean \pm S.E.M. values for each experiment. Differences in mean values between experimental groups were analyzed by unpaired 't' test. A probability values less than 0.05 ($p < 0.05$) was defined to be significant.

3. Results and Discussions

In this study, we have chosen Cu(II) and Cr(III) as intercalating metals with metronidazole and mebendazole, since copper and chromium are essential trace elements and traces are present in various foods. Copper could facilitate iron absorption and stimulate enzymes that are involved in the heme and/or globin biosynthesis pathways [16]. On the other hand, chromium was found necessary for optimal growth of experimental animals, in larger quantities it is toxic [17]. In addition we also take multivitamins and minerals preparations containing copper and chromium.

In spectral observation analysis, each of the drugs studied showed absorption in UV-VIS region. The initial detection of complexation on metronidazole and mebendazole with Cu(II) and Cr(III) ions was done from the nature of the spectra of pure compounds as well as their 1:1 mixtures in different buffer solutions at fixed concentration (1×10^{-4} M). Metronidazole gave a sharp peak at 328 nm and a valley at 267 nm at pH 2.4, 6.4 and 7.4. The peak intensities decreases at pH 2.4. Whereas, it gave a sharp peak at 277 nm and a valley at 245 nm at pH 0.8 and 1.4. When Cu(II) and Cr(III) were mixed with metronidazole in 1:1 ratio, the intensities of peaks of metronidazole change remarkably i.e absorption characteristics are altered due to interaction but the λ_{max} remained unchanged. (Fig. 1a). This is may be due to the interaction between two compounds which may lead to form complex(es) having different light absorption capacity. A peak is observed at 212 nm due to the solvent ethanol.

On the other hand, mebendazole gave a sharp peak at 290 nm and a valley at 264 nm at pH 0.8, 1.4 and 2.4. But when mixed with Cu(II) and Cr(III) in 1:1 ratio the intensity of

these peaks changed remarkably due to the interaction with metals (Fig. 2b). A peak is observed at 212 nm due to the solvent acetic acid.

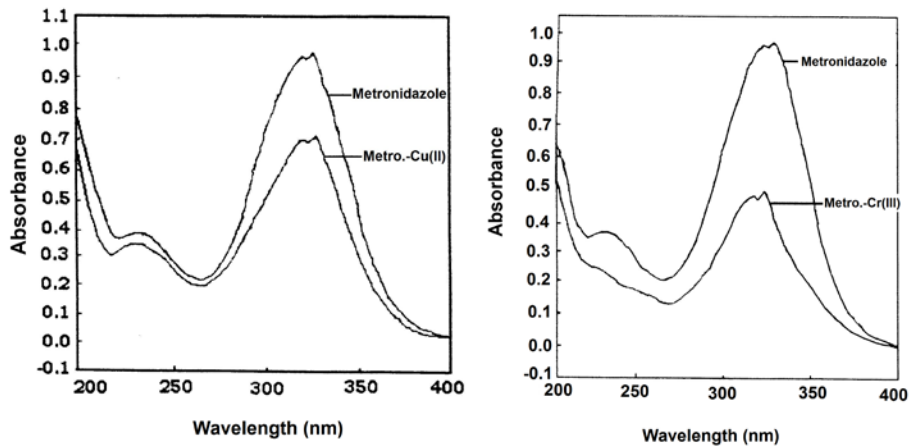


Fig. 1a. Influence of Cu(II) and Cr(III) on metronidazole at pH 7.4.

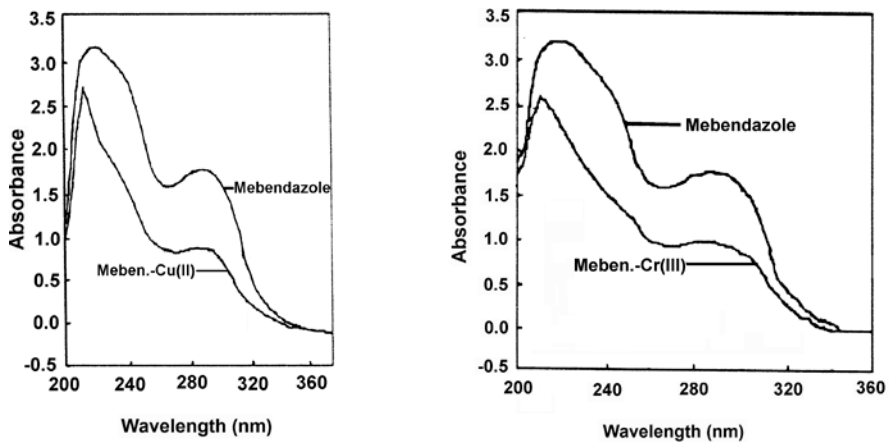


Fig. 1b. Influence of Cu(II) and Cr(III) on mebendazole at pH 1.4.

Conductometric measurement in ethanol-deionized water showed that both the drug molecules interact with Cu(II) and Cr(III) ions at various molar ratios including 1:1. This is a proof of complex formation of the drug molecules with the metal ions [18]. When metronidazole solution was titrated with Cu(II) it showed two distinct breaks at ratios 2:3 and 1:4 and reverse titration gave break at ratios 1:1 and 7:2. This indicate that metronidazole form stable complex with Cu(II) at 1:1 molar ratio and unstable complexes or intermediates at ratio 1:4 and 7:2. When metronidazole solution was titrated with

Cr(III) it showed one distinct break at ratios 2:3 and reverse titration gave two breaks at ratios 5:6 and 2:9 (Fig. 2a, b). This indicates that metronidazole form less stable complex with Cr(III) ion at 1:1 molar ratio and unstable complex or intermediate at 2:9.

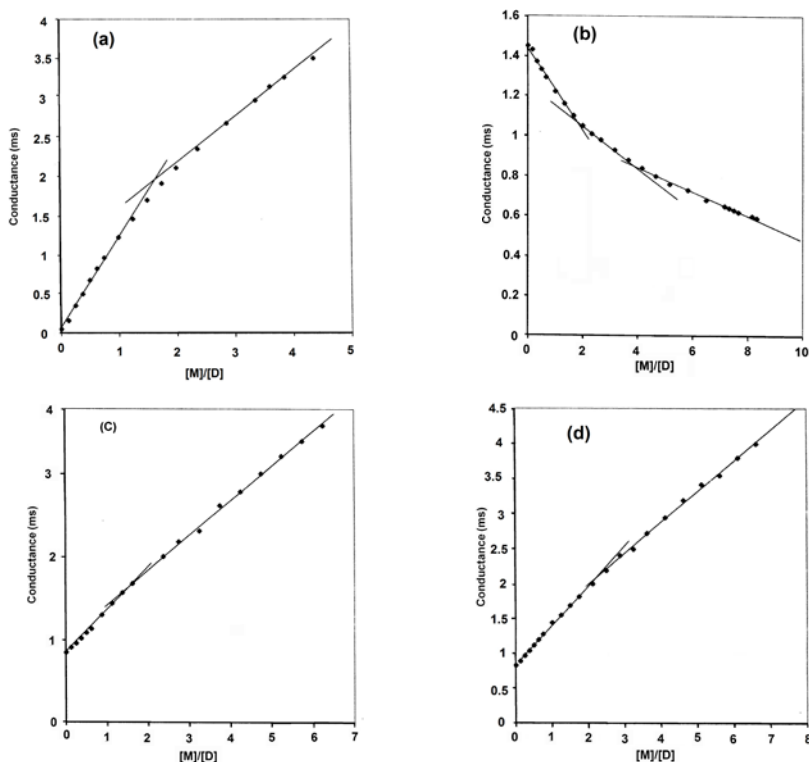


Fig. 2. Conductometric titration of metronidazole and mebendazole with Cu(II) and Cr(III); (a) metronidazole with Cr(III), (b) Cr(III) with metronidazole, (c) mebendazole with Cu(II), and (d) mebendazole with Cr(III).

But in the case of conductometric titration of mebendazole with Cu(II) and Cr(III) ions, no clear indication of interaction and formation of stable 1:1 complex was obtained, though formation of many intermediates were noticed. But the reverse mebendazole-Cu(II) and mebendazole-Cr(III) system showed the formation of less stable 1:1 complex. When mebendazole solution was titrated with Cu(II) it showed one faint break at ratio 2:3 and reverse titration gave two distinct breaks at ratios 7:10, 31:10 and one faint break at ratio 8:5 (Fig. 2c). So, it can be inferred that the intermediates lead to 1:1 complex, which are seemed to be unstable. But when mebendazole solution was titrated with Cr(III) it showed one faint break at ratio 4:9. It appears that this complex is very unstable and the reverse titration gave three distinct breaks at ratios 1:5, 3:4 and 13:10 indicate that mebendazole form less stable complex with Cr(III) ion at 1:1 molar ratio and unstable complexes or intermediates at ratios 51:20 and 4:9 (Fig. 2d).

The molar ratios of the complexes of metronidazole and mebendazole with Cu(II) and Cr(III) ions were estimated by Job's spectroscopic methods of continuous variation. The Job's plots were obtained by plotting absorbance differences against the mole fraction of the each drugs. At, pH 2.4, metronidazole (Fig. 3a) and mebendazole (Fig. 3b) formed 1:1 stable complex with Cr(III) ion. On the other hand, mebendazole-Cu(II) formed 1:1 stable complex but metronidazole-Cu(II) formed 2:3 complex at pH 2.4.

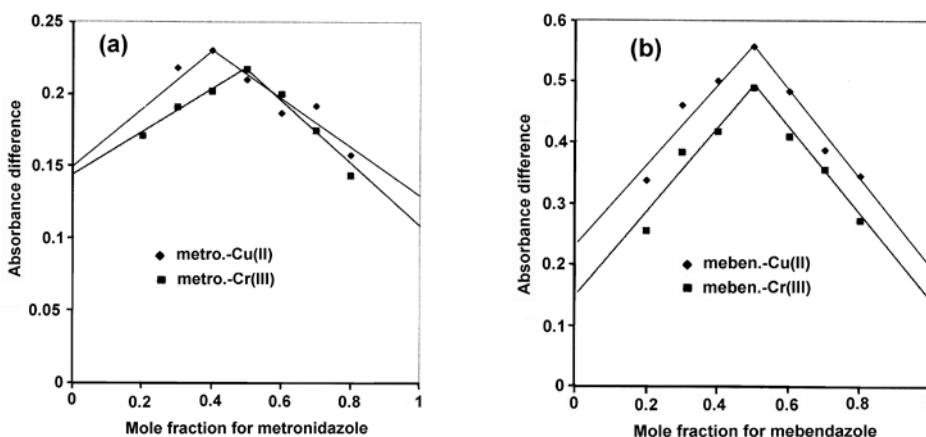


Fig. 3. Job's plot for complexation. (a) metro.-Cu(II) & metro.-Cr(III) at pH 6.4 and (b) meben.-Cu(II) and meben.-Cr(III) at pH 2.4.

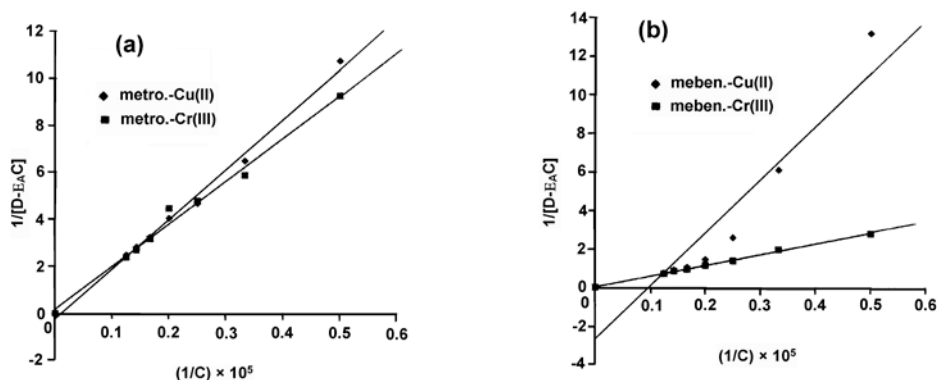


Fig. 4. Ardon's plot. (a) metro.-Cu(II) and metro.-Cr(III) at pH 2.4 and (b) meben.-Cu(II) and meben.-Cr(III) at pH 2.4.

Ardon's plots confirmed the formation of 1:1 complexes of metronidazole and mebendazole with Cu(II) and Cr(III). The data for Ardon's plots was given straight lines with different intercepts, indicating the formation of 1:1 complexes for all systems. Stability constants for Metro.-Cu(II), Metro.-Cr(III), Meben.-Cu(II) and Meben.-Cr(III)

systems were obtained from the Ardon's plots, which are straight lines. The stability constants were calculated from the slopes and intercepts of these plots and shown in Table 1. The Ardon's plots for Metro.-Cu(II), and Metro.-Cr(III) systems (Fig. 4a) and Meben.-Cu(II) and Meben.-Cr(III) (Fig. 4b) are shown at pH 2.4.

Table 1. The stability constants for different systems.

Systems	Stability constants ($K \times 10^{-2}/\text{mole}$)				
	pH 0.8	pH 1.4	pH 2.4	pH 6.4	pH 7.4
Metro. + Cu(II)	1.7	3.59	0.99	0.62	4.28
Metro. + Cr(II)	0.296	0.164	1.1	4.21	0.78
Meben. + Cu(II)	0.293	0.35	0.4	-	
Meben. + Cr(III)	2.156	1.31	9.64	-	

The stability constants obtained from the Ardon's plot shows that Metro.-Cu(II) system forms stable complex at pH 1.4 and pH 7.4. But, Metro.-Cr(III) forms stable complex at pH 6.4 having high stability constants. On the other hand, the stability constant for meben.-Cr(III) system is higher than all other systems at all pH values. So, we can conclude that metronidazole forms stable complex with both Cu(II) and Cr(III). However, mebendazole forms stable complex with Cr(III) ion and less stable complex with Cu(II) ion at lower pH. An inspection of the structures of the two ligands also indicate the justification of these observations.

4. Conclusion

It has been observed that the stability constants for metro.-Cu(II) system and metro-Cr(III) systems at higher pH were higher than that of metro.-Cu(II) system and metro.-Cr(III) system at lower pH conditions, indicating a weak complexation at a lower pH. Again, it was also observed that, complexation between metro-Cr(III) system at lower pH is too weak than metro.-Cu(II) system. The stability constant for meben.-Cr(III) system at lower pH was higher than that of meben.-Cu(II) system, indicating a stable 1:1 complexation between meben.-Cr(III) at lower pH.

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