International Journal of Research and Reviews in Pharmacy and Applied science

www.ijrrpas.com

IN VITRO EFFECTS OF COPPER (II) AND CHROMIUM (III) ON THE PROTEIN BINDING OF METRONIDAZOLE AND MEBENDAZOLE IN AQUEOUS MEDIA

Fouzia Siraji[1], A.T.M. Zafrul Azam[1], Md. Gias Uddin[1], Rehana Begum[1], Md. Shah Amran[1], Jamal Nazrul Islam[2] and Md. Amjad Hossain[1]*

Department of Pharmaceutical Chemistry, Faculty of Pharmacy

University of Dhaka, Dhaka-1000, Bangladesh

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

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

ABSTRACT

An in vitro study of protein binding of metronidazole and mebendazole and their 1:1 mixtures with Cu(II) and Cr(III) have been carried out by equilibrium dialysis method using spectrophotometer at 37± 0.5 °C and at pH 7.4 and 2.4. It has been found that the interaction of species with Cu(II) results into lowering the affinity and the percentage of protein binding of metronidazole to bovine serum albumin. On the other hand, interaction of Cu(II) with mebendazole increase the affinity and the percentage of protein binding of mebendazole to bovine serum albumin. But the interaction with Cr(III) has very little impact on protein binding of metronidazole and mebendazole. The values for the affinity constants, number of binding sites and the nature of protein binding curves have been investigated for these species. Careful consideration is needed during concurrent administration of metronidazole or mebendazole with Cu (II) and Cr (III).

KEYWORDS Metronidazole; Mebendazole; Cu(II); Cr(III); Protein-binding.

INTRODUCTION

Several factors influence the pharmacologic response of drugs administered into the body. The binding of drugs to plasma proteins is one of those important parameters that impacts both pharmacokinetics and pharmacodynamics of the drug molecules.

The study on interaction and complexation of drug molecules with other drugs species and various metal ions is an important field of research in chemistry, biochemistry and medicine that remarkably influences the bioavailability 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 number of metals in the dosage forms, diet, drinks and other ways which come in close contact with different drugs and bio-molecules 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. Meanwhile, Haider and co-workers investigated the complexation of thiamine hydrochloride with Co(II), Cu(II), Zn(II), Cd(II) and Mg(II) in the aqueous media. Accordingly, we have investigated the effect of 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.

Protein binding of a drug is a limiting factor for drug effect. Simultaneous administration of two or more drugs into the systemic circulation can modify the affinity of the drug to bind with plasma protein and thus percentage of protein binding. Due to this modification, the combined therapy can change the volume of distribution, renal and hepatic clearance, and thereby modulates the efficacy of drug.

Metronidazole, an antiprotozoal agent and mebendazole, an important anthelmentic drug are frequently prescribed for the treatment of giardiasis . Very recently, we have reported *in vitro* interaction and 1:1 complex formation between metronidazole and mebendazole, with Copper (II) and Chromium (III) in aqueous media. As part of our ongoing studies on the interactions of drug molecules with metals ions, we herein, report the preliminary results of interaction of metronidazole and mebendazole, with Cu(II) and Cr(III) ions in relation to their interactions with plasma proteins.

MATERIALS AND METHODS

Materials

Metronidazole and mebendazole (product grade) used in this study have been collected from Drug International, Dhaka, Bangladesh. 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.

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. A Dunbuff metabolic shaking incubator (GCA corporation,USA) was used to shake the plasma-drug mixtures for the attainment of equilibrium.

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 vatiation and Ardon's spectrophotometric methods. Then equilibrium dialysis method was utilized for the determination of protein binding of metronidazole & mebendazole and its 1 :1 mixture with Cu (II) and Cr (III).

Equilibrium Dialysis

Equilibrium dialysis is a frequently used method developed by E. Singlas in 1987 for the determination of protein binding of drugs and other substances. The method consists of dialysing the unbound fraction of the compound contained in a protein (bovine serum albumin, for example) solution through a semipermeable membrane. In this work, the membranes were activated first by digestion with 1.0M sodium bicarbonate at 70ºC for four hours and washing thoroughly with deionized water and immersing in 1/15M (or, 0.067M) phosphate buffer of pH 7.4. Activated membrane bags (12 cm long, 3.0 ml capacity) were filled with solutions of serum bovine albumin with different concentration of the drugs, metronidazole and mebendazole and their 1:1 mixtures. The membrane bags were immersed in a fixed amount (60 ml) of phosphate buffer and the system was shaken gently for six hours in Dubnoff metabolic shaking incubator at 37 ± 0.5ºC. After completion of dialysis the absorbance of the buffer (outside the membrane bags) was measured at 328 nm for metronidazole and 290 nm for mebendazole and the concentrations of the bound and unbound drugs were found using a standard curve.

Preparation of standard curves

For the spectrophotometric detremination of conc. of drugs in the buffer compartment, two separate standard curves were generated for metronidazole and mebendazole as shown in Figure 1a and 1b. To prepare these curves, two treated buffers (phosphate buffer, pH 7.4 and chloride buffer, pH 2.4) were used. Then the solutions of different concentrations of metronidazole were prepared in buffer (pH 7.4) and standard curve was obtained (Figure 1) by plotting the absorbance values (measured at 328 nm) against respective concentrations. Standard curve for mebendazole was prepared (Figure 2) in the same manner using the identical buffer system (pH 2.4) and absorbance measured at 290 nm.

Calculation of percentage of protein binding

The percentage of protein binding (F) is given by:

$$F = \frac{[B] - [A]}{[B]} \times 100$$

Where, [A] = Molar concentration of unbound drug in buffer compartment

[B] = Molar concentration of total drug in protein compartment

Calculation of affinity constants and number of binding sites

Number of binding sites and affinity constants of metronidazole, mebendazole and their 1:1 complexes with Cu (II) and Cr (III) ions were calculated separately by Scatchard method [20] was used for this purpose and a curve was thus produced by plotting 'r/[D]' versus 'r' using the equation:

$$r = \frac{[B] - [A]}{[Protein]} \times 100$$

where, r = the ratio between the molar concentration of the bound drug and the molar concentration of protein. And [D] is the concentration of unbound drug *i.e.* [A].

The curve thus obtained called Scatchard plot. The Scatchard plot when extrapolated on Y axis, gave an intercept nK, the intersection on X-axis representing n and the slope of line AB being k. Here, k is the affinity constant and n is the number of binding sites of protein binding.

Statistical analysis

The results were expressed as mean ± 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.

RESULTS AND DISCUSSION

The aim of this study is to investigate the effects of Copper (II) and Chromium (III) on the protein binding of Metronidazole and Mebendazole. We have chosen Cu(II) and Cr(III) as intercalating metals with metronidazole and mebendazole, since copper and chromium are essential trace elements present in various foods. Copper could facilitate iron absorption and stimulate enzymes that are involved in the heme and/or globin biosynthesis pathways. On the other hand, chromium was found necessary for optimal growth of experimental animals but in larger quantities it is toxic.

The protein binding pattern of metronidazole at various concentrations has been displayed in Figure 2a. As it is shown that at a low plasma concentration, the percentage of protein binding of metronidazole increases but at higher concentration range, it attains a steady plateau state. This may be the saturation zone for this drug. N. F. Wood studied the protein binding of metronidazole and found that it is only 1 to 4% bound to human serum albumin. Later Eric Singlas found that it was around 10 to 15%. But in our case we found the highest percentage (about 25%) of binding of metronidazole with bovine serum albumin at low concentration range and about 15% at saturated zone. It might be due to concentration effect or some inherent differences between BSA and HAS.

The effect of Cu(II) and Cr(III) on the plasma protein binding of metronidazole has been studied on their 1:1complexes in aqueous media by equilibrium dialysis. Results have been shown in Figure 2b and 2c. It is found that Cu(II) has very moderate impact on the protein binding of metronidazole which might be due to strong interaction between them. Copper causes a decrease in protein binding of metronidazole leading to the formation of 1:1 complex. This means that the decrease in protein binding may be due to capture of binding sites in the protein by Cu(II) ions or Metro-Cu(II) complex. Thus possibility of metronidazole adverse effects may become prominent in presence of Cu(II) or similar ions in the body systems. Compared to metronidazole alone, its 1:1complex with Cr(III) shows a significant increase by 15 to 17% in protein binding as it shown in Figure 2c. This may be due to weak interaction of metronidazole with Cr(III) leading to the formation of less stable complex thereby lowering the affinity of drug or drug-metal system for the protein. Complex formation between metronidazole and Cr(III) can decrease its unbound fraction in plasma at higher conc levels and increase with Cu(II). This may alter the therapeutic response, toxic effects, and pharmacological properties of the drug.

Mebendazole is highly bound to plasma protein. But in our experiment the highest percentage of binding of mebendazole to BSA was found to be only 15% (Figure 3a). It might be due to concentration effect (both drug and BSA) or some inherent differences between BSA and HSA.

The percentage of protein binding of mebendazole and Cu(II) complex (1:1) increases at low concentration range (about 15 to 28%) and decreases at high conc. This increment is due mainly to weak interaction between Cu(II) and mebendazole (Figure 3b). On the other hand, Cr(III) causes decrease in protein binding of mebendazole. Here the highest percentage of binding of mebend is 27.9% compared to 15.3% for mebendazole alone. This increase in percentage of protein binding is perhaps due to weak interaction between Cr(III) and mebendazole at low concentration and comparatively good interaction between them at saturation zone(Figure 3c).

The Scatchard plots show at least two classes of binding sites (class I and class II, the warfarin and the diazepam sites, respectively). The number of binding sites n_1 and n_2 for class I and class II, and affinity constants k_1 and k_2 for these classes have been calculated from Scatchard plots. Numbers of binding sites were

obtained by dividing the intercept (nk) by slope (k) of the straight lines. The values for affinity constants associated with respective class of binding sites were obtained directly from the slope of the straight lines.

From the Scatchard plot (Figure 4a) it is observed that number of binding sites for metro alone in BSA found to be $1.73x10^2$ and $4.53x10^2$ for class I and class II respectively. The affinity constants k_1 and k_2 associated with these respective classes of binding sites were found to be 0.266 and 0.062 respectively. For metro-Cu(II) complex these figures were $9.1x10^2$ and $3.19x10^2$ respectively. Whereas as the k1 and k2 values were 0.077 and 0.0766 respectively (Figure 4b). In case of the complex between metro and Cr(III) the number of binding sites in BSA found to be $1.69x10^2$ and $1.16x10^2$ for class I and class II respectively. The affinity constants k_1 and k_2 associated with these respective classes of binding sites were found to be 0.192 and 0.077 respectively (Figure 4c).

In the study involving mebendazole, the Scatchard plot (Figure 5a) shows that number of binding sites for mebendazole alone in BSA found to be $0.109x10^2$ and $1.56x10^2$ for class I and class II respectively. The affinity constants k_1 and k_2 associated with these respective classes of binding sites were found to be 1.75 and 0.039 respectively. For mebendazole-Cu(II) complex these figures were $1.92x10^2$ and $6.16x10^2$ respectively. Whereas as the k_1 and k_2 values were 0.277 and 0.0568 respectively (Figure 5b). In case of the complex between mebendazole and Cr(III) the number of binding sites in BSA found to be $0.829x10^2$ and $1.33x10^2$ for class I and class II respectively. The affinity constants k_1 and k_2 associated with these respective classes of binding sites were found to be 0.945 and 0.206 respectively (Figure 5c).

According to Table 1, in class I binding sites, it is obvious that values of affinity constants for metronidazole (0.266) alone is higher than its 1:1 complexes with Cu(II) (0.07) and Cr(III) (0.025)that is, the presence of Cu(II) and Cr(III), with metronidazole, at physiological temperature and pH conditions, cause a decrease in values of affinity constant, at higher concentration range. Again in class II binding sites it is obvious that values of affinity constants for mebendazole (0.0398) alone is lower than that of its 1: l complexes with Cu(II) (0.0568) and

Cr(Ill)(0.206) that is, the presence of Cu (II) and Cr (III) with mebendazole cause an increase in values of affinity constant specially at lower concentration and also higher concentration range. Due to this increase in affinity of the drug to plasma protein binding, the volume of distribution (Vd) of the drug (mebendazole) may decrease, because affinity of a drug for protein binding is a limiting factor of distribution of the drug. The values of affinity constants in class I binding sites for mebendazole (1.75) is higher than that of its 1:1 complexes with Cr (III) (0.945) may cause a decrease in affinity of the drug to plasma protein. The values of affinity constants in class II binding sites for metronidazole (0.062) and its 1:1 complexes with Cu(II) (0.077) and Cr(III) (0 .077) at higher concentration range that is at saturation zone, reveals the same results, i.e. increase in volume of distribution of metronidazole.

It is also seen that affinity constants in class II binding sites for metronidazole (0.062) is lower than that of metronidazole with Cr(III) (0.077) may cause a higher affinity to plasma. It is known that metronidazole is metabolized mainly in the river, into a relatively inactive acid metabolite and a hydroxy metabolite active against anaerobes. It is excreted through urine and its half-life is about 8 to 10 hr. It is observed that the affinity for protein is lower for 1:1 complexes of metronidazole with Cu (II) and is higher with Cr (III) than that of metronidazole alone. Again, it is observed that the affinity for protein is higher for 1:1 complexes of mebendazole with Cu (II) at lower and higher concentration ranges and also higher with Cr (III) at lower concentration ranges and lower with Cr(III) at higher concentration ranges than that of mebendazole alone. As a result, the intake of mebendazole as meben.-Cu(II) and meben.-Cr(III) complex or the concurrent therapy can decrease both hepatic and renal clearance of the drug as well as it can increase or decrease the half-life of the drug.

One thing is noticeable from these affinity constant data that the value of affinity constant of Cu(II) in crass I and crass II binding sites are same but the affinity constant of Cr (III) is lower from the drug alone in class I and significantly higher than drug and other interacting species. Thus due to increase in concentration of free drug or decrease in affinity for protein, the pharmacological effects of metronidazole will involve far-reaching pharmacological effects.

CONCLUSION

Polypharmacy, i.e. prescribing many drugs at a time is a common practice in case of patients undergoing a major operation, hospitalized patients, and also in geriatric patients. Results obtained in this study show that percent of protein binding increases after complexation of metronidazole and mebendazole with Cr(III) and Cu(II) respectively whereas a significant decrease in binding pattern was obtained after complex formation with the aforesaid metals in the reverse order. It can be inferred that careful consideration is needed during concurrent administration of metronidazole or mebendazole with Cu(II) and/or Cr(III) ions.

REFERENCES

1. Vuignier K, Veuthey J, Carrupt P, Schappler J. Characterization of drug-protein interactions by capillary electrophoresis hyphenated to mass spectrometry. Electrophoresis, 2012; 33: 1-10.

2. Martell AE, Smith RM. Critical Stability Constants (First Supplement Press, NY, USA, 1982).

3. Botton S, Ellin RI. Quantitative study of chelates of pyridine-2-aldoxime. J Pharm Sci 1962; 51: 533-539.

4. Hansten PD, Horn JR. Drug Interactions: Clinical Significance of Drug-drug Interaction, 6th ed. (Philadelphia, 1989).

5. Martin A, James S, Arthur C. Physical Pharmacy, Lea and Febiger, 3rd ed. (Philadelphia, USA, 1993) pp. 341-342.

6. Haider SZ, Introduction to Modern Inorganic Chemistry, 5th ed. (Students publication, Dhaka, Bangladesh, 1977) pp. 98, 278, 638, 653.

7. Haider SZ, Malik KMA. J Bang Acad Sci 1987; 2: 117.

8. Amran MS, Baki MA, Hossain MA. In vitro and in vivo effects of glipizide and gliclazide on the protein binding and plasma concentration of ibuprofen. Presented at 31st Annual Conference of Bangladesh Chemical Society, University of Dhaka, Dhaka, Bangladesh (2009).

9. Amran MS, Morshed SN, Khandakar MJA, Rahman MM, Hossain MA. The In vitro Effects of Atenolol and Zinc Chloride on the Protein Binding of Amlodipine in Aqueous Medium, Dhaka Univ J Pharm Sci, 2008; 7: 15-21.

10. Amran MS, Bari AHMR, Hossain MA. The in vitro and in vivo interactions of diltiazem with ibuprofen and naproxen in aqueous medium and rabbits. Dhaka Univ J Pharm Sci 2006; 5: 25-28.

11. Amran MS, Rahatuzzamn MM, Hossain MA. The synergism of diltiazem and nifedipine in their antihypertensive functions in the animal model and the case of coadministration of ketotifen fumerate and potassium nitrate with them. J Bio Sci 2006; 14: 61-67.

12. Amran MS, Hossain MA. Evaluation of in vitro interaction of diltiazem HCl with iron (II) in the aqueous media. Rajshahi Univ Studies, Part B. 1998; 26: 35-43.

13. Bari AHMR, Azam ATMZ, Amran MS, Hossain MA. In vivo effects of ibuprofen and naproxen on the plasma concentration of diltiazem in rabbits. Pak J Biol Sci 2000; 3(4): 555-557.

14. Cadwallader DE. Biopharmaceutics and druginteractions, 3rd edition, (Raven press, New York 1985), pp107-143.

15. Singlass E. Protein binding of drugs, F. Hoffman La Roche & Co. Ltd., (Basle, Switzerland, 2nd edition, 1987), pp17-32.

16. Kiser JD, Paulson CP, Nichols W. J Fam Prac 2008; 57**(**4): 270-272.

17. Siraji F, Azam ATMZ, Amran MS, Islam JN, Amjad FM, Hossain MA. In vitro Interaction of Metronidazole and Mebendazole with Copper (II) and Chromium (III) in Aqueous Media. J Sci Res 2012; 4 (1): 173-181.

18. Vogel AI. A Textbook of Quantitative Inorganic Analysis. 3rd ed. (Longmans, London, 1961) p. 93.

19. Ardon M. Oxidation of ethanol by ceric perchlorate. J Chem Soc 1957; 1811-1815.

20. Scatchard G. The attractions of proteins for small molecules and ions, Ann NY Acad Sci 1949; 660-673.

21. John HB, Edward BR, Charles OW, Taito OS. Inorganic Medicinal and Pharmaceutical Chemistry (Henry Kimpton, London, 1974) p. 213.

22. Pal B, Mukherjee J. J Appl Nutr 1978; 30: 14.

23. Wood NF. GLC analysis of metronidazole in human plasma. J Pharm Sci 1975; 64: 1048-1049.

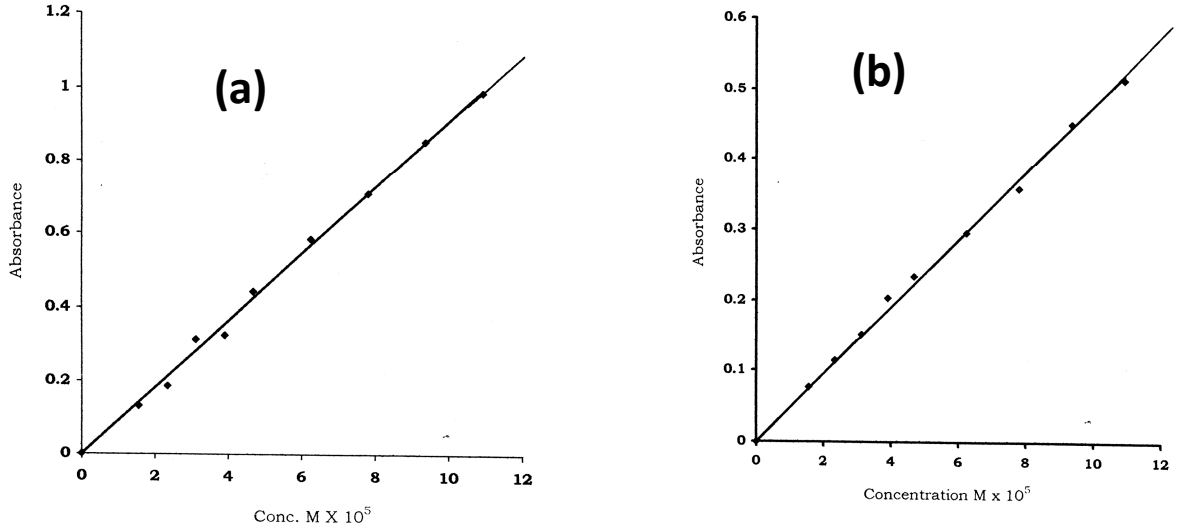

Figure 1: Standard curve for determination of a) Metronidazole and b) Mebendazole.

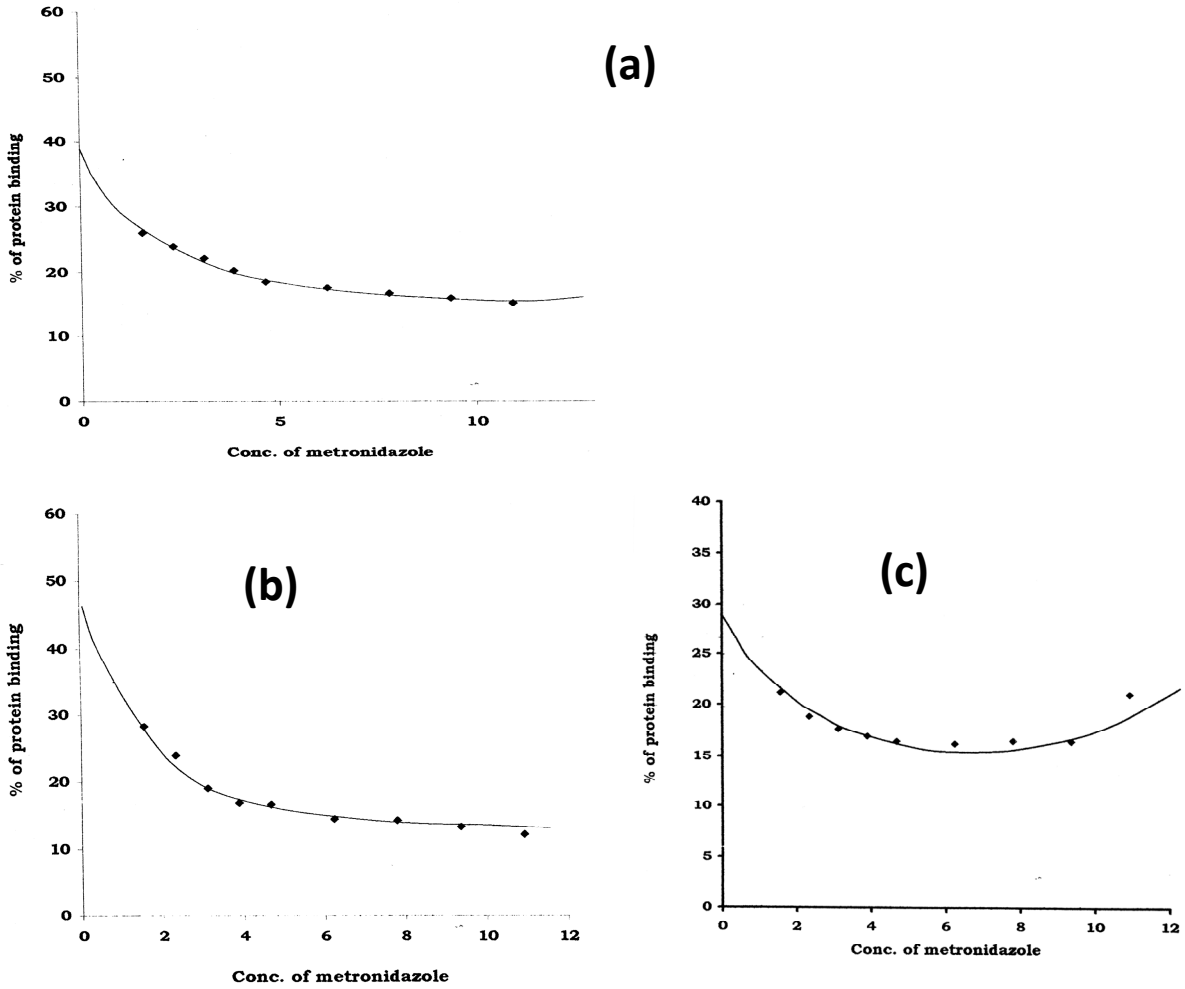

Figure 2: a) Percentage of protein binding of free metronidazole to BSA at pH 7.4; b) Percentage of protein binding of free metronidazole in presence of Cu(II) to BSA at same pH and c) Percentage of protein binding of free metronidazole in presence of Cr(III) to BSA at same pH.

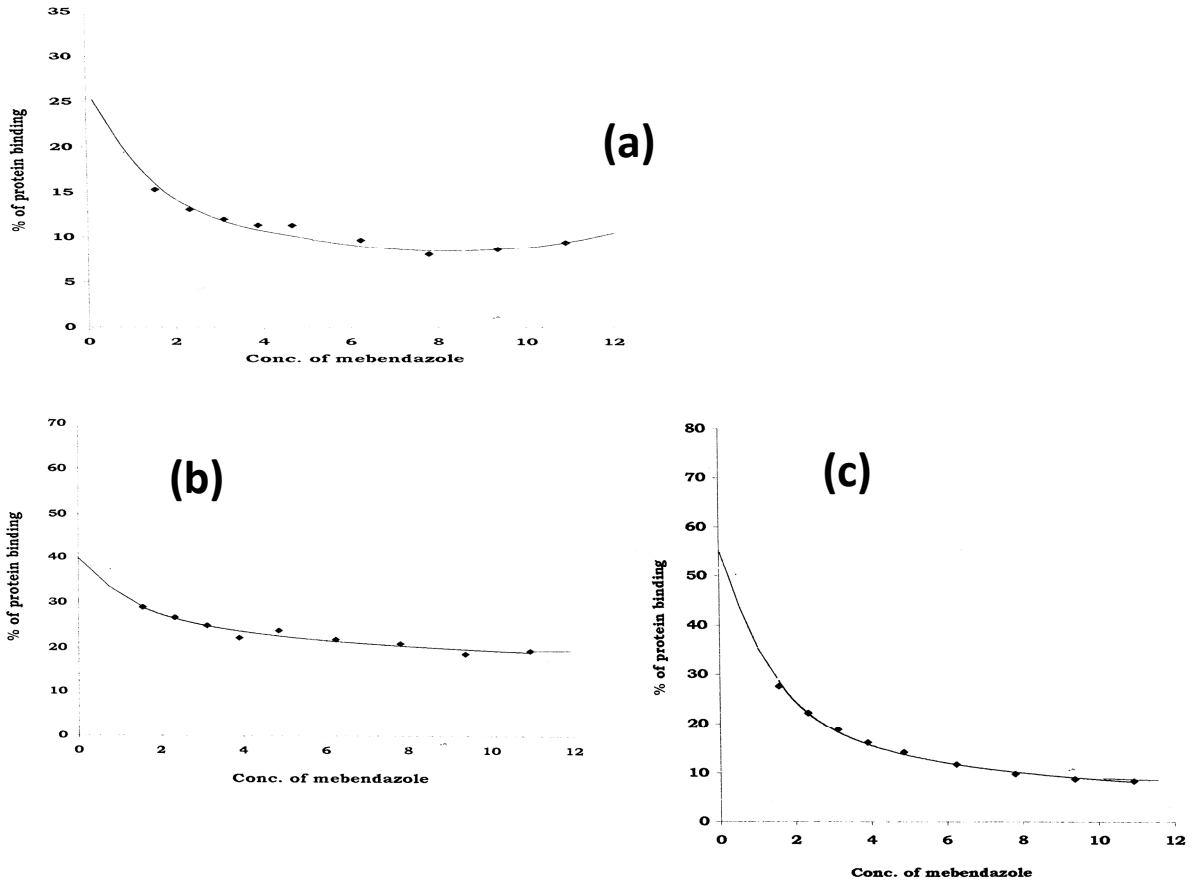

Figure 3: a) Percentage of protein binding of free mebendazole to BSA at pH 7.4; b) Percentage of protein binding of free mebendazole in presence of Cu(II) to BSA at same pH and c) Percentage of protein binding of free mebendazole in presence of Cr(III) to BSA at same pH.

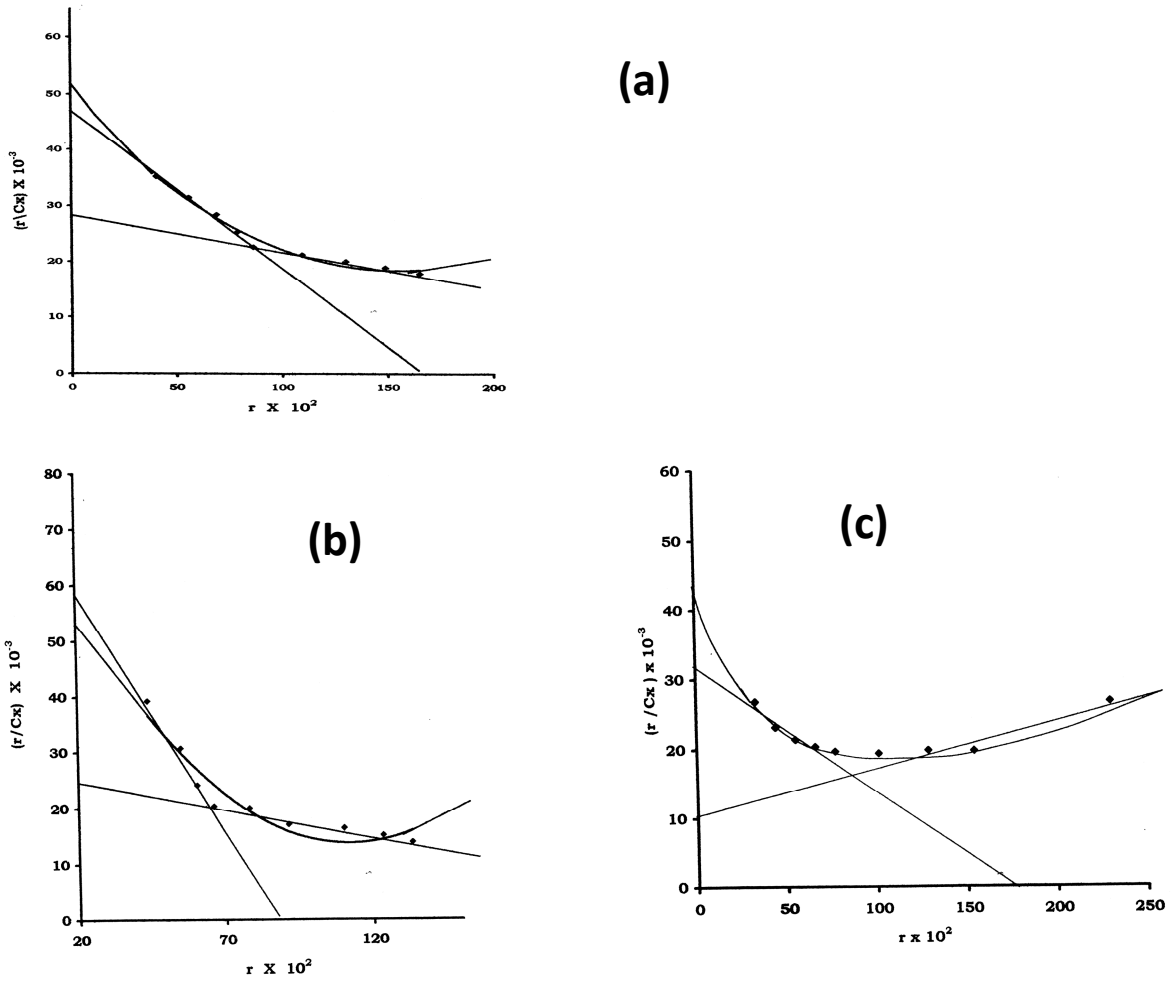

Figure 4: a) Scatchard plot for protein binding of metronidazole to BSA; b) Scatchard plot for protein binding of metronidazole in presence of Cu(II) (1:1 mixture); and c) Scatchard plot for protein binding of metronidazole in presence of Cr(III) (1:1 mixture).

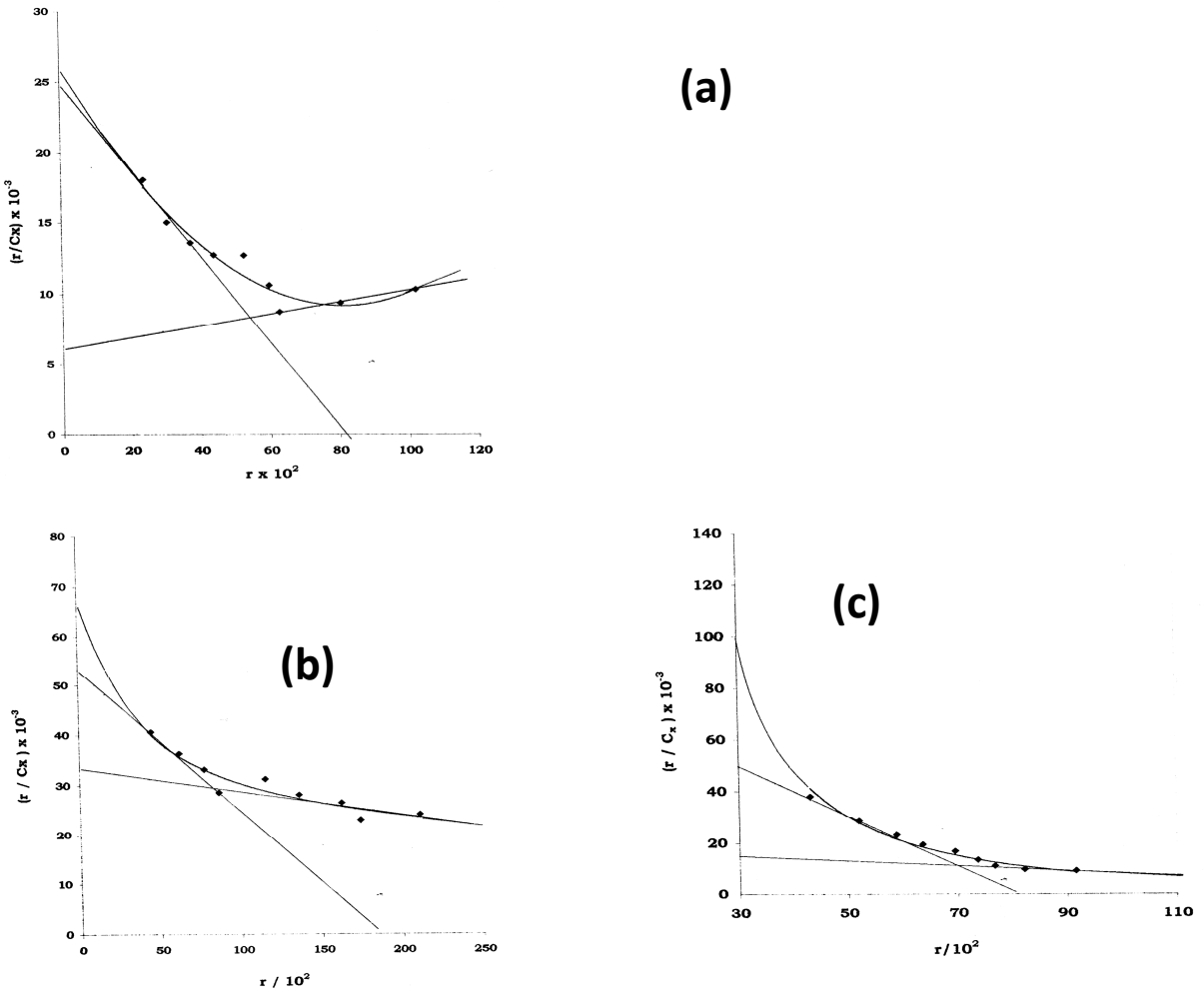

Figure 5: a) Scatchard plot for protein binding of mebendazole to BSA; b) Scatchard plot for protein binding of mebendazole in presence of Cu(II) (1:1 mixture); and c) Scatchard plot for protein binding of mebendazole in presence of Cr(III) (1:1 mixture).

Systems	Class I binding sites			Class II binding sites		
	$n_1.10^{-2}$	K_1	n_1K_1	$n_2.10^{-2}$	K_2	n_2K_2
Metronidazole	1.73	0.266	46.23	4.53	0.062	
Metro. – Cu(II)	9.1	0.077	70.09	3.19	0.077	
Metro. – Cr(III)	1.69	0.192	32.42	1.16	0.077	
Mebendazole	0.109	1.75	19.22	1.56	0.04	
Meben. – Cu(II)	1.93	0.277	53.49	6.16	0.057	
Meben. – Cr(III)	0.829	0.945	78.39	1.33		

Table 1:The values for number of binding sites and affinity constants.