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INTERACTION OF DIMETHINDENE MALEATE WITH BOVINE SERUM ALBUMIN AND PLASMA SUBSTITUTES

Bernd W. Muller*, M. Salama Mohamed, Fakhr A. Ghazy, Samir S. Abu-Zaid and Mohamed M. Abd El-Rahman

Department of Pharmaceutics, Faculty of Pharmacy, Zagazig University, Egypt.
**Department of Pharmaceutics, Pharmaceutics Institute, Christian Albrechts University, Kiel, Germany.*

ABSTRACT

Binding of dimethindene maleate to bovine serum albumin (BSA) was studied in isotonic Sorensen's phosphate buffer of pH 7.4 at 25°C, using equilibrium dialysis technique. Dimethindene maleate was bound to BSA, and the plot was curved indicating the presence of more than one binding site on the albumin molecule. At fixed protein concentration the percentage of bound drug to bovine serum albumin was inversely proportional to the concentration of the drug. However, the binding parameters were found to be increased as the albumin concentration increased. But, the binding parameters (binding constants as well as the number of binding sites) did not change by the change of pH. Phosphate buffer and Gomori's tris-ECl buffer have the same effect upon the binding of the drug to BSA while Walpole's acetate buffer decreases the binding of the drug to BSA. Also, it was found that chloride ions had no effect on the binding of the drug to BSA. The interaction of the drug with dextrans (40000, 266000 and 500000) in isotonic Sorensen's phosphate buffer of pH 7.4 was investigated. No interaction occurred between the drug and the tested dextrans under the condition of the experiment. Also, laevosan and hetastarch showed no significant effect on the binding process.

INTRODUCTION

Many drugs interact with plasma or tissue proteins to form a drug-protein complex, complexation of a drug with a protein or drug-protein binding can affect the therapeutic, pharmacodynamic and toxicological actions of drugs [1-5].

The fate of many drugs in the body is greatly influenced by their binding to serum albumin. Whatever the route of administration almost all therapeutic agents reach their sites of action via the systemic circulation. Only the fraction of the drug in the blood stream that does not bind to albumin can leave the circulation, distribute throughout the body, and reach the site of action. Because the equilibrium between bound and free drug is constantly maintained, some of the drug-albumin complex continuously dissociates as free drug diffuses out of the blood through capillary membranes [6-8].

Dimethindene Maleate is an alkylamine derivative with the properties and uses of the antihistamines. It is given by mouth for the symptomatic treatment of allergic conditions particulary to control pruritis.

The experimental work in this study deals with the binding of dimethindene maleate to BSA and their substitutes (dextrans, laevosan and hetastarch). In addition the factors that are commonly known to affect the binding process were also investigated. These factors include:

drug concentration, Albumin concentration, pH of buffer solution, buffer systems and chloride ion.

EXPERIMENTAL

Materials and reagents :

Dimethindene maleate (Zyma-Germany). Bovine serum albumin, purified and lyophilized, Fraction, V, molecular weight 67000 (Fluka, Switzerland). Dextran, molecular weight 40000, 266000, 50000 (Sigma U.S.A.). Laevonsan, molecular weight 180.2 (Haes- Germany). Hetastarch (2- hydroxyethyl strach) molecular weight 450000 (Fresenius AG, Germany). KH_2PO_4, Na_2HPO_4, NaCl, HCl, trihydroxyaminomethane, acetic acid and sodium acetate (Merck - Germany). Sorensen's phosphate buffer pH 5.6, 6.2, 6.6, 7.0 and 7.4 were prepared as described in Documenta Geigy [9]. Walpole's acetate buffer of pH 5.6 [9]. Gormori's tris-HCl buffer of pH 7.4 [9]. Cellulose dialyser membrane (Nadir dialyses schlauch, diameter 38 mm, pore size 25-80A°, Hochest- Germany). An amicon Diaflo ultrafiltration membranes (10 YM 10 25 mm Lot AD 05525 A, Amicon Corporation, Danvers, MA. 01923 - Irland).

Apparatus :

Shaker with teflon dialysis cells : Christian-Albrechts-University-Instit. Of Pharamcy-kiel, Germany [10]. pH meter : Microprocessor pH/ion meter PM x 2000, Germany. An Amicon Diaflo ultrafiltration apparatus (Model 3 Microvolume strirred ultrafiltration cell [(No. 5166)- England]. Uv spectrophotometer: Tegimenta AG. Type : Uvikon 810-No. 243616- Switzerland.

Methods :

Binding studies :

Binding studies were carried out using the dialysis technique method [10].

Determination of the equilibrium time:

A 2.5 ml of isotonic Sorensen's phosphate buffer pH 7.4 containing $5x10^{-4}$ M dimethindene maleate (initial concentration) was injected in the upper compartment of the cell, 7.5 ml of bovine serum albumin solution $8.96x10^{-4}$ M was injected in the middle compartment of the cell and 2.5 ml of isotonic Sorensen's phosphate buffer was injected in the lower compartment of the cell. The filling of each compartment of the cell was done through two stoppered side holes. The cells were rocked for specified periods at 25°C in order to determine the most appropriate period for carrying the experiment. Samples were withdrawn at a specified periods for analysis. The absorbance of the free drug was measured spectrophotometrically at 258 nm. against the same buffer and plotted against time. Results have demonstrated that 8 hours were found to be quite sufficient for carrying the binding parameters.

Binding studies of dimethindene maleate to bovine serum albumin and different plasma substitutes :

The binding of dimethindene maleate to bovine serum albumin was studied as a function of the concentration of the latter, pH, buffer system and chloride ion. Also, binding of dimethindene maleate to dextrans, laevosan and hetastrach were conducted. The experiments were carried out as it was described under binding studies [10].

Again, in order to verify the results obtained by the equilibrium dialysis technique, the ultrafiltration method was also used[10]. No significant differences between the results could be demonstrated. Accordingly the equilibrium dialysis technique was used.

RESULTS AND DISCUSSION

The equilibrium time was determined for one concentration of dimethin-

Table (1) : Equilibrium dialysis data of the binding of dimethindene maleate to bovine serum albumin in isotonic Sorensen's phosphate buffer pH 7.4 at 25° ± 1.

Starting concentration x 10^{-5} M	C_f x 10^{-5} (M)	C_b x 10^{-5} (M)	r	r/C_b (M^{-1})
1.2847	0.7393	0.5454	0.0061	825
1.4605	0.8605	0.5999	0.0067	779
1.6059	0.9696	0.6363	0.0071	732
1.8422	1.1514	0.6908	0.0077	689
2.0059	1.2787	0.7272	0.0081	633
2.9694	0.0846	0.8848	0.0099	475
4.0421	2.9997	1.0423	0.0116	387
5.0601	2.8481	1.2120	0.0135	351
5.8176	4.4359	1.3817	0.0154	347
6.9690	5.3934	1.5770	0.0176	326
7.8174	6.0721	1.7543	0.0195	321
9.3930	7.3932	1.9998	0.0223	302
9.9990	7.8780	2.1210	0.0237	301

Bovine serum Albumin Concentration 8.96 x 10-4 M

C_f = Free drug concentration

C_b = Bound drug concentration.

r = Moles of drug bound per mole of albumin.

Table (2) : Binding parameters of dimethindene maleate to bovine serum albumin in isotonic Sorensen's phosphate buffer pH 7.4 at 25° ± 1 as a function of protein concentration.

Albumin concentration x 10^{-5} M	K_1 x 10^4 (M^{-1})	n_1	K_2 x 10^4 (M^{-1})	n_2
2.99 x 10^{-4}	5.80	0.03	0.51	0.087
5.97 x 10^{-4}	7.70	0.020	0.54	0.060
8.96 x 10^{-4}	9.42	0.015	0.56	0.085

Drug concentration range from 1.2 x 10^{-5}M to 1.0 z 10^{-4}M.

dene maleate 5 x 10^{-4} M (intial concentration). The experiment was conducted at 25°C in sorensen's phosphate buffer pH 7.4.

Fig. (1) shows that the equilibrium was attained within 8 hours. Table 1 and Fig. 2 show the equilibrium dialysis data of the interaction of dimethindene maleate with bovine serum albumin in Sorensen's phosphate buffer pH 7.4 at 25°C. It is clear that dimethindene maleate bound to bovine serum albumin, and the extent of binding depends on drug concentration.

Fig. (3) shows that at a fixed protein concentration the percentage of dimethinden maleate bound to bovine serum albumin decreases as the concentration of the drug increases. To determine the binding parameters k (the association constant) and n (the number of binding sites available on bovine serum albumin) the equilibrium dialysis data were plotted according to Scatchard[11] (Fig. 2).

Since the plot is curved so the data were analysed in terms of two classes of binding sites using the linear regression. The intercepts on the abscissa represents n_1 (number of primary binding sites) and Σ n respectively from which n_2 (number of secondary binding sites) can be calculated. The slope of the first line represents k_1 (the primary association constant) and the slope of the second line represents k_2 (the sencondary association constant). The primary association constant k_1 was found to be 9.42×10^{-4} M^{-1} and the number of primary binding sites n_1 is 0.015, while the secondary association constant k_2 is 0.56×10^{-4} M^{-1} and n_2 is 0.075.

These results indicate that dimethindene maleate is bound to bovine serum albumin. This is an important factor in predicting drug kinetics in the body[8]. Substances with a binding constant higher than 10^{-4} M^{-1} have a pharmacokinetics behaviour that is dependent on the binding phenomena[8,12] especially when the volume of distribution is small. The effect of the concentration of bovine serum albumin on the binding of dimethindene maleate is shown in Fig. (4). The calculated k_1, n_1, k_2, and n_2 at different bovine serum albumin concentration are shown in Table 2. It is clear that the binding of dimethindene maleate is signficantly affected by changing the concentration of bovine serum albumin. The binding of the drug increases as the albumin concnetration increases. Fig. (5 and 6) show that at a given drug concentration, the percentage drug bound, k_1 and k_2, increases with increasing the concentration of bovine serum albumin. The percentage of bound drug was found to be linearly related to the logarithm of albumin concentration and similar results were obtained by M.A. Mahdy [13].

In the present work the binding of dimethindene maleate to bovine serum albumin appeared to be not affect by the change of pH (Figs. 7,8,9 and 10). This finding is in agreement with that of Bennet and Kirby [14] in their work on new penicillins. On the other hand, this results is in controversy to the finding of Abd El-Bary et al.[15] in their work on phenylbutazone and exophenbutazone where the binding decreases with increasing pH. Newbould and Kilpatrick[16] also reported that the binding of sulfonamides to HSA is pH dependent.

The effect of buffer components was investigated by replacing Sorensen's phosphate buffer by Gomori's tris-HCL buffer pH 7.4[9] and Walpole's acetate buffer pH 5.6[9]. All buffer solutions were made isotonic with sodium chloride. It was reported that different buffer systems vary in their interference with the binding of benzylpenicillin and phenoxymethlypenicillin to bovine serum albumin [17]. But, Fig. (11) shows that Sorensen's phosphate buffer and Gomori's tris-HCL buffer have the same effect on

the binding of the drug to bovine serum albumin. This finding is in agreement with that of Oroszlan and maengwyn-Davies[18] working on the binding of atropine to bovine serum albumin, but Walpole's acetate buffer decreases the binding of the drug to bovine serum albumin as shown in Fig. (12). This finding is in agreement with that of Naoki Nambu and Tsuneji Nagai [19] who reported that the effect of ion species on the binding of 13 kinds of phenothiazines to bovine serum albumin (BSA) was as follows: citrate > succinate > phosphate >acetate.

Also, it was reported that chloride ion affects the plasma protein binding of many drugs. Wilting et al [20] investigated the effect of chloride ion on the binding of warfarin to human serum albumin, the results revealed that the affinity of warfarin for albumin was decreased in the presence of chloride ion. Similar finding was observed by Abd El-Bary et al. [15], who reported that chloride ion reduces the binding of phenylbutazone and oxyphenbutazone to human serum albumin. In the present work Fig. 13 shows that the binding of dimethindene maleate to bovine serum albumin was not affected by the presence of chloride ion. This finding is in agreement with that Momburg et al. [21], who studied the binding of cisplatin to human serum albumin in presence of choride ions and reported that the binding was not affected by chloride ions.

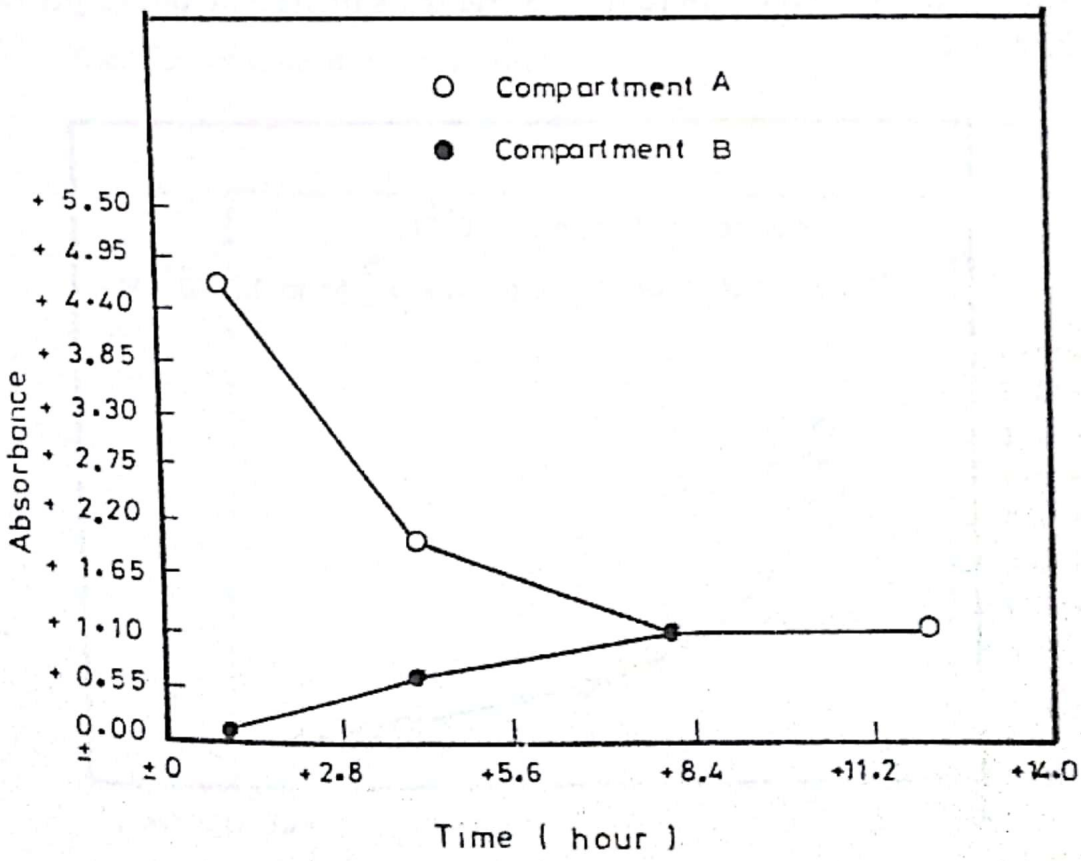

Fig. (1) :The equilibrium time for the binding of dimethindene maleate to BSA .
Drug concentration 5.0×10^{-4} M
BSA concentration 8.96×10^{-4} M.

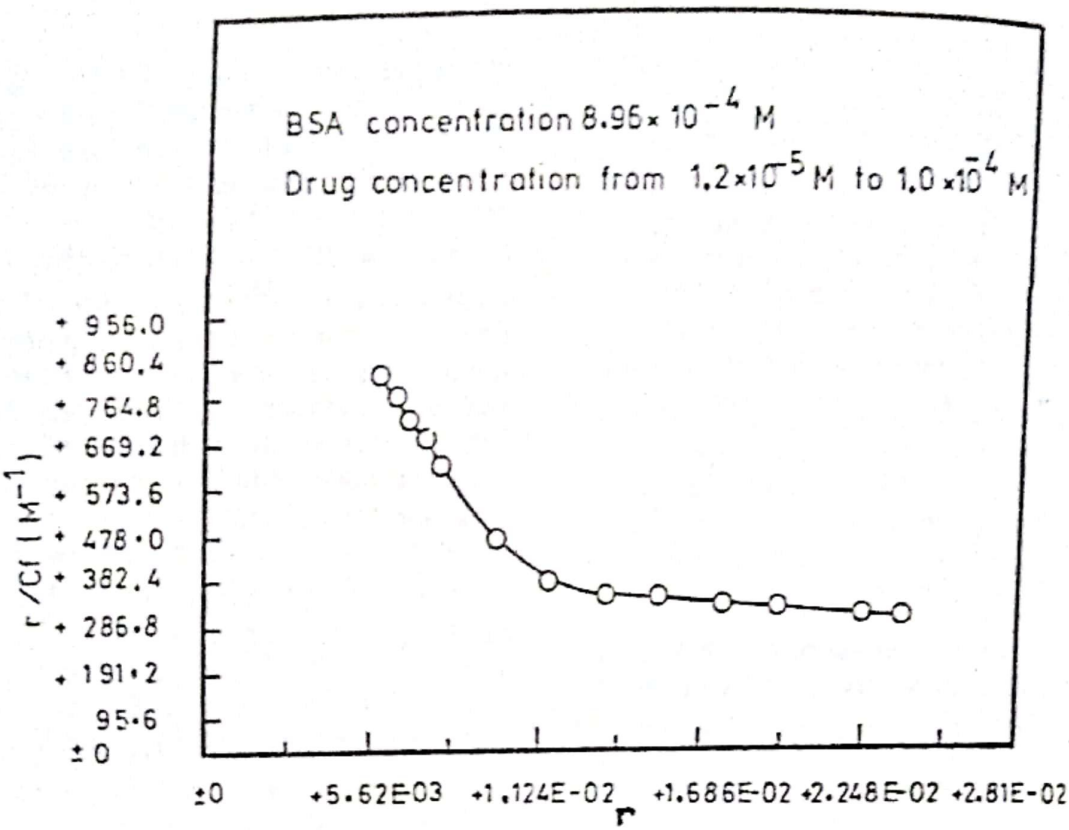

Fig. (2) : Scatchard plot of dialysis data for the binding of dimethindene maleate to bovine serum albumin in isotonic Sorensen's phosphate buffer pH 7.4 at 25°C ± 1.

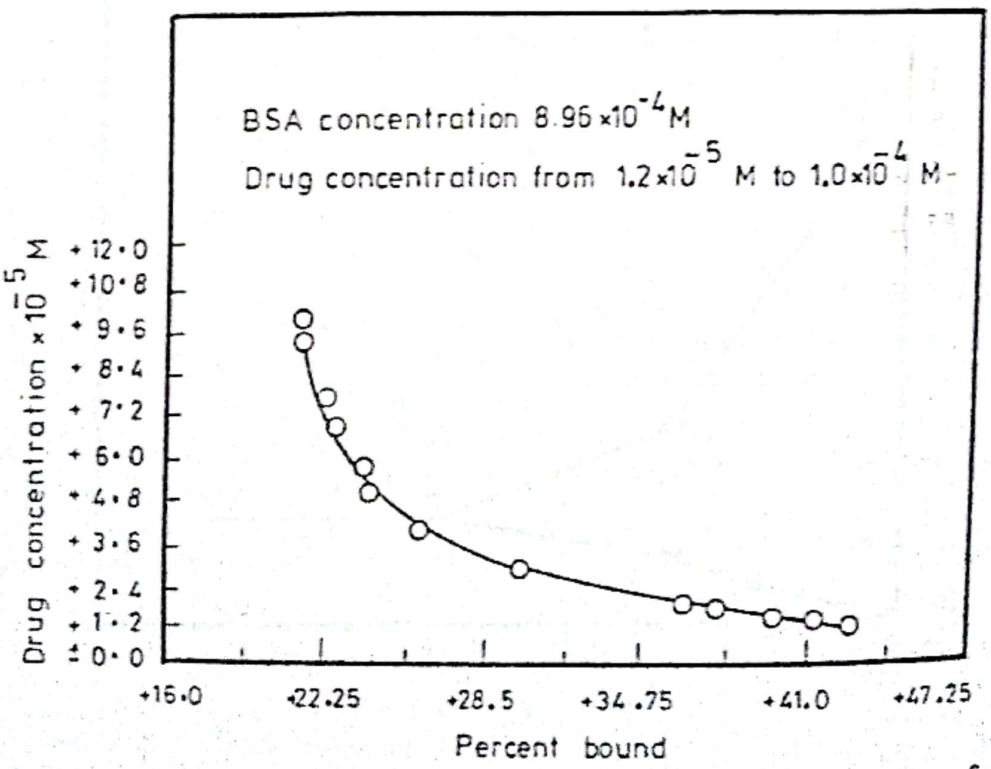

Fig. (3) : Binding of dimethindene maleate to bovine serum albumin as a function of drug concentration.

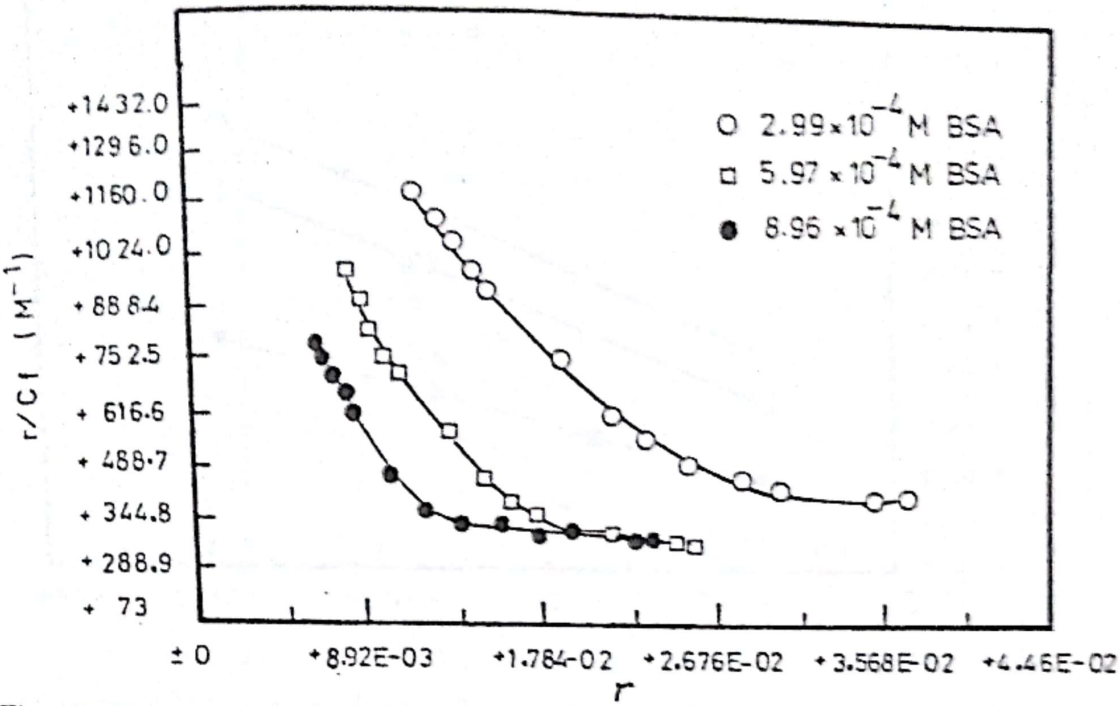

Fig. (4) : Scatchard plot of dialysis data for the binding of dimethindene maleate to bovine serum albumin in isotonic sorensen's phosphate buffer pH 7.4 as a function of albumin concentration.

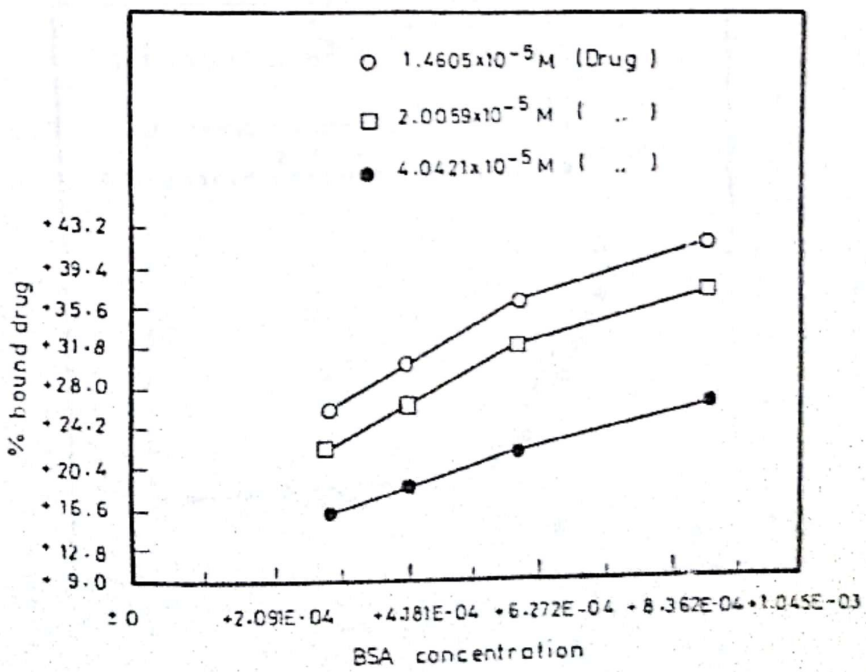

Fig. (5) : Effect of bovine serum albumin concentration of the binding of dimethindene maleate in isotonic Sorensen's phosphate buffer of pH 7.4 at 25°C ± 1.

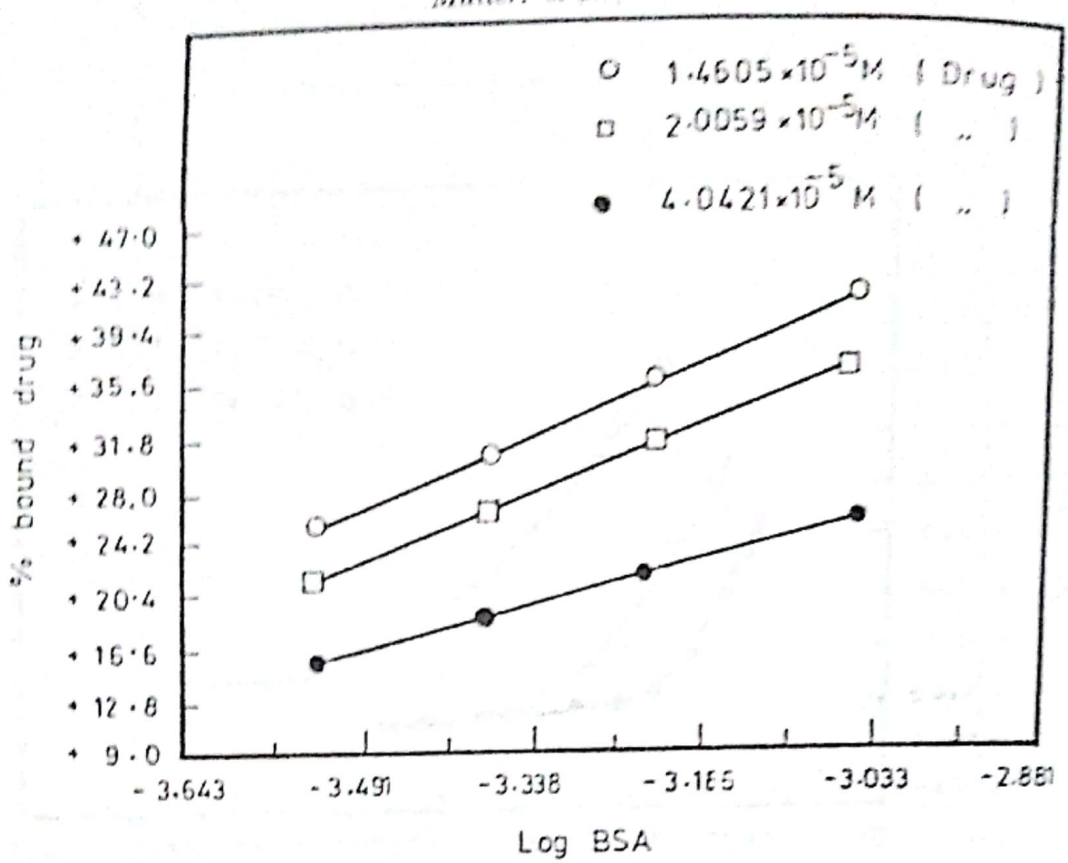

Fig. (6) : The relation between the percentage of bound drug and the logarithm of BSA concentration.

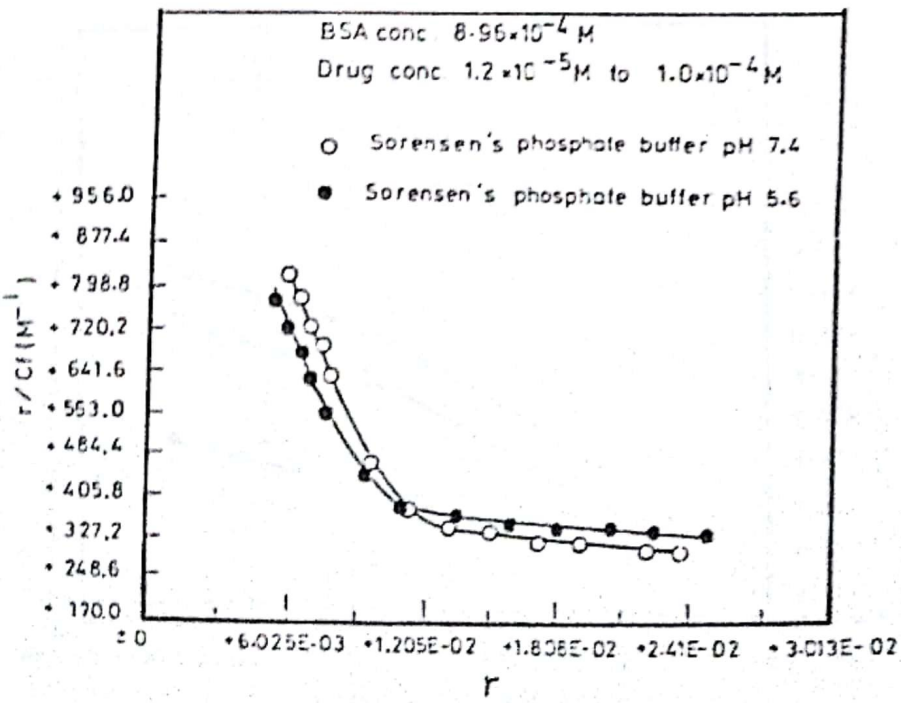

Fig. (7) : Scatchard plot of dialysis data for the binding of dimethidene maleate to bovine serum albumin in isotonic Serensen's phosphate buffer pH 5.6 at 25° ± 1.

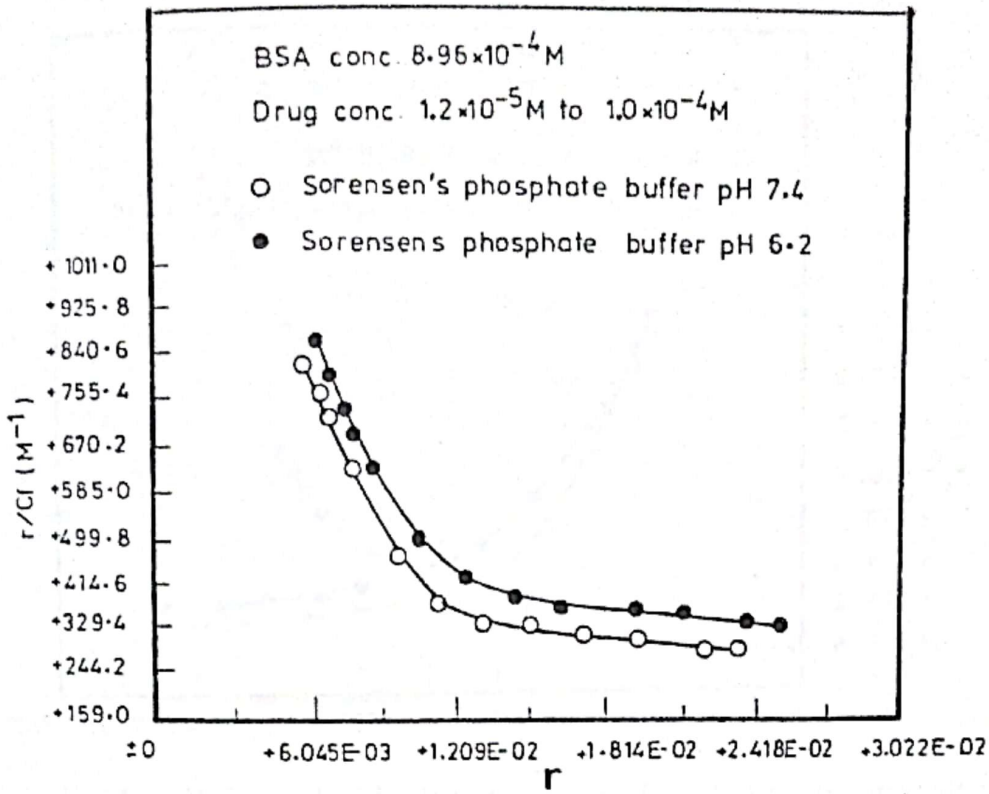

Fig. (8) : Scatchard plot of dialysis data for the binding of dimethindene maleate to bovine serum albumin in isotonic sorensen's phophate buffer pH 6.2 at 25°C ± 1.

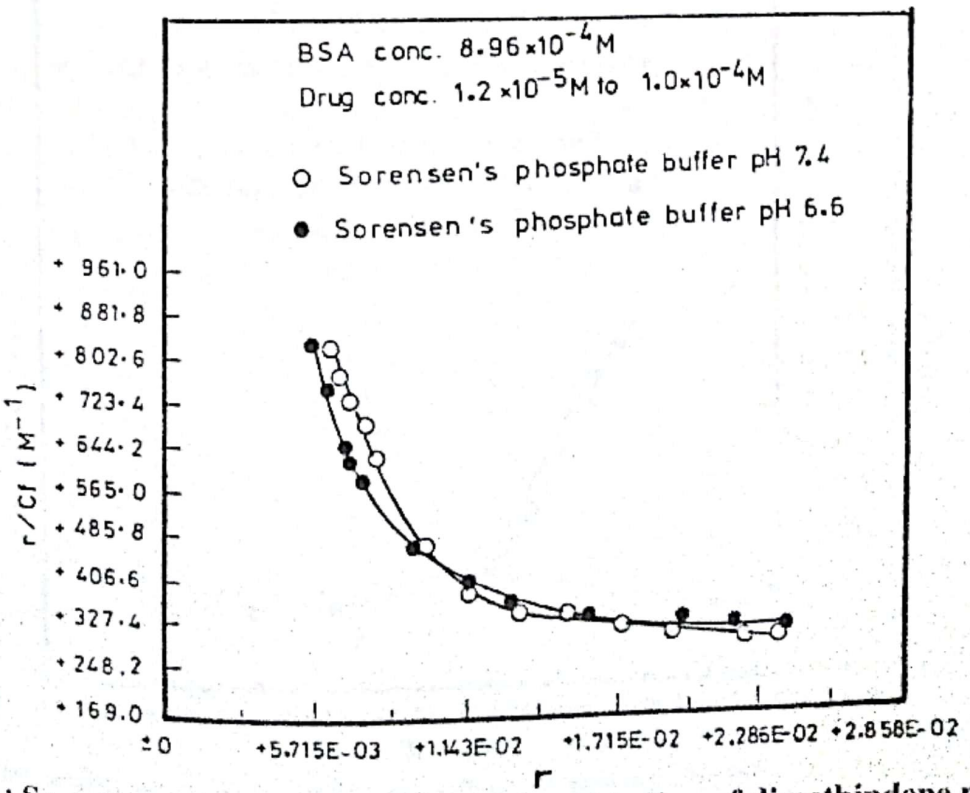

Fig. (9) : Scatchard plot of dialysis data for the binding of dimethindene maleate to bovine serum albumin in isotonic Sorensen's phosphate buffer pH 6.6 at 25° ±1.

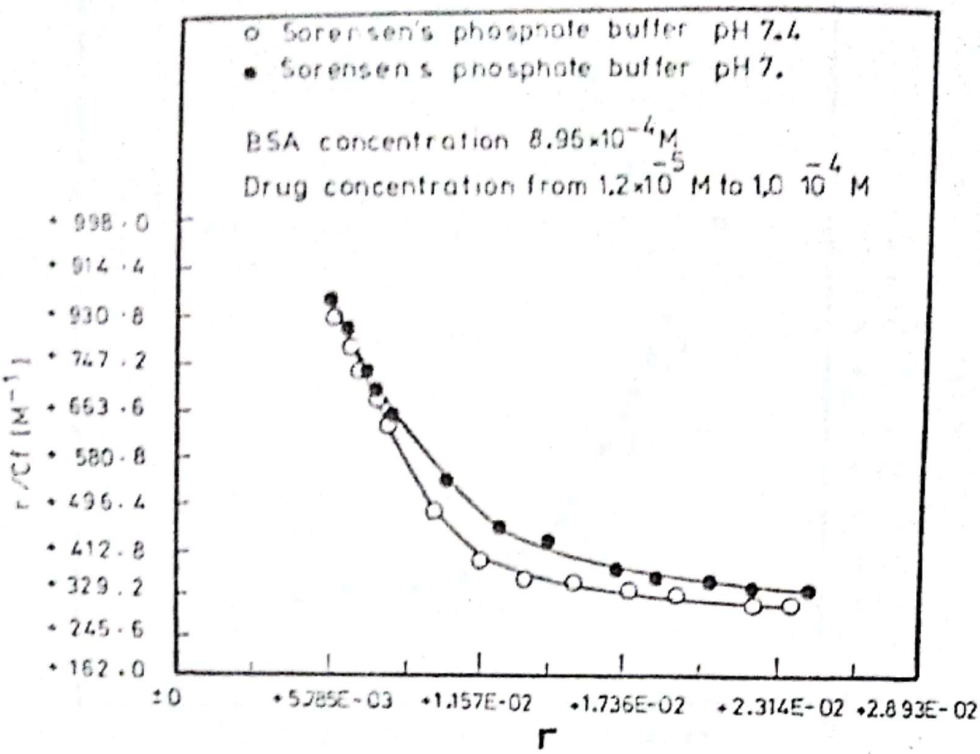

Fig. (10) : Scatchard plot of dialysis data for the binding of dimethindene maleate to BSA in isotonic Sorensen's phosphate buffer pH 7.0 at 25°C ± 1.

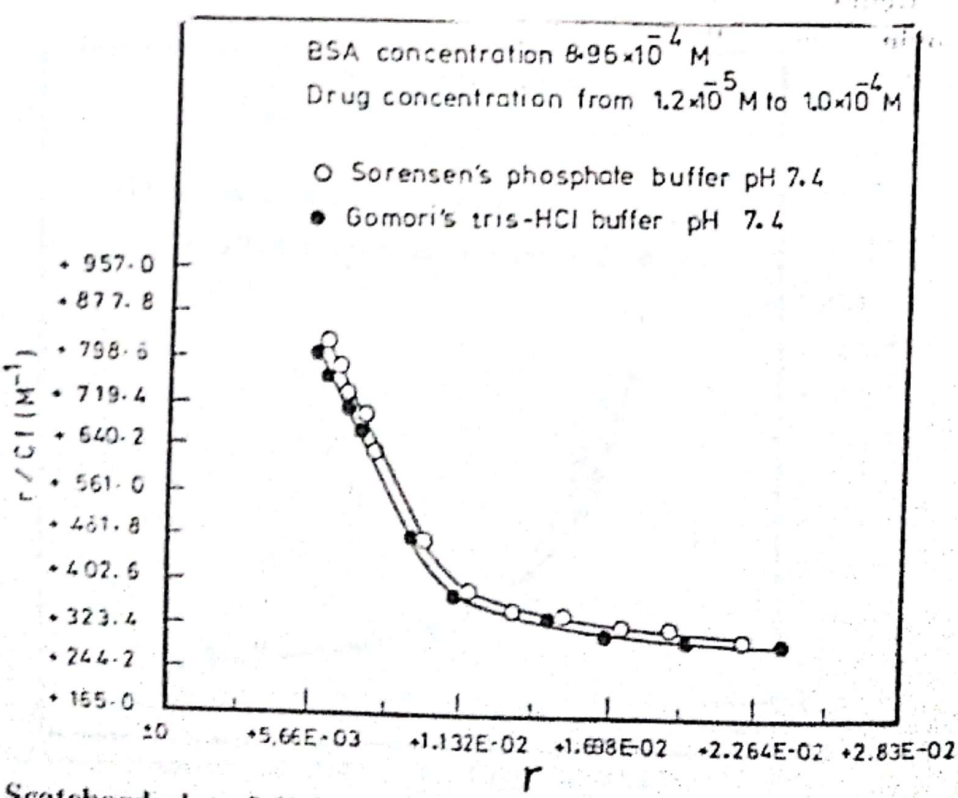

Fig. (11) : Scatchard plot of dialysis data for the binding of dimethindene maleate to bovine serum albumin in isotonic Gomori's tris-HCl buffer pH 7.4 at 25°C ± 1.

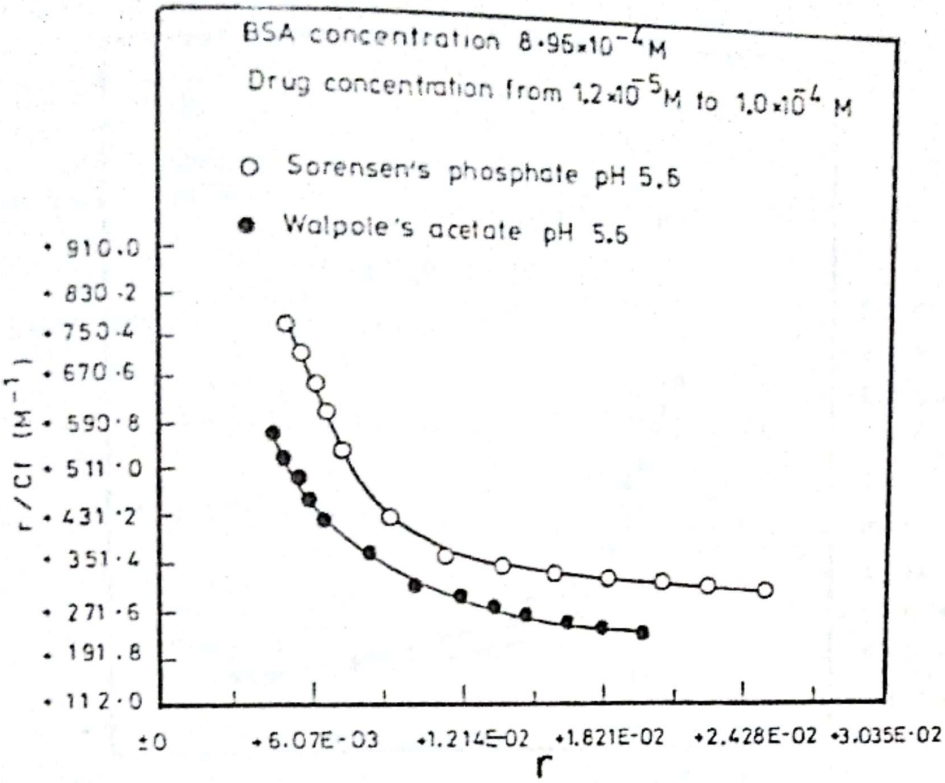

Fig. (12) : Scatchard plot of dialysis data for the binding of dimethindene maleate to bovine serum albumin in isotonic Walpole's acetate buffer pH 5.6 at 25°C ± 1.

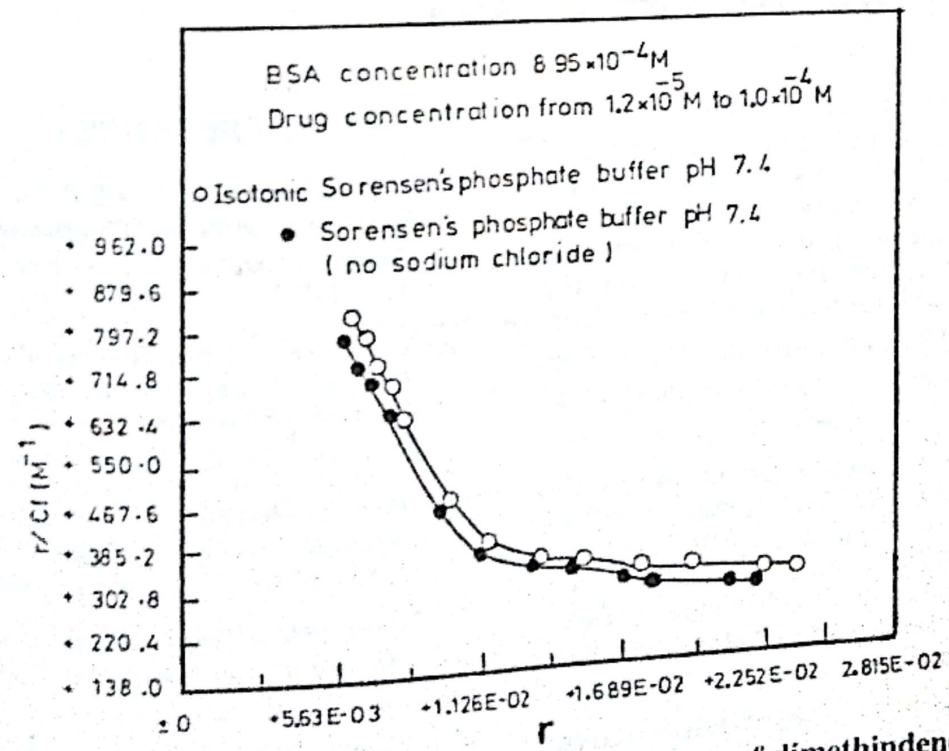

Fig. (13) : Scatchard plot of dialysis data for the binding of dimethindene maleate to bovine serum albumin in Sorensen's phosphate buffer pH 7.4 containing no sodium chloride at 25°C ± 1.

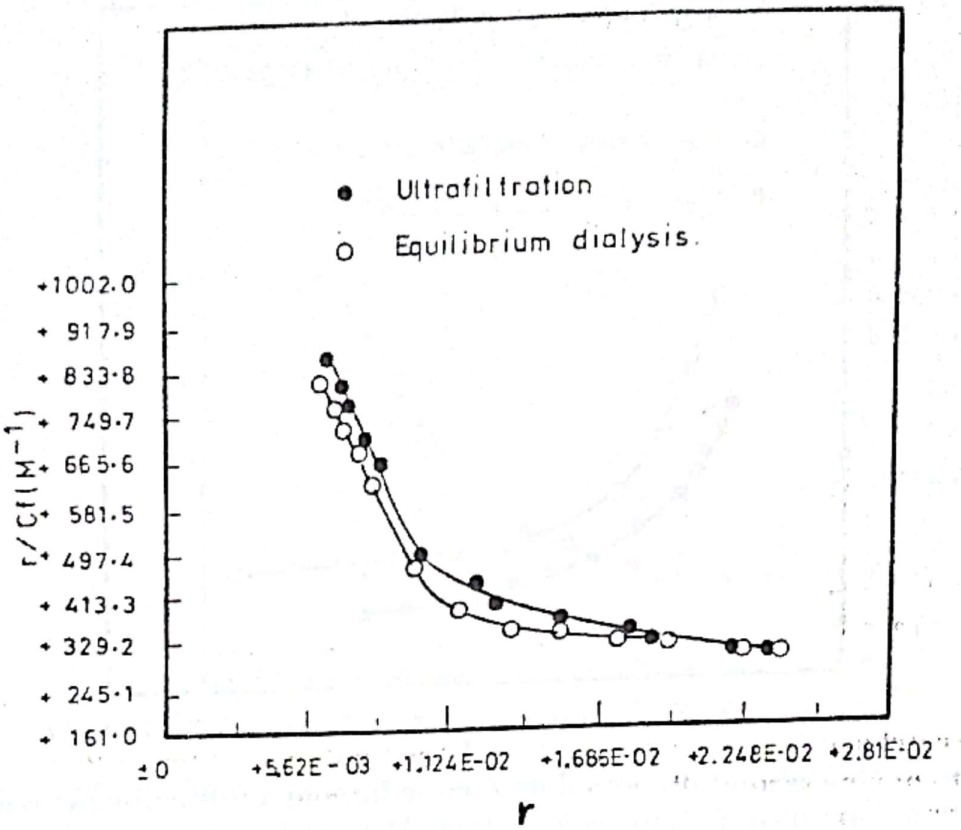

Fig. (14) : Scatchard plot of dialysis data and ultrafiltration for the binding of dimethindene maleate to bovine serum albumin in isotonic sorensen's phosphate buffer pH 7.4 at 25°C ± 1.

interaction of dimethindene maleate with dextran 40000, dextran 266000 and dextran 500000 was studied. Results revealed that no interaction of dimethindene maleate with any of these macromolecular compounds, at any of the tested concentrations, was occurred. Borchardt et al.[22], demonstrated the interaction of many drugs with dextran of different grades.

In the present investigation the binding of drugs to plsama substitute viz. Dextran was negligible and not of clinical relevance (< 20%). Also, no interaction of dimethindene maleate with laevosan and hetastarch was demonstrated.

Comparison of equilibrium dialysis and ultrafiltration techniques for measuring the free fraction of dimethindene maleate gave the same results as shown in Fig. (14).

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تفاعل ماليات الدايميتندين مع زلال بلازما البقر وبدائلها

برند مولر * - محمد سلامة محمد - فخر الدين سليمان غازى - سمير سيد أبو زيد و محمد محمود عبد الرحمن

قسم الصيدلانيات - كلية الصيدلة - جامعة الزقازيق - مصر

** قسم الصيدلانيات - معهد الصيدلة - كيل - المانيا*

تم دراسة ارتباط ماليات الدايميتندين مع دلال بلازما البقر باستخدام طريقة الديلزة المتوازنة فى وجود محلول الفوسفات المنظم بعد ضبط الضغط الاسموزى مع الدم باستخدام كلوريد الصوديوم عند درجة ٢٥°م وقد تم تمثيل النتائج بيانيا حسب طريقة سكاتشارد وقد دلت النتائج على أن ماليات الدايميتندين يرتبط مع زلال البلازما حيث كانت قيمة معامل الربط الاولى k1 والثانوي k2 تساوى ٩ر٤٢ x ٤١٠ م-١، ٥٦ر٠ x ٤١٠ م-١ على التوالى وعدد مواقع الربط n2, n1 تساوى ١٥ر٠، ٠٠ر٠، ٧٥ر٠ على التوالى. وقد اظهر الرسم البيانى وجود اكثر من موقع ربط على جزئي الزلال للدايميتندين، وقد وجد انه عند ثبات تركيز الزلال المستخدم فإن النسبة المئوية للعقار المتحد مع زلال بلازما البقر تتناسب تناسبا عكسيا مع تركيز العقار المستخدم وزن قيم معاملات الربط لم تتأثر كثيرا بتغيير درجة الاسم الايدروجينى فى المدى المذكور زو فى وجود محلول التريس فى حين أن محلول الخلات قد قلل عامل الربط بين العقار وزلال بلازما البقر ولا يتأثر بغياب أيون الكلوريد. وكذلك تمت دراسة ارتباط ماليات الدايميتندين مع الداكستران (ذو الوزن الجزيئى ٤٠٠٠٠، ٦٦٠٠٠، ٥٠٠٠٠٠) وكذلك الليفوزان والهيتاستارش فى وجود محلول الفوسفات المنظم وقد دلت النتائج على أن العقار لا يرتبط بأى من هذه المواد.