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A CONTRIBUTION TO THE INTERACTION BETWEEN CHLORAMPHENICOL AND NONIONIC SURFACTANTS.

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ABSTRACT

The interaction between chloramphenicol and some nonionic surfactants has been investigated using both the dynamic and equilibrium dialysis techniques. The surfactants tested were Tweens, Myrjs and Brijs. The permeation of chloramphenicol across a standard cellophane membrane either in absence or presence of increasing concentrations of those surfactants was found to follow first order kinetics. The data revealed that, the presence of nonionic surfactants resulted in a reduction of the corresponding permeation rate constant. The extent of reduction was parallel to the surfactant concentration.

The antimicrobial activity of chloramphenicol against B. subtilis (ATTC 663) was examined in presence of these nonionic surfactants. The data revealed that the presence of those surfactants has insignificantly affected the antimicrobial activity of chloramphenicol especially when they were used in concentrations above their respective CMC values.

INTRODUCTION

It is well known that nonionic surfactants are characterized by having the property of increasing the aqueous solubility of those slightly water soluble compounds[1-5]. The mechanism of solubilization is assumed to be attributed to an interaction between the slightly water soluble compound and the surfactant which was oriented in a micellar configuration. The phenomenon of drug macromolecule interaction has received a great attention in view of their relevance to drug absorption, transport and overall availability[6].

The marked reduction in drug absorption that is frequently observed in the presence of surfactants is attributed to micellar solubilization[6-8]. The release of salicylic acid from micellar solutions containing different concentrations of Tween 20 and Tween 80 was estimated using the dynamic dialysis method. In all case, the effect of surfactant was to reduce the amount of salicylic acid transferred[9]. The presence of Tween 20 in solutions of salicylic acid reduced its absorption from the stomach[10]. The influence of nonionic surfactants on the permeation of organic compounds across cellulose acetate membrane was previously studied[11-13]. In all experiments, the presence of surfactants reduced the amount of each compound permeated across the membrane. Several articles pertaining the interaction between preservatives and macromolecules have been reported[14-20]. Evidence given in these reports supports the theory that the antimicrobial activity of the preservative is diminished as a result of preservative macromolecule interaction. Recently, the interaction between rifampicin and polysorbates[21] as well as Myrjs[22] has been investigated using the dynamic dialysis technique.

The aim of the present investigation was to evaluate, quantita-

tively, the permeation rate of chloramphenicol across a standard cellophane membrane in presence and absence of some selected nonionic surfactants. The study was also directed to determine the physical parameters associated with the interaction between these surfactants and chloramphenicol. The surfactants tested were Tweens, Myrjs and Brijs. The effect of surfactant concentration on the antimicrobial activity of chloramphenicol was also studied.

EXPERIMENTAL

Chloramphenicol (CID Co., Egypt). Tween 20, Tween 40, Tween 80, Myrj 52, Myrj 59, Brij 35 and Brij 58 (Atlas Chemical Co., USA), Standard cellophane membrane 30/32 (Fischer Sci. Co., Pool, UK), Double beam spectrophotometer (Shimadzu, Japan), Shaker water bath (GFI, UK) and incubator (Memmert, Germany).

Description of the Dialysis Apparatus :

1-The Inner Compartment (Donor):

A piece of a standard cellophane membrane (5x5 cm) was rinsed and soaked in distilled water for 12 hours before use. The membrane was placed between two filter papers, and the water was squeezed out completely. The membrane was stretched at the lower end of a glass cylinder of int. diameter 1.1 cm and height 10 cm, by means of a cotton thread. A 5 ml sample of chloramphenicol solution either in absence or presence of the tested surfactants was introduced into the donor.

2-Outer Compartment (Acceptor)

A 100 ml beaker containing 20 ml of distilled water as a sink solution. It was placed in a constant temperature water bath shaker adjusted at 37°C and 25 shake/minute.

Dynamic Dialysis Study :

The inner compartment was placed into the outer one in a manner that the two liquids, in the donor and acceptor, were in the same level. At suitable time intervals, a one ml sample of the sink solution was pipetted and the cumulative amount of the drug permeated from the donor into the acceptor was calculated by measuring the absorbance at 333 nm. The mechanism of transfer across the membrane was checked according to zero and first order kinetics as well as the controlled diffusion mechanism.

Equilibrium Dialysis Study :

The dialytic units were prepared as mentioned before. They were placed into a water bath shaker and allowed to be shaked for 16 hours, the equilibrium time, under the same experimental conditions. In each experiment the surfactant concentration was varied from 0.3095 to 0.5571 mM. The data obtained were treated according to Scatchard equation to calculate the binding parameters associated with the interaction between chloramphenicol and the tested surfactants.

Estimation of the Antimicrobial Activity :

The antimicrobial activity of chloramphenicol solution either in presence or absence of the tested surface active agents was investigated against B. subtilis (ATTC 6633) as a test microorganism. The microorganism was allowed to grow overnight on nutrient agar medium at 37°C. Nutrient broth was then inoculated with the tested microorganism containing 1% yeast extract and the broth was incubated for 24 hours at 37°C. The antimicrobial activity was measured using the cup plate method[23-24]. In this method the nutrient agar medium was sterilized by autoclaving at 121°C for 20

minutes. When the temperature of the melted sterile agar dropped to 50°C, it was inoculated with 1% v/v of the microorganism which was suspended in the broth. A 25 ml of the melted medium was introduced into the sterile Petri dish. The inoculated medium was allowed to solidify at 37°C before use. A hole of 8 mm in diameter was made in the medium and 0.1 ml of chloramphenicol solution either alone or in the presence of surfactant solution was introduced. The prepared dishes were incubated for 24 hours at 37°C. The diameter of the inhibition zone was measured for the drug alone or in the presence of surfactants. The percentage of inhibition in relation to that produced by control was estimated. It is worthy to note that each experiment was carried out in triplicate and the average diameter of the inhibition zone was calculated. It should be noticed that the tested surface active agents did not exhibit any anti-microbial activity against the tested microorganism when they were used alone.

RESULTS AND DISCUSSION

Figures 1-3 show the results of permeation rate experiments in which the drug was used alone or in presence of Tweens, Fig. 1, Myrjs, Fig. 2, and Brijs, Fig. 3. As shown from these Figures, in presence of increasing concentrations of the tested surfactants a linear relationship between log % concentration remained and time was always obtained. It could be observed that all the tested nonionic surfactants exhibit the same permeation pattern for the drug. The lines show progressive decreasing slopes with increasing the surfactant concentration. This decrease in the slopes provide an evidence for the interaction of chloramphenicol and surfactants. The permeation rate constants of the drug through the cellophane membrane, were calculated using the linear regression analysis and are collected in Table 1. From this table, it can be observed that 1% Brij 58 reduced the permeation rate constant to about 54% in relation to the control. The data also revealed that Myrjs have the least retarding effect towards the permeation rate of chloramphenicol. It is worthy to note that, all the tested surfactants exhibited the same pattern for the permeation of chloramphenicol across the membrane.

Figure 4 is a Langmuir's plot for the interaction between chloramphenicol and nonionic surfactants. The data are plotted in such manner to calculate the limiting binding capacity of each surfactant at infinitely chloramphenicol concentration. In all cases, a linear relationship was obtained when m/x was plotted versus the reciprocal of the equilibrium concentration, $1/c_e$; where , m/x is the amount of the drug bound by one gram of the tested surfactant. When these lines were extrapolated to the Y-axis, an intercept was existed. The presence of such intercepts is of a particular interest; since it suggests that a complex formation is the main factor in the binding of chloramphenicol by these surfactants. If the interaction is mainly due to the partitioning of the drug into the micellar phase the expected curve will be a straight line passing through the origin[25].

From the Langmuir's plots, the limiting binding capacity of each surfactant used at infinitely high chloramphenicol concentration can be calculated and collected in Table 2 the Y-intercept is the reciprocal of the limiting quantity (mole) of chloramphenicol bound per one gram of the tested surfactant. From this table it could be observed that, the higher limiting binding capacity was obtained by Tween 20 ($=816 \times 10^{-7}$ mole/gm). It is of an interest to

find that the permeation rate constant of chloramphenicol in presence of the selected surfactants is inversely proportional to its corresponding limiting binding capacity, Table 2. The data also indicate that, the extent of the interaction is dependent on the type of the surfactant used especially in case of Tween and Brijs. On the other hand, the limiting binding capacity of Myrj 52 and Myrj 59 is the same. This finding indicates that, the interaction takes place between chloramphenicol molecule and both the hydrophilic and lipophilic moieties of the surfactant molecule.

Figures 5-7 are Scatchard plots for the interaction between chloramphenicol and Tweens, Myrjs and Brijs respectively. From these figures, it could be observed that, a straight line was obtained in case of Tweens and Brijs as depicted in Figures 5 and 7 respectively. However, in case of Myrjs, Fig. 6, the plots are curved. This curvature is an indication of the presence of more than one binding site for interaction. On the other hand, the straight lines obtained in case of Tweens and Brijs indicate that the interaction takes place via one site, as shown in Table 3.

The antimicrobial activity of chloramphenicol in presence and absence of the tested surfactants was investigated. The results showed that, the surfactants did not exhibit any antimicrobial activity against the tested microorganism when they are used alone. The antimicrobial activity was measured as a zone of inhibition around the walls after 24 hours of incubation at 37°C. The effect of surfactant concentration on the antimicrobial activity of chloramphenicol is collected in Table 4. The data revealed that, the percent of inhibition exhibited by the drug in presence of different concentrations of the surfactants, was insignificant. This could be explained on the basis that as the surfactant concentration was increased, more drug molecules will be partitioned into the micellar core resulting in a decrease in the amount of the free drug concentration present in the inner micellar phase.

Table 1:Effect of Surfactant Concentration on the Permeation Rate Constant (K, x 10^{-2}) of Chloramphenicol Through Cellophane Membrane.

Surfactant	% w/v		
	0.2	0.6	1
Tween 20	12.39	11.69	8.76
Tween 60	13.05	11.67	9.09
Tween 80	11.73	11.52	10.02
Brij 35	12.23	12.04	9.78
Brij 58	12.26	10.55	8.19
Myrj 52	11.33	10.31	10.29
Myrj 59	15.05	12.16	11.85

N.B.The rate constant for the control=15.13x$10^{-2}hr^{-1}$

Table 2:Limiting Binding Capacity (mole / g) for the Interaction Between Chloramphenicol and Different Surfactants, Calculated from the Langmuir's Plots.

Surfactant	Limiting Binding Capacity (mole / g x 10^5)
Tween 20	8.16
Tween 60	2.58
Tween 80	1.34
Brij 35	1.54
Brij 58	2.99
Myrj 52	1.60
Myrj 59	1.60

Table 3:Binding Parameters for the Interaction Between Chloramphenicol and Surfactants 1% w/v Calculated from Scatchard Plots.

Surfactants	n1	n2	$K_1 \times 10^3$	$K_2 \times 10^3$
Tween 20	12.42	--	0.74	--
Tween 60	3.16	--	7.40	--
Tween 80	1.71	--	10.73	--
Brij 35	1.80	--	5.76	--
Brij 58	3.28	--	8.73	--
Myrj 52	1.87	1.72	0.39	9.5
Myrj 59	3.59	6.4	0.18	4.8

Table 4:Effect of Different Surfactant Concentration on Percent Inhibition Zone of Chloramphenicol Using Streptococcus Subtilis.

Surfactant	% w/v			
	0.2	0.5	1.0	1.5
Tween 20	100	100	100.0	95.5
Tween 60	100	100	97.5	92.7
Tween 80	100	100	95.7	87.0
Brij 35	100	100	100.0	100.0
Brij 58	100	100	98.5	98.5
Myrj 52	100	100	100.0	96.0
Myrj 59	96.2	96.2	96.2	95.1

* Chloramphenicol was used in a concentration of 50 ug/ml.

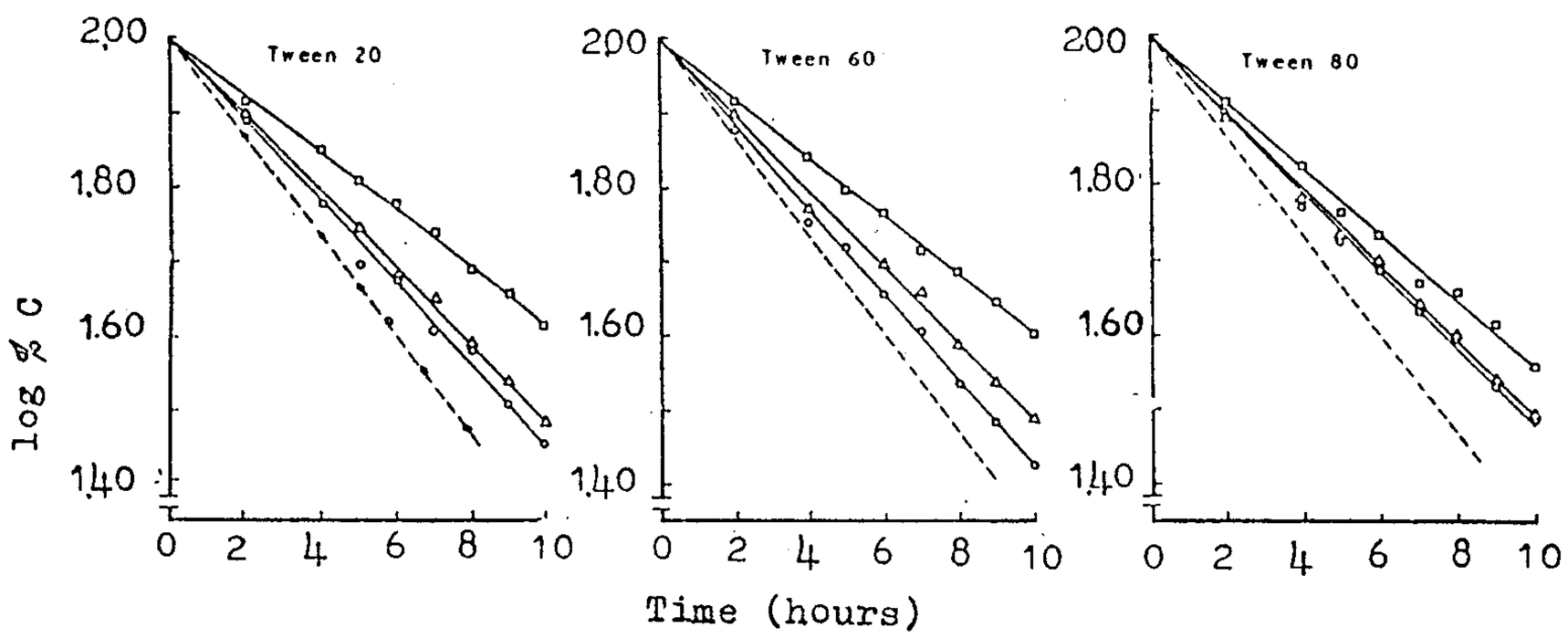

FIGURE 1
First Order Plots for Permeation of Chloramphenicol through Standard Cellophane Membrane in Presence of Different Concentrations of Tweens at 37°C.
Key:---, Control; O, 0.2%; □ 0.6% and △ ,1% w/v.

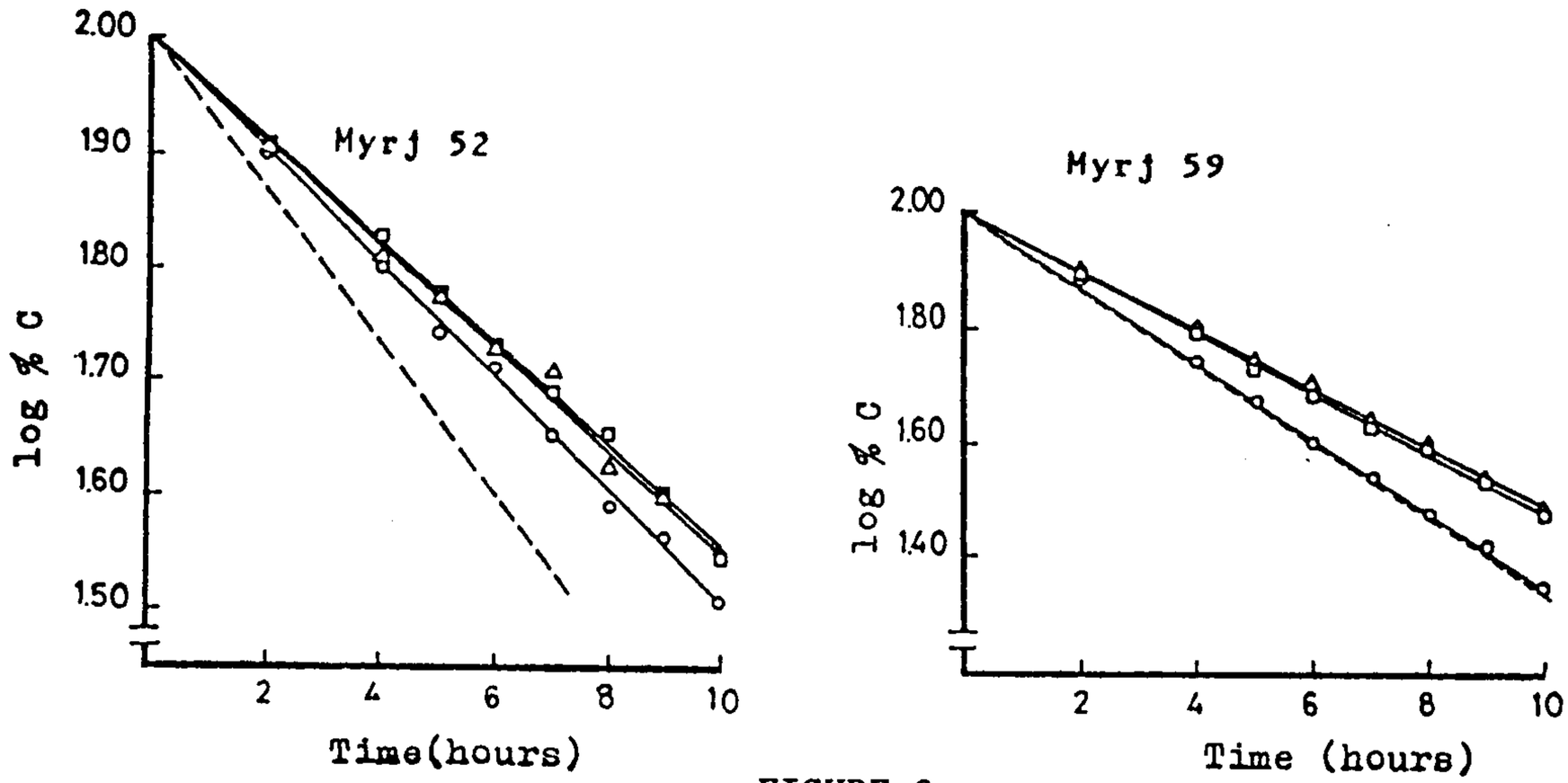

FIGURE 2
First-Order Plots for the Permeation of Chloramphenicol through Standard Cellophane Membrane in Presence of Different Concentrations of Myrjs at 37 °C.
Key: ----, Control; O, 0.2%; □ ,0.6% and △ ,1% w/v.

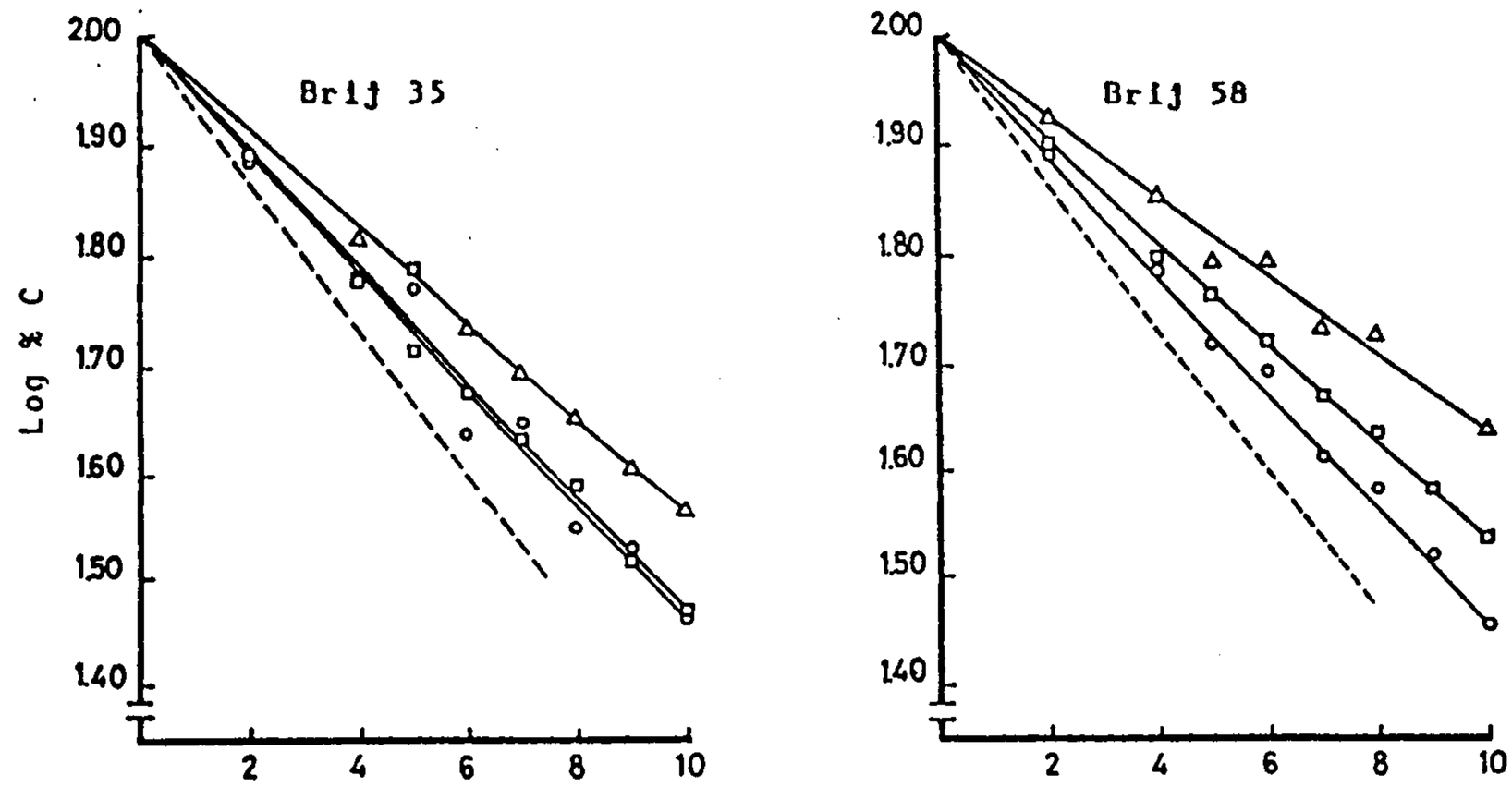

FIGURE 3
First-Order Plots for the Permeation of Chloramphenicol through A Standard Cellophane Membrane in Presence of Different Concentrations of Brijs at 37°C.
Key: ----, Control; O, 0.2%; □ ,0.6% and △ ,1% w/v.

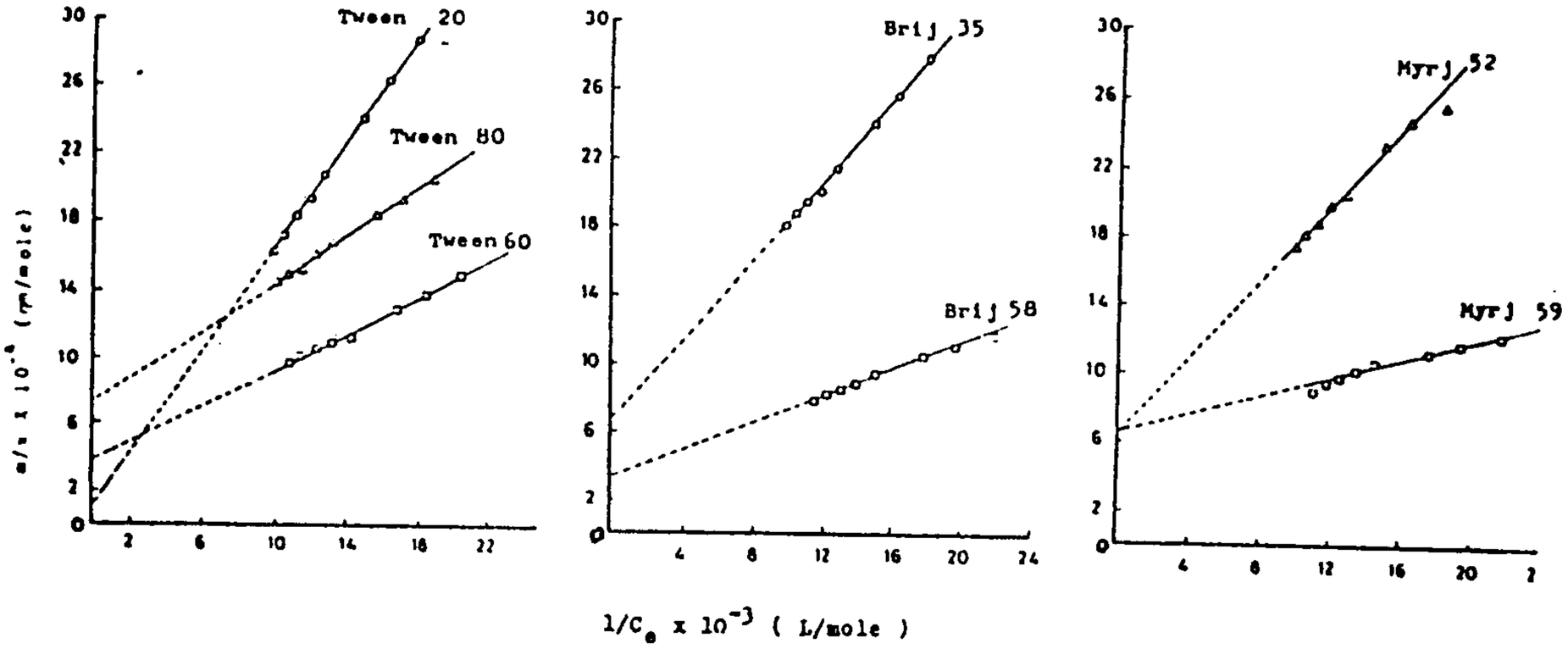

FIGURE 4
Langmuir's Plots for the Interaction between Chloramphenicol and Nonionic Surfactants

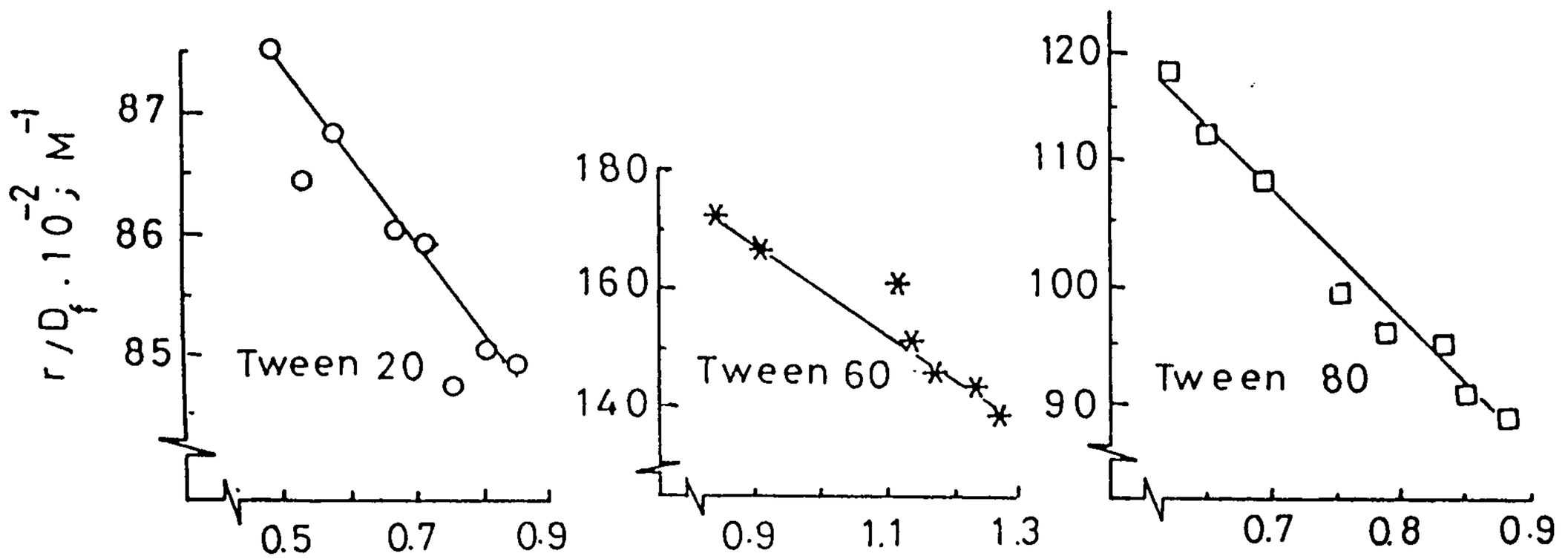

FIGURE 5
Scatchard Plots for the Interaction between Chloramphenicol and Tweens

r = moles of drugs bound per mole macromolecule

D_f= free drug concentration

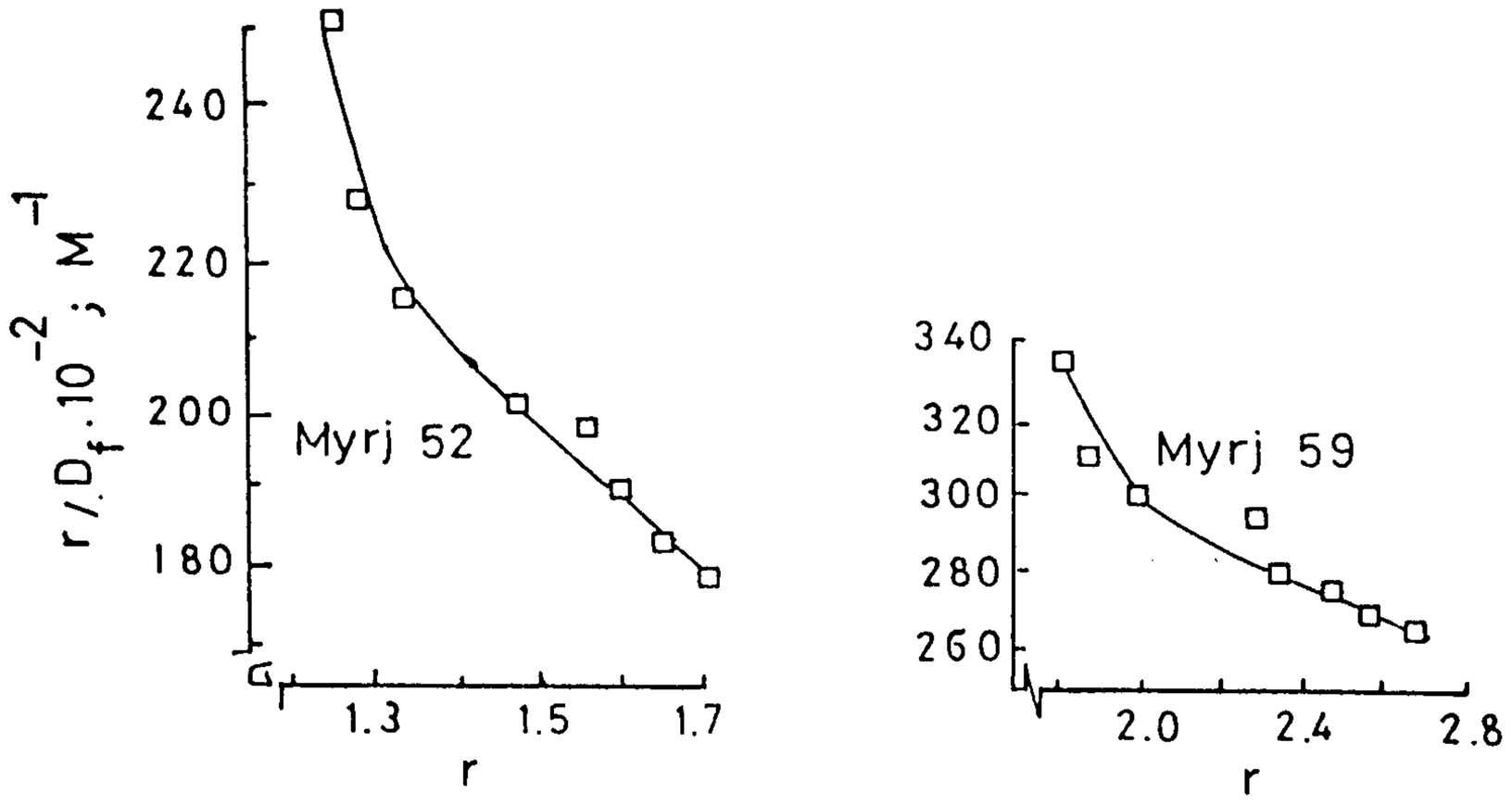

FIGURE 6
Scatchard Plots for the Interaction between Chloramphenicol and Myrjs.

r = moles of drugs pound per mole macromolecule

D_f = free drug concentration

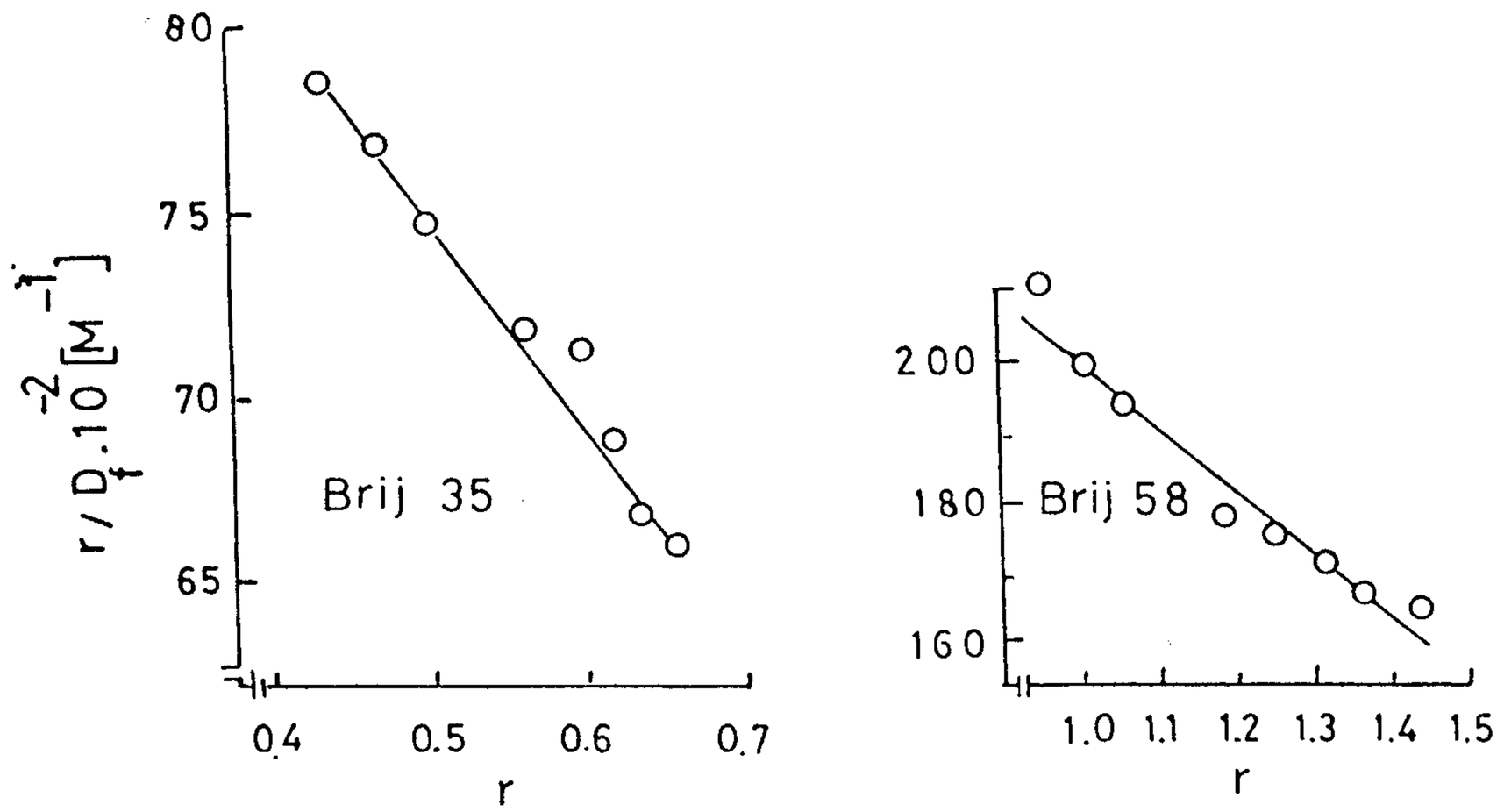

Figure 7
Scatchard Plots for the Interaction between Chloramphenicol and Brijs.

r = moles of drugs pound per mole macromolecule

D_f = free drug concentration

REFERENCES

1-S.Ismail, F.S.Habib and M.A.Attia, Acta Pharm. Fenn., 94, 163 (1985).

2-A.E.Abu-Table and A.Abd El-Zaher; Bull. Pharm., Sci., Assiut Univ. 3, 87 (1980).

3-H.Krasowska; Pharm. Ind., 40, 1381 (1978).

4-H.Tomido, T.Yotsuyanagi and K.Ikeda; Chem Pharm. Bull., 26, 2824 (1978).

5-V.F.Naggar and A.R.Ebian; Pharm. Ind., 47, 1089 (1985).

6-M.Gibaldi and S.Feldman; J. Pharm. Sci., 59, 579 (1970).

7-N.A.Allawala and S.Riegelman; J. Amer. Pharm. Assoc. Sci., Ed., 42, 267 (1953).

8-K.J.Humphreys, G.Richardson and C.T.Rhodes; J. Pharm. Pharmac., 20, 4s (1968).

9-J.H.Collett and N.A.Dickinson; J. Pharm. Pharmac., 32, 805 (1980).

10-J.H.Collett, R.Withingoton and B.Cox; J. Pharm. Pharmac., 26, 34 (1974).

11-P.M.Short, E.T.Abbs and C.T.Rhodes; J. Pharm. Sci., 59, 995 (1970).

12-B.W.Barry and D.I.D. Eini; J. Pharm. Pharmac., 28, 219 (1976).

13-S.W.Barry and A.R.Brace; J. Pharm. Pharmac., 29, 397 (1977).

14-P.P.Deluca and H.B.Kostenbauder; J. Amer. Pharm. Assoc., 49, 430 (1960).

15-N.K.Patel and N.E.Foss; J. Pharm. Sci., 53, 94 (1964).

16-C.K.Bahal and H.B.Kostenbauder; J. Pharm. Sci., 53, 1027 (1964).

17-N.K.Patel and N.E.Foss; J. Pharm. Sci., 54, 1495 (1965).

18-N.K.Patel; Canad. J. Pharm. Sci., 2, 77 (1967).

19-K.F.Brown and M.J.Crooks; Pharm. Acta Helv., 48, 494 (1973).

20-S.J.A.Kazmi and A.G.Mitchell; Canad. J. Pharm. Sci., 11, 10 (1976).

21- F.S.Habib, S.Ismail, S.El-Shanawany and E.A.Fouad; Bull. Pharm. Sci., Assiut Univ. 9, 144 (1986).

22-S.Ismail, F.S.Habib, S.El-Shanawany and E.A.Fouad; Bull. Pharm. Sci., Assiut Univ., 10, 95 (1987).

23-"The United States Pharmacopoeia" United States Pharmacopeia Convention, Inc., P. 595 (1975).

24-F.S.Habib, S.Ismail, S.El-Shanawany and E.A.Fouad; Eur. J. Pharm. Biopham., 37, 38-41 (1991).

25-N.K.Patel and H.B.Kosten bauder, J. Amer. Pharm. Assoc., 47, 289 (1959).

مواصلة دراسة التفاعل بين الكلورامفينيكول والمنشطات السطحية الغير متأينه

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فى هذا البحث أستخدمت الديلزة الديناميكية والمتوازنة فى محاولة لاثبات وجود تفاعل بين الكلورامفينيكول وبعض المنشطات السطحيه الغير متأينه وهى التوينات والميرجات والبريجات وقد لوحظ أن وجود هذه المنشطات قد قلل من ثابت معدل النفاذ للدواء وأن معاملة النتائج بأستخدام معادلة لانجمير أثبتت وجود هذا التفاعل حيث أتضح ذلك من وجود جزء مقطوع من المحور الرأسى وأيضا تم رسم معادلة سكاتشارد لتعين معامل الربط وعدد مواقع الربط .

كما أوضحت الدراسة أن وجود هذه المنشطات لا يقلل من النشاط الحيوى للدواء حتى ولو أستخدمت بتركيزات عالية.

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