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THE USE OF MOLECULAR SIEVE TECHNIQUE FOR STUDYING UNDERSATURATED SYSTEMS CONTAINING CHLORAMPHENICOL

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Molecular sieve technique was carried out using sephadex G 25 fine for investigating the under-saturated systems containing chloramphenicol. This drug was solubilized by a series of commerical non-ionic surfactants which include: Tween 20, Tween 40, Tween 60, Tween 80, Emulgin C1000, Emulgin C1500, Myrj 52, Myrj 53 and Myrj 59. At two different temperatures 25 and 35° it was found that the amount of chloramphenicol incorporated within the micelle as calculated by the molecular sieve technique was greater for Emulgin than Emulgin 1500, on the other hand for Tween series the following sequence was obtained : Tween 80> Tween 40> Tween 60> Tween 20. For Myrj series Myrj 52 was greater than Myrj 53 which in turn was higher than Myrj 59.

The results obtained by investigating the under-saturated systems using this technique agreed with those determined by solubility method and gave further support to the partition model of solubilization and prove the validity of using this technique for investigating undersaturated solubilized systems containing non-ionic surfactants.

Ashworth and Heard[1] applied the molecular sieve technique for investigating the mechanism of binding of methyl P-hydroxybenzoate to polysorbate 80 solutions.

Gelotte[2] showed that most aromatic compounds were adsorbed, reversibly, on sephadex and that a linear adsorption isotherm was obtained.

Aboutaleb et al[3-5] employed the molecular sieve technique for investigating solubilized systems containing salicylic acid and salicylamide in pure non-ionic surfactant solutions. They concluded that the adsorption isotherm for both solutes in non-ionic surfactant solutions were linear

and intersect with the points obtained from saturated solubility measurements. Furthermore, the distribution coefficient calculated from the solubility measurements for both solutes agreed with those obtained by the molecular sieve and equilibrium dialysis techniques.

Donbrow et al[6] investigated solubilized systems containing benzoic acid using this technique.

Aboutaleb et al[7] also used this method together with the ultrafiltration technique for studying other solubilized systems containing benzoic acid.

The purpose of this work is to study undersaturated solubilized systems containing chloramphenicol in order to evaluate the system and determine the activity of solubilized chloramphenicol(amount free in solution).

Furthermore the mechanism of incorporation of this solute within the micelles of different non-ionic surfactants can be clarified and the validity of this technique for investigating other solubilized systems.

EXPERIMENTAL

Materials:

Chloramphenicol[1] was analytical grade.Sephadex G 25 fine[2]. Bule dextran[2].

Non-ionic surfactants:

Tweens[3] : Polyoxyethylene sorbitan monolaurate(Tween 20), polyoxyethylene sorbitan monopalmitate(Tween 40), polyoxyethylene sorbitan monostearate(Tween 60) and polyoxyethylene sorbitan monoleate(Tween 80).

Myrjs[3] : Polyoxyl 40 stearate (Myrj 52), polyoxyl 50 stearate (Myrj 53) and polyoxyl 100 stearate (Myrj 59).

1-El-Nasr chemical Co. Egypt

2-Pharmacia uppsala, Sweden.

3-Atlas Chemical Industries, Delaware U.S.A.

Emulgins[4]: Cetyl stearyl alcohol with 20 ethylene oxide units (Emulgin ^{C}1000) and cetyl stearyl alcohol 30 ethylene oxide units(Emulgin ^{C}1500). The number between brackets denotes the number of ethylene oxide units present in the surfactant.

a) Determination of the water regain of the gel:

1g. dry sephadex G 25 fine, was swollen with 15 ml. distilled water for three hours in a glass stoppered tube at 25°. The gel was transferred quantitatively to a previousely weighed filter stick (seitz filter number 3), then centrifuged at 1000 r.p.m. for ten minutes in order to remove the water in the external phase of the gel. The filter stick was then weighed. The gel showed a water regain of 2.5 ml. per g. of the dry gel.

b) Determination of the void volume of the gel:

For the purpose of the present study, it is important to determine accurately the void volume for the batch of sephadex used. This was performed by using a high molecular weight polysaccharide(blue dextran). In this experiment 4 g. of the dry gel was accurately weighed in a dry glass stoppered tube. The gel was swollen with 15 ml. distilled water for three hours at room temperature. Then, 10 ml. of 0.1% w/v solution of blue dextran in water was added, and the gel equilibrated for one hour at the required temperature. After equilibration, about half of the upper layer was centrifuged gently for 10 minutes in order to remove any suspended gel particles. The solution was analyzed for its blue dextran content spectrophotometrically at 260 nm. The mean void volume values of the gel(4g., 10 determinations) were 15.87 and 16.6 at 25° and 35° respectively.

Determination of adsorption isotherm of chloramphenicol on sephadex G25 fine in absence of surfactant:

4 g. dry gel was swollen with 15 ml. distilled water as described in the determination of the void volume. 10 ml. of varying concentration of chloramphenicol in distilled water was added and the gel equilibrated for one hour at the required temperature e.g, 25°

4. Henkel international, Dusseldorf West Germany

and 35°. The supernatant liquid was centrifuged and assayed for its chloramphenicol content spectrophometrically at 278 nm after appropriate dilution with distilled water. The results obtained at 25°and 35° are shown in Figure 1.

Determination of the degree of interaction between Chloramphenicol and different concentrations of the non-ionic surfactant solutions at 25° and 35°:

Four grams of the dry gel was swollen with 15 ml. of distilled water or 0.2%w/v chloramphenicol solution in water The gel was allowed to swell for three hours at room temperature. 10 ml. of different concentrations of chloramphenicol in the required surfactant concentration was added to the swollen gel. After equilibration for one hour at the required temperature, the upper liquid phase was separated, centrifuged and analyzed for its chloramphenicol content as before. The results obtained at 25° and 35° in 2% w/v and 5% w/v surfactant solutions are shown in Tables 1-4 and Figures 2-5.

RESULTS AND DISCUSSIONS

Gelotte[2] showed that most aromatic compounds were adsorbed, reversibly, on sephadex and that a linear adsorption was obtained. It was therefore, anticipated that chloramphenicol would also reversibly adsorbed to the gel. The plots of concentration of chloramphenicol in the external phase against the weight of chloramphenicol associated with the gel were also linear as shown in Figure 1. The amount of chloramphenicol bound to the gel in this case is the sum of that present in solution in the internal phase and the quantity actually adsorbed on the gel matrix. It is required neither to distinguish between them, nor necessary to know the volume of the internal phase since the quantity of chloramphenicol adsorbed is dependent on its concentration in the internal phase, the latter must be equal to the external concentration. Thus the quantity of chloramphenicol associated with the gel in this case is dependent on the concentration

of this medicament in the external phase. The quantity of chloramphenicol associated with the gel, also decreases by increasing the temperature as shown in Figure 1.

In the presence of surfactants, determination of this solute in the external phase by direct spectrophotometric measurements enables the quantity of chloramphenicol associated with the gel to be determined, as the total amount of the solute added to the system is known. The concentration of solute free in the external phase can, then, be calculated from the experiments performed in absence of surfactants as shown in Figure 1.

The amount of solute bound to micelles can be also obtained as the amount of solute in the external phase includes those free in solution and those bound to micelles.

The results obtained are shown in Figures 2-5. and Tables 1-4. From previous experiments, the linear relationship between concentration of chloramphenicol free in solution(mole/L.)and those bound to micelles(m mole per gram)for the different non-ionic surfactant solutions (2% w/v) at25^{o} was shown in Figure 2. This linearity in the adsorption isotherm of this solute between the micellar and aqueous phases gave further support to the partition model of solubilization.

It is very obvious from the results that micelles formed by Emulgin ^{C}1000 can incorporate more chloramphenicol than those of Emulgin ^{C}1500. and thus the binding capacity of the different non-ionic surfactant micelles for chloramphenicol can be arranged in the following sequence at the two different temperatures used: Emulgin ^{C}1000> Emulgin ^{C}1500 > Tween 80> Tween 40> Tween 60> Myrj 52> Myrj 53> Myrj 59.

It is interesting to notice that the solubilizing capacity of the different non-ionic surfactant micelles were in the same order as obtained before from the saturated solubility measurements. On raising the temperature from 25^{o} to 35^{o}, it was found that the ability of the different non-ionic

micelles to incorporate chloramphenicol was increased. This can be shown on comparing Figure 2 and Figure 4 or Figure 3 and Figure 5, as well as form the corresponding Tables 1-4. The gel experiments were also performed using different non-ionic surfactant solutions of concentrations 5% w/v, it was found that the amount of solute bound to micelles increased by increasing the surfactant concentration, but the distribution coefficients obtained were the same all over the saturation range as shown in Figure 5 and Table 4. Furthermore, they were not affected by change in surfactant concentration. This agreed with the results obtained from solubility measurements and gave further support to the partition model of solubilization proposed by McBain[8], which was originally proposed for saturated systems containing ionic surfactants and was extended by Mulley[9] for systems containing non-ionic surfactants.

Some of the points on the adsorption isotherms were found to deviate from linearity especially at small solute concentratations but most of the points obtained were found to be linear all over most of the saturation range. This slight deviation may be due to one or more factors arised during the experiments. In gel experiments, it is difficult to tell whether, or not, significant amount of surfactants could pass into the internal phase of sephadex gel because of the difficulty of complete removing the external phase.

Ashworth and Heard[1], reported, for Tween 80(molecular weight 1300) that there was only 0.009% w/v present in the internal phase of the gel. Aboutaleb[3-5] suggested that cetomacrogol, which is a very similar material, did not penetrate the internal phase significantly. He reported that even lower molecular weight non-ionic surfactants can remain almost completely in the external phase of the gel.

Some of the solute is obviously bound to the gel and the method depends on the assumption that this is reversible and

unchanged by the presence of surfactant. There appears to be a not satisfactory way for checking this assumption.

Some changes in the void volume were observed at higher temperature.

For good resulte, void volume must remain constant whithin a set of experiments and since temperature changes inevitably occur during the hydration process(a marked temperature rise takes place when the gel is placed in contact with water) errors may arise in tempertature effects on the void volume which are not reversible. Although this possibility must be considered, there was no evidence that change in void volume occurred during the experiments performed at the same temperature. The linear relationship between the the amount of chloramphenicol free in the external phase and those bound by micelles suggests also that micellar structure is unchanged by the concentration of the surfactant and that there is one micellar species present. The behaviour is again consistent with two pseudo-phase theory of solubilization. This technique offers great advantage for estimating the activity of chloramphenicol or the amount free in solution within a short period which can be correlated with other bacteriological methods.

Table 1) Results of gel experiments using different concentrations of cholramphenicol in 2% w/v non-ionic surfactant solutions at 25°C.

Tween 20	A	15.0	13.5	10.0	7.40	3.00
	B	0.55	0.50	0.34	0.27	0.11
Tween 40	A	13.3	11.0	9.0	6.6	3.0
	B	0.84	0.71	0.54	0.41	0.2
Tween 60	A	14.1	12.5	10.0	7.0	2.0
	B	0.70	0.66	0.49	0.35	0.10
Tween 80	A	14.0	12.3	9.0	8.0	5.0
	B	1.16	1.0	0.75	0.63	0.40
Emulgin C_{1000}	A	11.3	9.0	5.6	4.0	3.0
	B	1.18	0.94	0.58	0.40	0.30
Emulgin C_{1500}	A	14.0	11.8	9.0	6.0	3.5
	B	1.28	1.07	0.82	0.53	0.30
Myrj 52	A	16.0	14.2	13.7	8.0	4.0
	B	0.38	0.35	0.32	0.19	0.10
Myrj 53	A	16.8	15.0	13.5	8.4	3.0
	B	0.24	0.22	0.19	0.11	0.04
Myrj 59	A	16.0	14.0	12.0	9.0	6.0
	B	0.09	0.08	0.07	0.05	0.03

A : Concentration of free chloramphenicol m Mole/L.

B : m Mole chloramphenicol bound per gram micelle.

Table 2) Results of gel experiments using different concentrations of chloramphenicol in 5% w/v non-ionic surfactant solutions at 25°C.

Tween 20	A	15.0	14.0	11.3	8.5	5.6
	B	0.6	0.57	0.47	0.34	0.23
Tween 40	A	15.0	11.0	10.0	7.4	5.0
	B	1.19	0.92	0.8	0.6	0.4
Tween 60	A.	15.0	10.0	8.4	4.8	3.0
	B	0.94	0.63	0.54	0.28	0.20
Tween 80	A	15.0	10.0	6.4	3.0	2
	B	1.53	1.07	0.67	0.31	0.19
Emulgin C_{1000}	A	15.0	10.0	7.0	5.6	3.0
	B	1.9	1.27	0.95	0.73	0.38
Emulgin C_{1500}	A	15.0	10.0	6.0	5.0	3.0
	B	1.71	1.14	0.69	0.6	0.35
Myrj 52	A	15.0	12.3	10.0	6.0	3.0
	B	0.45	0.4	0.33	0.2	0.1
Myrj 53	A	15.0	12.3	10.0	6.0	3.0
	B	0.45	0.4	0.33	0.2	0.1
Myrj 59	A	16.0	12.7	9.4	6.0	4.0
	B	0.15	0.12	0.1	0.06	0.04

A : Concentration of free chloramphenicol m Mole/L.

B : m Mole chloramphenicol bound per gram micelle.

Table 3) Results of gel experiments using different concentrations of chloramphenicol in 2% w/v non-ionic surfactant solutions at 35°C.

Tween 20	A	13.2	10.0	8.0	6.0	3.0
	B	0.86	0.65	0.52	0.4	0.2
Tween 40	A	10.0	8.9	7.8	5.5	3.0
	B	1.07	0.95	0.83	0.6	0.3
Tween 60	A	12.3	11.1	9.8	6.1	3.0
	B	1.10	0.89	0.8	0.5	0.24
Tween 80	A	12.0	10.2	9.1	6.0	3.0
	B	1.6	1.35	1.23	0.79	0.4
Emulgin C_{1000}	A	9.0	8.0	7.0	4.6	3.0
	B	1.54	1.4	1.27	0.75	0.52
Emulgin C_{1500}	A	12.0	10.0	8.0	6.0	3.0
	B	1.78	1.51	1.17	0.9	0.45
Myrj 52	A	14.3	10.0	7.2	6.0	3.0
	B	0.67	0.48	0.32	0.3	0.14
Myrj 53	A	15.0	10.0	7.4	6.0	3.0
	B	0.53	0.34	0.27	0.2	0.1
Myrj 59	A	15.0	13.0	10.0	7.0	3.0
	B	0.35	0.33	0.24	0.15	0.07

A : Concentration of free chloramphenicol m Mole/L.

B : m Mole Chloramphenicol bound per gram micelle.

Table 4) Results of gel expermints using different concentrations of chloramphenicol in 5% w/v non-ionic surfactant solutions at 35°C.

Tween 20	A	10.0	8.0	7.0	4.6	3.0
	B	0.73	0.56	0.51	0.3	0.22
Tween 40	A	10.0	8.0	6.8	3.3	2.0
	B	1.13	0.93	0.78	0.38	0.25
Tween 60	A	7.7	6.7	5.5	3.9	3.0
	B	0.71	0.65	0.6	0.35	0.3
Tween 80	A	8.0	7.0	5.7	5.0	2.7
	B	1.15	1.04	0.84	0.7	0.42
Emulgin C_{1000}	A	8.0	7.0	6.0	5.0	2.2
	B	1.6	1.4	1.2	1.0	0.46
Emulgin C_{1500}	A	8.0	6.5	5.2	4.0	2.0
	B	1.35	1.1	0.88	0.65	0.35
Myrj 52	A	9.0	7.8	6.8	5.7	4.0
	B	0.5	0.45	0.4	0.35	0.24
Myrj 53	A	11.1	10.0	8.8	5.5	3.0
	B	0.48	0.43	0.38	0.25	0.1
Myrj 59	A	14.3	13.2	12.2	7.2	6.0
	B	0.24	0.22	0.17	0.12	0.13

A : Concentration of free chloramphenicol in Mole/L.

B : m Mole chloramphenicol bound per gram micelle.

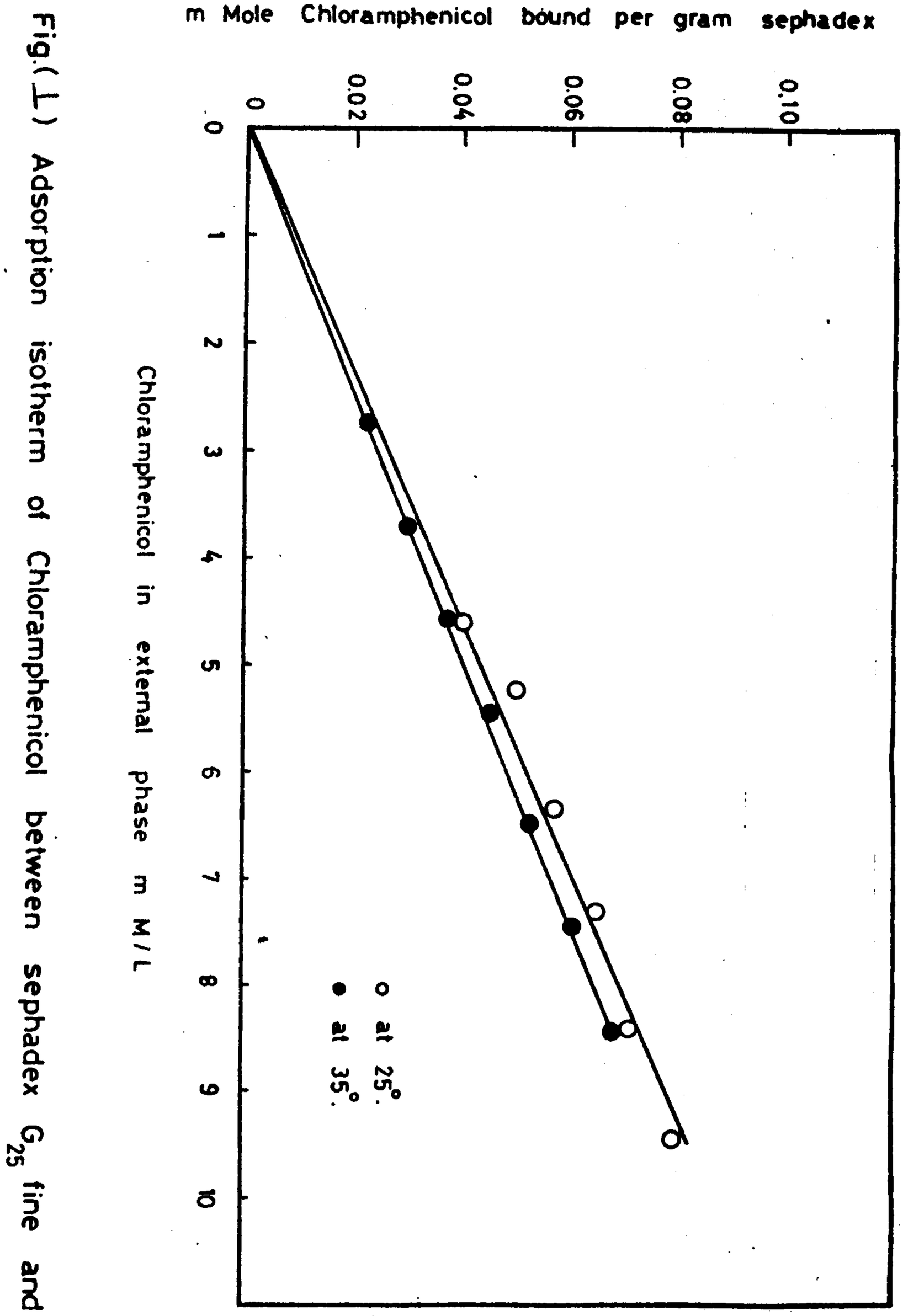

Fig.(1) Adsorption isotherm of Chloramphenicol between sephadex G_{25} fine and water at different temperatures.

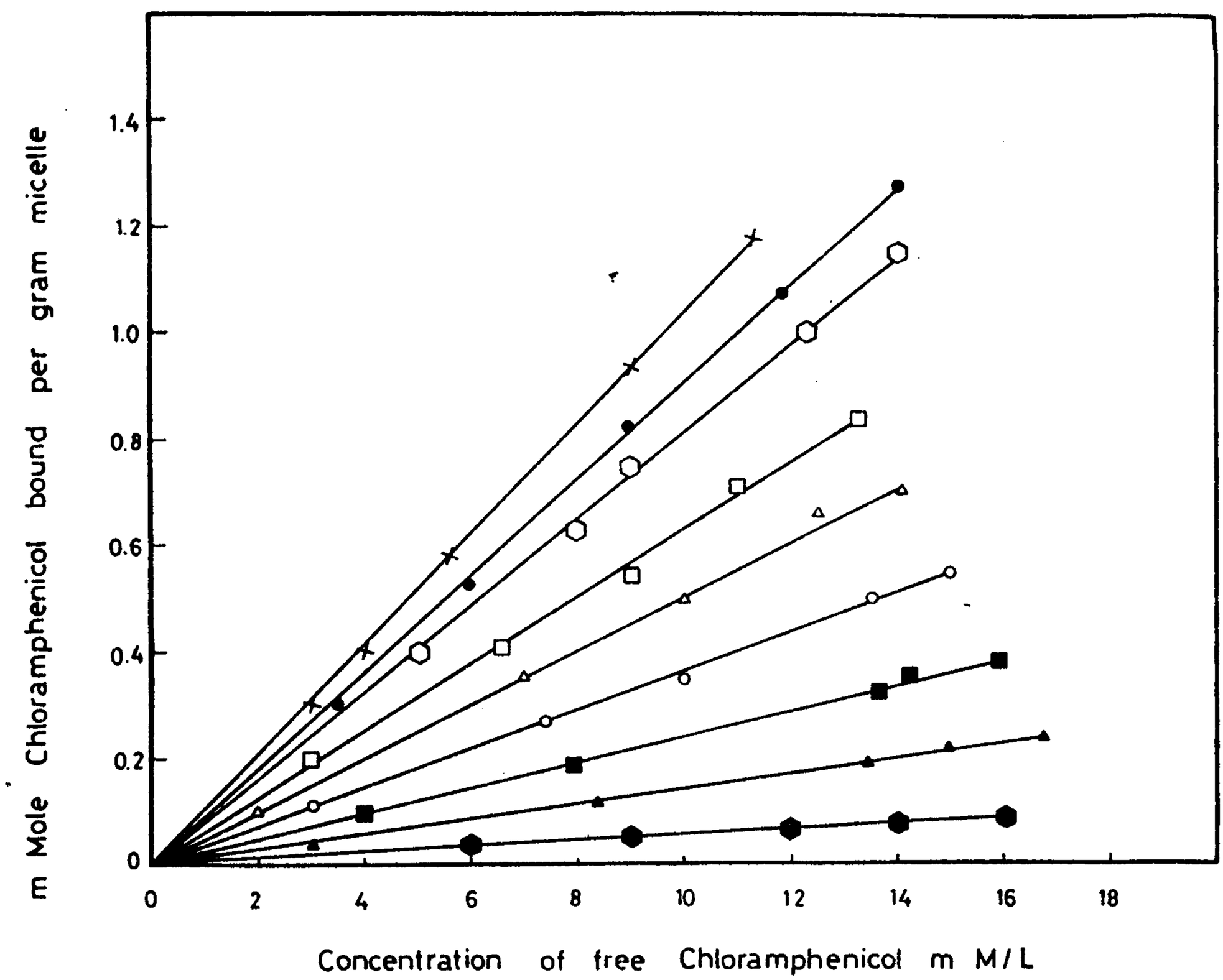

Fig.(2) Gel experiments using different concentrations of Chloramphenicol in 2% w/v non-ionic surfactant solutions at 25°.

Key: x Emulgin C1000. ● Emulgin C1500. ⬡ Tween 80. △ Tween 60.
□ Tween 40. o Tween 20. ■ Myrj 52. ▲ Myrj 53. ⬢ Myrj 59.

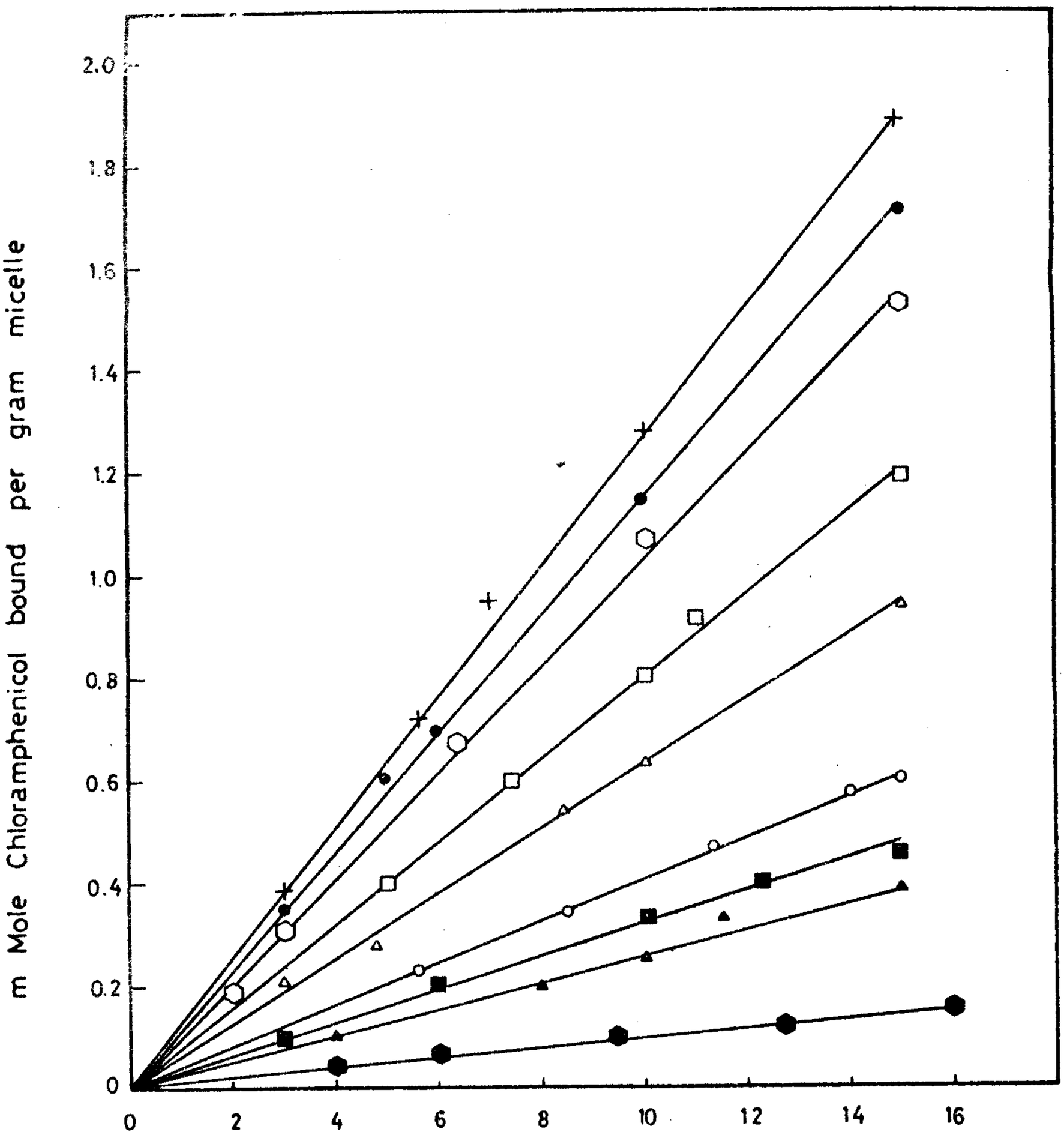

Fig.(3) Gel experiments using different concentrations of Chloramphenicol in 5% w/v non-ionic surfactant solutions at 25°.

Key: The same as Fig.(2).

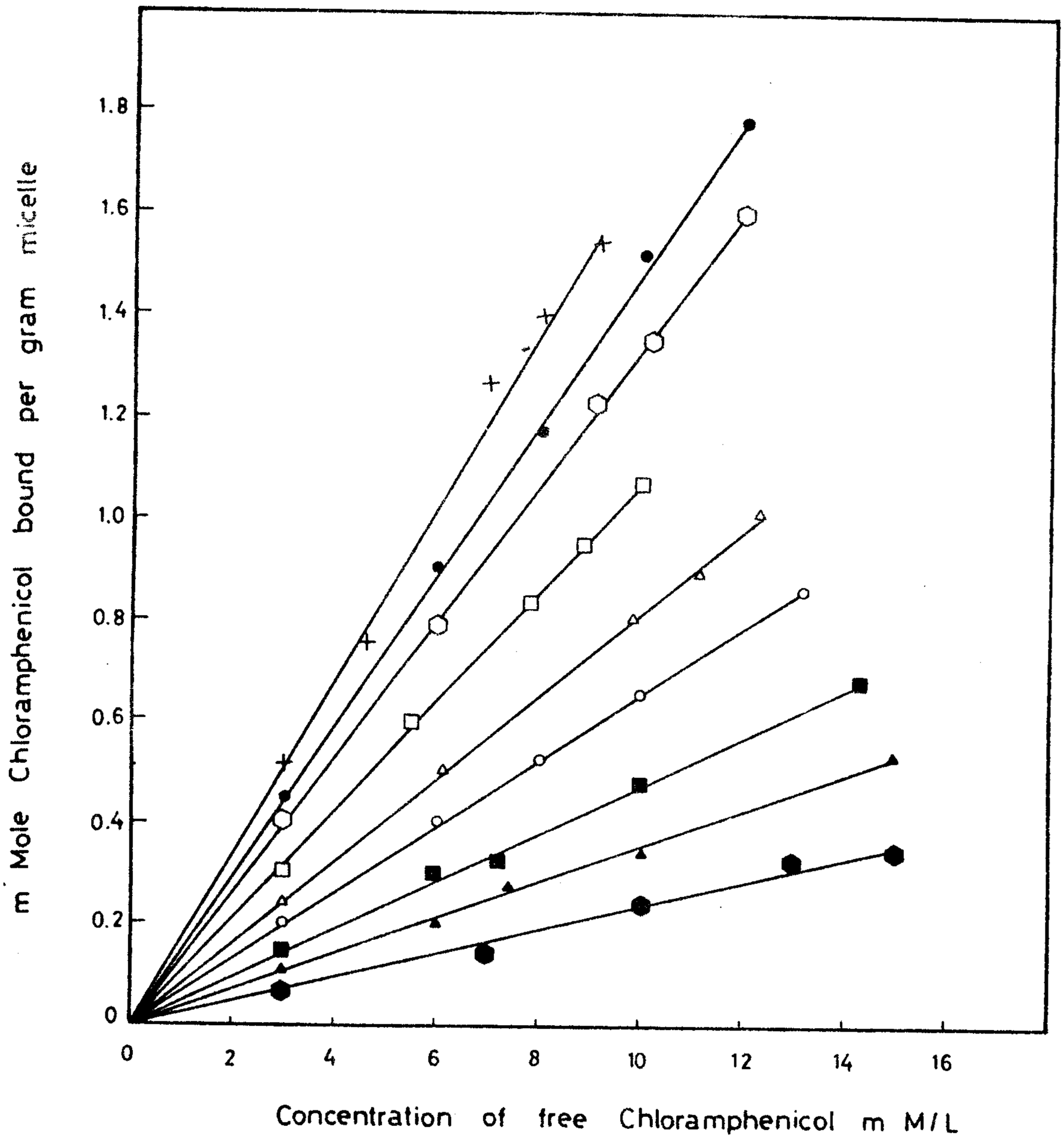

Fig.(4) Gel experiments using different concentration of Chloramphenicol in 2 % w/v non-ionic surfactant solutions at 35°.

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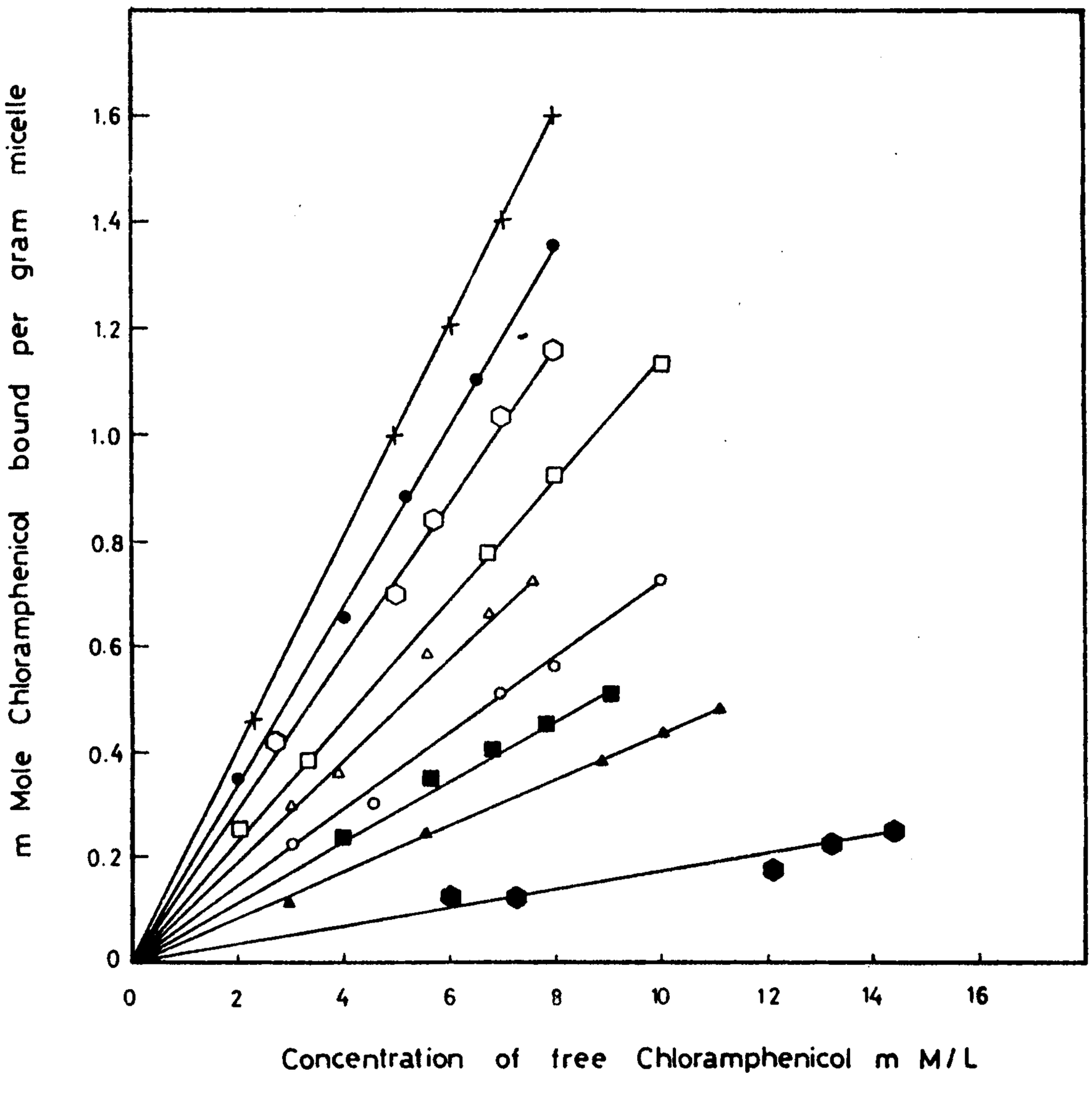

Fig.(5) Gel experiments using different Concentration of Chloramphenicol in 5% w/v non-ionic surfactant solutions at 35°.

Key: The same as Fig.(2).

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استخدام طريقة الغرملة الجزيئية لدراسة النظم تحت المشبعة التى تحتوى على الكلورامفينيكول

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أتبعت طريقة الغرملة الجزيئية باستخدام مادة السفادكس ج ٥٢ الدقيق لدراسة النظم تحت المشبعة التى تحتوى على الكلورامفينيكول . أذيب هذا الدواء بسلسلة من منشطات السطح غير المتأينة وهى, توين ٢٠ ، توين ٤٠ ، توين ٦٠ ، توين ٨٠ أملجين س ١٠٠٠ ، أملجين س ١٥٠٠ ، ميرج ٥٢ ، ٣٥ ميرج ، ميرج ٥٩.

ولقد وجدت أن كمية الكلورامفينكول فى درجتى حرارة ٢٥ ، ٣٥ درجة مئوية والتى متواجده داخل الشباك والتى حسبت بطريقة الغرملة الجزيئية كانت اكبر للاملجين س ١٠٠٠ من الاملجين س ١٥٠٠ ومن الناحية الاخرى وجد أن كمية الكلورامفينكول المتواجدة داخل شباك سلسلة التوين يمكن أن ترتب كالاتى : توين ٨٠ ، توين ٤٠ ، توين ٦٠ ، توين ٢٠. أما الميرج ٥٢ فلقد وجد أنه يذيب كمية كلورامفينكول اكبر من الميرج ٥٣ وهذا بدوره اكبر من الميرج ٥٩.

ولقد وجد ان هناك تطابق بين النتائج التى أخذت من هذه الطريقة وطريقة الاذابة واعطت مزيدا من التأييد لنظرية الاذابة بالتجزئ وبرهنت على قيمة هذه الطريقة لدراسة النظم تحت المشبعة التى تحتوى على الكلورامفينكول .