CHAPTER IV

IN VITRO STUDIES

4.1 INTRODUCTION

Physiological availability of a topically applied drug depends on both, the rate of release from the vehicle and permeability through the skin. *In vitro* methods are valuable for screening procedures and for deducing physicochemical parameters such as fluxes, partition coefficient and diffusion coefficient. A theoritical disadvantage of such a technique is that the method does not exactly duplicate the behaviour of living tissue *in situ*, particularly with respect to a capricious blood supply and metabolism. Topical steroids constrict the capillaries *in vivo* and may decrease their own clearances, which would not happen *in vitro*.

The dissolution test is the single most useful *in vitro* method for assuring batch-to-batch uniformity and bioavailability of solid oral dosage forms. However, at present, no such method exists for determining the *in vitro* release characteristics of topical products such as creams, ointments and lotions. In test cells which have been developed (1,2,3) for studying percutaneous absorption, the dosage form is generally applied to one side of a membrane (mostly skin) and the diffusion of drug is measured. Due to constraints in availability of human cadaver skin, excised skin from a variety of animals including rats, mice, rabbits, guinea pigs, hamsters, pigs, dogs and monkeys has been used in diffusion cells although mammalian skin varies widely in characteristics such as stratum corneum thickness and the number density of sweat glands and hair follicles (4).

Laboratory rodents are convenient for dermal studies because of ease of handling and low cost. Hairless mouse has similarity in skin to human skin in absorption of antiinflammatory steroids(5). Rat skin is more permeable than human skin but the difference is not large. The thickness of the stratum corneum of female rat back skin is equivalent to that of human skin while for male rat is almost twice as thick as that of female and hence presents a better barrier against percutaneous absorption.

In the present study, a verticle *in vitro* diffusion apparatus was designed. All the formulations prepared were subjected to permeation studies to short-list the promising formulations for *in vivo* studies.

4.2 EXPERIMENTAL

4.21 Apparatus and Equipments : Tablet Dissolution test apparatus (Veego TSC 2000), Spectrophotometer (Systronics UV-Visible spectrophotometer 108), Funnels (i.d. 4.8 cms), beakers (250ml) (Borosil).

4.22 Materials : Cellophane (gift sample from Sarabhai Chemicals Ltd.), isoniazid (Wilson Laboratories), sodium chloride, disodium hydrogen phosphate, potassium hydrogen phosphate, anhydrous sodium sulphate, methanol, chloroform (Qualigens). All the materials used were of analytical grade and were used without further purification.

4.23 Reagents

(i) pH 7.4 phosphate buffered saline I.P: This was prepared as per the procedure described in I.P. 1985.

(ii) Spiking solution : 10µg/ml solution of drug in methanol.

4.24 Methods :

The <u>in vitro</u> experimental set-up is shown in Fig.4.1. The membrane used for the <u>in vitro</u> study was rat skin. Wistar female rats weighing between 150-200g were sacrificed by a stunning blow on the head followed by cutting the jugular vein. The hair on the back was shaved carefully with a razor and the skin was excised. After removal of the subcutaneous fat by peeling, the skin was washed thoroughly with water followed by pH 7.4 phosphate buffered saline I.P. The skin was tied, with its epidermal surface upwards, to the mouth of a funnel, after applying 200mg of the formulation to be tested, on it. The sides were stuck to the surface of the funnel by means of a cellotape to prevent the permeation fluid from entering from the sides. Six such funnels were prepared per formulation and were plugged into the protrusion of the dissolution apparatus. The assembly was then lowered, such that, the dermal surface was just flush to the surface of the permeation fluid (pH 7.4 phosphate buffered saline, 50ml) kept in 250ml beakers. The dissolution apparatus was operated at 50 r.p.m. and 25ml of samples were withdrawn at 1,2,4,6 and 24 hours intervals with fresh 25ml buffer

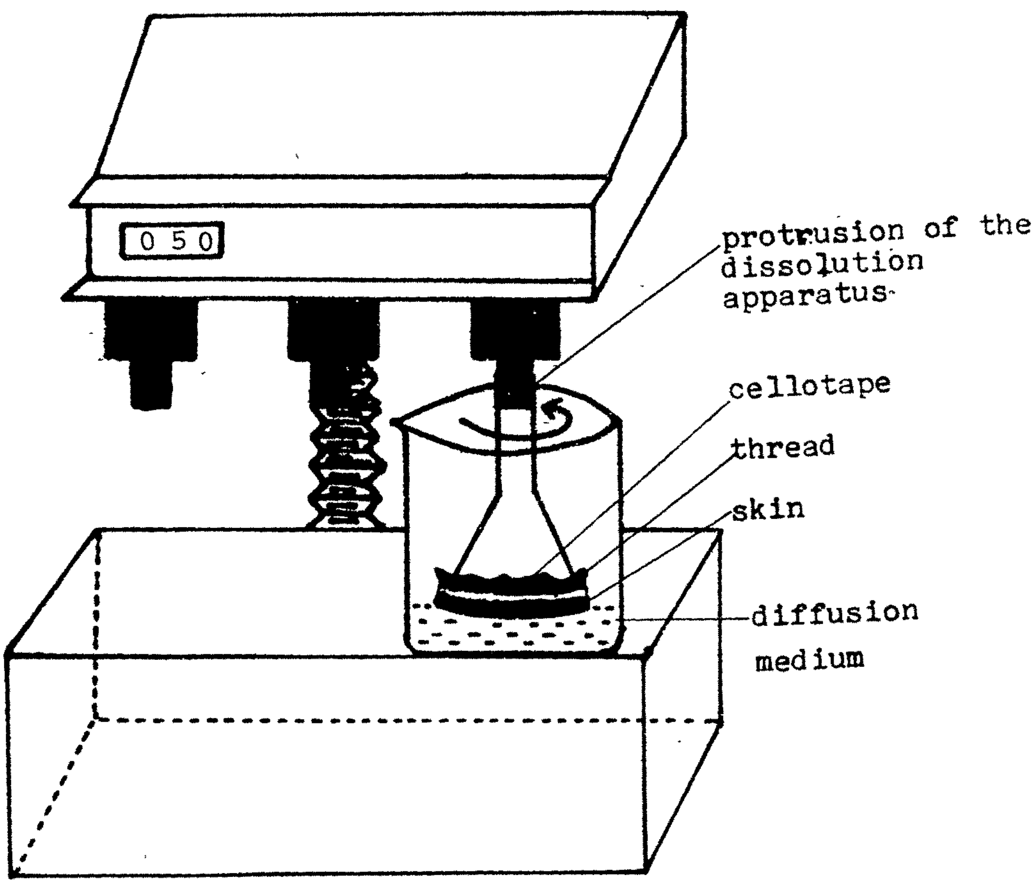

FIG. 4.1 : EXPERIMENTAL SET-UP FOR THE DIFFUSION STUDIES.

replacement after every sampling time point. Each 25 ml sample was extracted with 10,10 and 5ml chloroform after being saturated with sodium chloride. The combined chloroform extracts were passed through a bed of anhydrous sodium sulphate which was washed down with fresh chloroform. They were transferred to 25 ml volumetric flasks and the solvent evaporated to dryness at 65^{o}C on a water bath. The residue was dissolved in 2ml of methanol. All the methanolic solutions so obtained, were spiked with 10ml of the spiking solution of the drug and the contents estimated for the drug by using the method described in Sec. 3.23. Corrections were made for the sample volume withdrawn. The mean percentage released, of 6 runs for each formulation is recorded in Tables 4.2, 4.6 and 4.10.

The uniformity of the experimental set-up with respect to the 6 different attachment points, was tested, by conducting the <u>in vitro</u> test, in triplicate, using cellophane paper as a standard membrane. Formulation 'KTG' was used to validate the apparatus. The results of these runs were subjected to ANOVA to detect any differences between the 6 sampling ports.

In order to establish whether sink condition for each drug was achieved using 150 ml of the permeation fluid, the solubilities of the drugs in pH 7.4 phosphate buffered saline were determined at room temperature by shaking excess of the drug in it for 8 hours and analysing the filtrate for the quantity of drug dissolved per ml. These experiments were performed in triplicate for each drug and the results are shown in Table 4.1.

4.3 DATA ANALYSIS

The following sequence was followed to determine the various kinetic parameters governing the release of the drug from its vehicle, skin permeability and clinical efficacy.

(i) The data of Mean cumulative amount released ($\mu g/cm^2$) and square root of time ($sec^{1/2}$) was subjected to linear regression analysis to determine the relationship between these two variables (r). If a positive correlation was obtained i.e. $0.97 \leq r \leq 0.99$ the equation of the regression line was determined. The slope of this line, obtained by linear regression represents the release rate (R $\mu g/cm^2/sec^{1/2}$) (6).

(ii) Permeability coefficient of the drug through the membrane was calculated using the following equation :

$$R = PCd \qquad (7)$$

where R = release rate ($\mu g/cm^2/sec^{1/2}$)

Cd = drug concentration on the surface of the stratum corneum (mg/cm^3)

P = Permeability coefficient (cm/sec)

(iii) Diffusion coefficient of the drug in the barrier was calculated using the following equation :

$$R = 200 \left(\frac{Dt}{\pi h^2}\right)^{1/2} \qquad (8)$$

where R = % drug released

h = thickness of the skin (0.04cm)

t = time (sec)

D = diffusion coefficient (cm^2/sec)

(iv) Partition coefficient of drug between stratum corneum and vehicle was determined using the following equation :

$$K = \frac{Ph}{D} \qquad (9,10)$$

where K = partition coefficient between stratum corneum and vehicle

P = permeability coefficient (cm/sec)

D = diffusion coefficient (cm^2/sec)

h = thickness of skin (0.04 cm)

(v) Since the *in vitro* set up was a zero order flux case i.e. having a membrane separating two compartments with a concentration gradient operating during a run and sink conditions prevailing in the receptor compartment, the theoritical quantity of drug which enters the membrane upto any time t and the quantity which remains in the membrane was calculated using the following equations (2) :

$$Q^i(t) = \frac{KDCo'}{h} \left(t + \frac{h^2}{3D}\right)$$

$$Q^m = \frac{KhCo'}{2}$$

where $Q^i(t)$ = quantity of drug which enters the membrane upto any time t (dimensionless)

Q^m = quantity of the drug remaining in the membrane (dimensionless)

Co' = donor concentration (mg/g)

K = partition coefficient

D = diffusion coefficient (cm^2/sec)

h = thickness of skin (0.04cm)

t = time (sec)

(vi) The steady state blood concentrations following topical application in human beings can be calculated using the permeability coefficient from in vitro experiments on human skin by using the following equation :

$$Css = A.\ Co'P/\ Vd.ke \qquad (11)$$

where

Css = steady state blood concentration (mg/ml)

A = area of application to the skin (cm^2)

P = permeability coefficient (cm/sec)

Vd = Volume of distribution (ml)

ke = elimination constant (sec^{-1})

Co' = donor concentration (mg/g)

Since A and Vd and ke are constant for an experimental set up and animal respectively, rather than calculating Css, we calculated the factor for quantity of drug in the blood stream of rat following topical application by using the following equation :

$$Csf = Co' x P$$

Where Csf = factor for quantity of drug in rat blood stream following its topical application.

4.4 RESULTS AND DISCUSSIONS

Although the therapeutic doses of TRMA, FLU and CLO are 0.1% w/w, 0.025% w/w and 0.05% w/w respectively, a 1% w/w gel of each drug was used in the in vitro studies so that the sink contained estimatable quantities of the drug. For the same reason, a large surface area of the skin ($18.218 cm^2$) had to be used in the in vitro studies. Inspite of these two precautions, in some cases, concentration of drug emerging in the sink was still below the estimatable limit and hence the standard addition method was followed wherein all the samples were spiked with 10ml of the spiking solution prior to color development.

In the validation of the in vitro set-up, the calculated F value (3.01) was less than the table F value (3.74, $f_1=5$; $f_2=24$) at $P<0.01$ indicating that all the six sampling ports did not vary significantly with respect to their performance during the in vitro run.

From the solubility data of the drugs under test in pH 7.4 phosphate buffered saline I.P. (Table 4.1), it is clear that sink conditions are maintained when 150ml of the buffer is used as the permeation fluid and hence zero-order flux conditions are not violated (2).

Linear curves are not obtained by plotting the Amount of the drug released per square cm area vs time for TRMA, FLU and CLO in HPMC K4M gel base (Fig.4.2) indicating that the release is not zero order. (The error bars have been omitted in all such plots to avoid overlapping). However, the regression coefficients of the data of Q vs $t^{1/2}$ lie between 0.967-0.983 indicating that a linear relationship exists between these two parameters and proving that the release obeys Higuchi's diffusion controlled model (12). The lines of regression are shown in Fig.4.3. The release rate R, permeability coefficient P and diffusion coefficient D are shown in Fig.4.4; the partition coefficient K and the factor for quantity of drug in rat blood stream Csf following its topical application are shown in Fig.4.5; the quantity of drug entering into the membrane upto 6 hours $Q^i(6)$ and the quantity of drug remaining in the membrane Q^m are shown in Fig.4.6. From the figures it is seen that all the values are in the order FLU>CLO>TRMA. The higher K value for FLU indicates that it partitions in favour of the stratum corneum much more than the other two drugs. However, the greater solubility in the aqueous phase as compared to the other two drugs leads to a comparatively higher P and D value i.e. it

TABLE 4.1

SOLUBILITY OF TRMA, FLU AND CLO IN pH 7.4 PHOSPHATE BUFFERED SALINE AT ROOM TEMPERATURE.

Drug	**Solubility ($\mu g/ml$)**
TRMA	26.19
FLU	35.69
CLO	13.00

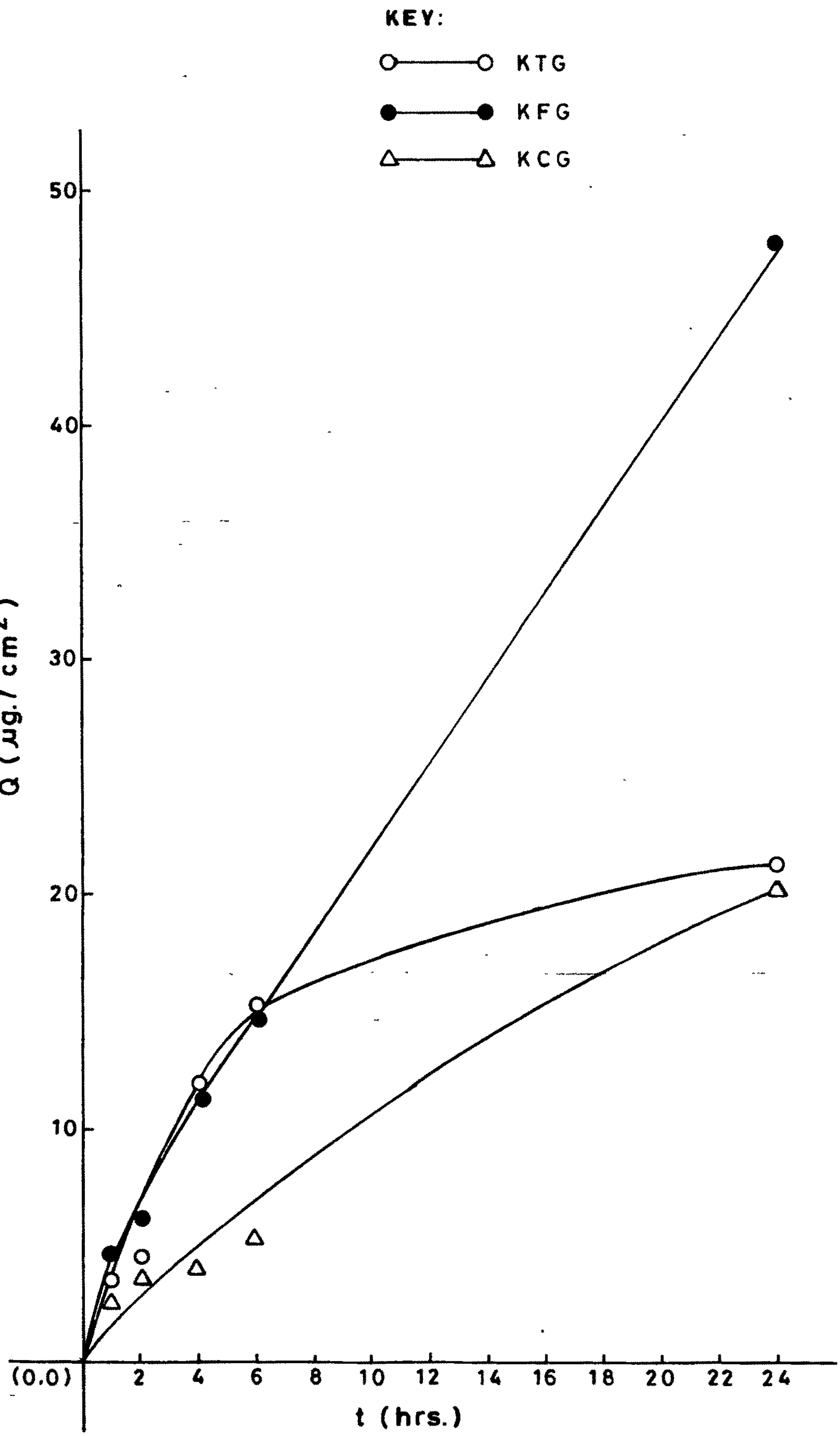

FIG. 4.2 : IN VITRO RELEASE PROFILES OF TRMA, FLU AND CLO FROM THEIR PLAIN DRUG GELS.

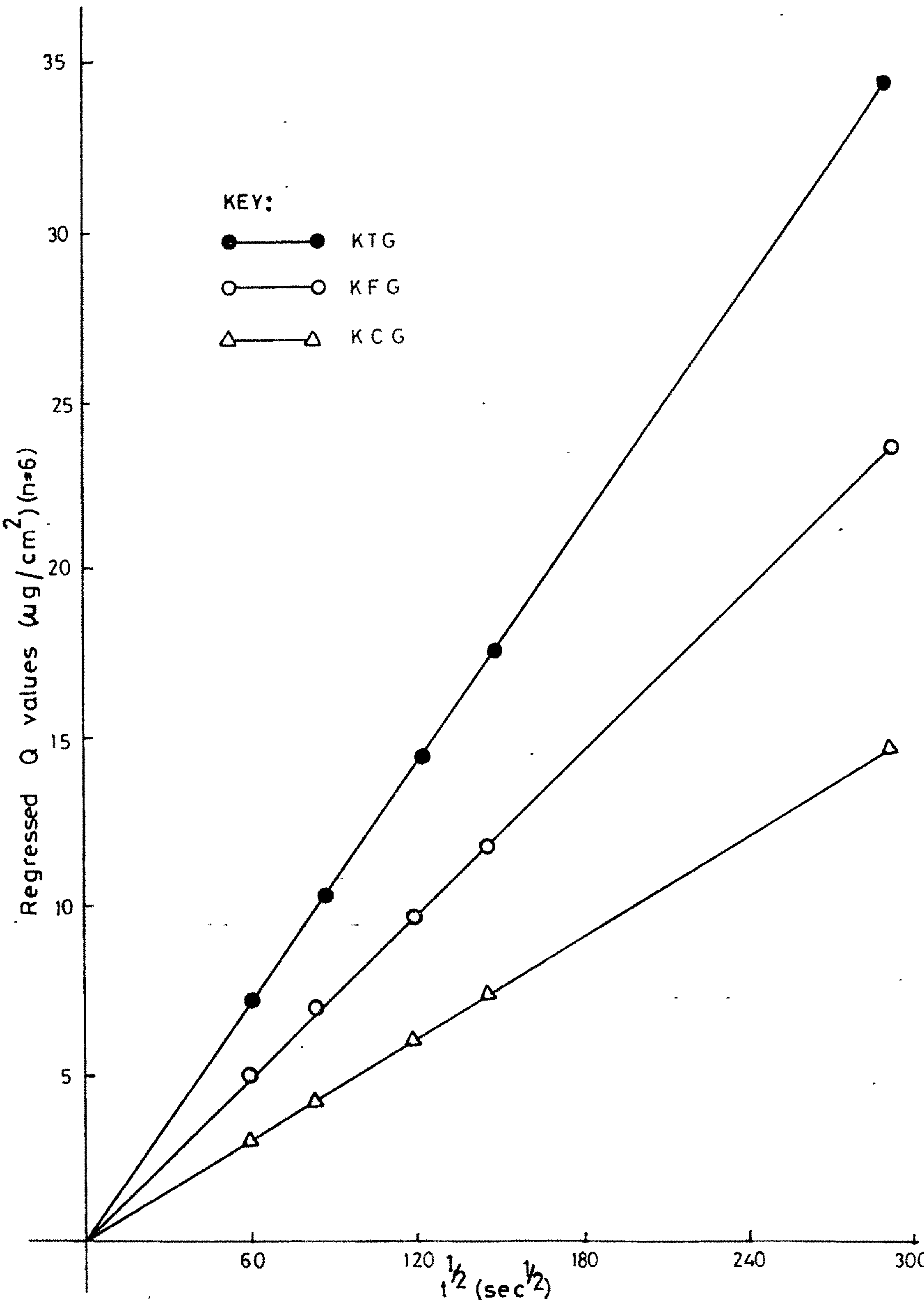

FIG. 4.3: Q Vs t½ PLOTS FOR RELEASE OF TRMA, FLU and CLO FROM THEIR PLAIN DRUG GELS.

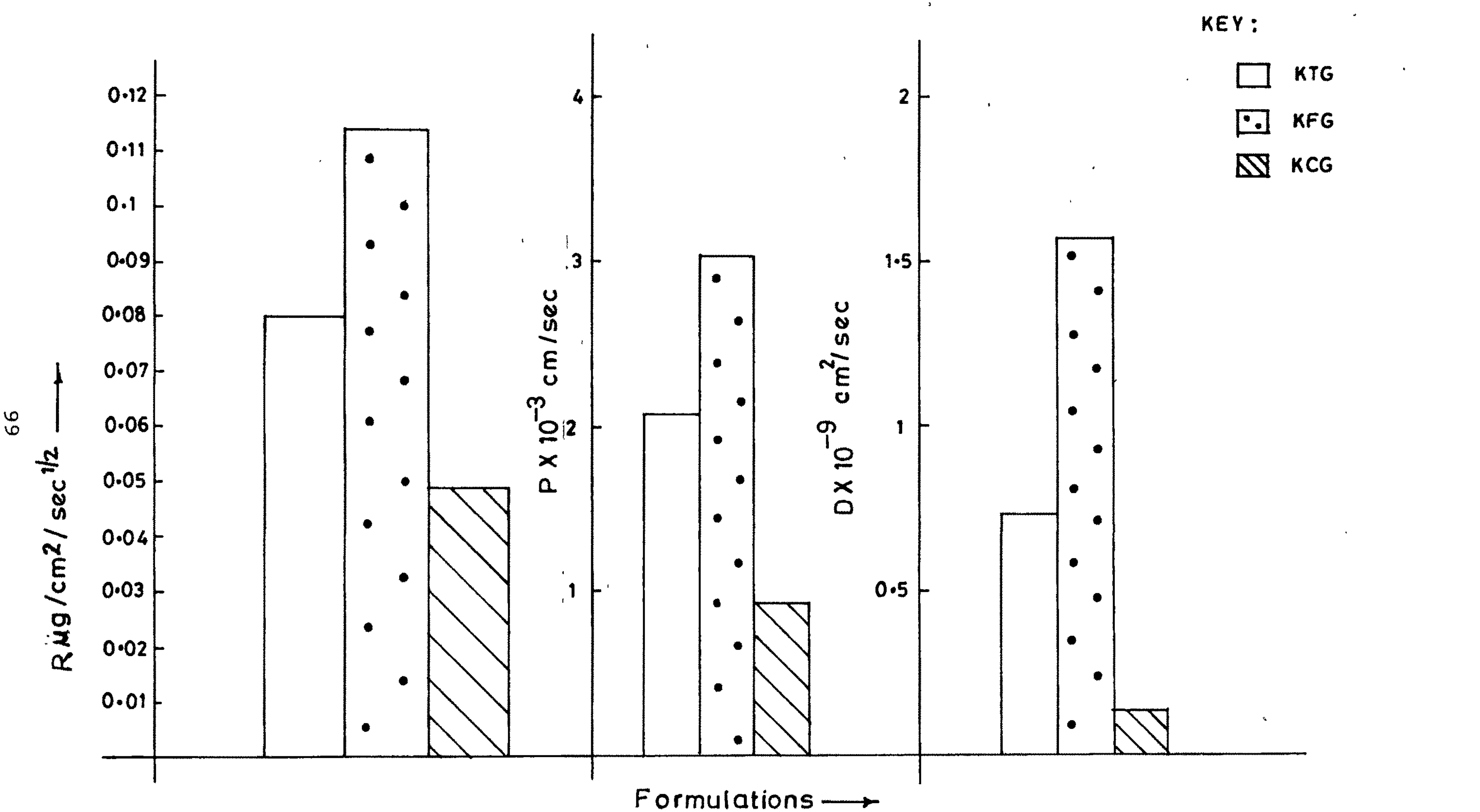

FIG. 4·4: COMPARISON OF R, P AND D VALUES OF TRMA FLU AND CLO FROM THEIR RESPECTIVE PLAIN DRUG GELS.

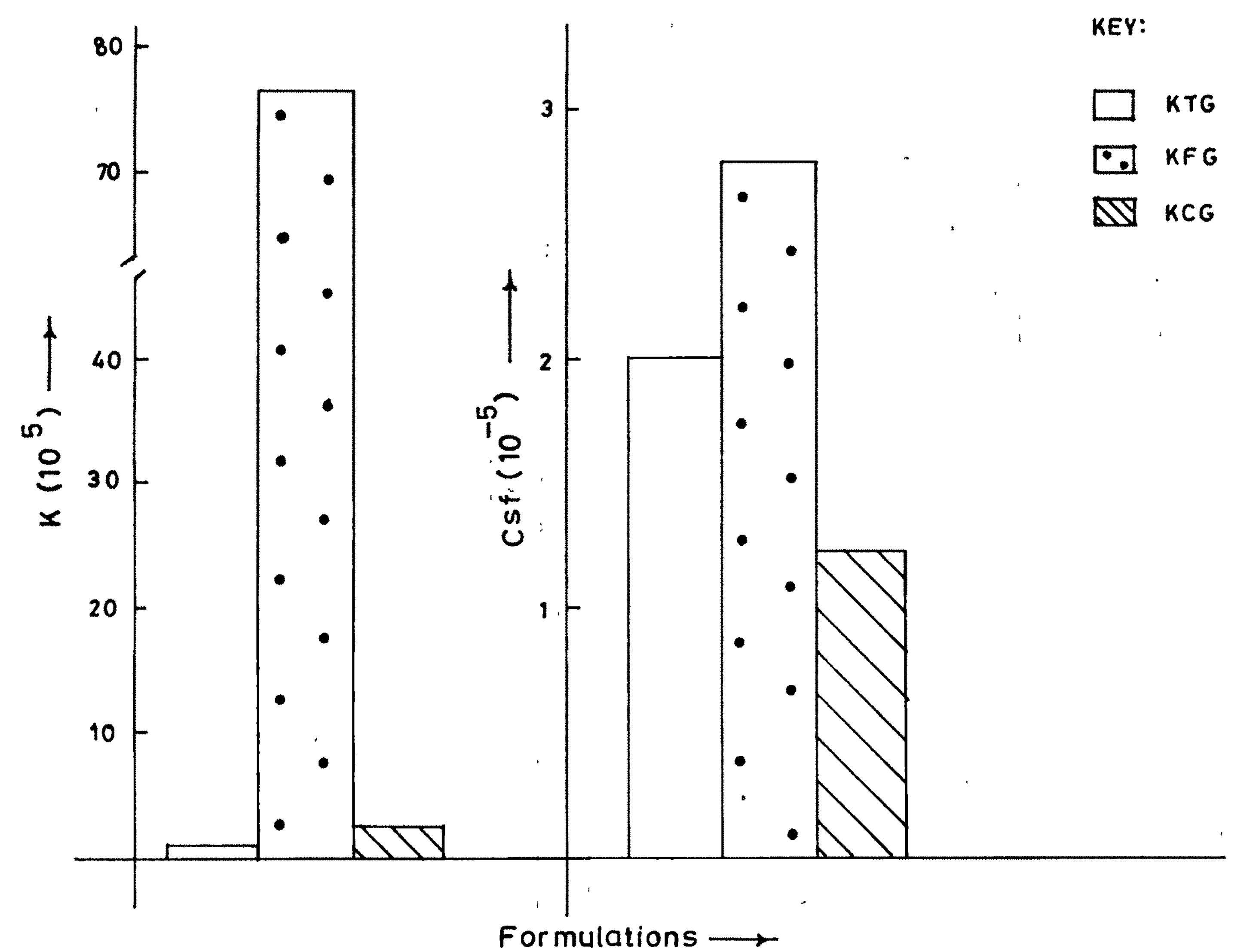

FIG. 4·5: COMPARISON OF K AND Csf VALUES OF TRMA, FLU AND CLO FROM THEIR RESPECTIVE PLAIN DRUG GELS.

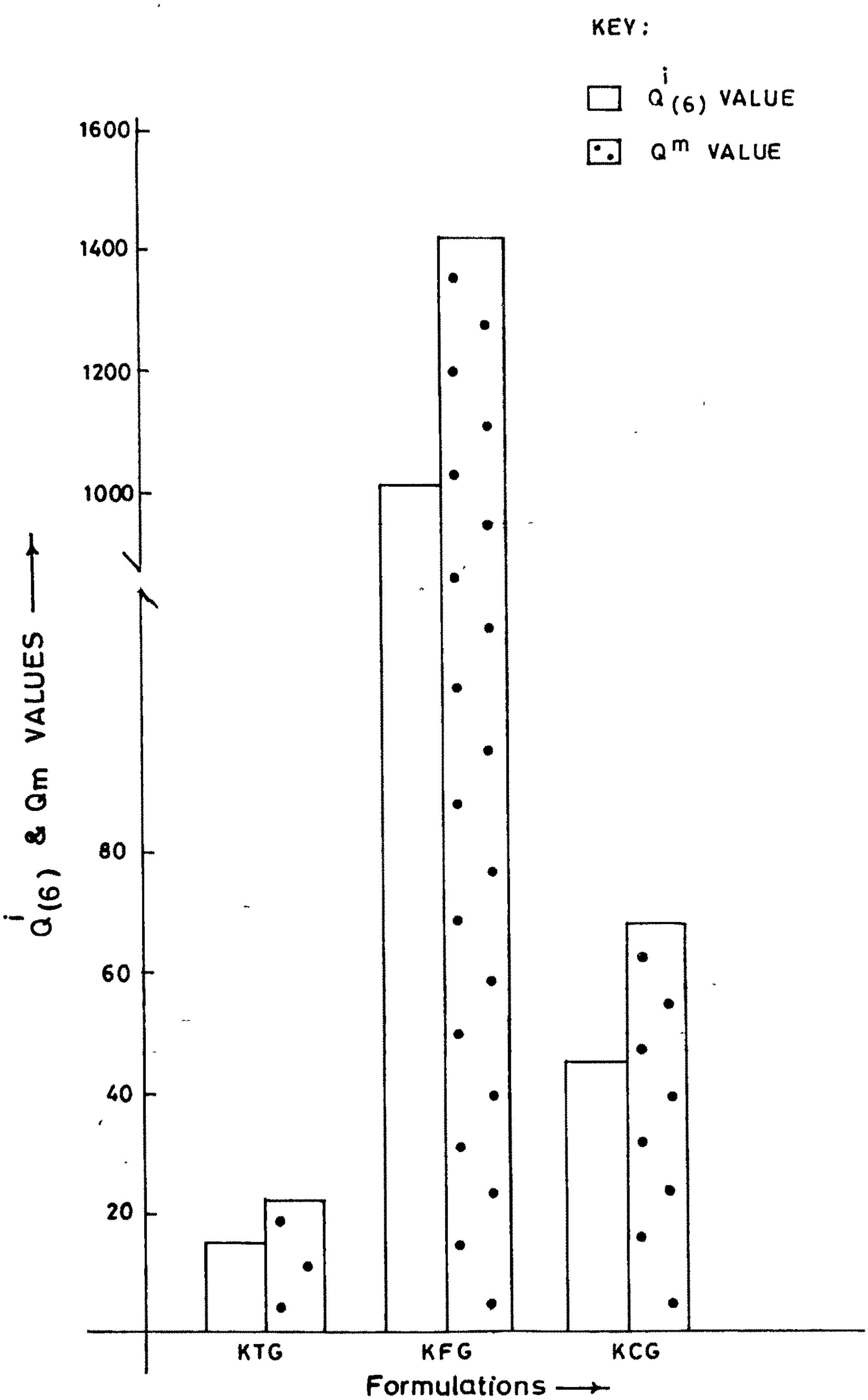

FIG. 4·6: COMPARISON OF $Q^{i}_{(6)}$ AND Q^{m} VALUES OF TRMA, FLU AND CLO FROM THEIR RESPECTIVE PLAIN DRUG GELS.

gets cleared from the skin faster and hence the higher R and Csf values. The higher $Q^i(6)$ value of this drug may be attributed to the higher K value. Although FLU gets cleared from the skin faster than TRMA and CLO, due to the 68.23 fold higher K value as compared to that of TRMA and 29.5 fold higher K value as compared to CLO, it achieves a high Q^m value as compared to that for the other two drugs. As per this study, the 3 steroids can be ranked in order of effect to side effects ratio following topical application as FLU>CLO>TRMA contrary to the reported ranking of CLO>FLU>TRMA when used in vivo (13). This is because of the fact that effect and side effects not only depend on the drug concentration at the site but also on the intrinsic activity of the drug moiety and the later is in the order CLO>FLU>TRMA. Hence, although concentration-wise FLU might be maximum in the membrane and in the receptor fluid, due to its lower intrinsic activity, the potency of anti inflammatory action and side effects may be lower than those of CLO.

The release profiles of TRMA from its liposomal gels as a function of time are shown in Table 4.2 and Figs. 4.7-4.11. From the figures it is evident that release of TRMA from its liposomal gels does not follow the zero order release kinetics. However, since the mean cumulative amount released Vs $t^{1/2}$ plots (Figs.4.12-4.16) are straight lines ($0.966<r<0.999$) except in the case of formulation T_3, the release obeys Higuchi's diffusion controlled model.

TABLE 4.2

IN VITRO RELEASE PROFILES OF FORMULATIONS OF TRMA IN HPMC K4M GEL BASE.

Formulation	*Mean cumulative amount released Q ($\mu g/cm^2$) $\pm$ SEM after				
	1hr	**2hrs**	**4hrs**	**6hrs**	**24hrs**
KTG	3.59 (0.055)	4.67 (0.058)	12.19 (0.078)	15.04 (0.081)	21.23 (0.070)
TPM	3.34 (0.034)	5.01 (0.052)	11.40 (0.081)	14.28 (0.101)	21.90 (0.032)
T1	2.19 (0.078)	3.23 (0.085)	5.82 (0.117)	7.70 (0.050)	21.66 (0.308)
T2	2.10 (0.01)	2.10 (0.043)	3.98 (0.057)	6.24 (0.082)	17.43 (0.065)
T3	2.49 (0.012)	3.61 (0.083)	4.10 (0.078)	5.62 (0.213)	17.30 (0.187)
T4	1.84 (0.028)	2.53 (0.082)	3.54 (0.088)	3.87 (0.031)	11.57 (0.072)
T5	2.09 (0.05)	2.26 (0.06)	3.32 (0.083)	3.85 (0.062)	11.87 (0.045)
T6	1.73 (0.033)	2.25 (0.088)	3.21 (0.067)	4.85 (0.125)	12.61 (0.170)
T7	1.88 (0.048)	2.38 (0.028)	4.91 (0.055)	6.64 (0.172)	17.78 (0.160)
T8	1.20 (0.028)	1.66 (0.052)	3.92 (0.095)	4.26 (0.127)	11.83 (0.102)
T9	1.04 (0.012)	1.18 (0.01)	2.27 (0.028)	2.70 (0.047)	9.77 (0.178)
T10	1.19 (0.008)	1.24 (0.012)	3.20 (0.04)	4.90 (0.072)	12.44 (0.057)
T11	1.74 (0.006)	1.83 (0.016)	4.02 (0.118)	5.47 (0.041)	14.83 (0.211)

Contd...

Formulation	*Mean cumulative amount released Q ($\mu g/cm^2$) $\pm$ SEM after				
	1hr	2hrs	4hrs	6hrs	24hrs
T12	1.67 (0.025)	1.86 (0.053)	3.60 (0.039)	4.61 (0.045)	16.03 (0.108)
T13	1.26 (0.025)	1.53 (0.010)	2.59 (0.07)	3.78 (0.150)	11.39 (0.070)
T14	1.56 (0.013)	1.81 (0.03)	2.99 (0.087)	3.98 (0.087)	12.18 (0.155)
T15	1.41 (0.023)	2.15 (0.010)	2.22 (0.032)	3.43 (0.18)	10.96 (0.23)
T16	1.87 (0.008)	2.97 (0.020)	3.40 (0.027)	3.90 (0.053)	13.95 (0.275)
T17	1.71 (0.007)	2.75 (0.032)	3.98 (0.017)	5.31 (0.053)	10.46 (0.05)
T18	1.79 (0.043)	2.12 (0.115)	2.99 (0.072)	4.18 (0.036)	10.84 (0.152)
T19	2.14 (0.073)	2.73 (0.078)	3.85 (0.056)	4.49 (0.072)	11.30 (0.143)
T20	1.95 (0.030)	2.63 (0.068)	3.78 (0.168)	3.79 (0.077)	11.86 (0.317)
T21	1.15 (0.057)	1.86 (0.108)	2.65 (0.018)	2.88 (0.04)	8.78 (0.10)

* n = 6

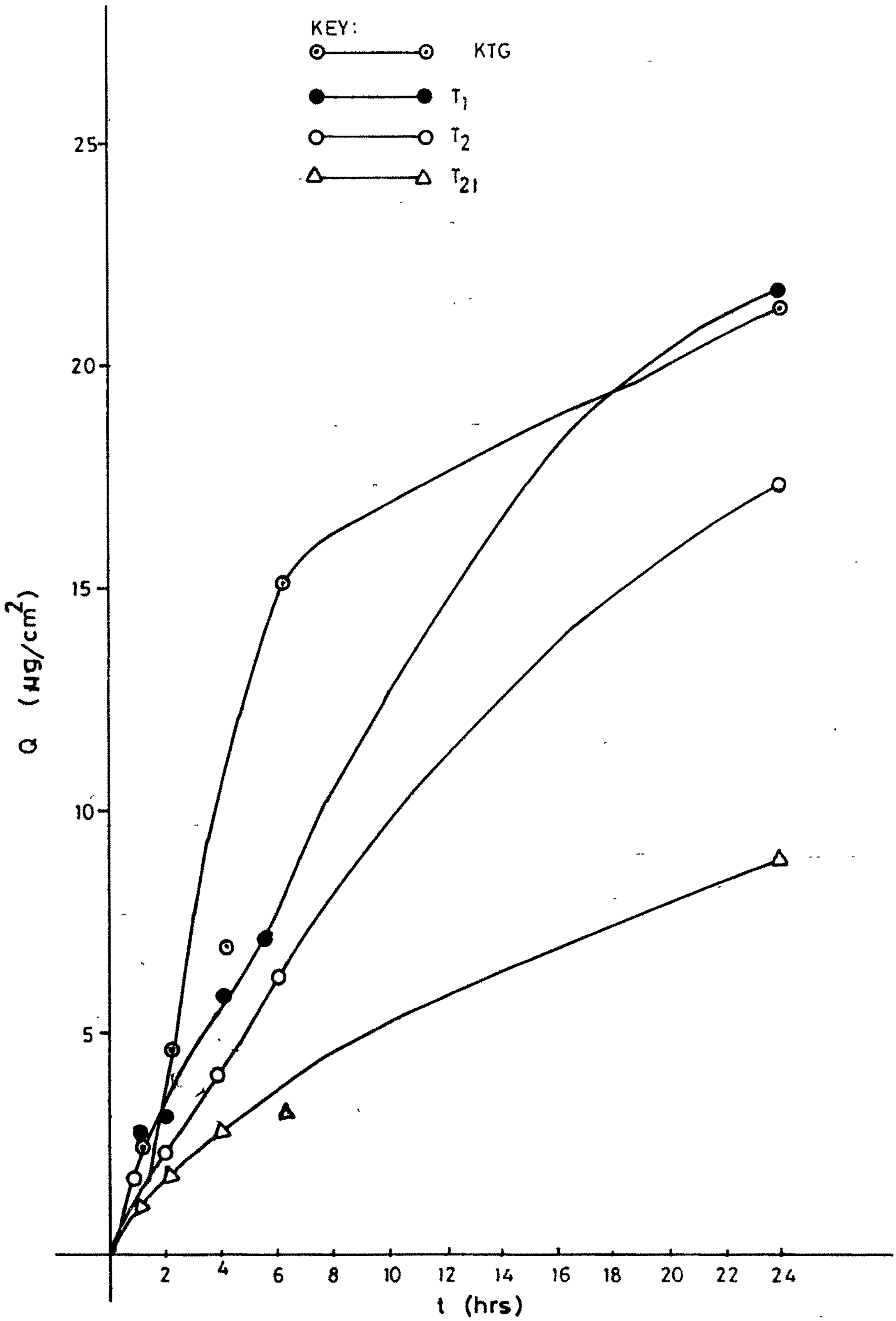

FIG. 47: IN VITRO RELEASE PROFILE OF TRMA FROM ITS LIPOSOMAL GELS AS COMPARED TO THAT FROM PLAIN TRMA GEL (KTG).

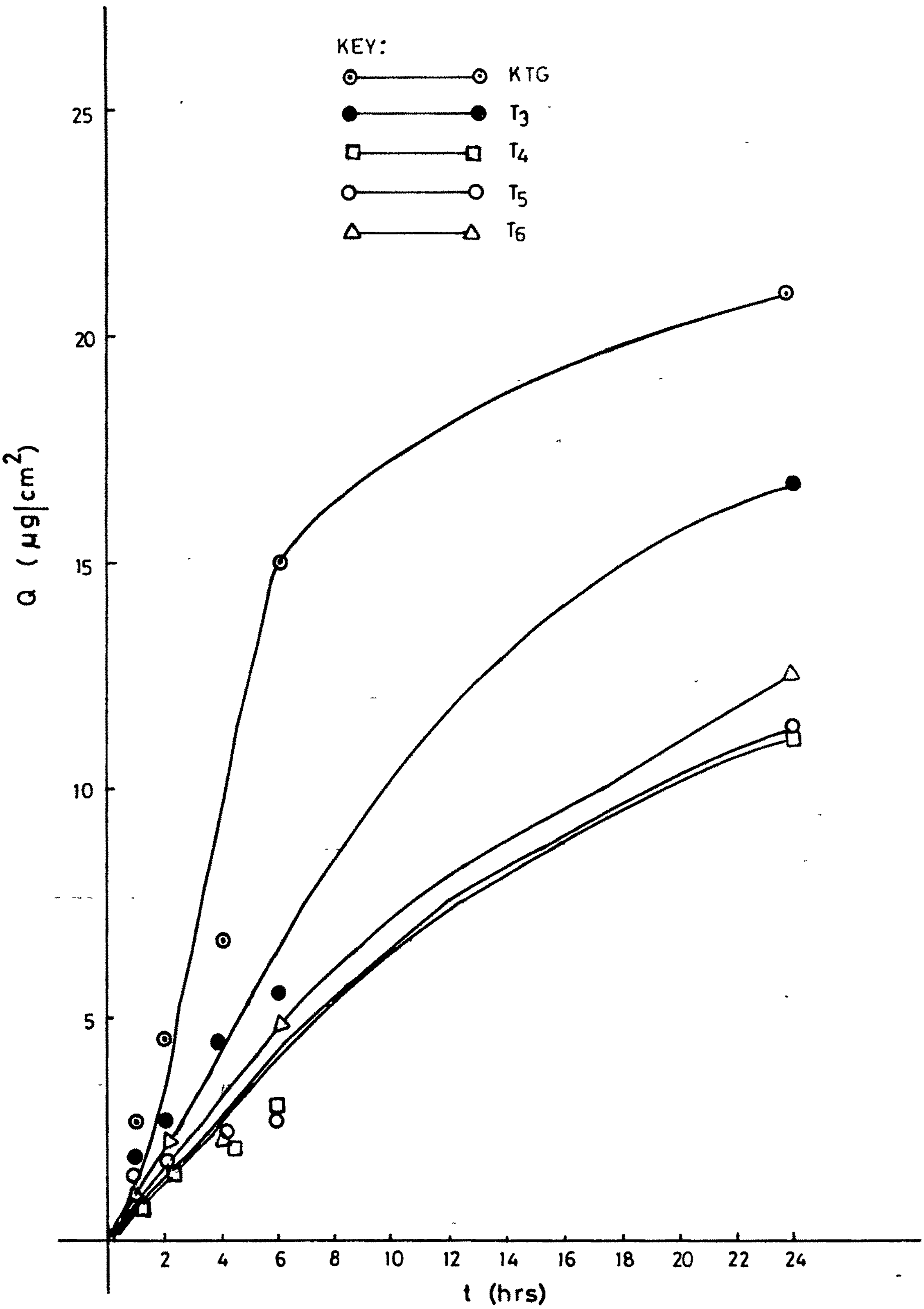

FIG. 4.8 : IN VITRO RELEASE PROFILE OF TRMA FROM ITS LIPOSOMAL GELS (TRMA: PC≡1:0.91) AS COMPARED TO THAT FROM PLAIN TRMA GEL (KTG).

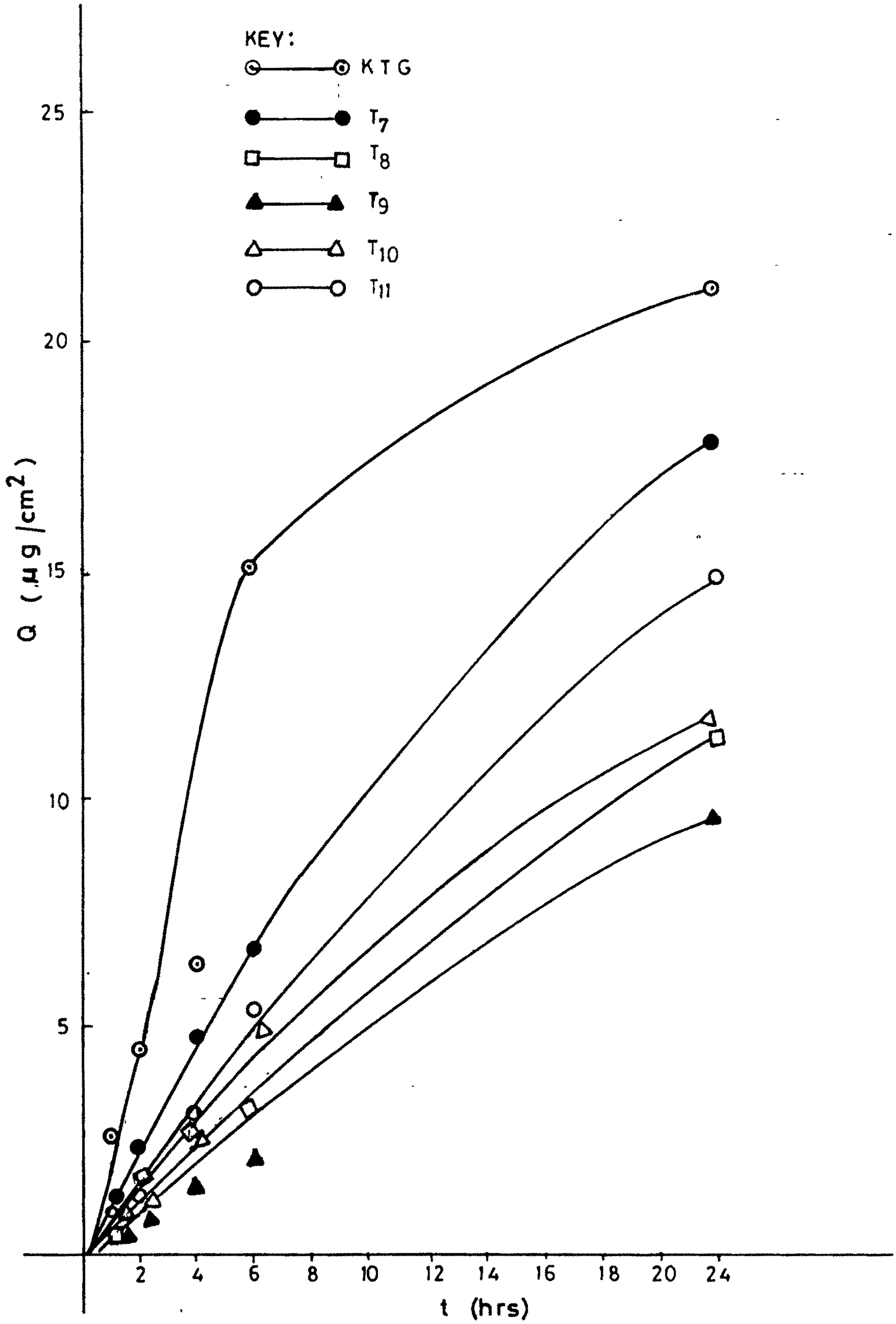

FIG. 4·9: IN VITRO RELEASE PROFILE OF TRMA FROM ITS LIPOSOMAL GELS (TRMA : PC = 1:1·81) AS COMPARED TO THAT FROM KTG.

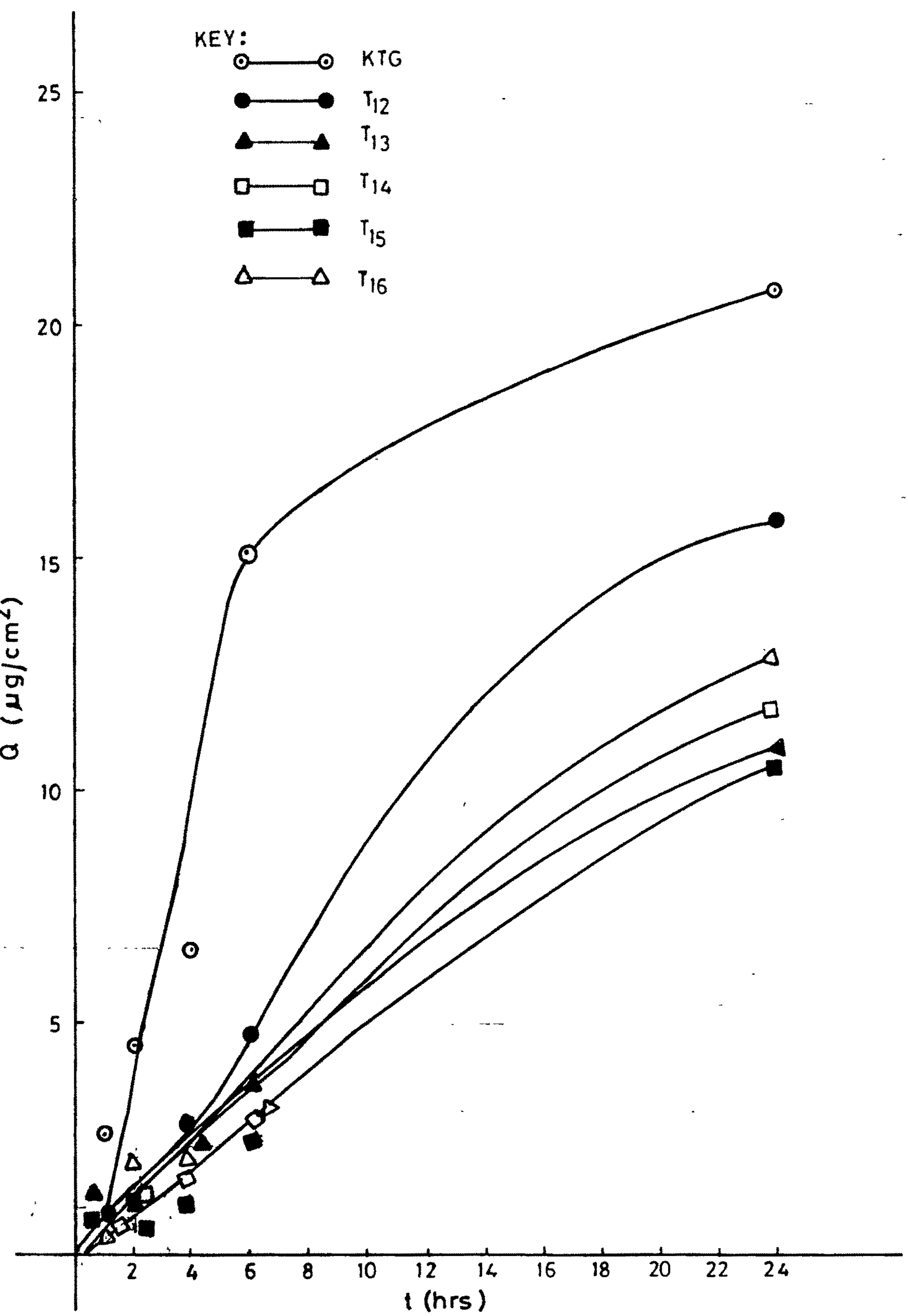

FIG. 4.10 : IN VITRO RELEASE PROFILE OF TRMA FROM ITS LIPOSOMAL GELS (TRMA: PC ≡ 1:3.62) AS COMPARED TO THAT FROM KTG.

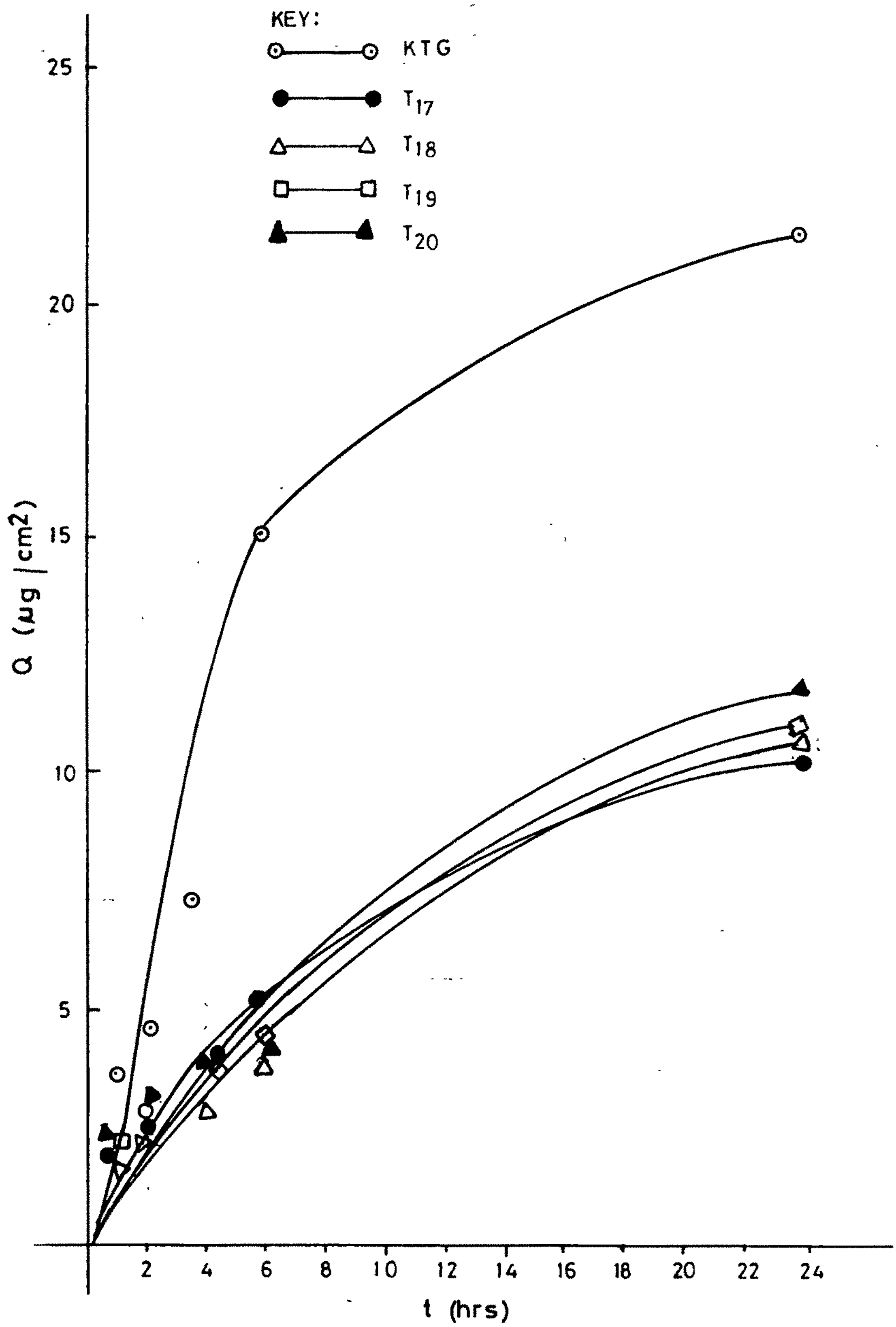

FIG. 4.11 : IN VITRO RELEASE PROFILE OF TRMA FROM ITS LIPOSOMAL GELS (TRMA : PC ≡ 1:5.43) AS COMPARED TO THAT FROM KTG.

TABLE 4.3

CORRELATION COEFFICIENTS AND 'a' AND 'b' VALUES OF THE LINES OF REGRESSION FOR THE DATA OF MEAN CUMULATIVE AMOUNT RELEASED VS SQUARE ROOT OF TIME FOR FORMULATIONS OF TRMA.

Formulation	Correlation coefficient r	*a	*b
KTG	0.983	0.048	0.080
TPM	0.989	0.039	0.079
T_1	0.981	0.357	0.055
T_2	0.975	0.314	0.043
T_3	0.860	-0.151	0.048
T_4	0.988	0.153	0.032
T_5	0.980	0.161	0.032
T_6	0.985	0.131	0.034
T_7	0.981	0.350	0.045
T_8	0.984	0.194	0.031
T_9	0.970	0.146	0.023
T_{10}	0.976	0.214	0.031
T_{11}	0.980	0.216	0.038
T_{12}	0.966	0.327	0.037
T_{13}	0.970	0.160	0.028
T_{14}	0.977	0.124	0.031
T_{15}	0.974	0.220	0.027
T_{16}	0.976	0.137	0.036
T_{17}	0.999	0.043	0.034
T_{18}	0.989	0.149	0.030
T_{19}	0.996	0.100	0.034
T_{20}	0.988	0.142	0.033
T_{21}	0.986	0.214	0.023

* Equation of regression line is $y = a+bx$

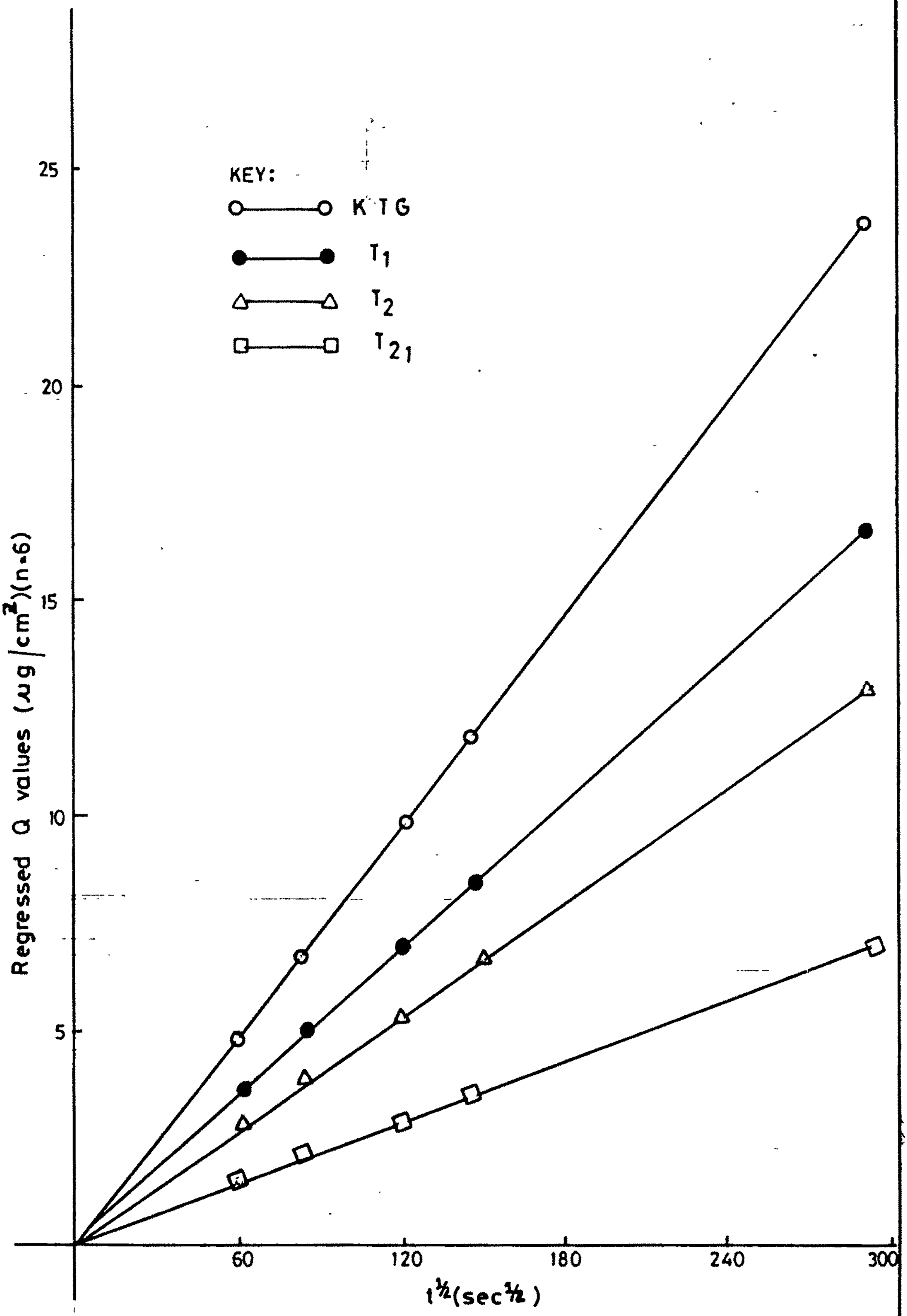

FIG. 4.12 : Q Vs $t^{1/2}$ PLOTS FOR RELEASE OF TRMA FROM ITS LIPOSOMAL GELS AS COMPARED TO THAT FROM KTG.

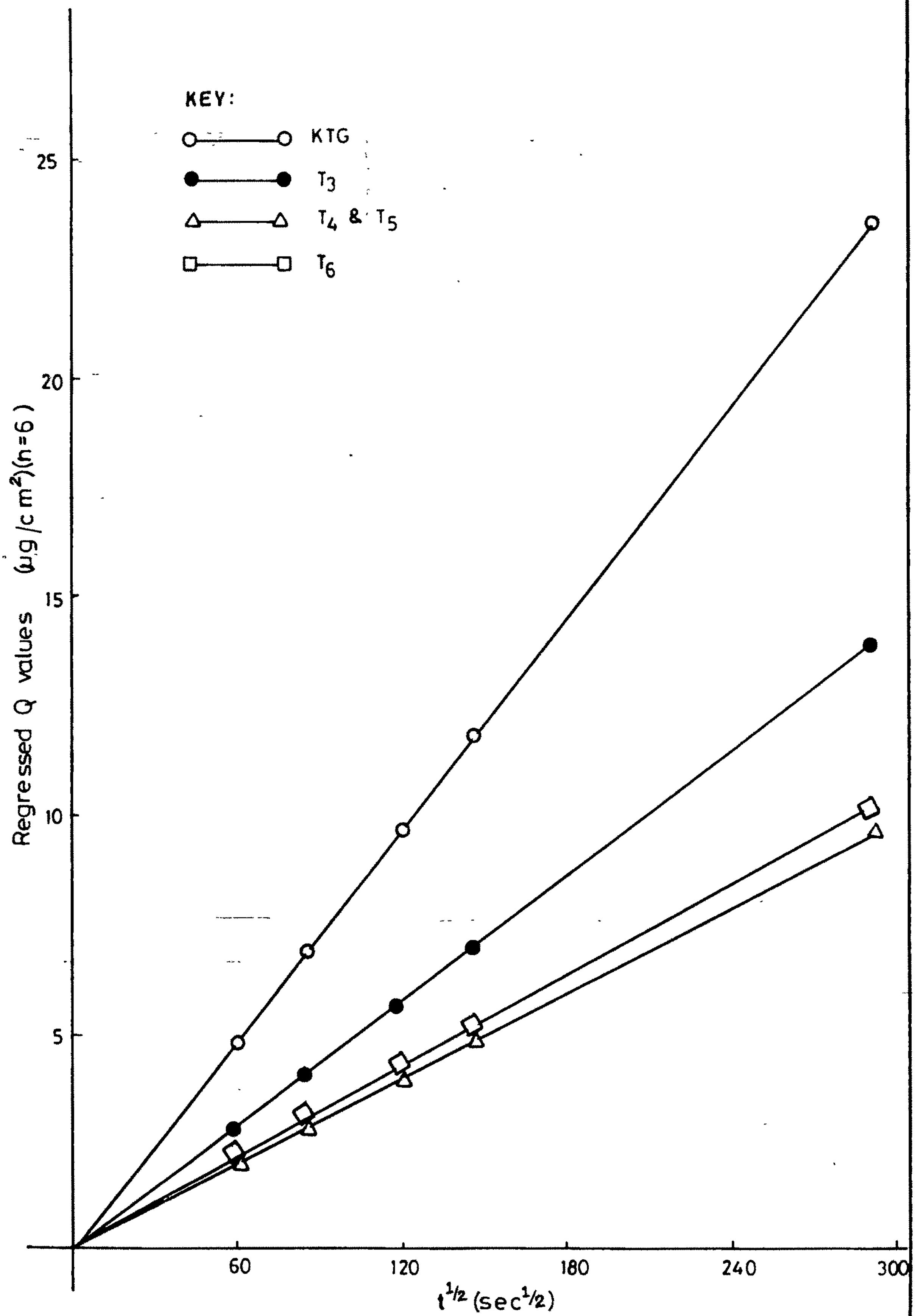

FIG. 4.13 : Q Vs $t^{1/2}$ PLOTS FOR RELEASE OF TRMA FROM ITS LIPOSOMAL GELS (TRMA: PC ≅ 1:0·91) AS COMPARED TO THAT FROM KTG.

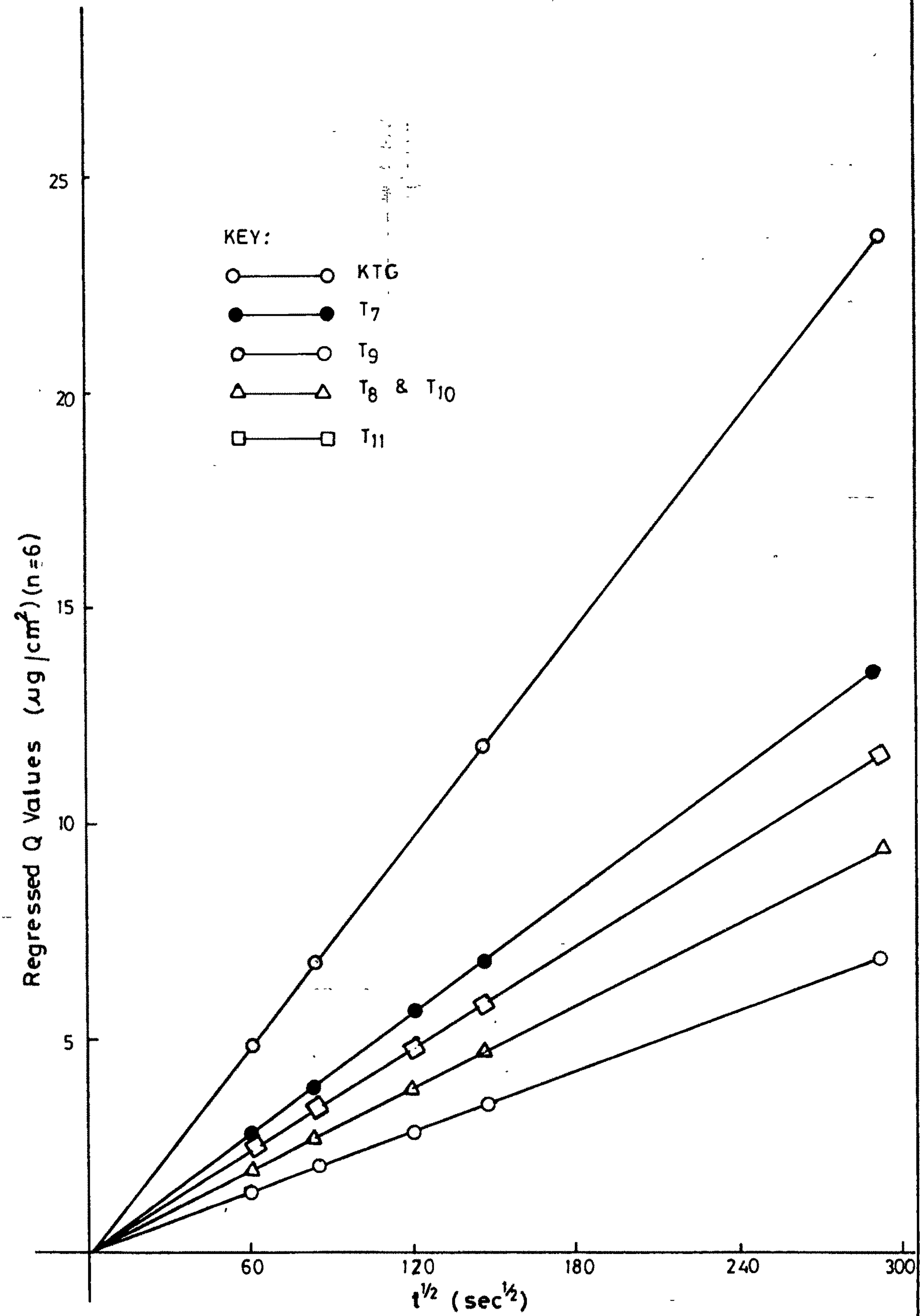

FIG. 4.14: Q Vs $t^{1/2}$ PLOTS FOR RELEASE OF TRMA FROM ITS LIPOSOMAL GELS (TRMA: PC ≡ 1:1.81) AS COMPARED TO THAT FROM KTG.

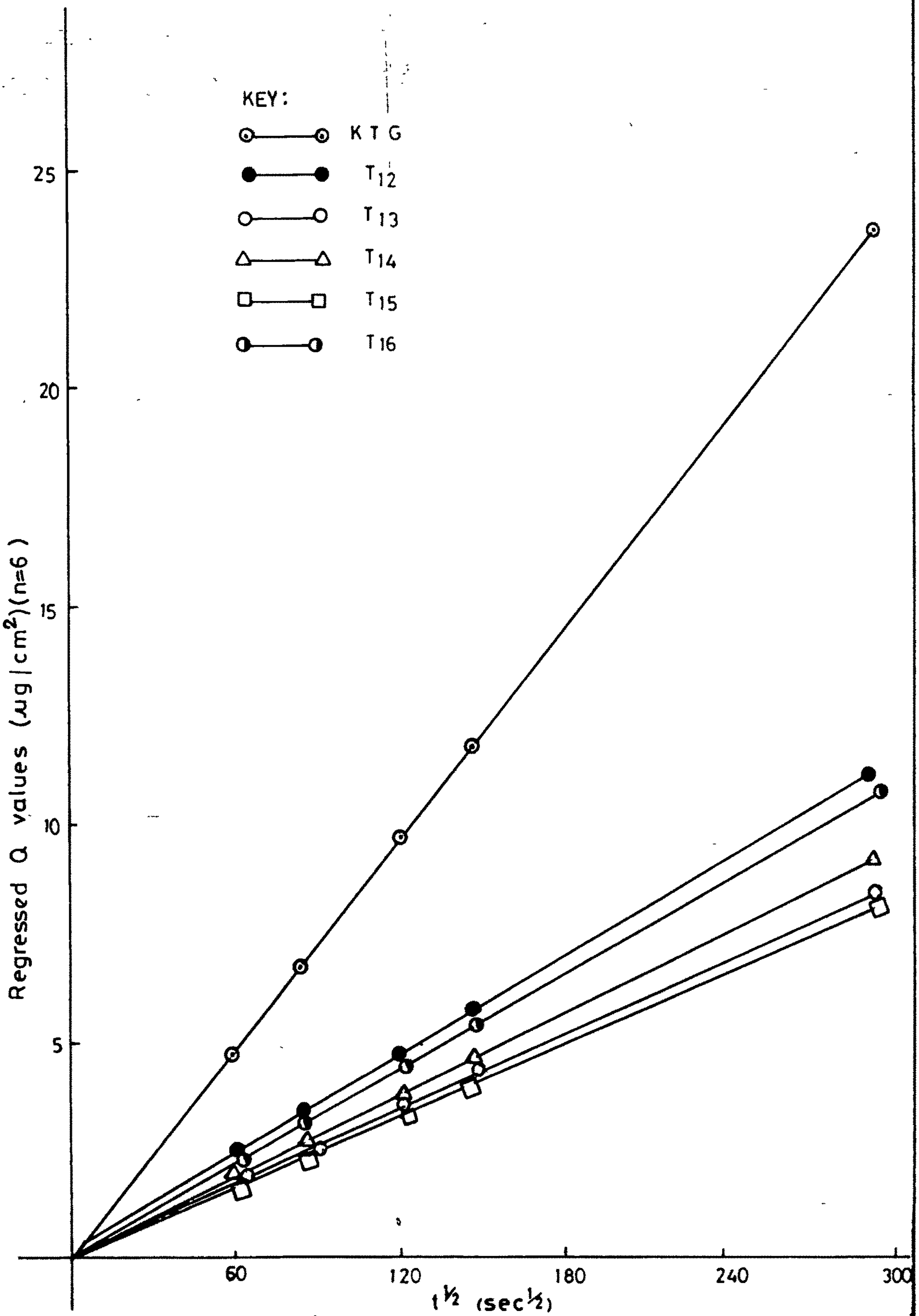

FIG. 4.15: Q Vs $t^{1/2}$ PLOTS FOR RELEASE OF TRMA FROM ITS LIPOSOMAL GELS (TRMA : PC ≡ 1:3.62) AS COMPARED TO THAT FROM KTG.

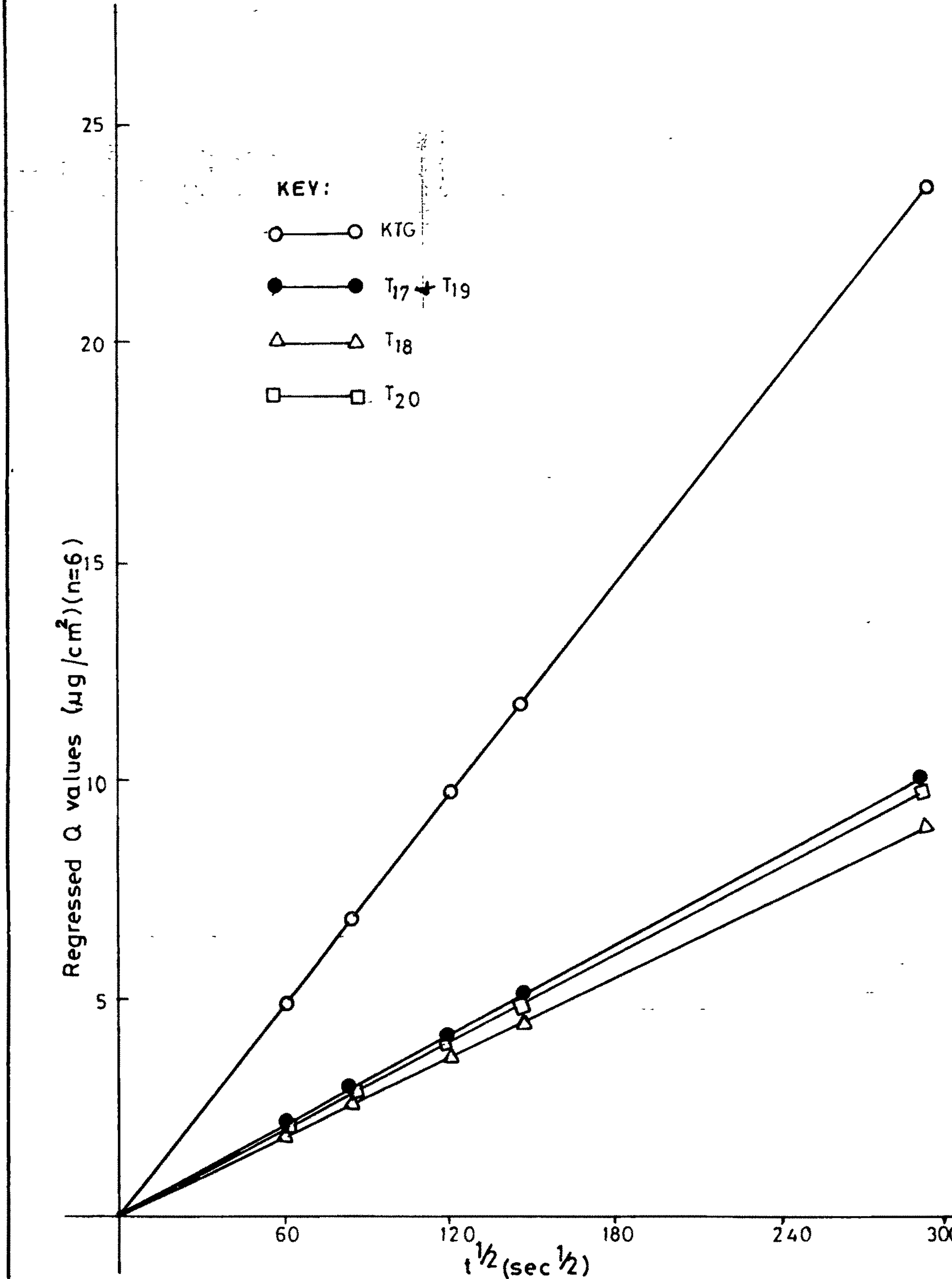

FIG. 4.16: Q vs $t^{1/2}$ PLOTS FOR RELEASE OF TRMA FROM ITS LIPOSOMAL GELS (TRMA : PC ≡ 1:5.43) AS COMPARED TO THAT FROM KTG.

The R, P, D, K, $Q^i(6)$, Q^m, Csf and Q^m/Csf values for all the formulations of TRMA in HPMC K4M gel base are shown in Tables 4.4 and 4.5. From the tables, it is seen that for all the liposomal formulations, there is a reduction in the R value, P value, D value and Csf value and enhancement in the $Q^i(6)$ value, Q^m value, Q^m/Csf ratio and K value as compared to the plain drug gel (KTG) (Figs. 4.17-4.22). In general, a reduction in the P and D values are indicative of decrease in the ability of the solute to cross the membrane and enter into the sink. Higher values of K indicate that the compound is so lipid soluble that it will remain dissolved in the stratum corneum and will not readily pass into the water rich viable tissues. A high K value coupled with a low R value is indicative of the fact that the drug is getting adsorbed on to the stratum corneum components. An increase in the $Q^i(6)$ and Q^m values is suggestive of increase in the efficacy due to the fact that more amount of drug enters the membrane and remains in the membrane which incidentally, is the site of action. A decrease in the Csf value indicates a possible reduction in the side effects due to decreased drug levels in the blood. A high Q^m/Csf ratio is indicative of enhanced efficacy coupled with decreased side effects.

For the purpose of discussion, the liposomal formulations of TRMA prepared are divided into 2 broad groups :

TABLE 4.4

DIFFUSION PARAMETERS TO ASSESS THE RELEASE OF TRMA FROM ITS FORMULATIONS IN HPMC K4M BASE.

Formulation	*P (10^{-4})	*D (10^{-10})	*R	*K (10^{5})
KTG	20.94	7.45	0.080	1.12
TPM	20.79	7.26	0.079	1.15
T_1	14.60	3.53	0.055	1.65
T_2	11.41	2.11	0.043	2.16
T_3	9.68	1.39	0.048	2.79
T_4	8.67	1.29	0.032	2.69
T_5	7.64	0.99	0.032	3.10
T_6	8.61	1.20	0.034	2.87
T_7	8.46	1.21	0.023	2.80
T_8	7.52	0.99	0.031	3.05
T_9	5.53	0.50	0.023	4.41
T_{10}	9.71	1.54	0.031	2.52
T_{11}	10.96	2.00	0.038	2.19
T_{12}	6.82	0.79	0.037	3.40
T_{13}	7.14	0.83	0.028	3.44
T_{14}	7.27	0.85	0.031	3.43
T_{15}	7.77	1.06	0.027	2.90
T_{16}	8.13	1.06	0.036	3.07
T_{17}	8.52	1.72	0.034	1.98
T_{18}	8.99	1.37	0.030	2.62
T_{19}	11.30	2.22	0.034	2.04
T_{20}	8.96	1.37	0.033	2.62
T_{21}	9.18	1.54	0.023	2.38

* P = Permeability coefficient (cm/sec)
D = Diffusion coefficient (cm^2/sec)
R = Release rate ($\mu g/cm^2$ / $sec^{1/2}$)
K = Partition coefficient

All above values are calculated from the data obtained after regression and hence SEM values are not shown.

TABLE 4.5

PARAMETERS RESPONSIBLE FOR THE EFFECT, SIDE EFFECT AND THEIR RATIO FOR FORMULATIONS OF TRMA IN HPMC K4M GEL BASE.

Formulation	Effect		Side effect	Q^m/Csf
	$Q^i(6)$	Q^{m*}	$Csf^*(10^{-5})$	
KTG	14.69	21.40	2.00	10.70
TPM	14.99	21.85	1.96	11.15
T_1	20.96	31.02	1.37	22.64
T_2	27.34	40.69	1.07	38.03
T_3	46.36	69.19	1.20	57.66
T_4	33.29	49.71	0.80	62.14
T_5	43.55	65.10	0.80	81.38
T_6	37.93	56.65	0.85	66.65
T_7	49.87	74.48	1.13	65.91
T_8	42.03	62.83	0.77	81.60
T_9	61.23	91.73	0.58	158.16
T_{10}	26.96	40.22	0.77	52.23
T_{11}	25.48	37.93	0.95	39.93
T_{12}	61.63	92.21	0.92	100.23
T_{13}	45.07	67.42	0.70	96.31
T_{14}	48.84	73.06	0.77	94.88
T_{15}	33.72	50.40	0.68	74.12
T_{16}	45.48	67.96	0.90	75.51
T_{17}	26.49	39.48	0.85	46.45
T_{18}	29.28	43.70	0.75	58.27
T_{19}	20.57	30.60	0.85	36.00
T_{20}	32.30	48.21	0.82	58.79
T_{21}	19.98	29.80	0.57	52.28

* $Q^i(6)$ is the quantity of drug which enters the membrane upto 6 hrs.

Q^m is the quantity of drug which remains in the membrane. C_{sf} is the factor for quantity of drug entering into the blood stream.

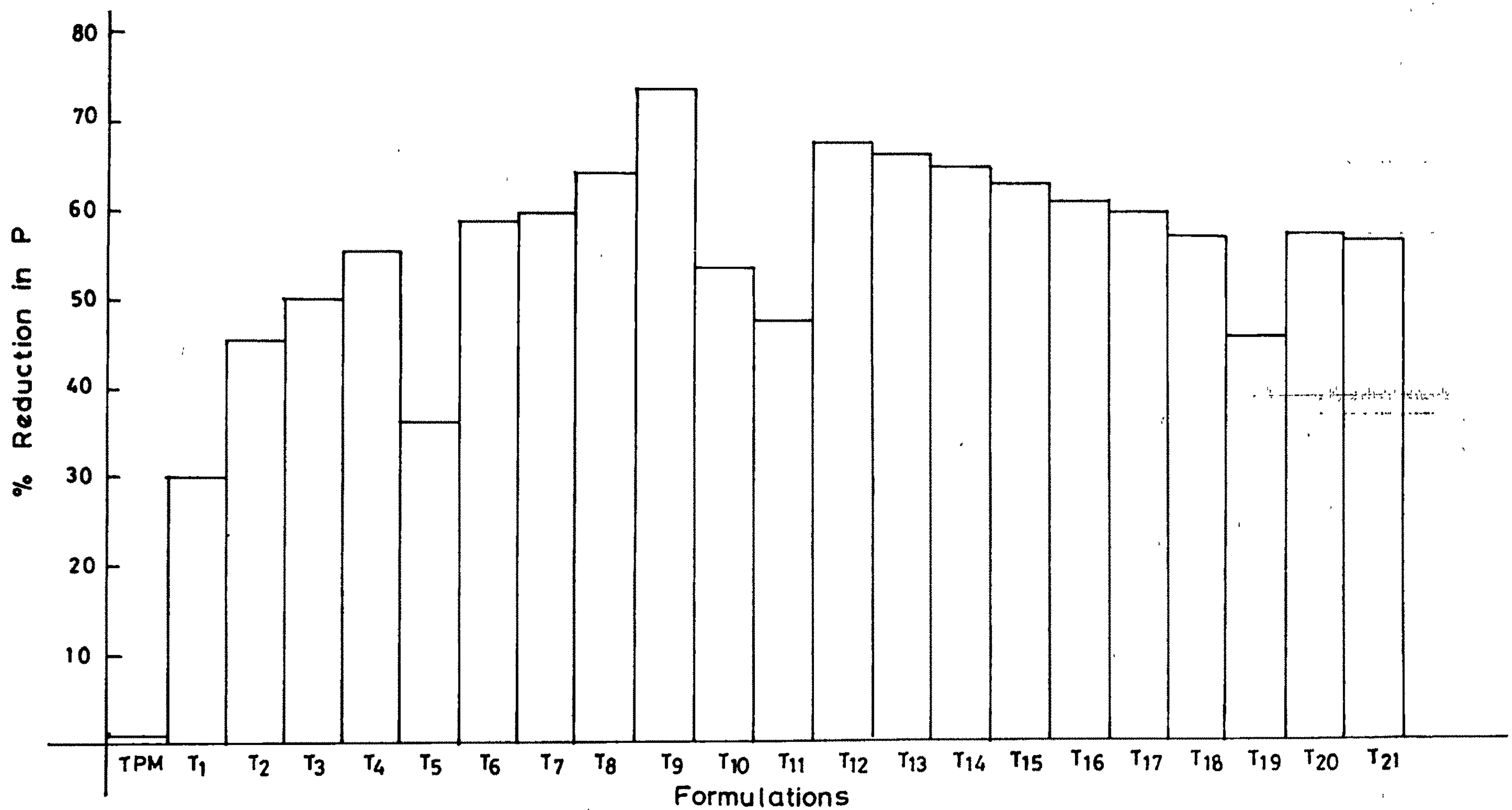

FIG. 4·17: % REDUCTION IN P VALUE OF TRMA FROM ITS TPM AND LIPOSOMAL GELS AS COMPARED TO THAT FROM KTG.

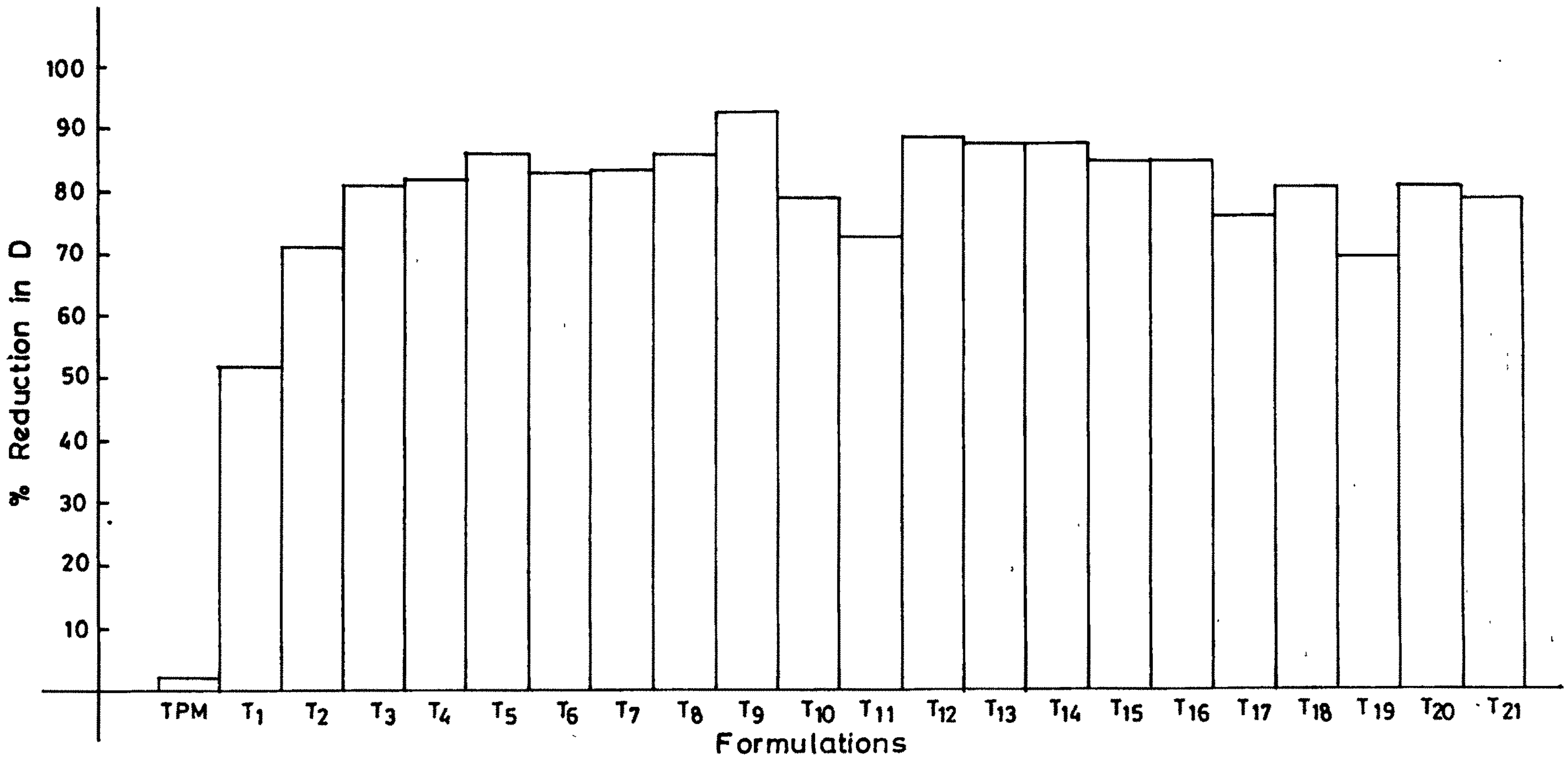

FIG. 4.18: % REDUCTION IN D VALUE OF TRMA FROM ITS TPM AND LIPOSOMAL GELS AS COMPARED TO THAT FROM KTG.

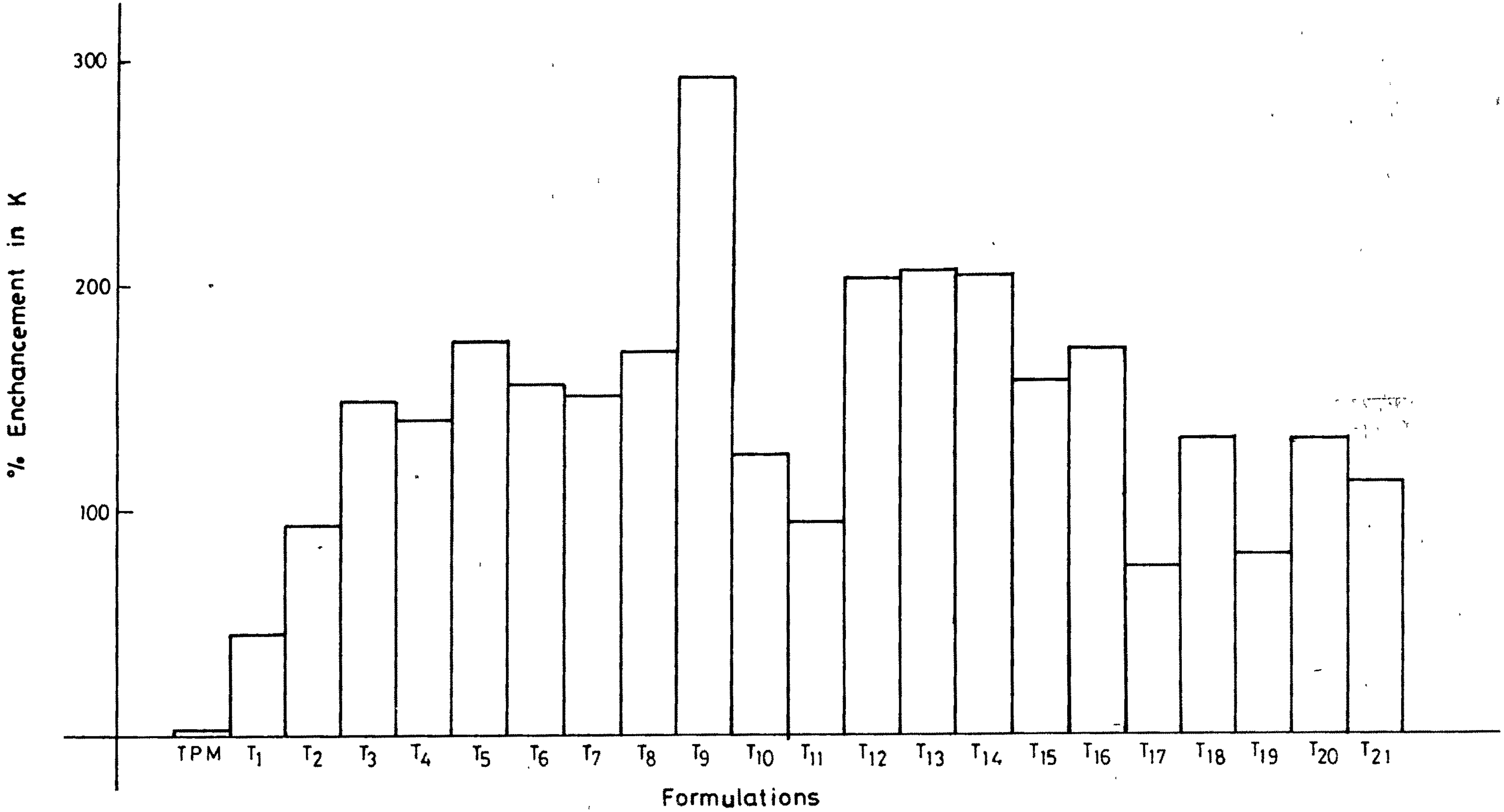

FIG. 4.19: %ENHANCEMENT IN THE K VALUE OF TRMA FROM ITS TPM AND LIPOSOMAL GELS AS COMPARED TO KTG.

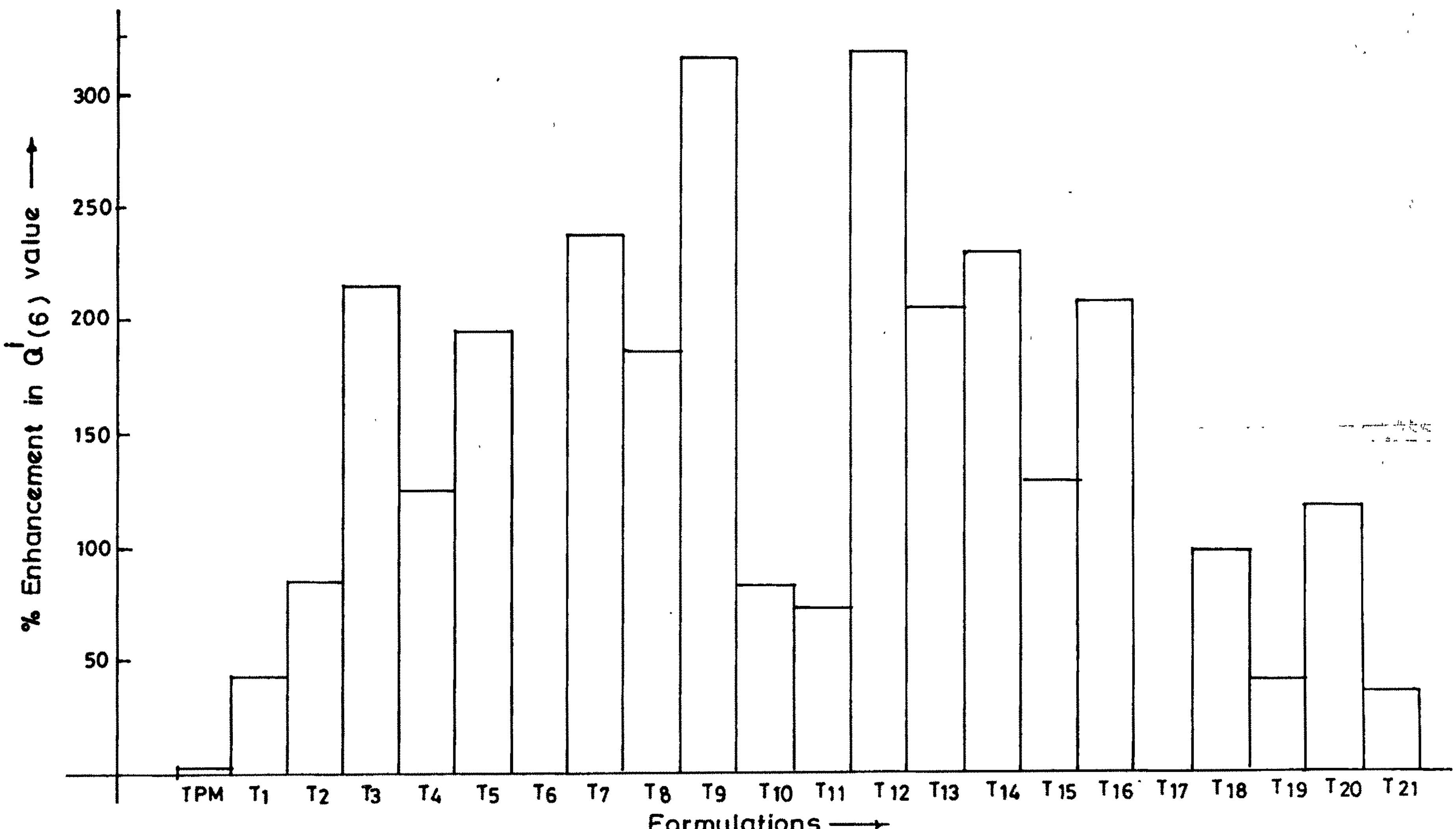

FIG.4.20: %ENHANCEMENT IN $Q^{i}_{(6)}$ VALUES OF TPM AND LIPOSOMAL GELS AS COMPARED TO KTG.

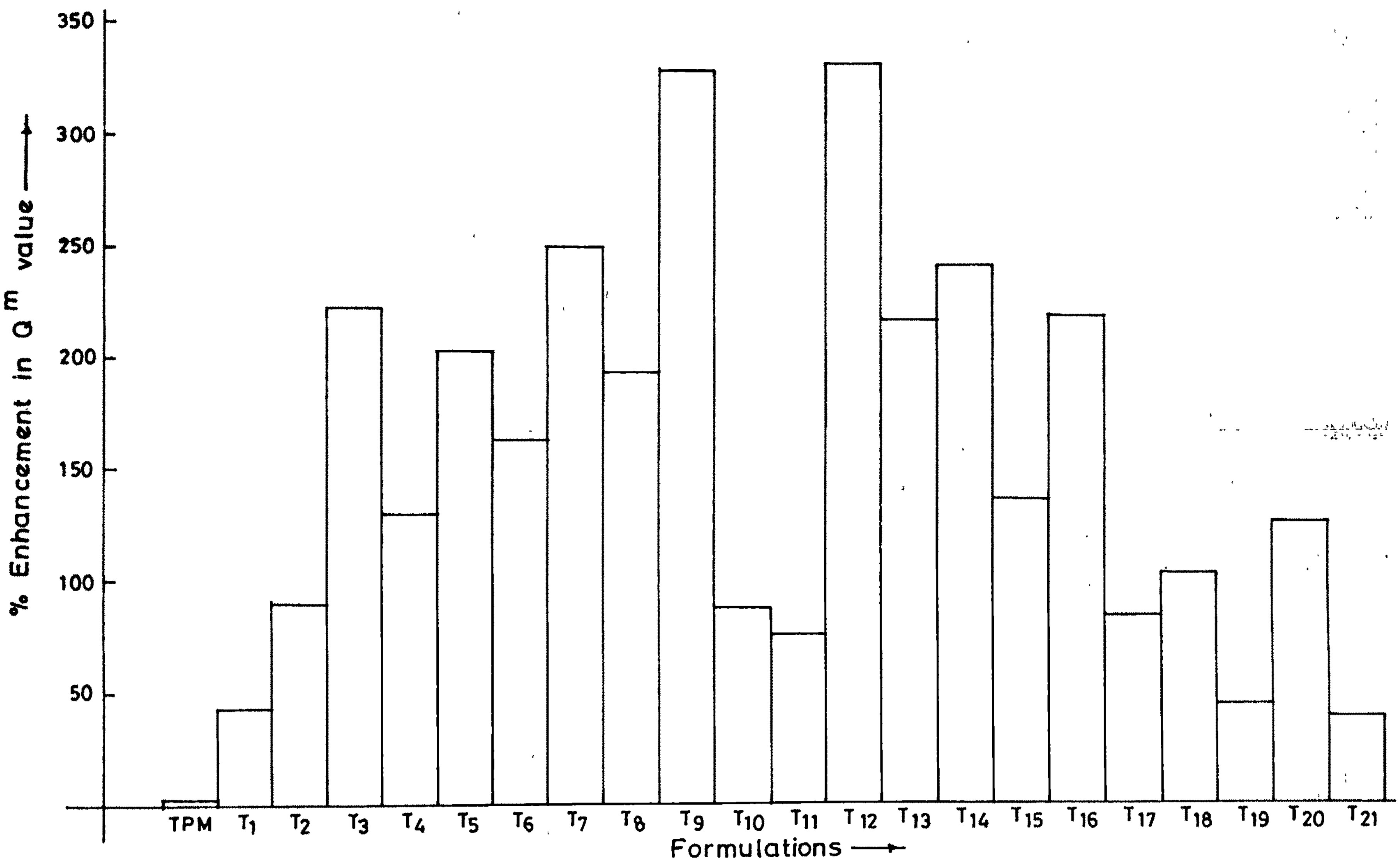

FIG. 4·21: % ENHANCEMENT IN Q^m VALUES OF TPM AND LIPOSOMAL GELS AS COMPARED TO KTG.

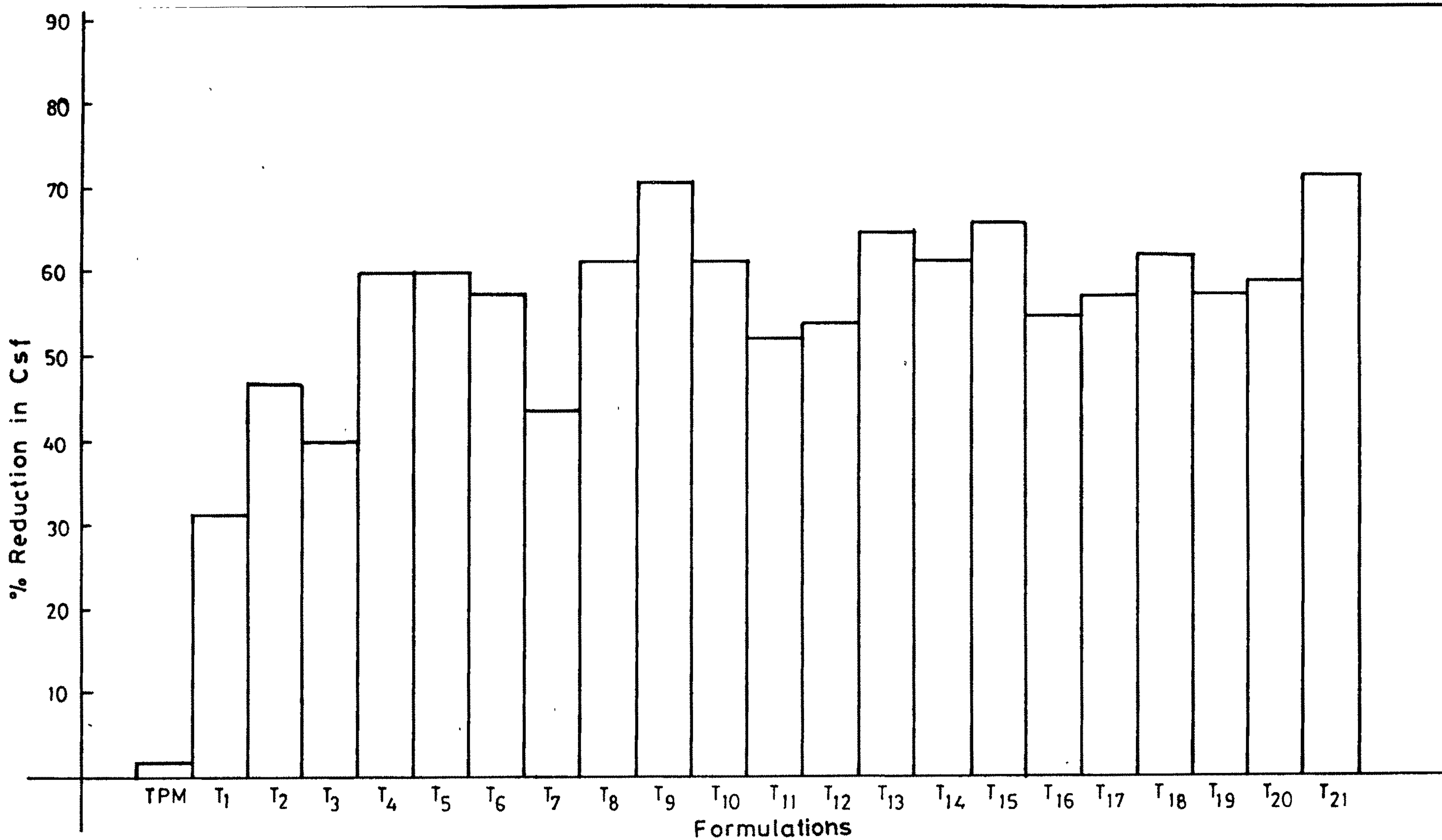

FIG. 4.22 : REDUCTION IN Csf OF TRMA FROM ITS TPM AND LIPOSOMAL GELS AS COMPARED TO KTG.

(A) Formulations having the same TRMA:PC ratio with different amounts of CHOL.

(B) Formulations having the same PC:CHOL ratio with varying amounts of TRMA.

(A)

(i) Formulation containing liposomes with minimum PC cover (T_1) i.e. TRMA:PC≡ 1:0.6: From Figs. 4.17-4.19 it is evident that this formulation has the highest release rate, lowest reduction in the P and D values and lowest percent enhancement in the K value as compared to all other liposomal formulations. Although it's $Q^i(6)$ and Q^m values exceed those of gel T_{21} which has the maximum PC cover, its Csf value is comparatively low. Besides, its Q^m/Csf ratio is lowest among all liposomal formulations and hardly twice that of KTG, indicating that the PC coverage of 1:0.6 is too low to give a cutting edge over KTG.

(ii) Formulations with TRMA:PC≡1:0.91 i.e. formulations T_3, T_4, T_5 and T_6: The release rate (R) is in the order $T_3 > T_6 > T_4 = T_5$ indicating that there is an optimum CHOL concentration below and above which the release rate increases. The P and D values are minimum for T_5 indicating that its CHOL concentration brings about decreased transfer of drug through the skin into the sink. In addition, maximum K value for this gel is indicative of higher transport of drug to the membrane. A random variation is observed in the $Q^i(6)$ and Q^m values as the CHOL concentration increases from gel T_3 to T_6 while the Csf value decreases and then increases. The Q^m/Csf ratio which ultimately decides the effect to side effect is highest for gel T_5 indicating that at TRMA:PC≡1:0.91, the CHOL ratio of 0.46 may be optimum.

(iii) Formulations with TRMA:PC≡1:1.81 i.e. formulations T_7, T_8, T_9, T_{10} and T_{11} : The release rate (R) is in the order $T_{11} > T_{10} = T_8 > T_7 = T_9$ indicating that the release rate is minimum at CHOL concentrations of gels T_7 and T_9 and increases with deviations above and below these concentrations. The P and D values are minimum for gel T_9 with the values increasing on either sides of gel T_9. The K value is maximum for gel T_9 as is the $Q^i(6)$ and Q^m value. The Csf is minimum for gel T_9 and hence the ratio of Q^m/Csf is maximum for this gel. So, at TRMA : PC≡1:1.81, the CHOL ratio of 0.91 may be optimum for maximum effect and minimum side effects.

(iv) Formulations with TRMA:PC≡1:3.62 i.e. formulations $T_{12}, T_{13}, T_{14}, T_{15}$ and T_{16} : The release rate is lowest for gel T_{15} with the value increasing on both sides of this gel.

The P and D values are minimum for gel T_{12} and increase with increasing CHOL concentration. The K value is almost equal for gels T_{12}, T_{13} and T_{14} and decreases for gels T_{15} and T_{16}. The $Q^i(6)$ and Q^m values are highest for gel T_{12} and progressively decrease while Csf is minimum for gel T_{12} and progressively increases with increase in CHOL concentration. The Q^m/Csf ratio is highest for gel T_{12} indicating that at TRMA:PC≡1:3.62 a CHOL ratio of 0.92 may be optimum for maximum effect and minimum side effects.

(v) Formulations with TRMA:PC≅1:5.43 i.e. gels T_{17},T_{18},T_{19} and T_{20} : The release rate for these gels is almost similar, with gel T_{18} having the minimum R. The P value is minimum for gel T_{17} while the D value for gels T_{18} and T_{20}. The K value is highest for gels T_{18} and T_{20}. Although the $Q^{i}(6)$ and Q^{m} values are highest for gel T_{20}, the Csf is lowest for gel T_{18}. From the Q^{m}/Csf ratio, gels T_{18} and T_{20} with CHOL in the ratio of 2.75 and 5.43 respectively, may have maximum effect and minimum side effects.

(vi) Formulation having maximum PC cover i.e. TRMA:PC≅1:10.86 i.e. gel T_{21}. The release rate R is minimum for this gel as compared to all other liposomal gels of TRMA prepared. However, it does not have the lowest P and D values or the highest K, $Q^{i}(6)$ or Q^{m} values. Rather, its $Q^{i}(6)$ and Q^{m} /Csf ratio is just 5 fold greater than that for the plain drug gel.

Comparing the above 6 groups of formulations amongst themselves, the following aspects emerge :

As the PC cover increases from 1:0.6 to 1:1.81, the R,P,D and Csf values go on decreasing and when it is increased from 1:1.81 to 1:10.86, these values go on increasing indicating that TRMA:PC ratio of 1:1.81 is optimum as far as these values are concerned. The K, $Q^{i}(6)$ Q^{m} and Q^{m}/Csf ratio values increase when TRMA:PC ratio is increased from 1:0.6 to 1:1.81 and decrease when the ratio is further increased from 1:1.81 to 1:10.86 indicating that

TRMA:PC≡1:1.81 is optimum for maximum effect and minimum side effects. Hence, 3 formulations of this group were subjected to a 45 days stability study at room temperature. The results of the <u>in vitro</u> release studies conducted on these gels for the study period in terms of R,P and D values are shown in Figs. 4.23-4.25. There is no marked change in these values over a period of 45 days indicating that storage does not adversely affect the permeation profile of TRMA from these liposomal gels.

(B)
(i) Formulations with PC:CHOL≡1:1 i.e. gels T_{11},T_{16},T_{20} and T_{21}: The histograms comparing the R,P,D,K,Csf, $Q^i(6)$ and Q^m values are shown in Figs. 4.26-4.28. From Fig.4.26 it is seen that as the PC:CHOL cover increases the release rate R decreases but the P and D values are minimum for gel T_{16}. The K, $Q^i(6)$ and Q^m values are maximum for the same batch although the Csf is minimum for the batch with highest PC:CHOL cover i.e. gel T_{21}. The Qm/Csf ratio is highest for gel T_{16} indicating that at PC:CHOL ratio of 1:1, the optimum concentration of TRMA:PC:CHOL may be 1:3.62:3.62 for maximum effect and minimum side effects.

(ii) Formulations with PC:CHOL≡2:1 i.e. gels T_5, T_9, T_{14} and T_{18}: The histograms comparing the R,P,D,K,Csf, $Q^i(6)$ and Qm values of these gels are shown in Figs. 4.29-4.31. From Fig.4.29, it is seen that the R, P and D values are minimum for gel T_9. Besides, this batch has the highest K, $Q^i(6)$ and Q^m values and lowest Csf value. The Qm/Csf ratio too is

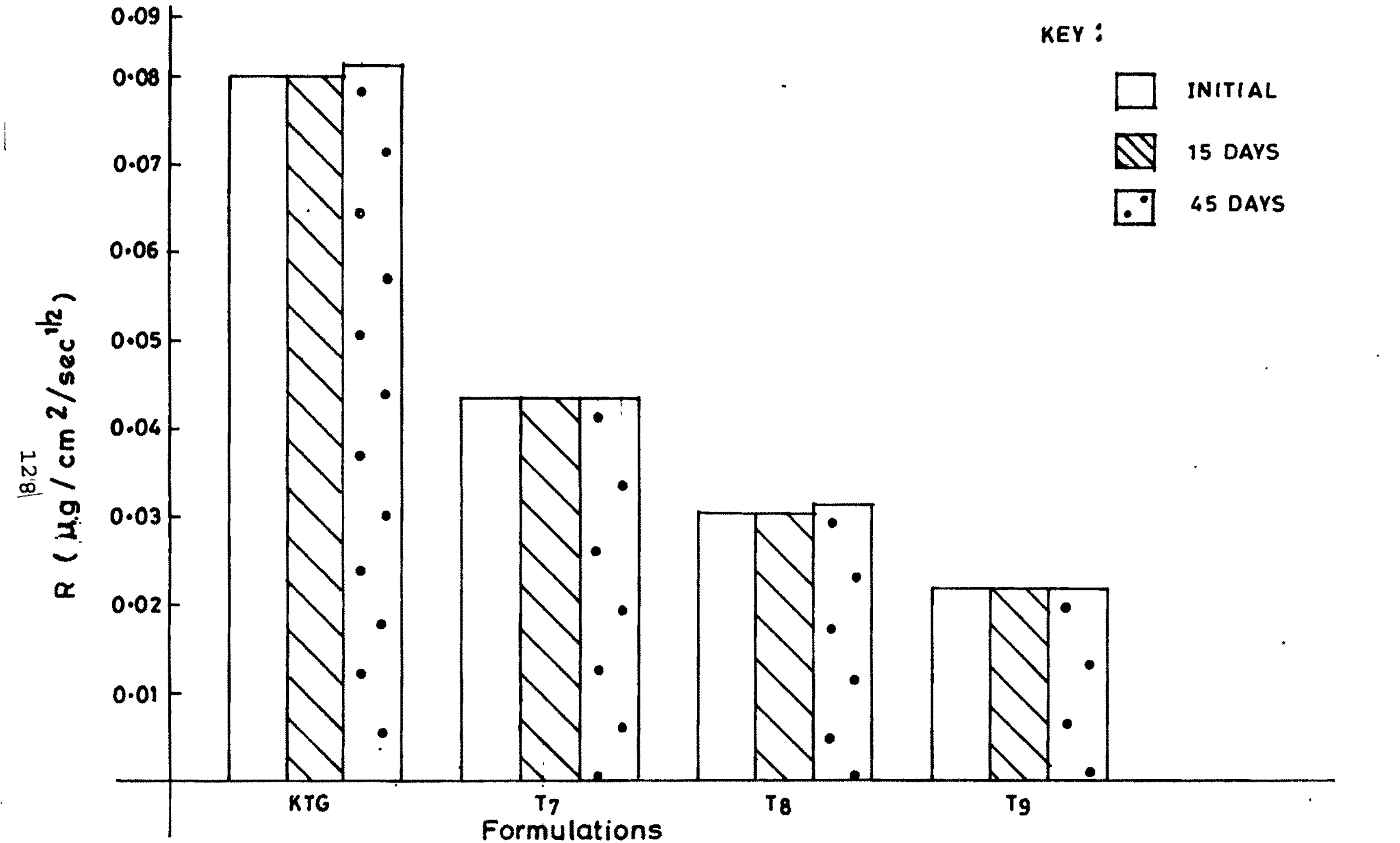

FIG. 4·23 : COMPARISON OF THE R VALUES OF TRMA FROM THE GELS OVER THE PERIOD OF THE STABILITY STUDY.

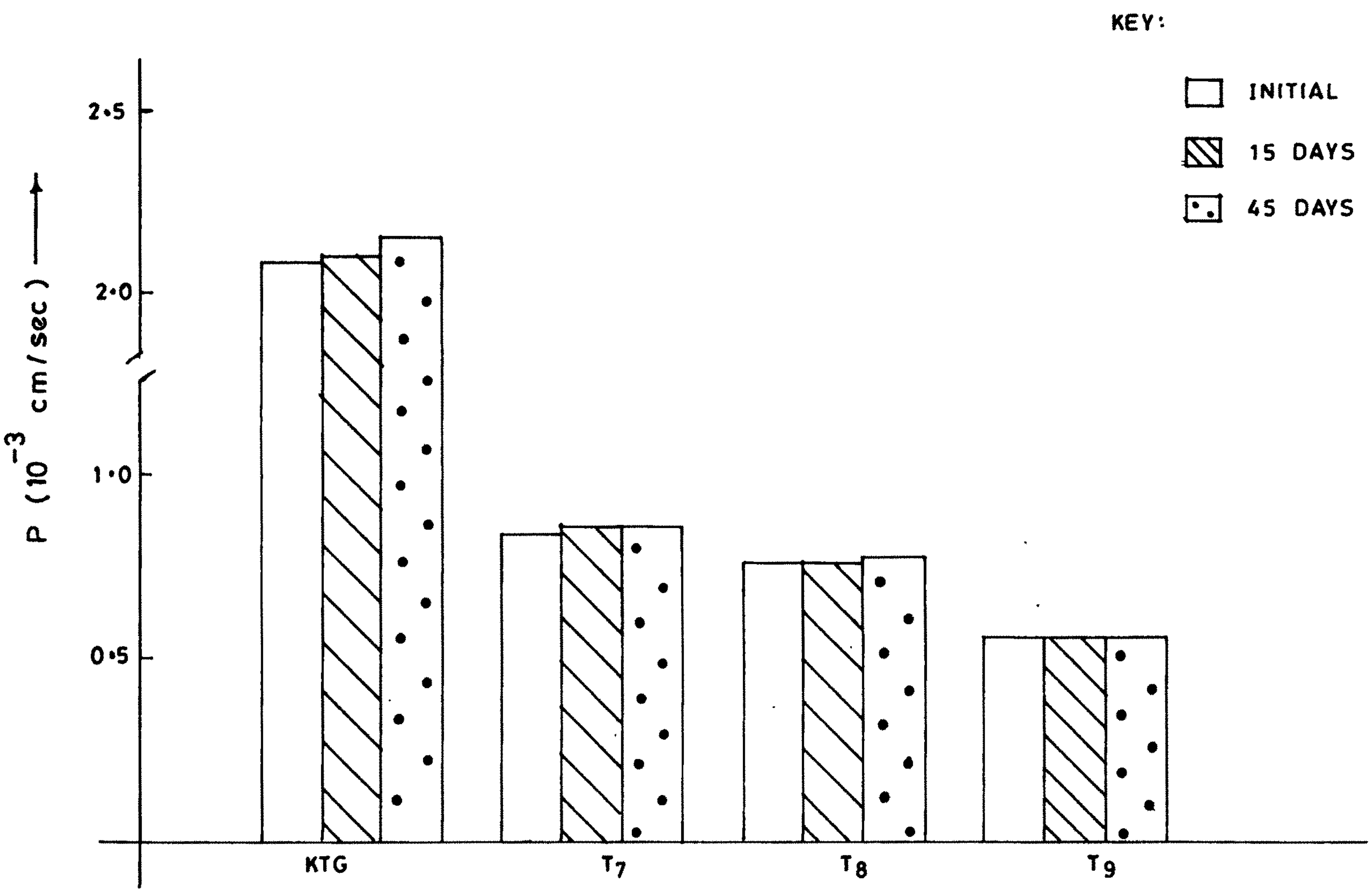

FIG. 4·24 : COMPARISON OF THE P VALUES OF THE GELS OVER THE PERIOD OF THE STABILITY STUDY.

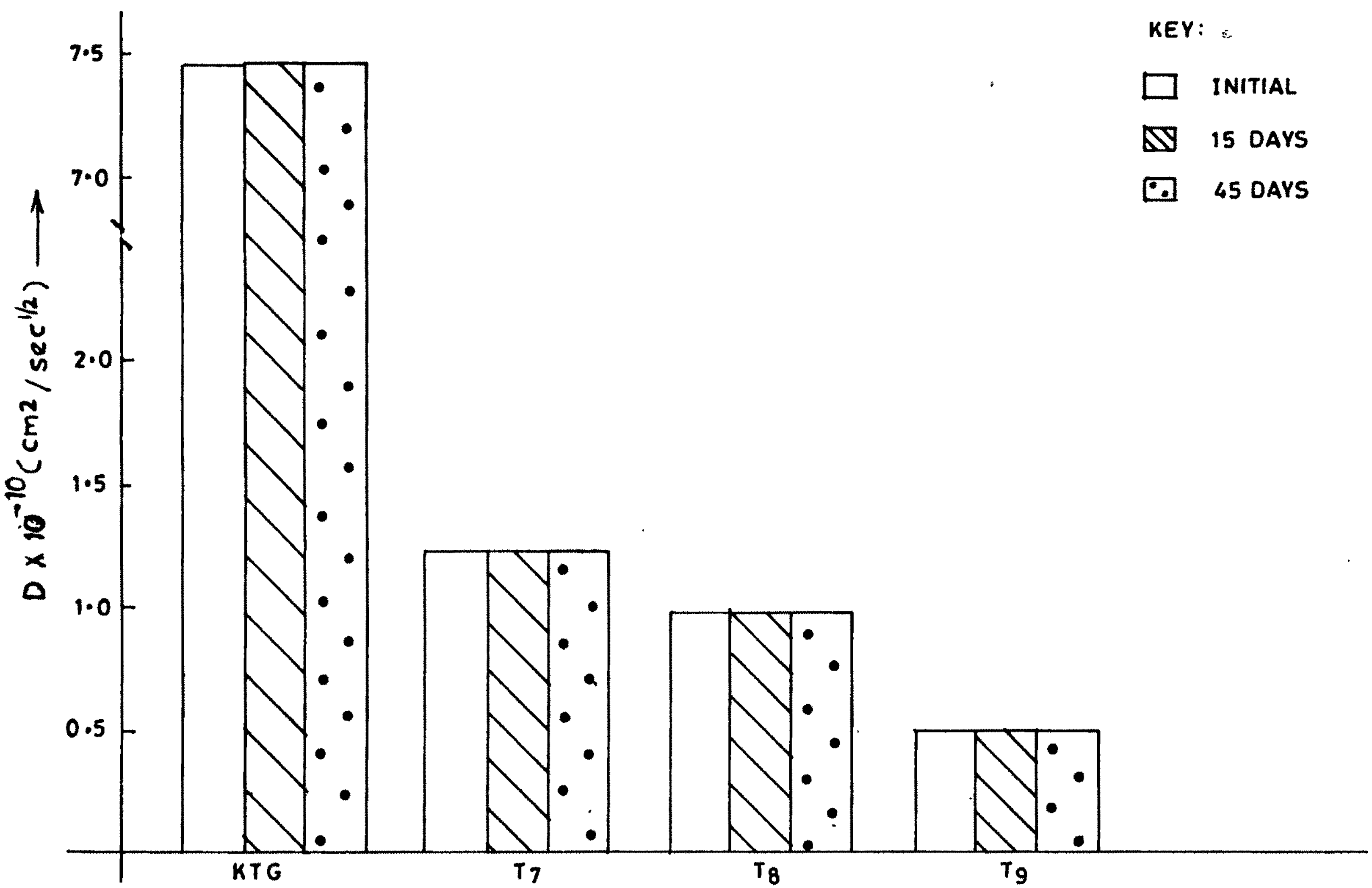

FIG. 4·25: COMPARISON OF THE D VALUES OF THE GELS OVER THE PERIOD OF THE STABILITY STUDY.

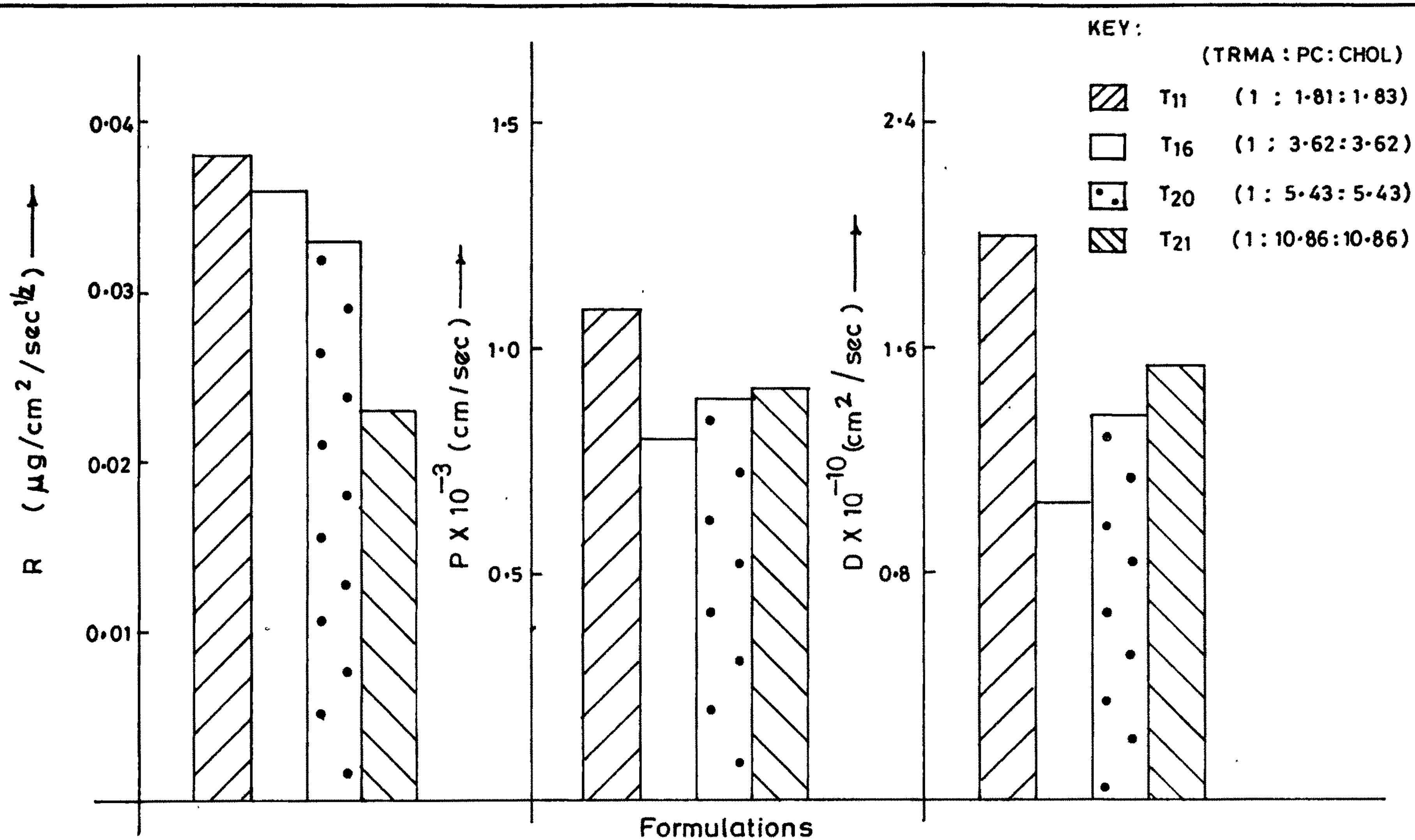

FIG. 4·26 : HISTOGRAMS SHOWING COMPARISON OF R, P AND D VALUES FOR TRMA FROM LIPOSOMAL GELS CONTAINING LIPOSOMES WITH PC:CHOL≡1:1.

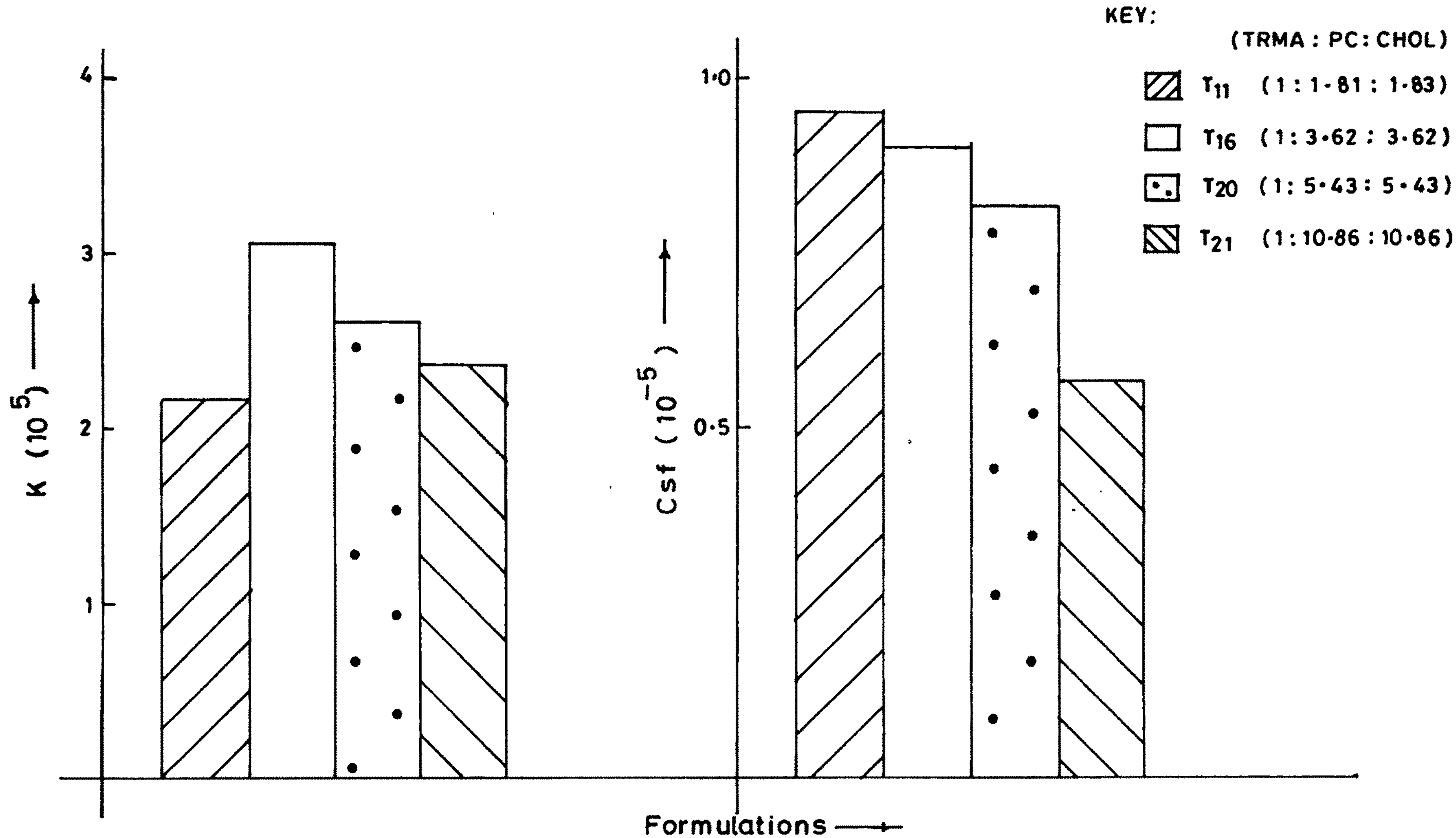

FIG. 4·27: HISTOGRAMS SHOWING K AND Csf VALUES OF TRMA FROM LIPOSOMAL GELS CONTAINING LIPOSOMES WITH PC : CHOL≡1:1.

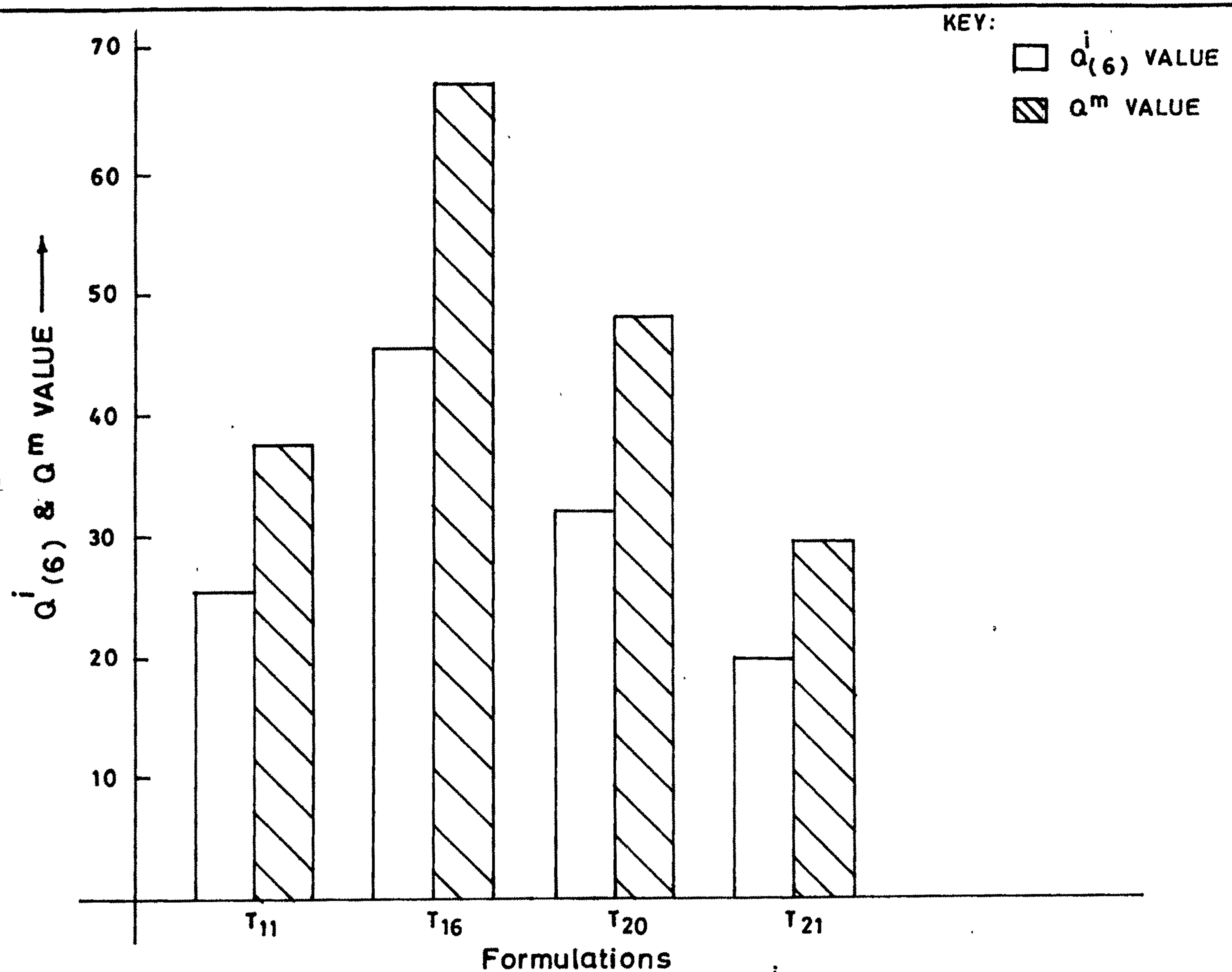

FIG. 4·28: HISTOGRAMS SHOWING THE $Q^{i}_{(6)}$ AND Q^{m} VALUES OF TRMA FROM LIPOSOMAL GELS WITH PC:CHOL≡1:1.

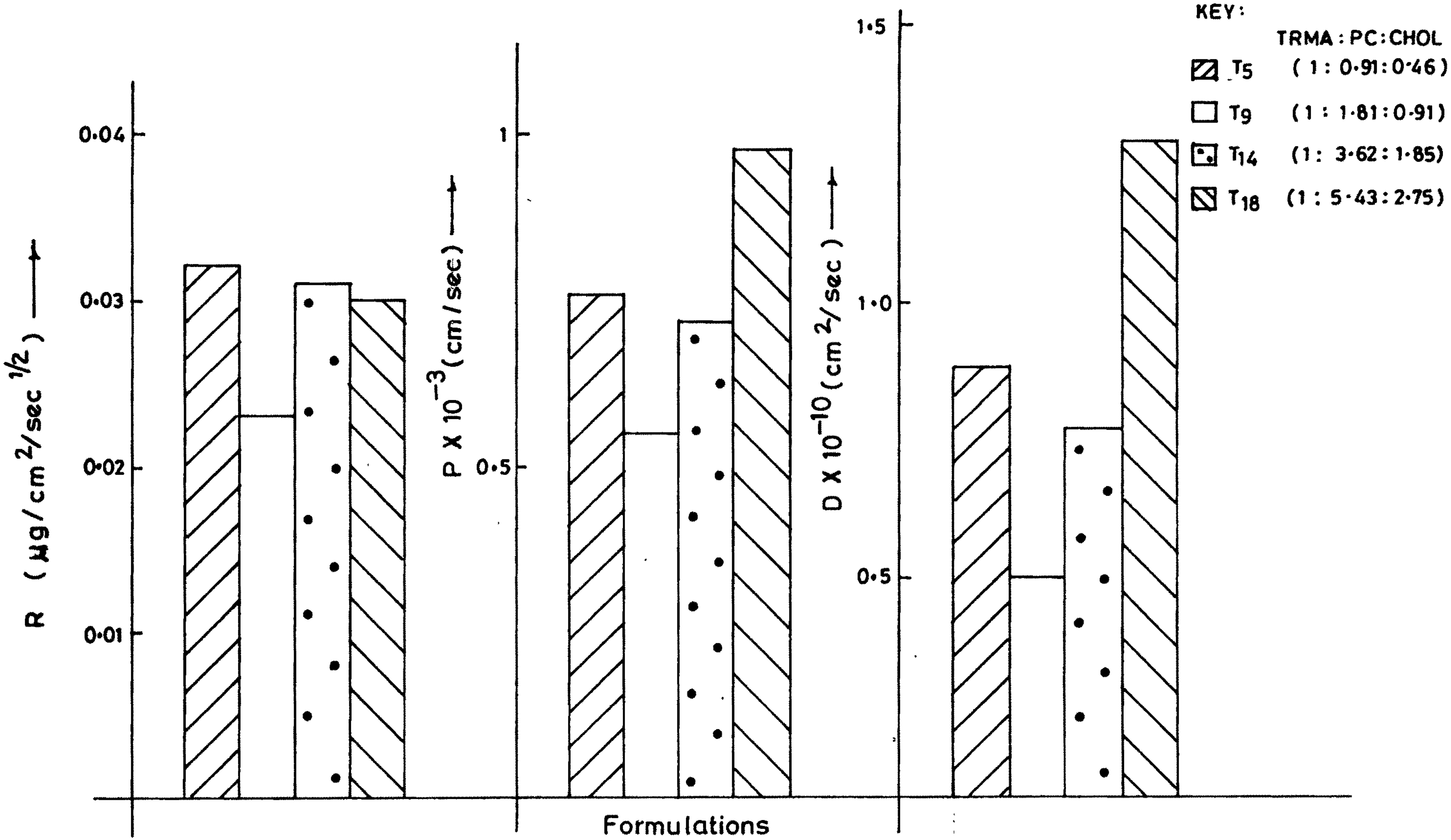

FIG. 4·29: HISTOGRAMS SHOWING R, P AND D VALUES OF TRMA FROM LIPOSOMAL GELS CONTAINING LIPOSOMES WITH PC : CHOL≡2:1.

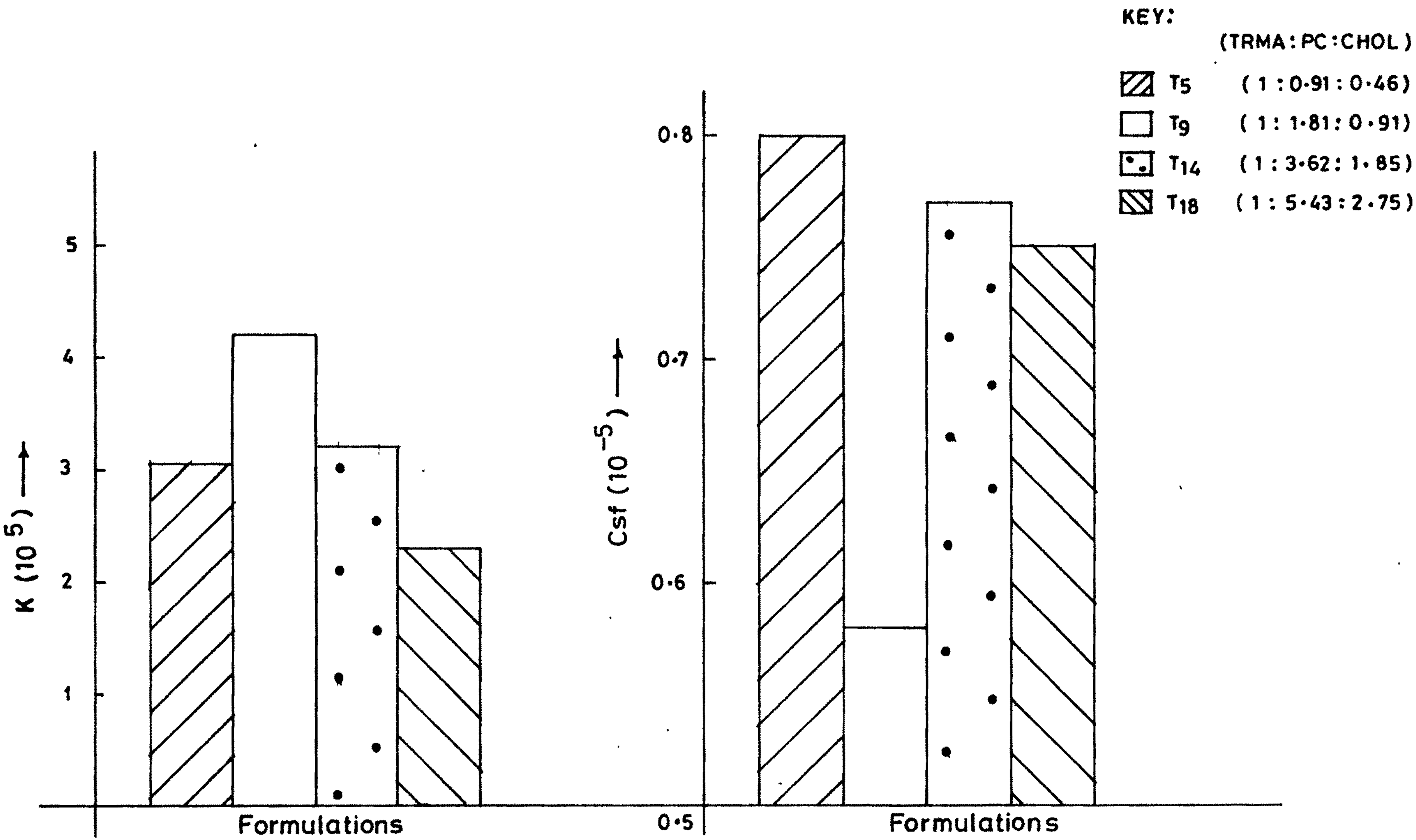

FIG. 4·30: HISTOGRAMS SHOWING K AND Csf VALUES OF TRMA FROM LIPOSOMAL GELS CONTAINING LIPOSOMES WITH PC:CHOL≡2:1.

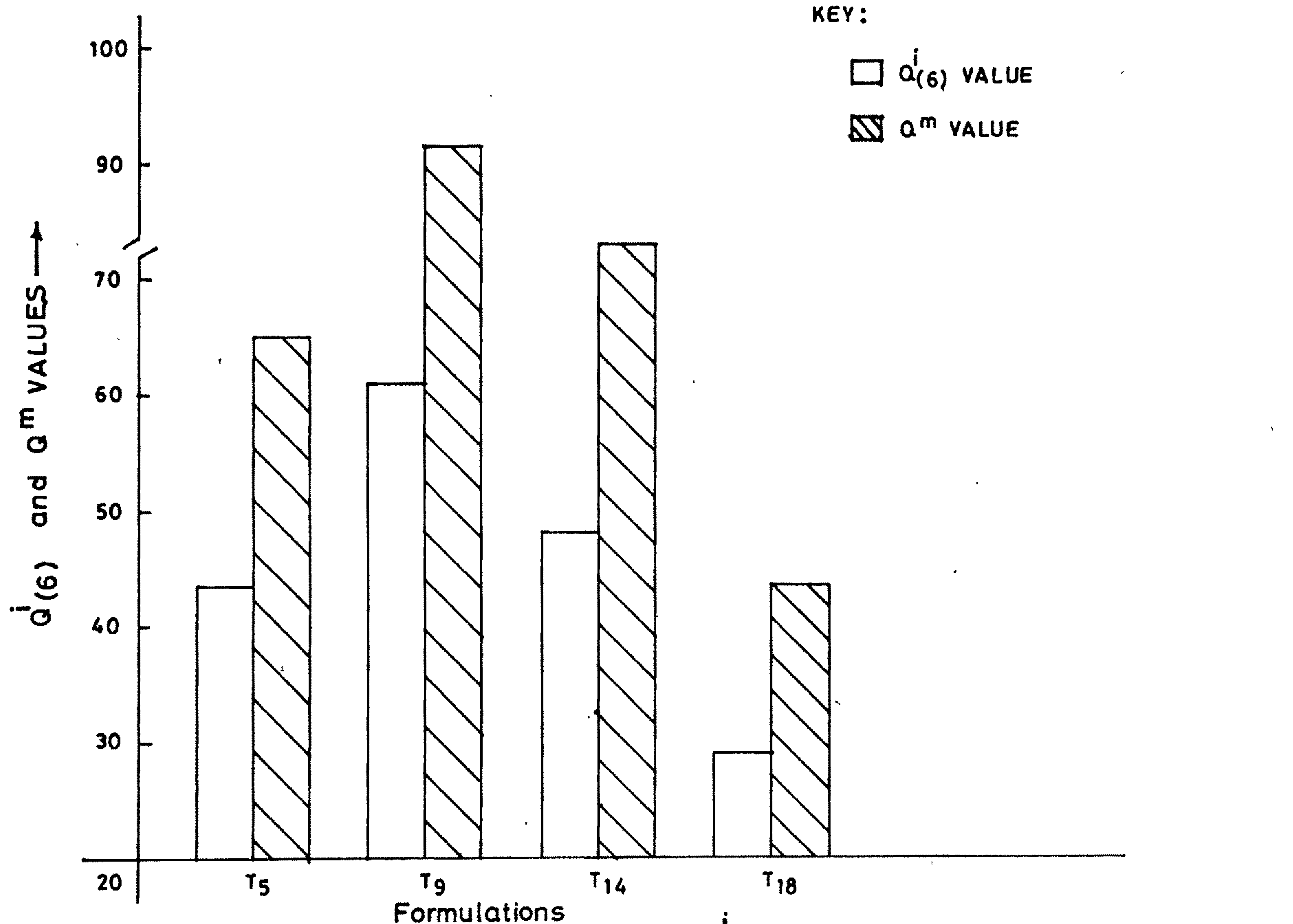

FIG. 4.31: HISTOGRAMS SHOWING THE $Q^{i}_{(6)}$ AND Q^{m} VALUES OF TRMA FROM LIPOSOMAL GELS WITH PC:CHOL≡2:1.

highest for the same gel indicating that at PC:CHOL ratio of 2:1, the optimum concentration of TRMA:PC:CHOL maybe 1:1.81:0.91.

(iii) Formulations with PC:CHOL≡4.1 i.e. gels T_1, T_2,T_7 and T_{17} : The histograms comparing the R, P, D, K, Csf, $Q^i(6)$ and Q^m values of these gels are shown in Figs. 4.32-4.34. From Fig. 4.32, it is seen that the R value is minimum for gel T_{17} while the P and D values for gel T_7. Gel T_7 has the highest K, $Q^i(6)$ and Q^m values but the Csf value is lowest for gel T_{17}. The Q^m/Csf ratio is highest for gel T_7 indicating that at PC:CHOL ratio of 4:1, the optimum ratio of TRMA:PC:CHOL may be 1:1.81:0.459.

Comparing the above 3 groups of formulations amongst themselves, the following aspects emerge :

The R,P,D, and Csf values are minimum for gel T_9 i.e. PC:CHOL≡2:1 and the $Q^i(6)$, Q^m and Q^m/Csf ratio values are maximum for the same gel indicating that PC:CHOL≡2:1 might be optimum for maximum effect and minimum side effects.

Fig.4.35 shows the _in vitro_ release profile of TRMA from its plain drug gels prepared by using different gel bases. From the curves it is evident that the release of TRMA from its gel bases is not zero order. A linear relationship ($0.969<r<0.997$) exists between Q and $t^{1/2}$ for plain TRMA gels and liposomal TRMA gels in different bases indicating that the release of TRMA from these gels obeys the

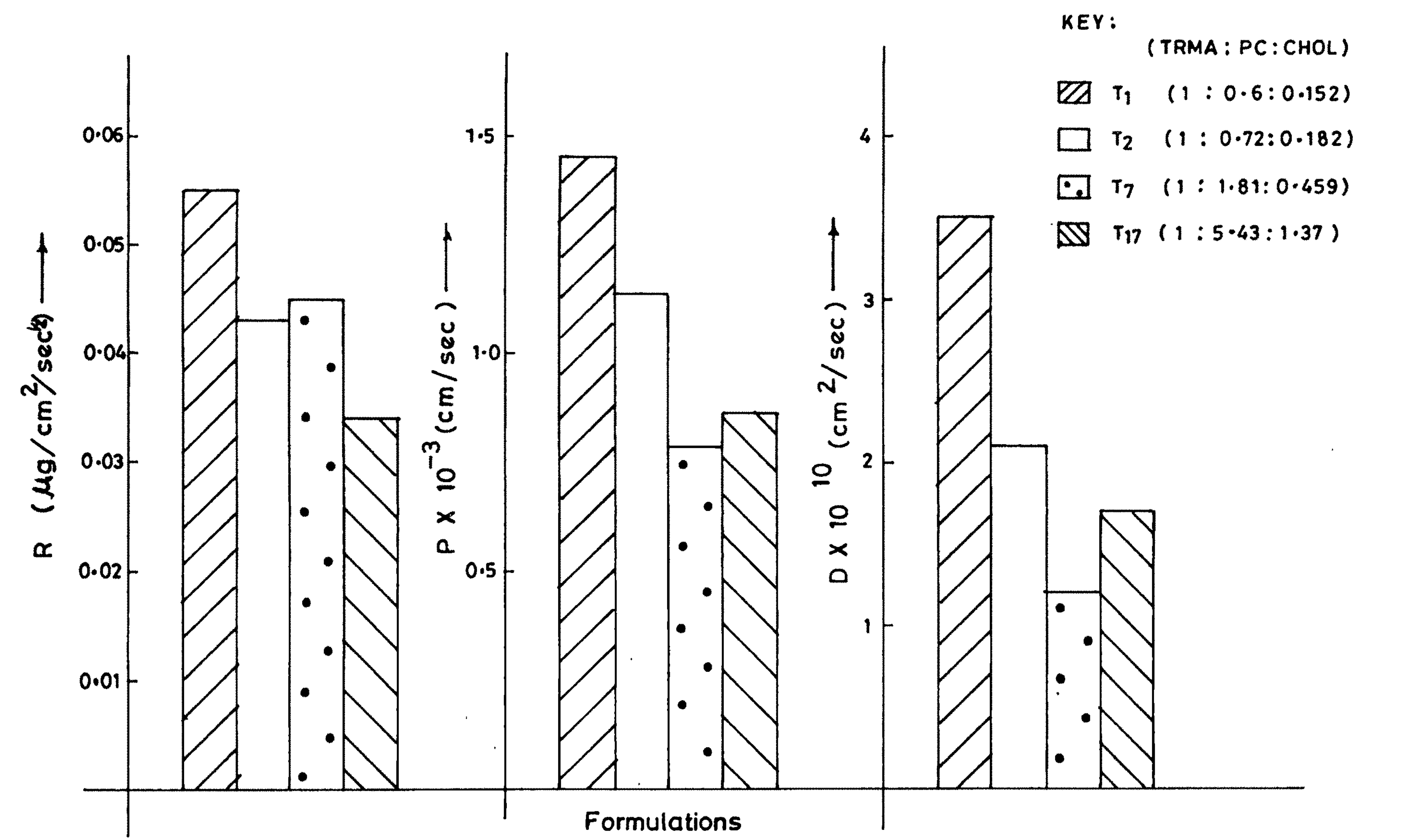

FIG. 4·32 : HISTOGRAMS SHOWING R, P AND D VALUES OF TRMA FROM LIPOSOMAL GELS CONTAINING LIPOSOMES WITH PC : CHOL≡4:1.

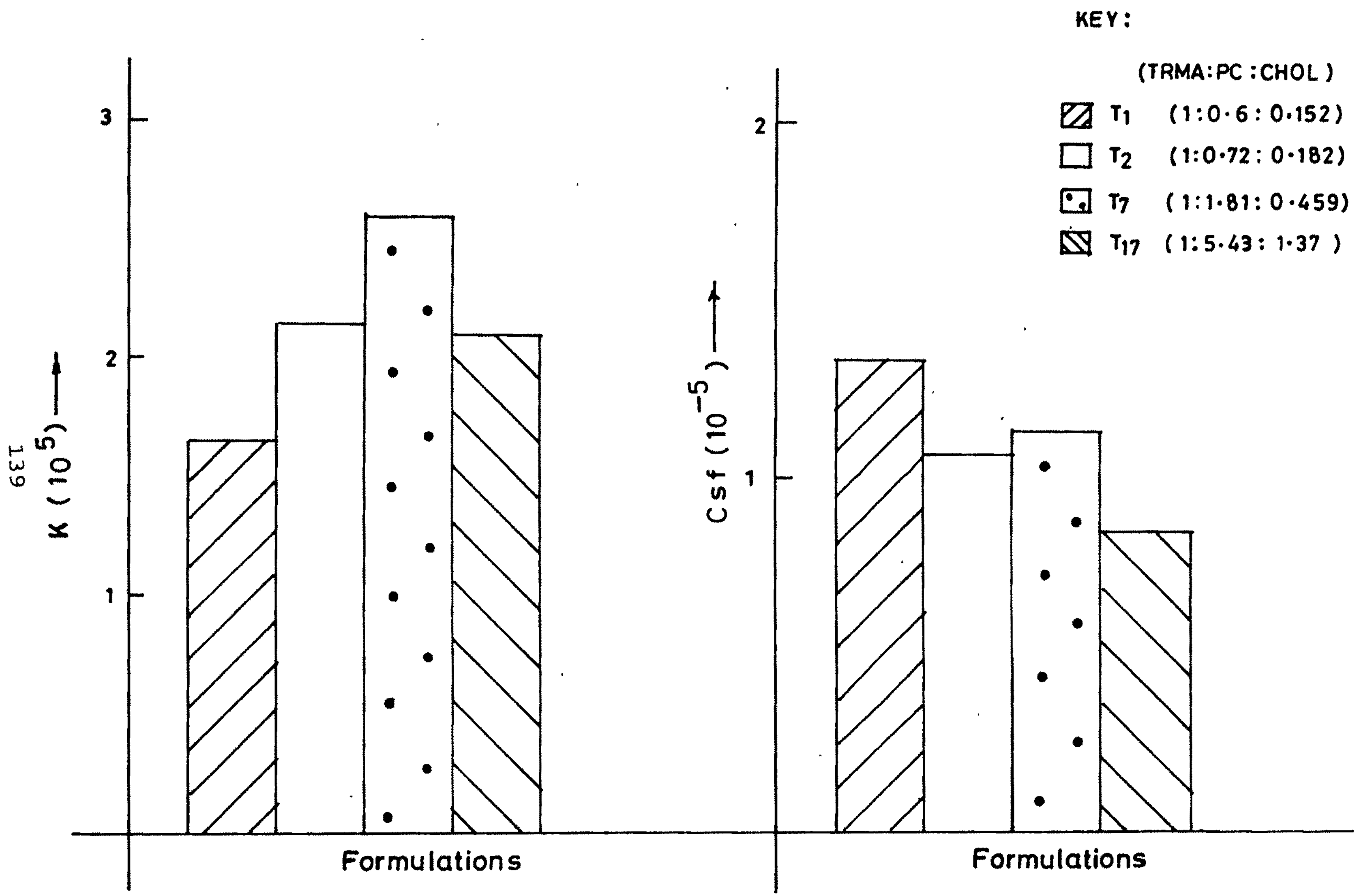

FIG. 4·33: HISTOGRAMS SHOWING K AND Csf VALUES OF TRMA FROM LIPOSOMAL GELS CONTAINING LIPOSOMES WITH PC : CHOL ≡ 4:1.

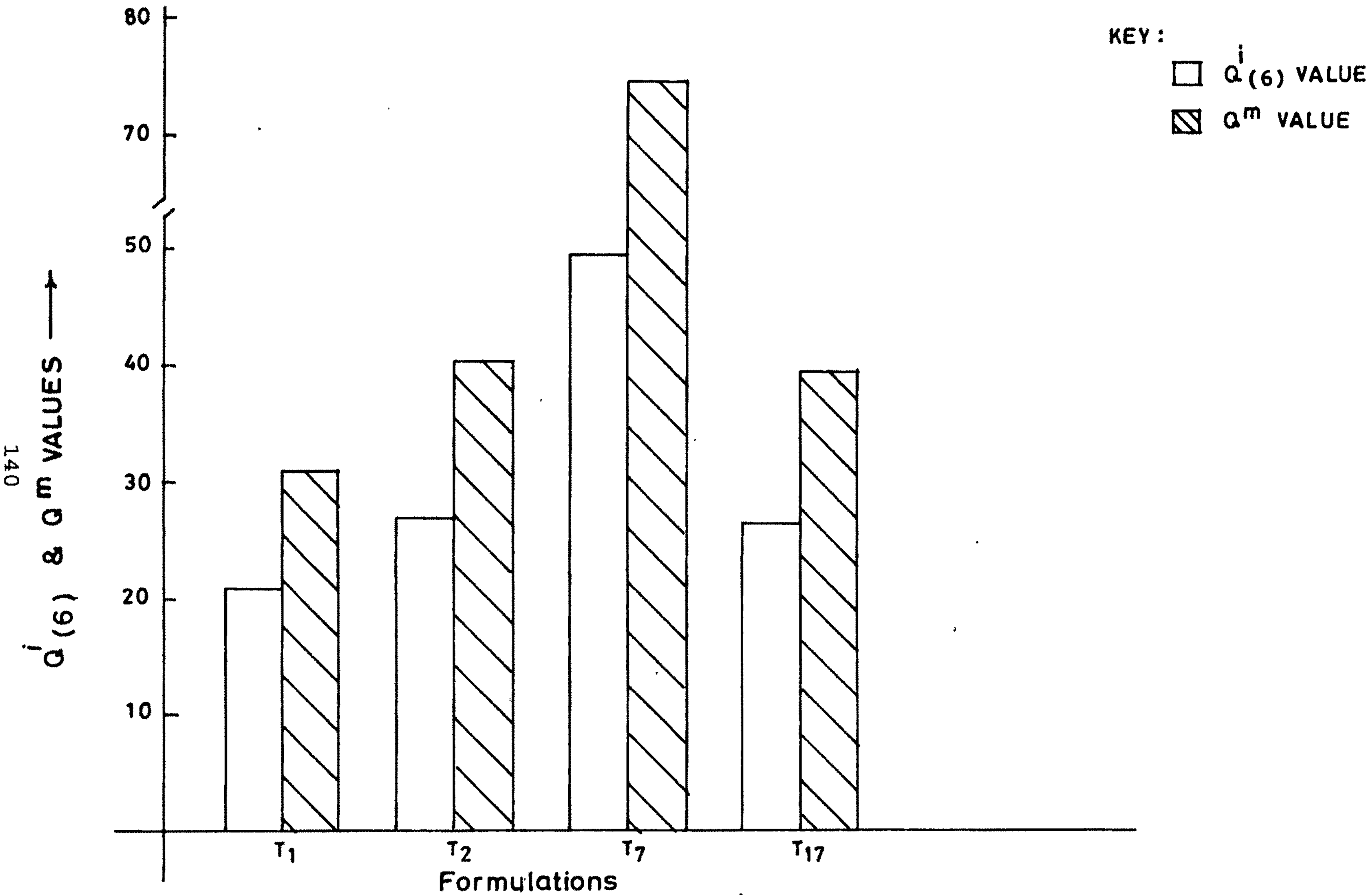

FIG. 4·34: HISTOGRAMS SHOWING THE $Q^{i}_{(6)}$ AND Q^{m} VALUES FOR TRMA FROM LIPOSOMAL GELS WITH PC : CHOL≡ 4:1.

TABLE 4.6

IN VITRO RELEASE PROFILE OF FORMULATIONS OF TRMA IN DIFFERENT GEL BASES.

Formulation	***Mean cumulative amount released Q(µg/cm²) ± SEM after**				
	1hr	**2hrs**	**4hrs**	**6hrs**	**24hrs**
KTG	3.59 (0.055)	4.67 (0.058)	12.19 (0.078)	15.04 (0.081)	21.23 (0.07)
KLG	1.88 (0.048)	2.38 (0.028)	4.91 (0.055)	6.64 (0.172)	17.78 (0.160)
ETG	2.13 (0.078)	2.93 (0.068)	4.23 (0.103)	7.46 (0.48)	16.37 (0.51)
ELG	1.50 (0.007)	1.78 (0.033)	3.08 (0.023)	4.06 (0.038)	13.69 (0.13)
CTG	2.59 (0.152)	3.31 (0.073)	4.67 (0.112)	5.76 (0.028)	15.20 (0.123)
CLG	2.47 (0.033)	3.14 (0.163)	4.33 (0.733)	6.08 (0.033)	15.02 (0.238)
PTG	2.80 (0.125)	4.17 (0.212)	8.20 (0.033)	8.71 (0.277)	19.33 (0.558)
PLG	2.52 (0.045)	3.70 (0.043)	4.61 (0.085)	5.18 (0.148)	13.01 (0.23)

* n = 6

(For key to formulations refer Table 3.11)

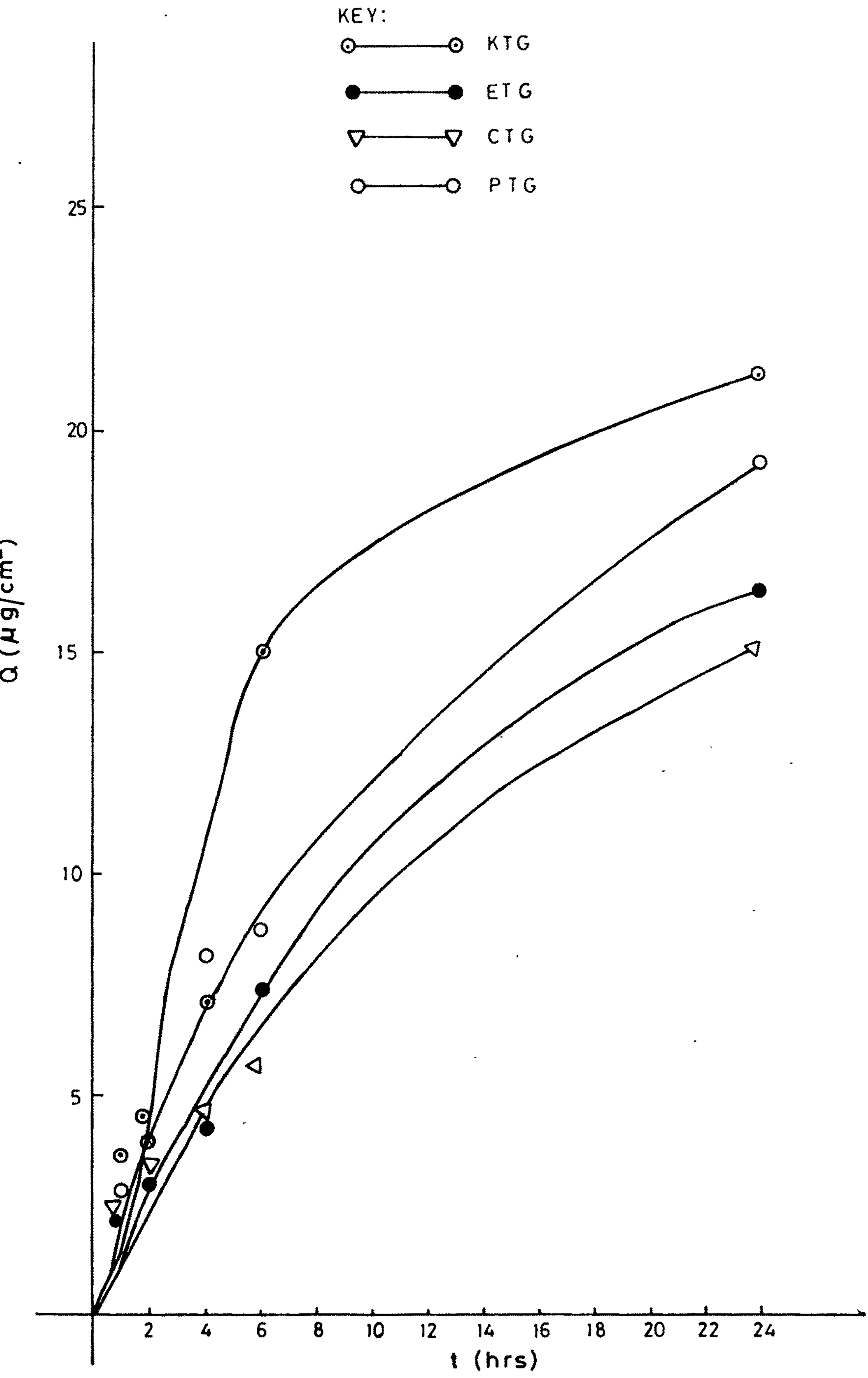

FIG. 4·35: IN VITRO RELEASE PROFILE OF TRMA FROM ITS PLAIN DRUG GELS PREPARED BY USING DIFFERENT GEL BASES.

Higuchi's diffusion controlled model. The parameters for the regression lines are shown in Table 4.7 and the lines of regression in Fig.4.36. The R,P,D,K, Csf, $Q^i(6)$ and Q^m values and the Qm/Csf ratio for the gels compared are shown in Tables 4.8 and 4.9 and in Figs. 4.37-4.39. In general, the R,P,D and Csf values are lower while K, $Q^i(6)$, Q^m values and Q^m/Csf ratio higher for liposomal TRMA in all gel bases as compared to plain TRMA in these gel bases.

From Table 4.10 it is seen that when plain TRMA was incorporated in different gel bases, the TRMA gel prepared using carbopol base showed the minimum R,P,D and Csf value and maximum K, $Q^i(6)$, Q^m and Q^m/Csf ratio values indicating that it may be the best base for a plain TRMA formulation to give maximum effect and minimum side effects. However, for liposomal TRMA, HPMC K4M gel base shows all these properties. Hence it can be clearly seen that the effect and side effect of TRMA are dependent on the gel base also.

A hydrophilic drug is released slowly from a hydrophilic base than from a hydrophobic base and vice versa. Incorporation of a hydrophobic drug like TRMA into liposomes imparts hydrophilicity to the drug surface and hence the release from the same base is altered, once the drug is incorporated into liposomes. It is a known fact that HPMC E4M is more hydrophobic than HPMC K4M. The release of free TRMA from HPMC E4M gel base is slower than that from HPMC K4M gel base while the reverse is the case for liposomal TRMA since

TABLE 4.7

CORRELATION COEFFICIENT AND 'a' AND 'b' VALUES OF THE LINES OF REGRESSION FOR THE DATA OF MEAN CUMULATIVE AMOUNT RELEASED VS SQUARE ROOT OF TIME FOR FORMULATIONS OF TRMA IN DIFFERENT GEL BASES.

Formulation	**Correlation coefficient r**	***a**	***b**
KTG	0.983	0.048	0.080
KLG	0.981	0.350	0.040
ETG	0.988	0.268	0.045
ELG	0.969	0.303	0.032
CTG	0.990	0.099	0.044
CLG	0.992	0.140	0.062
PTG	0.997	0.171	0.060
PLG	0.997	-0.700	0.041

* Equation of regression line is $y = a + bx$

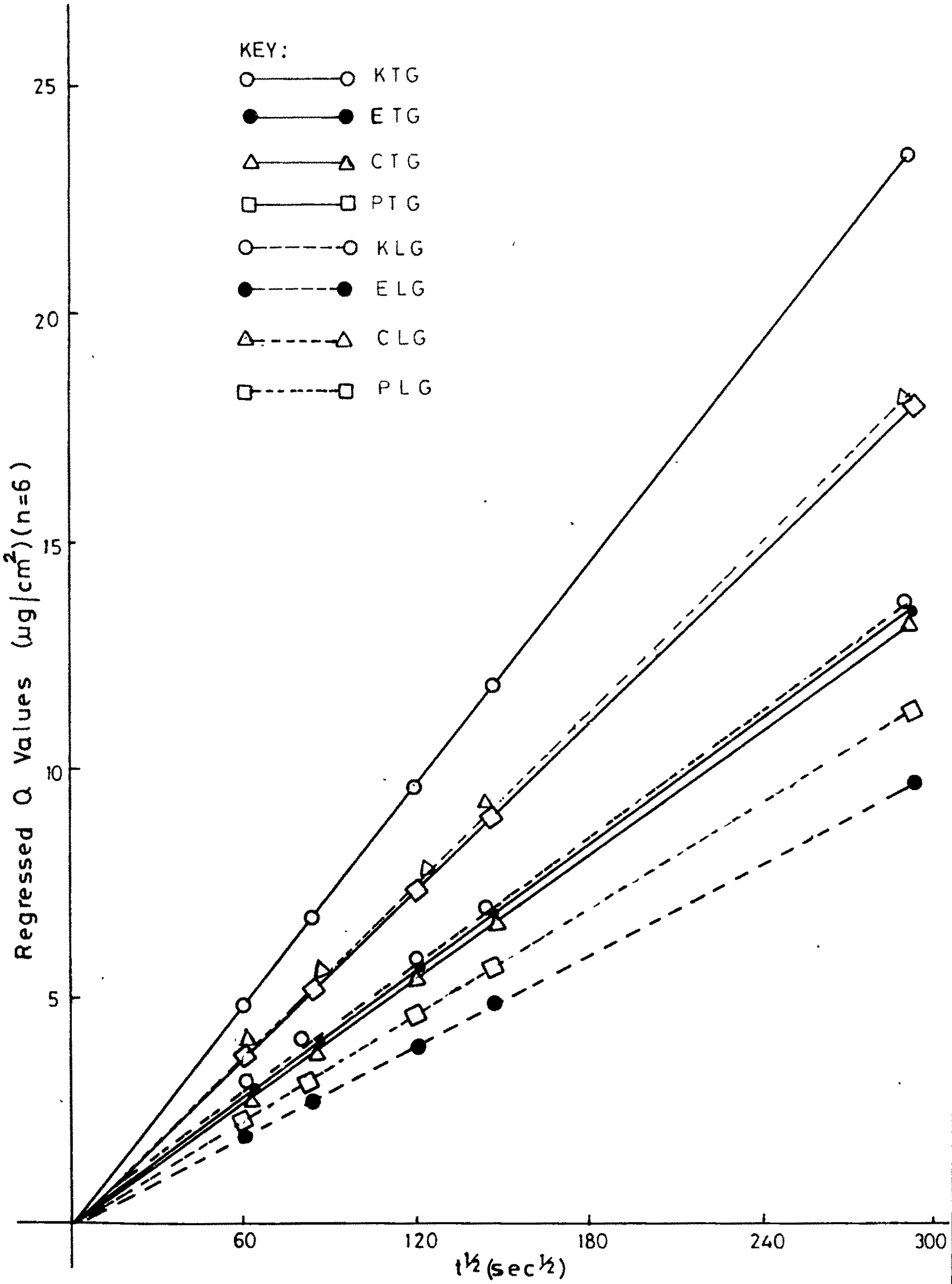

FIG. 4.36: Q Vs $t^{1/2}$ PLOTS FOR RELEASE OF TRMA FROM ITS LIPOSOMES IN DIFFERENT GEL BASES (-----) AS COMPARED TO THAT FROM PLAIN TRMA IN DIFFERENT GEL BASES (——).

TABLE 4.8

DIFFUSION PARAMETERS TO ASSESS THE RELEASE OF TRMA FROM ITS FORMULATIONS IN DIFFERENT GEL BASES.

Formulation	*P (10^{-4})	*D (10^{-10})	*R	*K (10^{5})
KTG	20.94	7.45	0.080	1.12
KLG	4.32	1.21	0.023	2.80
ETG	12.60	2.77	0.045	1.82
ELG	5.58	0.55	0.032	1.82
CTG	10.66	1.91	0.044	2.23
CLG	9.37	1.45	0.062	2.58
PTG	18.20	5.64	0.060	1.29
PLG	9.91	1.63	0.041	2.43

* P = Permeability coefficient (cm/sec)

D = Diffusion coefficient (cm^2/sec)

R = Release rate ($\mu g/cm^2/sec^{1/2}$)

K = Partition coefficient

All above values are calculated from the data obtained after regression and hence SEM values are not shown.

TABLE 4.9

PARAMETERS RESPONSIBLE FOR THE EFFECT, SIDE EFFECT AND RATIO THEREOF FOR FORMULATIONS OF TRMA IN DIFFERENT GEL BASES.

Formulation	Effect		Side effect	Q^m/C_{sf}
	$Q^i(6)^*$	Q^{m*}	$Csf^*(10^{-5})$	
KTG	14.69	21.40	2.00	10.70
KLG	49.87	74.48	1.13	65.91
ETG	21.83	32.40	1.12	28.93
ELG	34.76	52.05	0.80	65.06
CTG	30.90	46.03	1.10	41.85
CLG	39.91	59.55	1.08	55.14
PTG	14.16	21.26	1.50	14.17
PLG	33.70	50.25	1.02	49.26

* $Q^i(6)$ is the quantity of drug which enters the membrane upto 6 hrs.

Q^m is the quantity of drug which remains in the membrane.

C_{sf} is the factor for the quantity of drug in the blood stream.

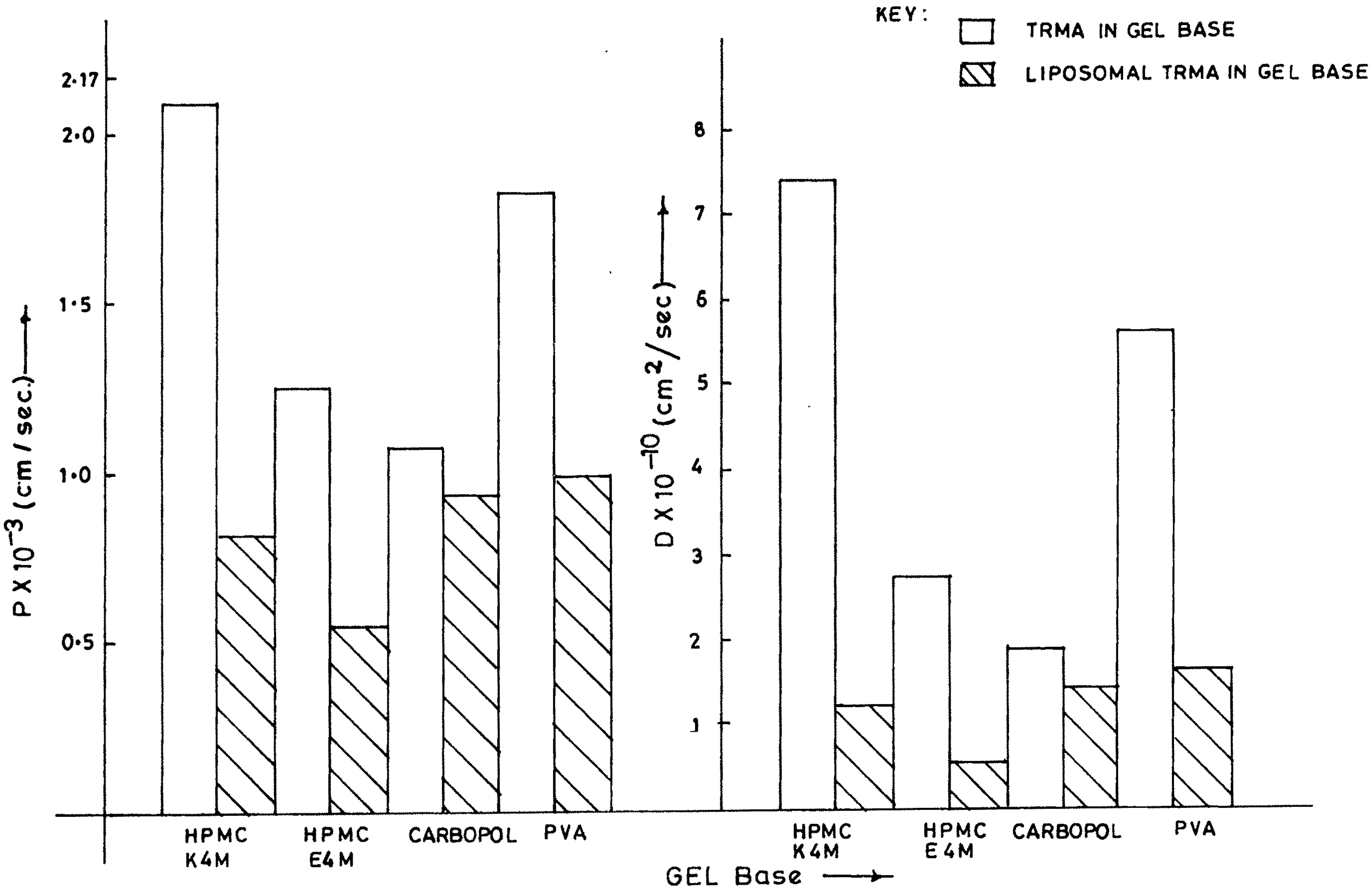

FIG. 4·37: COMPARISON OF P AND D VALUES FOR TRMA BY INCORPORATING IT AND ITS LIPOSOMES IN DIFFERENT GEL BASES.

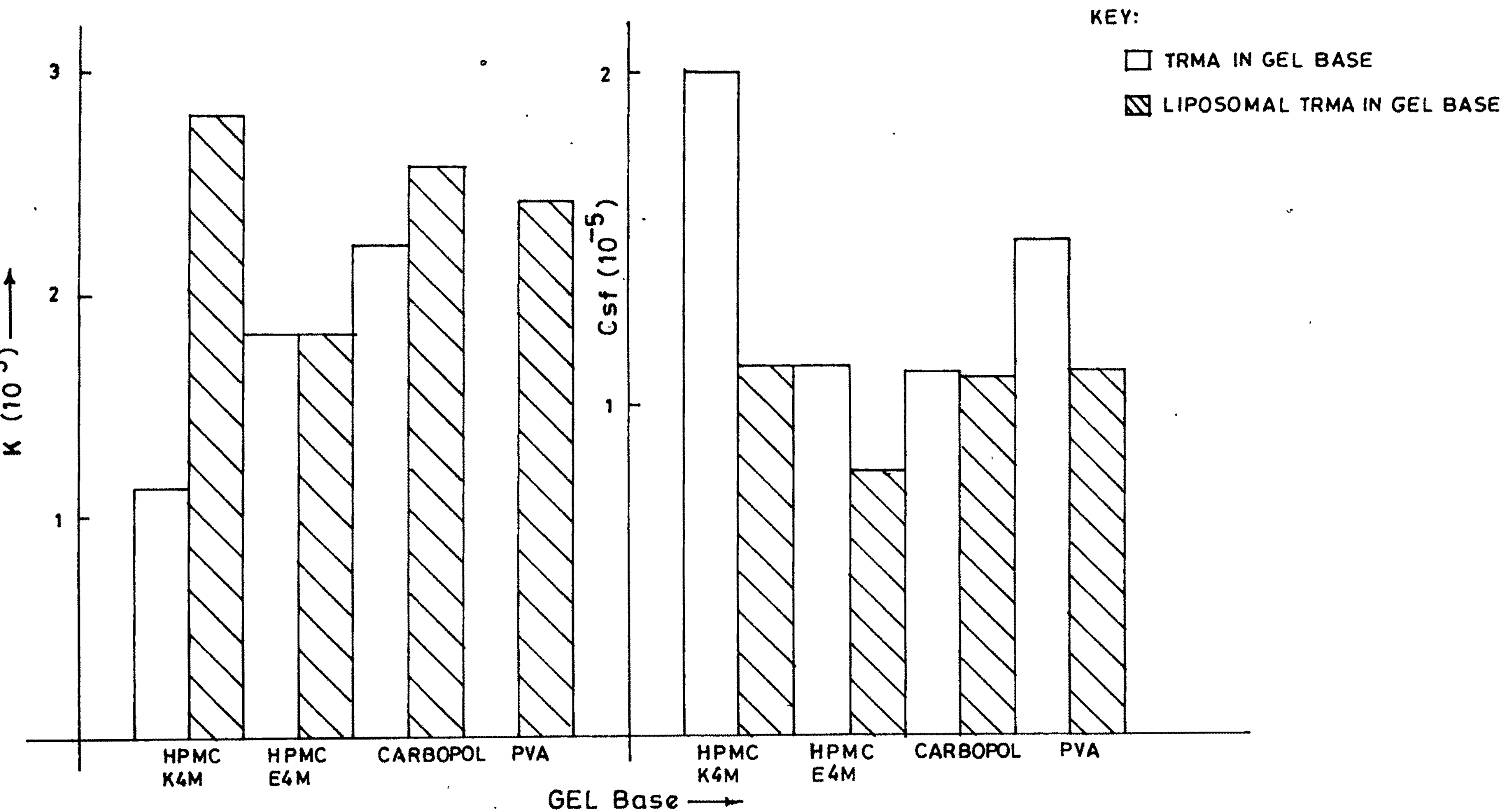

FIG. 4·38: COMPARISON OF K AND Csf VALUES OF TRMA OBTAINED USING DIFFERENT GEL BASES AND LIPOSOMES (TRMA 7) IN THESE GEL BASES.

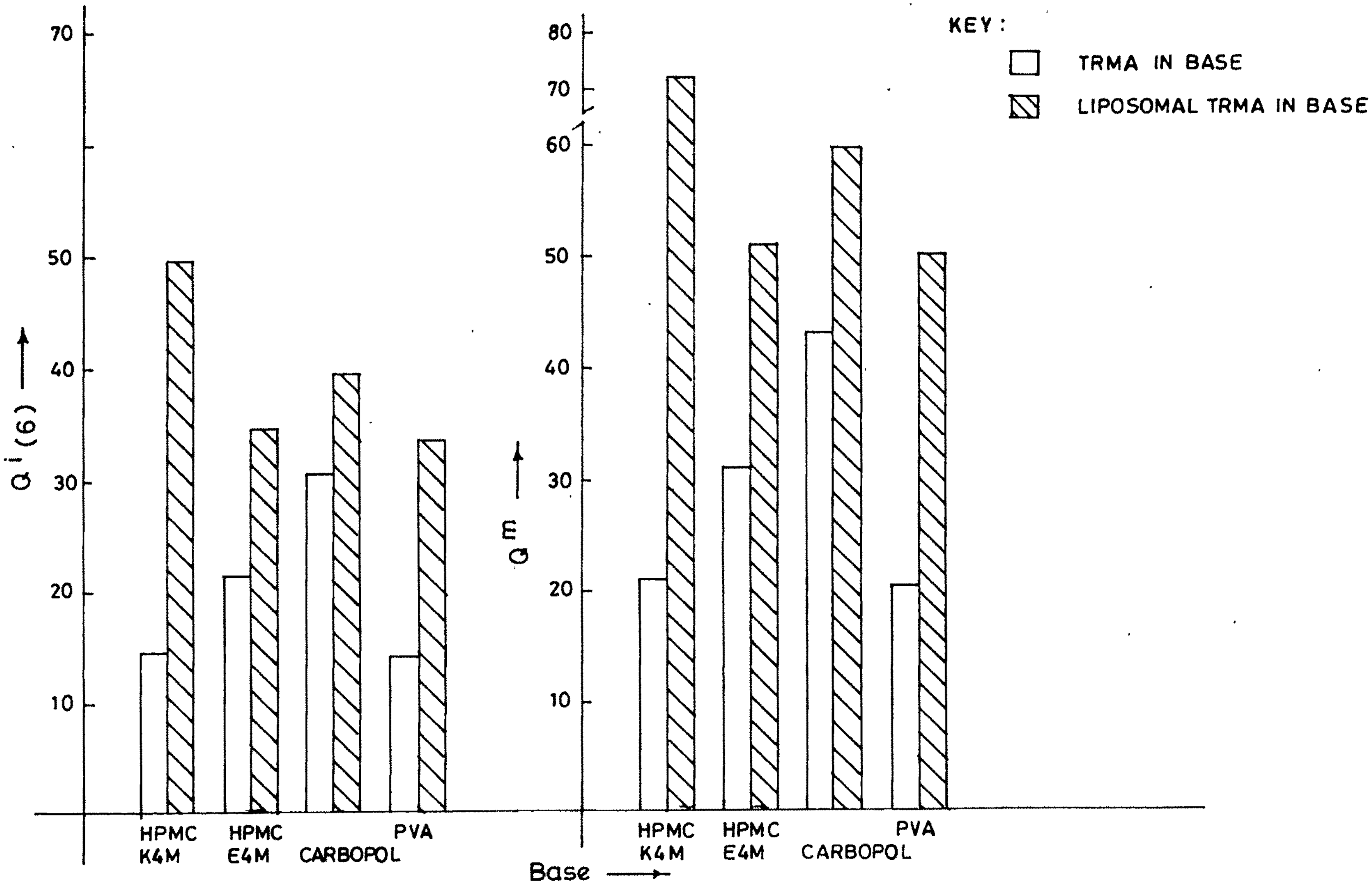

FIG 4·39: HISTOGRAMS SHOWING $Q^{i}(6)$ AND Q^{m} VALUES OF TRMA AND LIPOSOMAL TRMA IN DIFFERENT BASES.

TABLE 4.10

RANK ORDER VARIATION IN THE VARIOUS PERMEATION PARAMETERS FOR PLAIN TRMA AND LIPOSOMAL TRMA FORMULATIONS USING DIFFERENT GEL BASES.

Parameter	**Rank order for**	
	Plain drug gel	**Liposomal drug gel**
R	K4M>PVA>E4M = Carbopol	Carbopol>PVA>E4M>K4M
P	K4M>PVA>E4M> Carbopol	PVA>Carbopol>E4M>K4M
D	K4M>PVA>E4M Carbopol	PVA>Carbopol>K4M>E4M
K	Carbopol>E4M>PVA>K4M	K4M>Carbopol>PVA>E4M
$Q^i(6)$	Carbopol>E4M>K4M=PVA	K4M>Carbopol>E4M>PVA
Q^m	Carbopol>E4M>K4M=PVA	K4M>Carbopol>E4M>PVA
Q^m/Csf	Carbopol>E4M>K4M>PVA	K4M>E4M>Carbopol>PVA

the later is more hydrophilic and hence may be retained in HPMC K4M base more than in HPMC E4M gel base. From this, we can suggest that since the free drug is released slowest and the liposomal drug fastest from the carbopol gel base, it must be more hydrophobic than HPMC E4M gel base. Hence, the rank order of preference in case of selecting a suitable gel base for plain TRMA may be Carbopol>HPMC E4M > HPMC K4M while for liposomal TRMA it may be HPMC K4M>HPMC E4M > Carbopol. However, this should be confirmed by in vivo studies.

The release rate of TRMA from PVA gels is high, irrespective of whether TRMA is in the free or the liposomal form indicating that some factor, other than the hydrophilicity of the base must be involved in the release of the drug in this case. PVA gels, both free and liposomal, exhibit the minimum Q^m/Csf ratio and so it is not recommended for topical steroidal formulations.

Release profiles for the various formulations of FLU and CLO studied are shown in Figs. 4.40 and 4.41 and Table 4.11. It is clear that the release is not zero order. A linear relationship ($0.967<r<0.997$) exists between Q and $t^{1/2}$ for all these formulations indicating that the release obey's the Higuchi's diffusion controlled model. The parameters of the regression lines for formulations of FLU and CLO studied are listed in Table 4.12 and the lines are shown in Figs. 4.42 and 4.43.

TABLE 4.11

IN VITRO RELEASE PROFILES OF FORMULATIONS OF FLU AND CLO.

Formulation	*Mean cumulative amount released Q ($\mu g/cm^2$) $\pm$ SEM after				
	1hr	**2hrs**	**4hrs**	**6hrs**	**24hrs**
KFG	4.71 (0.03)	6.34 (0.275)	11.55 (0.305)	14.82 (0.323)	47.79 (0.597)
FPM	4.36 (0.06)	6.60 (0.23)	12.38 (0.401)	15.02 (0.082)	51.96 (0.055)
F_1	4.36 (0.035)	6.85 (0.07)	10.49 (0.16)	11.53 (0.107)	28.82 (0.428)
F_2	4.80 (0.052)	6.80 (0.08)	10.65 (0.088)	11.52 (0.045)	30.46 (0.618)
F_3	4.44 (0.06)	6.58 (0.36)	7.72 (0.123)	9.51 (0.06)	33.61 (0.30)
KCG	2.85 (0.087)	3.80 (0.053)	4.05 (0.103)	5.27 (0.10)	20.19 (0.063)
CPM	3.02 (0.032)	3.68 (0.048)	4.17 (0.055)	5.32 (0.062)	20.10 (0.101)
C_1	2.67 (0.047)	3.77 (0.157)	3.62 (0.15)	7.03 (0.07)	9.20 (0.072)
C_2	1.78 (0.037)	2.02 (0.022)	2.20 (0.027)	5.06 (0.027)	8.89 (0.082)
C_3	1.63 (0.007)	1.98 (0.005)	2.37 (0.075)	4.03 (0.022)	7.15 (0.063)

* n = 6

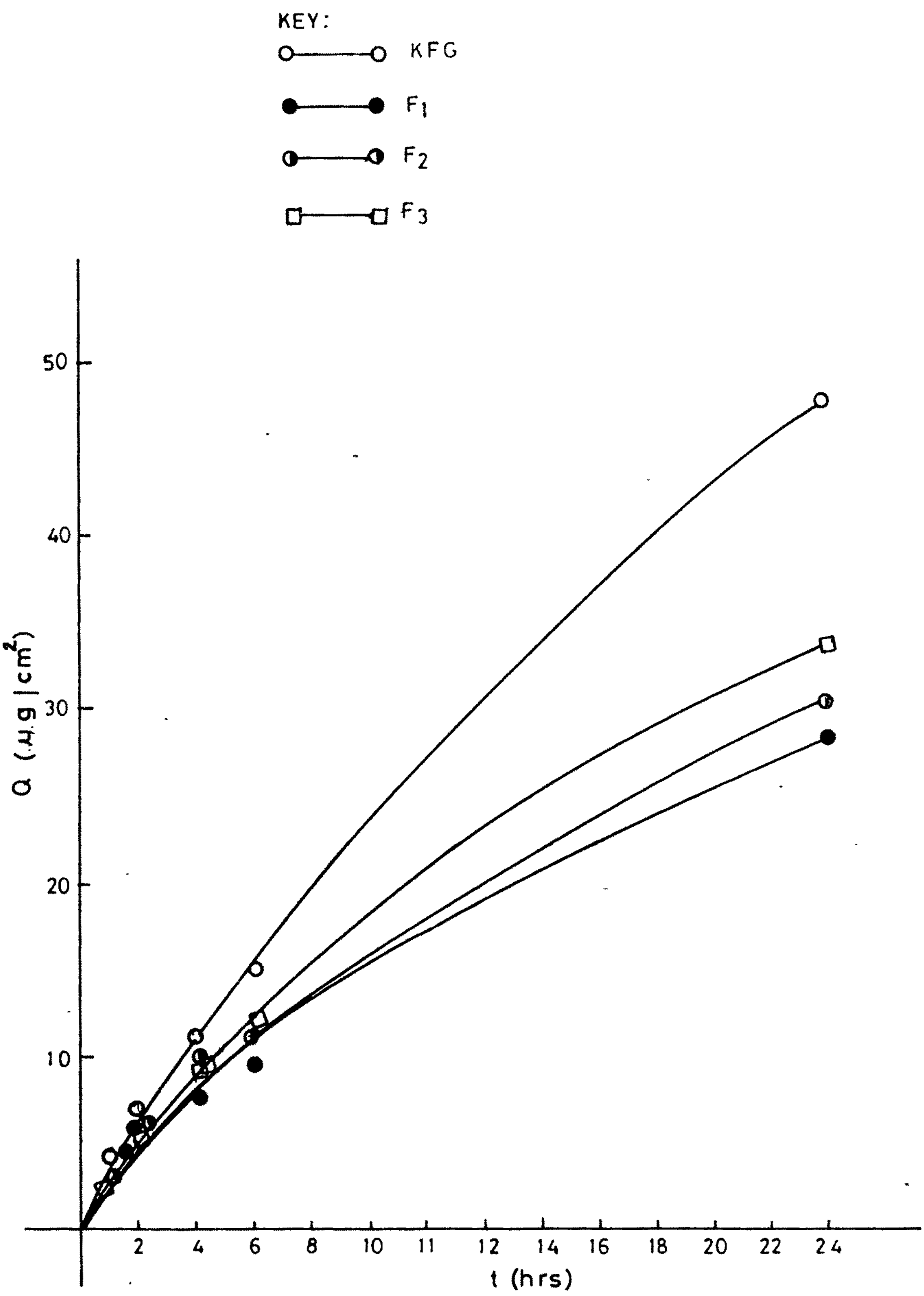

FIG. 4.40 : IN VITRO RELEASE PROFILE OF FLU FROM ITS LIPOSOMAL GELS AS COMPARED TO THAT FROM KFG.

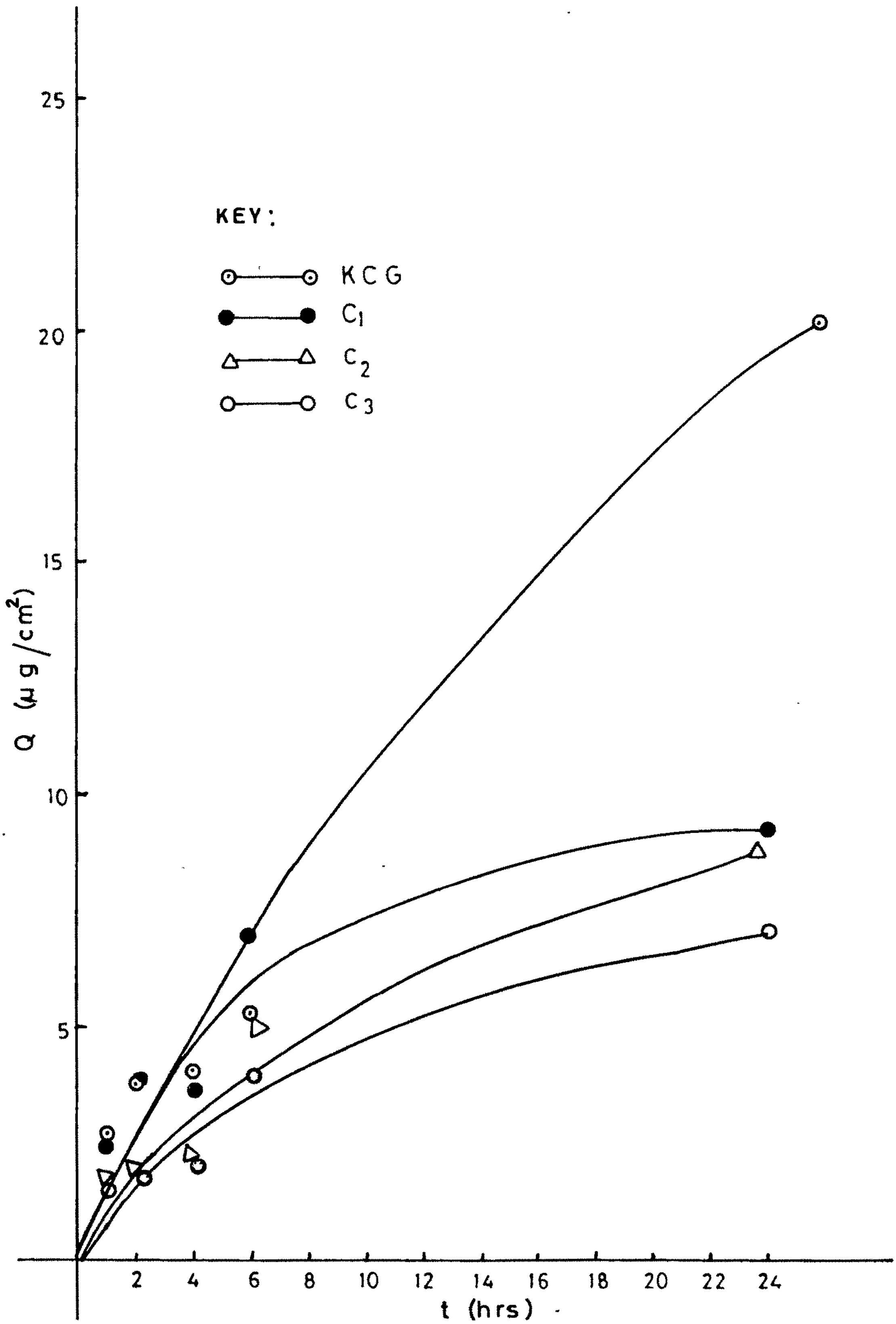

FIG. 4·41 : IN VITRO RELEASE PROFILE OF CLO FROM ITS LIPOSOMAL GELS AS COMPARED TO THAT FROM KCG.

TABLE 4.12

CORRELATION COEFFICIENT AND 'a' AND 'b' VALUES OF THE LINES OF REGRESSION FOR THE DATA OF MEAN CUMULATIVE AMOUNT RELEASED Vs SQUARE ROOT OF TIME FOR FORMULATIONS OF FLU AND CLO.

Formulation	**Correlation coefficient r**	***a**	***b**
KFG	0.972	0.949	0.114
FPM	0.967	1.120	0.110
F_1	0.996	0.271	0.086
F_2	0.994	0.227	0.089
F_3	0.974	0.513	0.084
KCG	0.967	0.314	0.049
CPM	0.969	0.342	0.049
C_1	0.982	-0.104	0.038
C_2	0.989	0.038	0.028
C_3	0.997	0.042	0.024

* Equation of regression line is $y = a+bx$

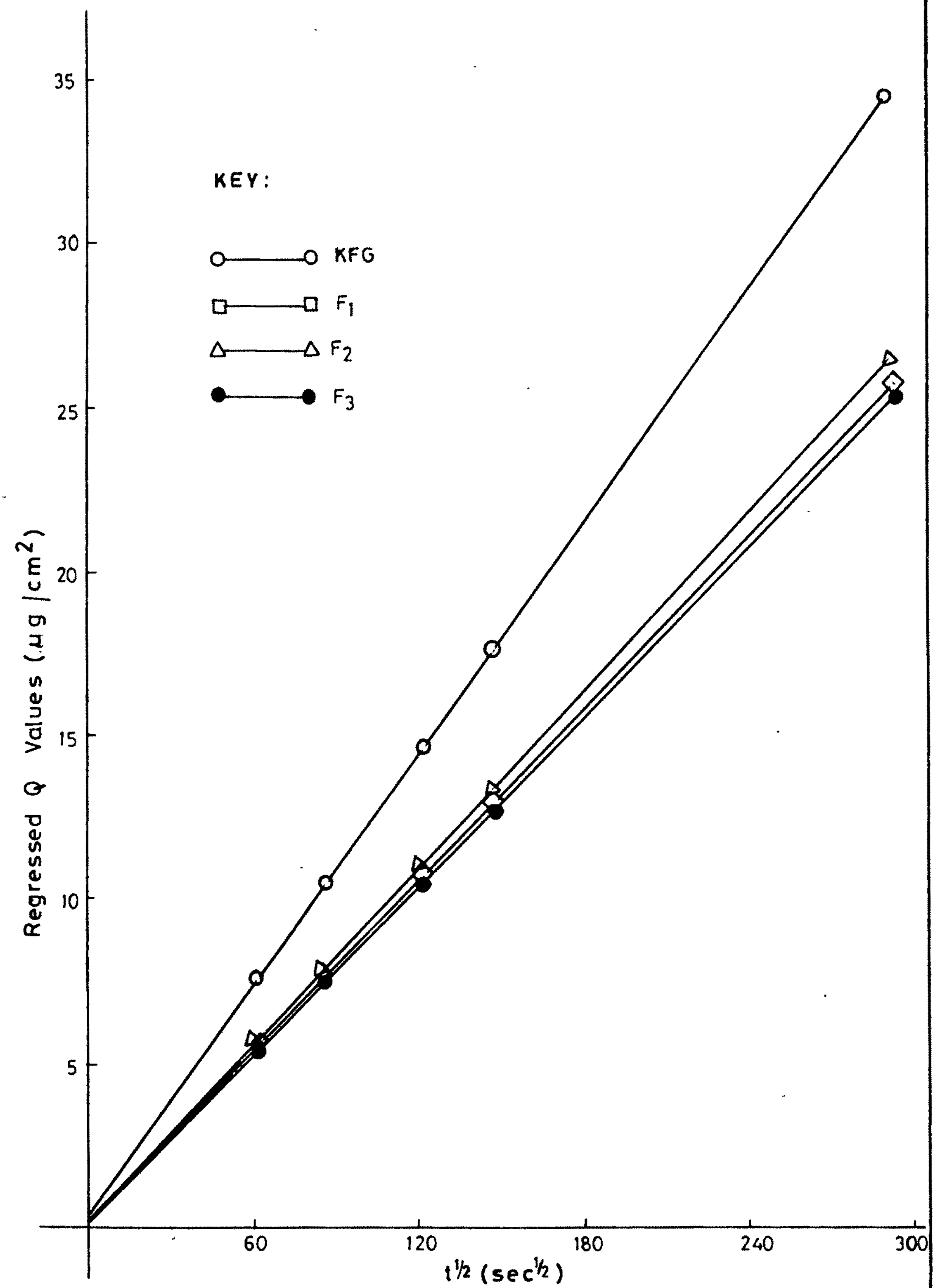

FIG. 4.42 : Q Vs $t^{1/2}$ PLOTS FOR RELEASE OF FLU FROM ITS LIPOSOMAL GELS AS COMPARED TO THAT FROM KFG.

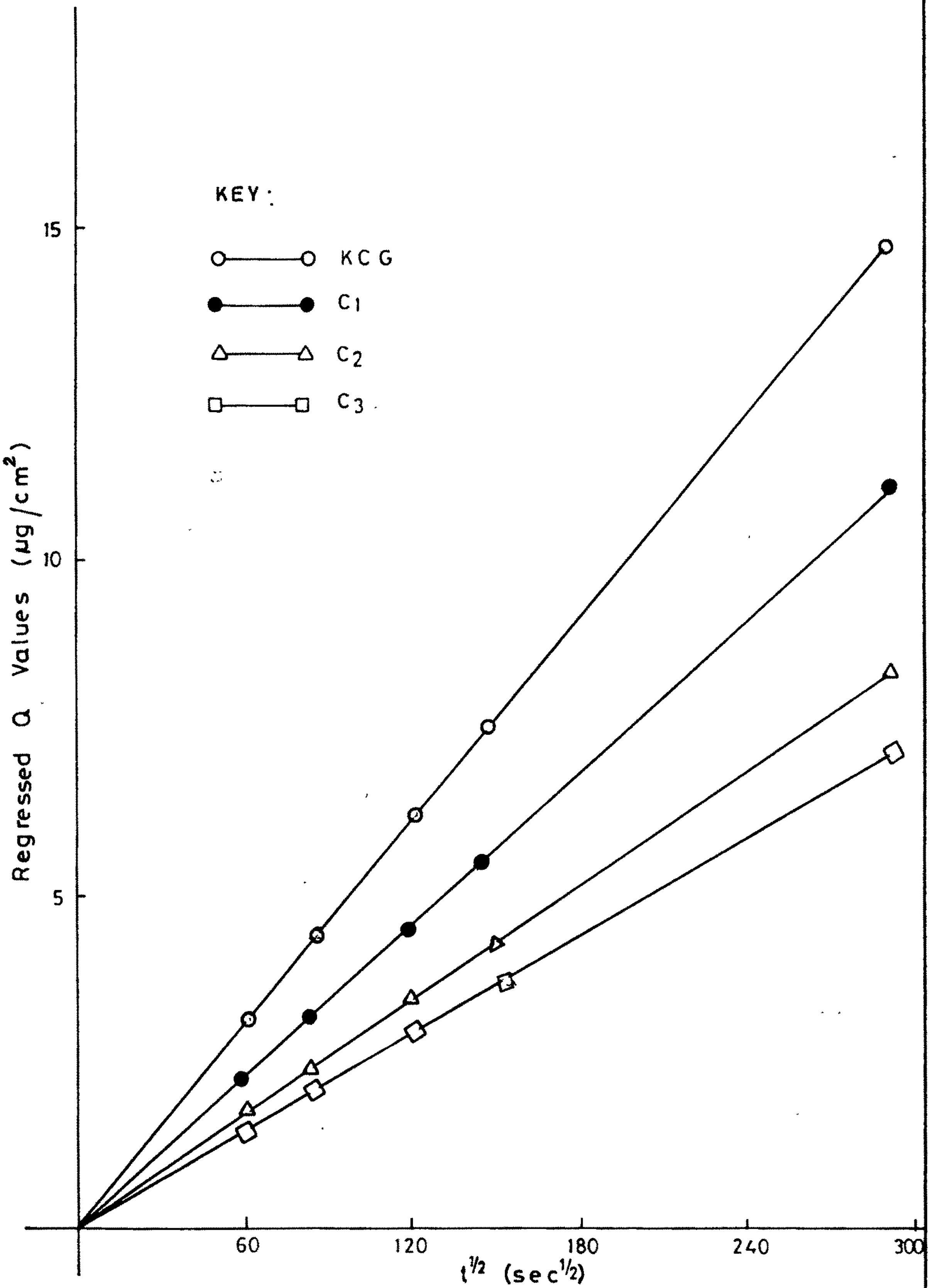

FIG. 4.43 : Q Vs $t^{1/2}$ PLOTS FOR RELEASE OF CLO FROM ITS LIPOSOMAL GELS AS COMPARED TO THAT FROM KCG.

The R,P,D,K, $Q^i(6)$, Q^m, Csf values and Q^m/Csf ratios for various formulations of FLU and CLO are listed in Tables 4.13 and 4.14 and compared in Figs. 4.44-4.46. The release rate decreases when FLU is incorporated into liposomes with the formulation F_3 having the minimum release rate. The P and D values also decrease when FLU is incorporated into liposomes with formulation F_3 having the lowest values. Incorporating FLU into liposomes, increases the K, $Q^i(6)$ and Q^m values with formulation F_1 having the highest K and $Q^i(6)$ values and formulation F_3 having the highest Q^m value. The Csf value decreases when FLU is incorporated into liposomes with formulation F_3 having the lowest value. The Q^m/Csf ratio for formulation F_3 is highest amongst all the formulations of FLU studied indicating that it may give the best efficacy and least side effects.

Although the release rate is minimum for formulation C_3, the P,D and Csf values are minimum and K, $Q^i(6)$ and Q^m values maximum for formulation C_2. Besides, this formulation has the maximum Q^m/Csf ratio indicating that it may be the CLO formulation which may give the best efficacy and least side effects.

From the results of the in vitro studies, it was decided that liposomal TRMA formulations, namely T_1,T_3,T_7,T_8, T_9,T_{10},T_{11},T_{17} and T_{21} and all the formulations of FLU and CLO may be subjected to in vivo studies to confirm their performance in vivo.

TABLE 4.13

DIFFUSION PARAMETERS TO ASSESS THE RELEASE OF FLU AND CLO FROM THEIR FORMULATIONS.

Formulation	***P (10^{-4})**	***D (10^{-10})**	***R**	***K (10^{5})**
KFG	30.30	15.80	0.114	76.70
FPM	29.74	14.90	0.120	79.80
F_1	21.80	7.64	0.086	162.52
F_2	19.51	6.33	0.089	123.31
F_3	14.87	3.66	0.084	114.10
KCG	9.42	1.45	0.049	2.60
CPM	9.35	1.45	0.049	2.58
C_1	7.20	0.85	0.038	3.39
C_2	4.71	0.36	0.028	5.19
C_3	6.17	0.66	0.024	3.72

* P = Permeability coefficient (cm/sec)

D = Diffusion coefficient (cm^2/sec)

R = Release rate ($\mu g/cm^2/sec^{1/2}$)

K = Partition coefficient

All above values are calculated from the data obtained after regression and hence SEM values are not shown.

TABLEE 4.14

PARAMETERS RESPONSIBLE FOR THE EFFECT, SIDE EFFECT AND RATIO THEREOF FOR FORMULATIONS OF FLU AND CLO.

Formulation	Effect	Side effect		Q^m/C_{sf}
	$Q^i(6)$	Q^{m*}	$C_{sf}{}^*$	
KFG	1010.25	1425.09	2.80	508.96
FPM	1062.14	1503.43	2.70	556.83
F_1	2196.91	3198.39	2.15	1487.62
F_2	1921.19	2811.47	2.22	1266.42
F_3	2175.54	3217.62	2.10	1532.20
KCG	45.30	67.60	1.22	55.41
CPM	45.30	67.08	1.22	54.98
C_1	59.83	89.50	0.95	94.21
C_2	103.19	154.66	0.70	220.94
C_3	48.31	72.32	0.60	120.53

* $Q^i(6)$ is the quantity of drug which enters the membrane upto 6 hrs.

Q^m is the quantity of drug which remains in the membrane.

C_{sf} is the factor for the quantity of drug in the blood stream.

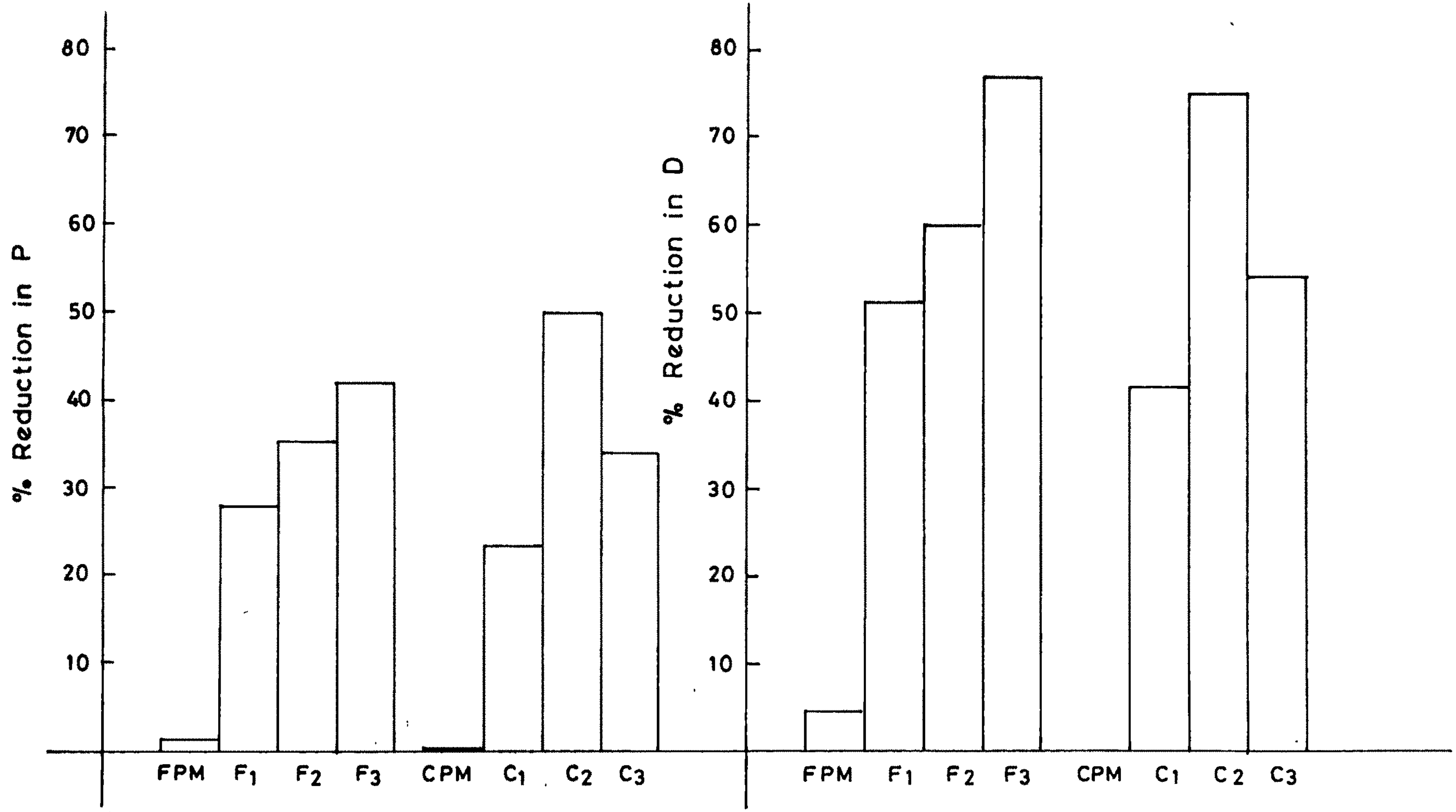

FIG. 4·44 : % REDUCTION IN P AND D VALUES OF FLU AND CLO FROM THEIR PHYSICAL MIXTURE GELS AND LIPOSOMAL GELS AS COMPARED TO THAT FROM THEIR RESPECTIVE PLAIN DRUG GELS.

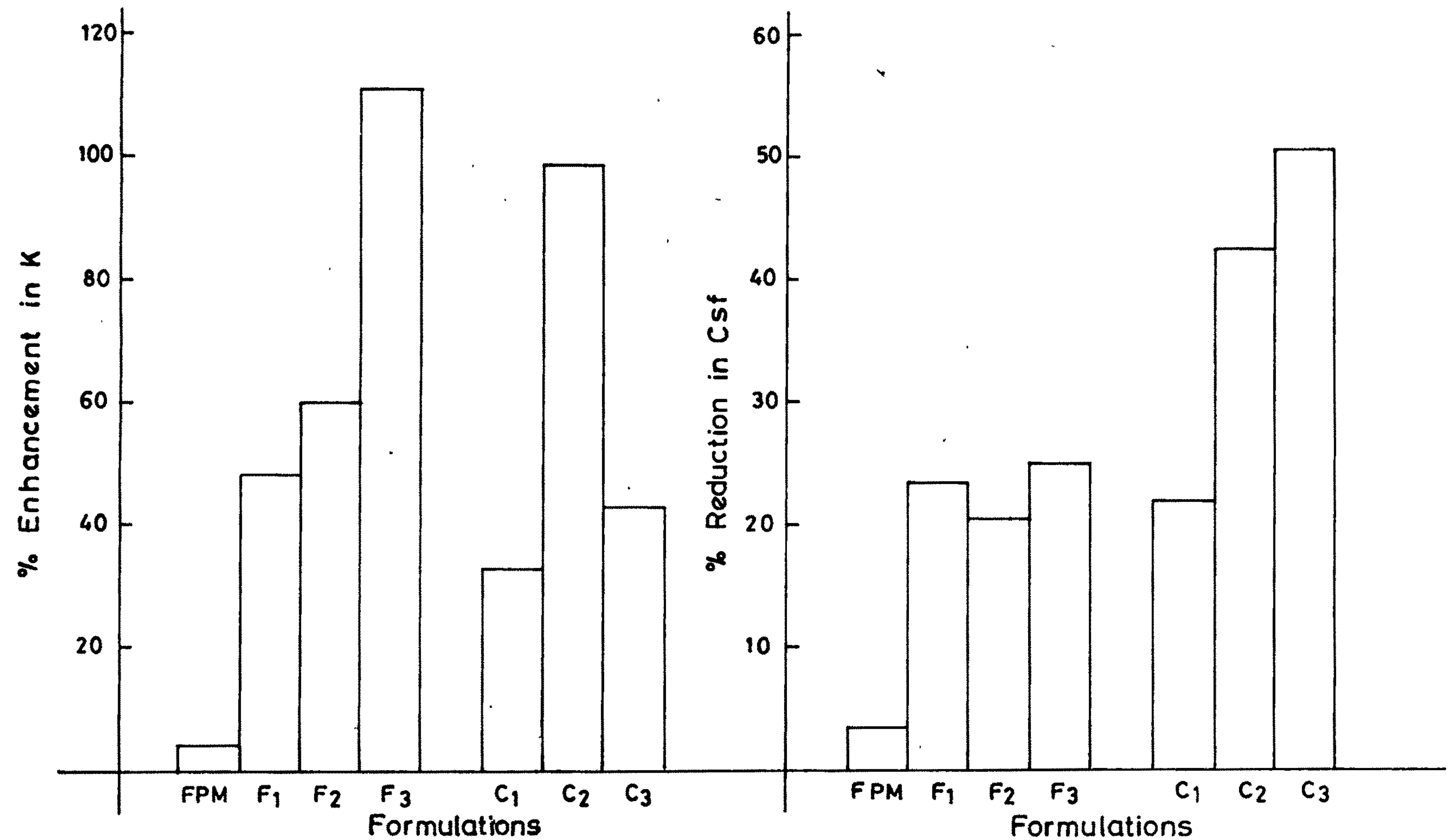

FIG. 4·45 :% ENHANCEMENT IN K AND % REDUCTION IN Csf VALUE OF FLU AND CLO FROM THEIR PHYSICAL MIXTURE GELS AND LIPOSOMAL GELS AS COMPARED TO THAT FROM THEIR RESPECTIVE PLAIN DRUG GELS.

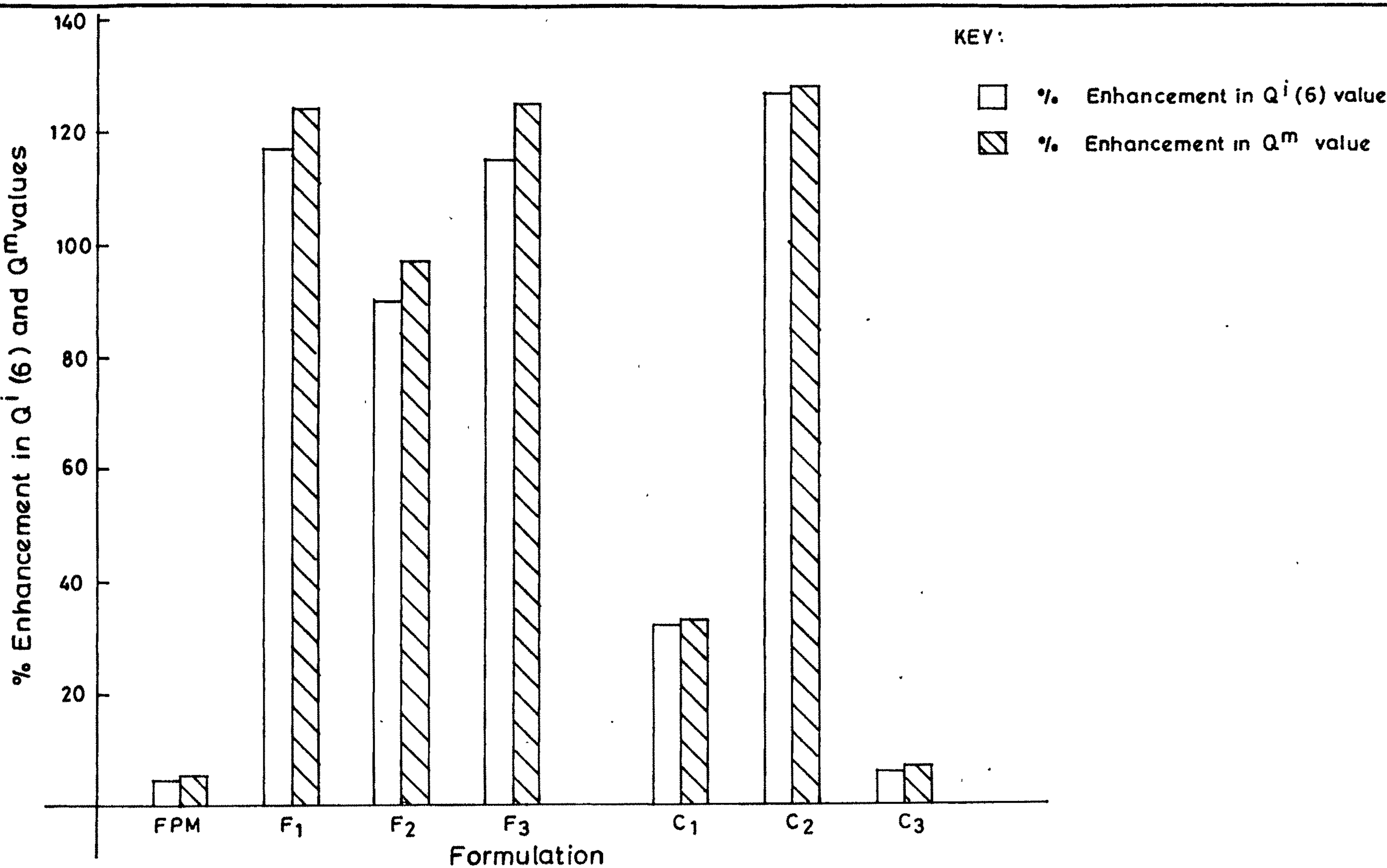

FIG. 4·46 : % ENHANCEMENT IN Q^i (6) AND Q^m VALUES OF FPM & LIPOSOMAL GELS OF FLU AND CLO AS COMPARED TO RESPECTIVE PLAIN DRUG GELS.

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