CHAPTER IV

DISCUSSION AND CONCLUSION

4.1 Tonicity of Formulations

Isotonic solution is 0.9% NaCl solution which is equivalent to 300 milliosmoles (Appendix B). The tonicity range that the eyes can tolerate is 0.5-2.0% NaCl solution or 160-630 milliosmoles (Appendix B).

All additives increased the tonicity. It was slightly increased when HPMC or PVP was added. Moreover it was increased when increasing the concentration of both additives. HPMC is usually added in the eye preparation to increase viscosity (35,36,39,40), probably due to its low tonicity.

PEG series extensively increased the tonicity. Some produced higher tonicity than upper tolerance limit. Some produced solution of unmeasurable. There were two reasons for the unmeasurable. The first was the tonicity of solution which was more than upper limit that the osmometer could measure (>3,000 milliosmoles). The second reason was the unfrozen sample solution by the freezing compartment of the osmometer. The freezing compartment contained a bath of liquid PEG which was to be frozen and freezed the sample solution. If sample solutions were PEG solutions , it was possible that a bath of liquid PEG could not freeze the sample solution.

If the concentrations of PEG_{400} in the formulations were plotted againts tonicity, and extrapolated to predict the unmeasurable tonicity, the result was not more than 3,000 milliosmoles. Figure 29 showed the extrapolated tonicity of the formulation containing 25% PEG_{400}. The predicted tonicity of the formulations containing 25% PEG_{1500}, 25% PEG_{4000}, 35% PEG_{6000} and 30% PEG_{20000} were also not more than 3,000 millosmoles as shown in Figures 30-33 respectively. Therefore, the second reason for the unmeasurable tonicity would be appropriate. The relationships between the tonicity and the concentration of PEG and PF_{407} in the formulations were illustrated in Figures 29-34. The relationships were curve lines. When plotting the logarithm of the tonicity against the concentration of PEG and PF in the formulations rather straight lines were obtained, as shown in Figures 35-40. The linear regression was calculated and the coefficients of determination (r^2) were listed in Table 38. The coefficients of determination were between 0.9877-0.9999 , thus the correlations were linear. The tonicity of the formulations containing PEG and PF_{407} at other concentrations could be estimated by these relationships.

The tonicity of formulations containing PEG series was interesting in many ways. At the same concentrations but difference molecular weight ,the tonicity of formulation was difference. PEG of low molecular weight showed higher tonicity than those of high molecular weight. Table 39 and Figure 41 showed the relationship between molecular weight and tonicity. The profile of all

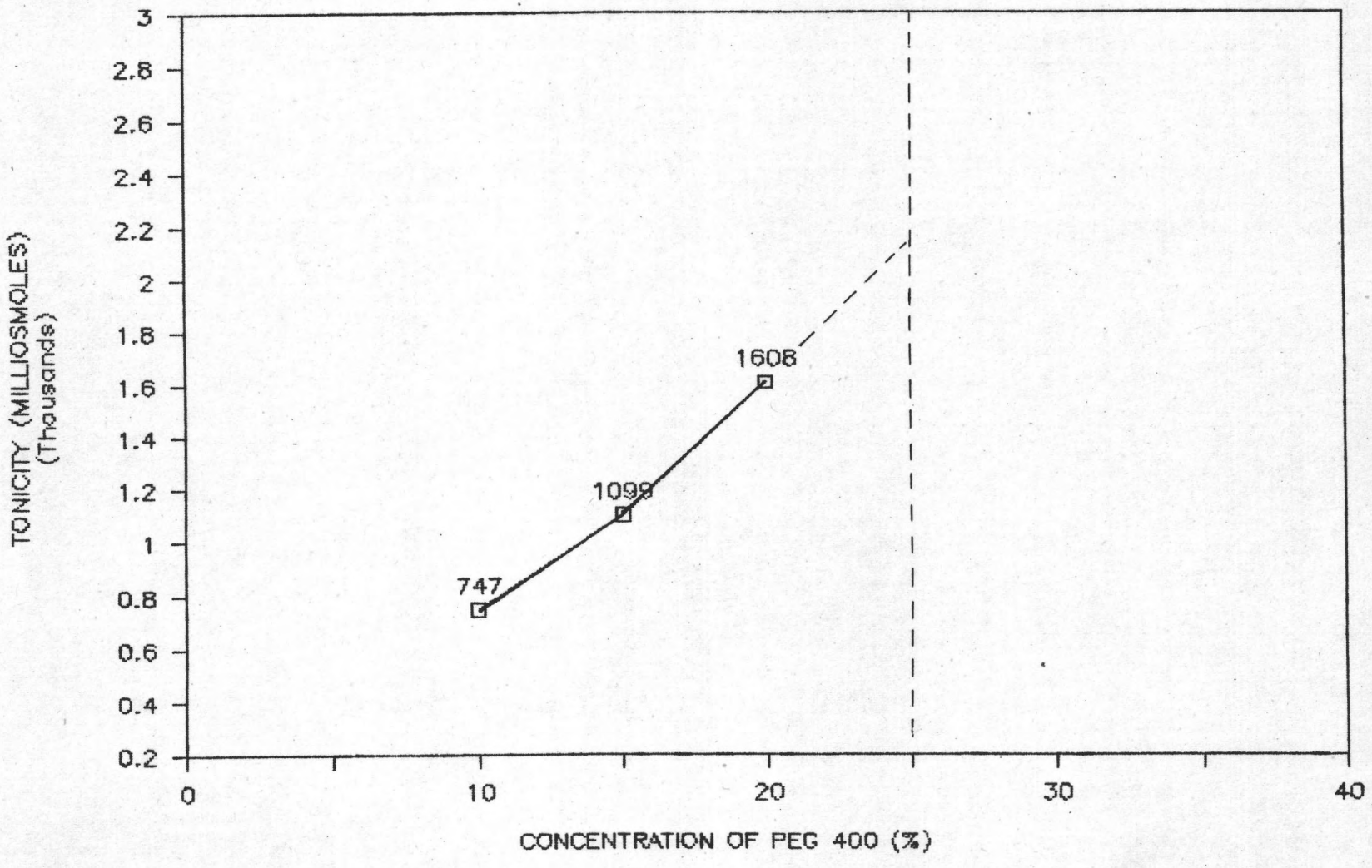

Figure 29 The concentrations of PEG_{400} in the formulations were plotted against tonicity, and extrapolated to predict the unmeasurable tonicity of the formulation containing 25% PEG_{400}.

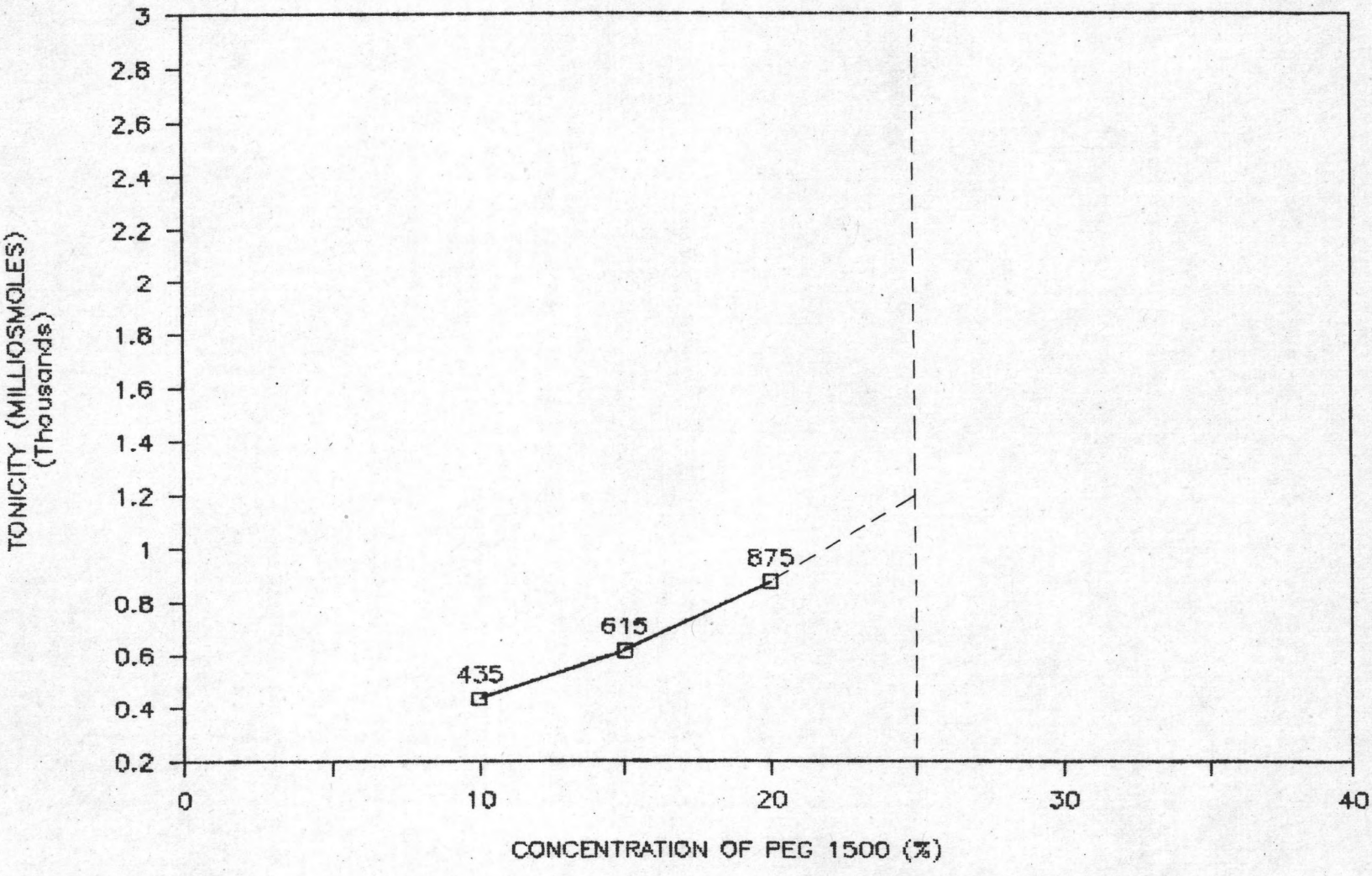

Figure 30 The concentrations of PEG_{1500} in the formulations were plotted against tonicity, and extrapolated to predict the unmeasurable tonicity of the formulation containing 25% PEG_{1500}.

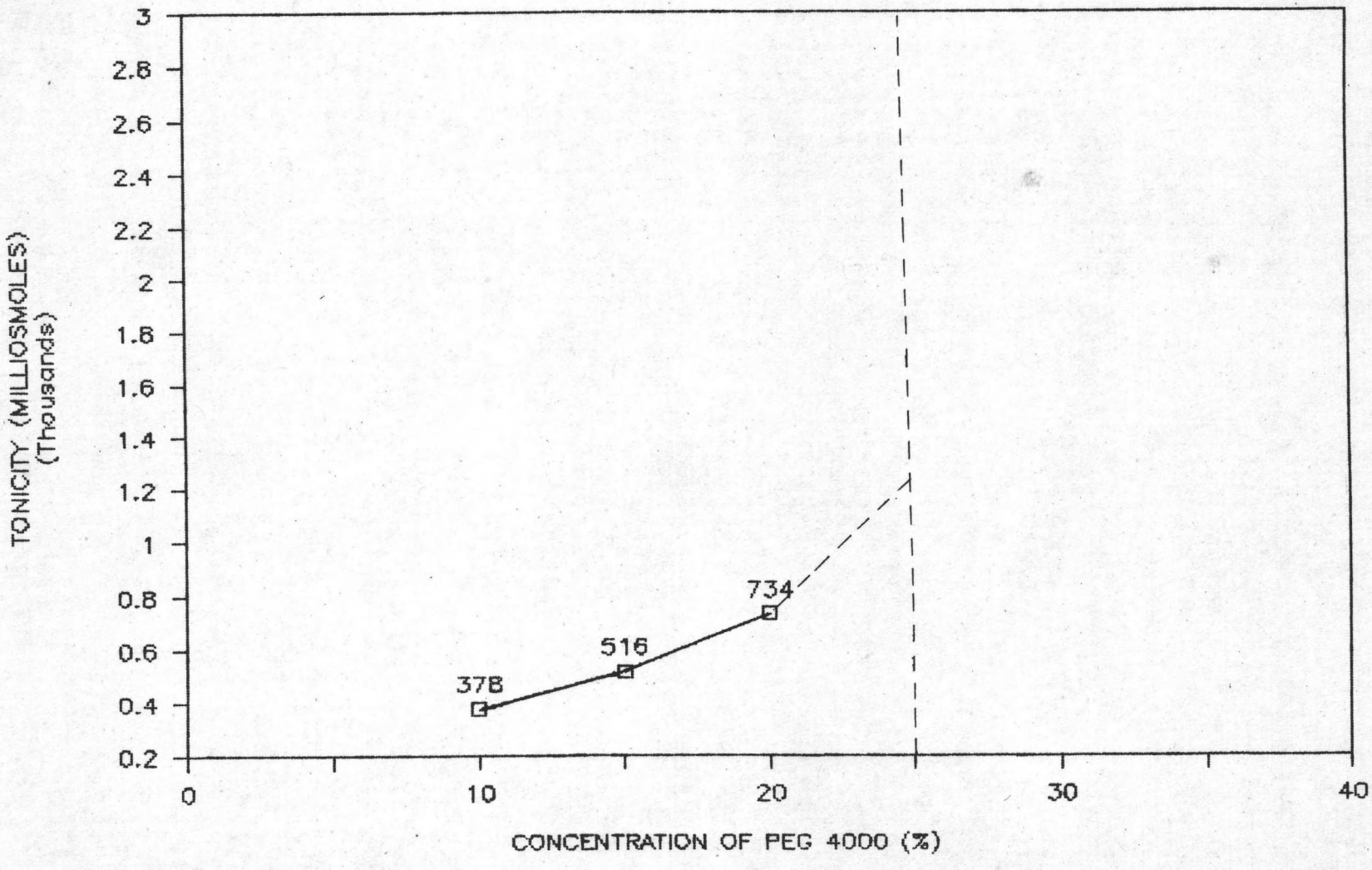

Figure 31 The concentrations of PEG_{4000} in the formulations were plotted against tonicity, and extrapolated to predict the unmeasurable tonicity of the formulation containing 25% PEG_{4000}.

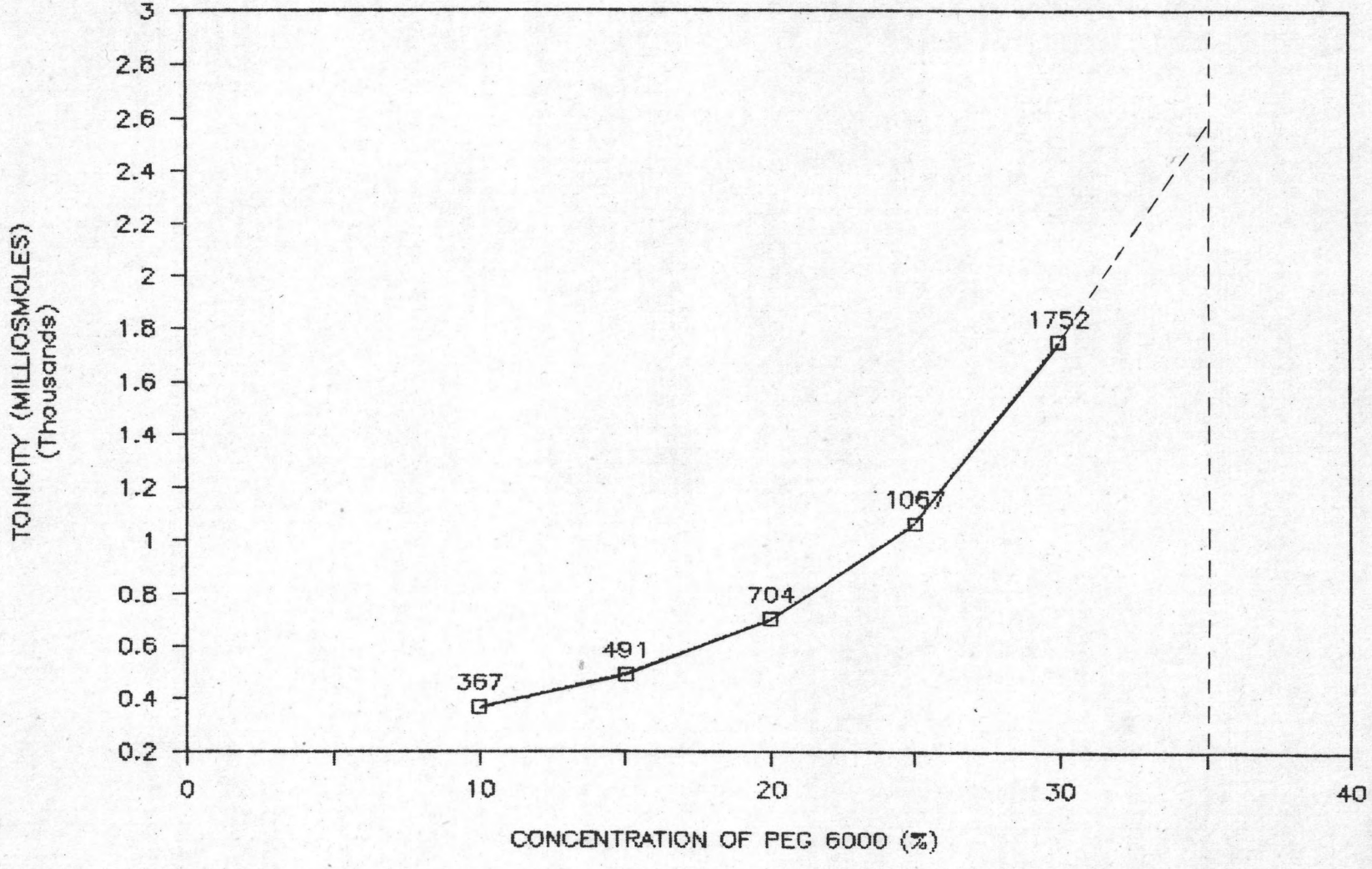

Figure 32 The concentrations of PEG_{6000} in the formulations were plotted against tonicity, and extrapolated to predict the unmeasurable tonicity of the formulation containing 35% PEG_{6000}.

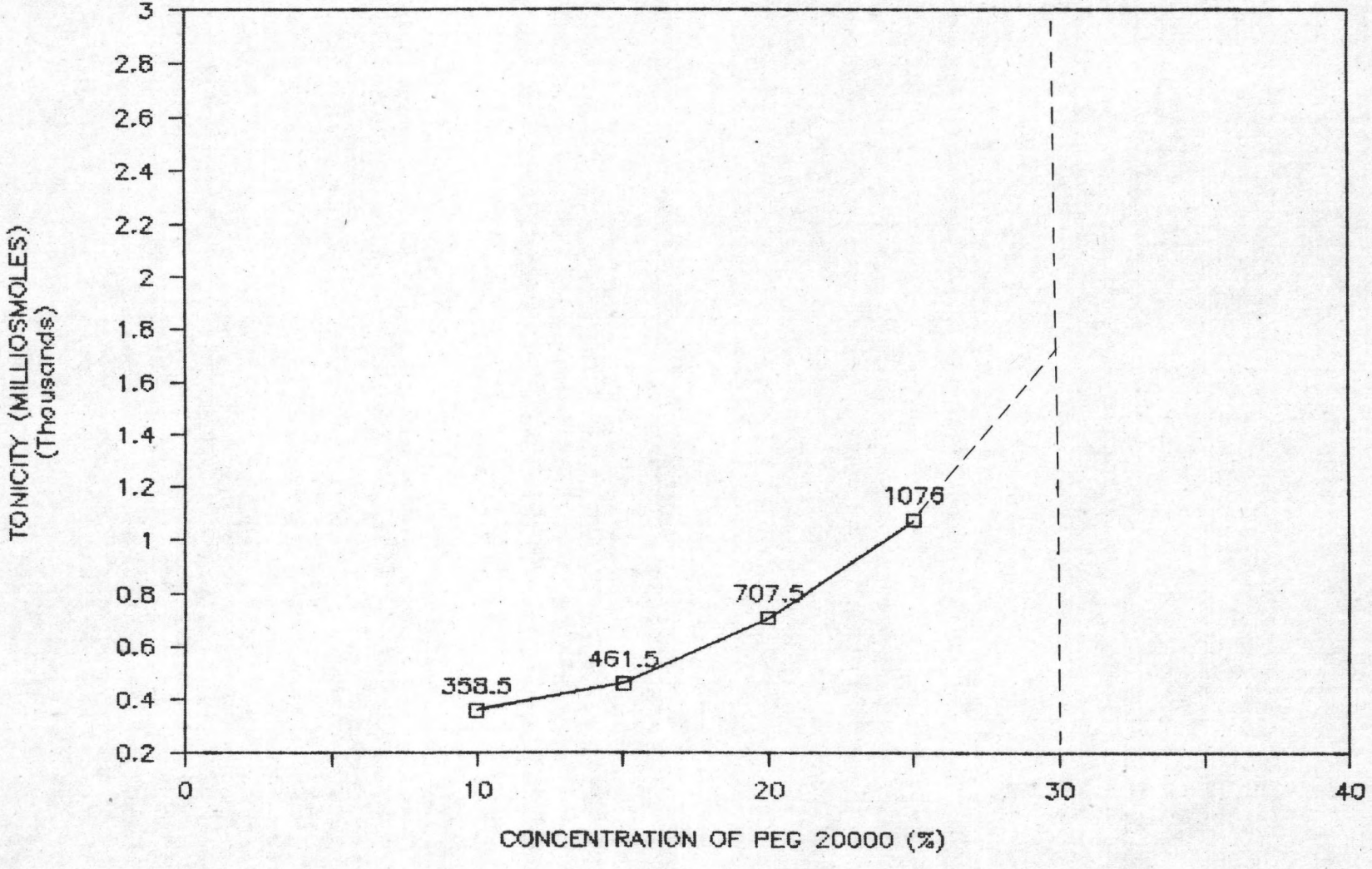

Figure 33 The concentrations of PEG_{20000} in the formulations were plotted against tonicity, and extrapolated to predict the unmeasurable tonicity of the formulation containing 30% PEG_{20000}.

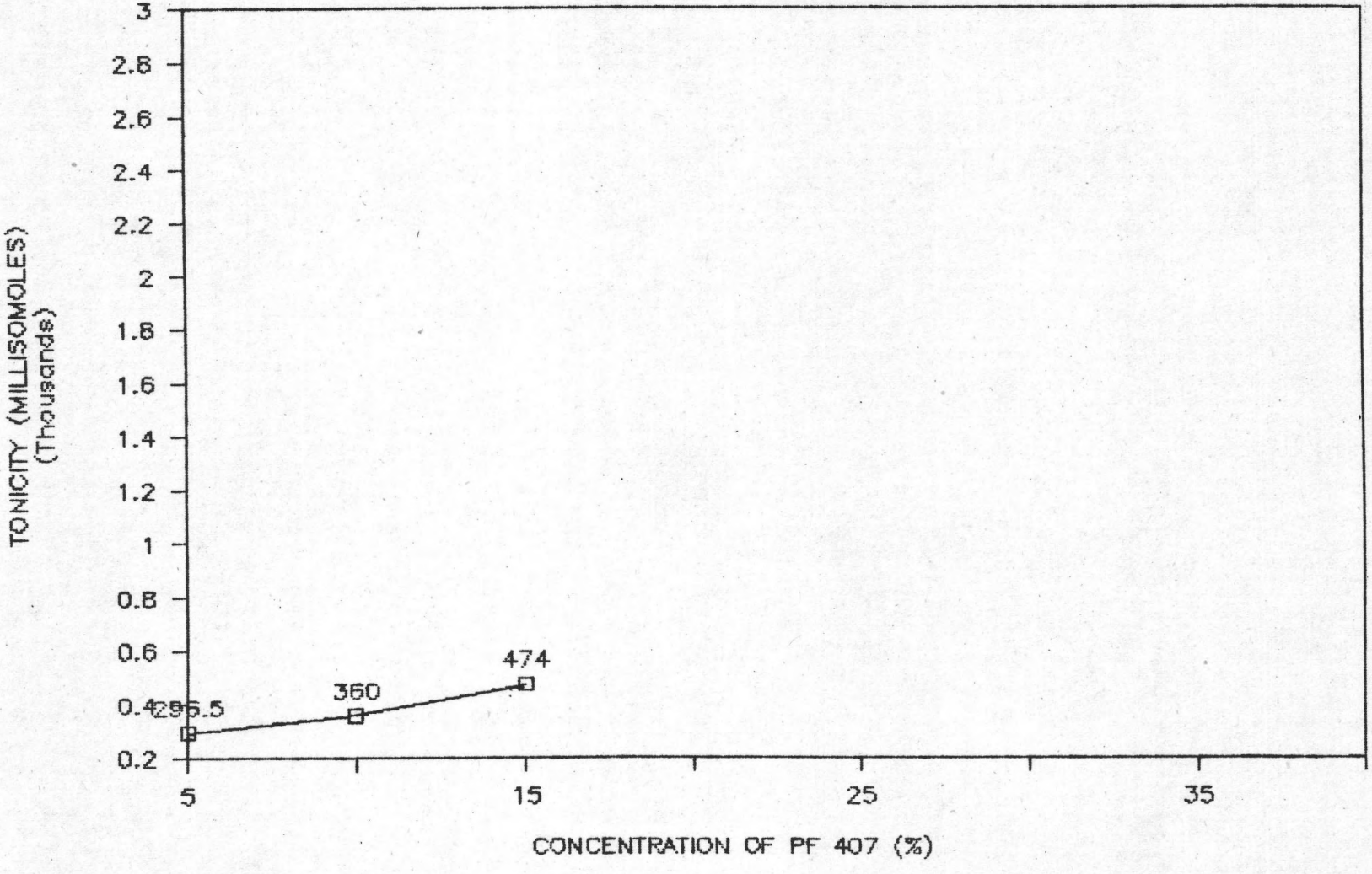

Figure 34 The relationships between tonicity and concentrations of PF_{407} in the formulations

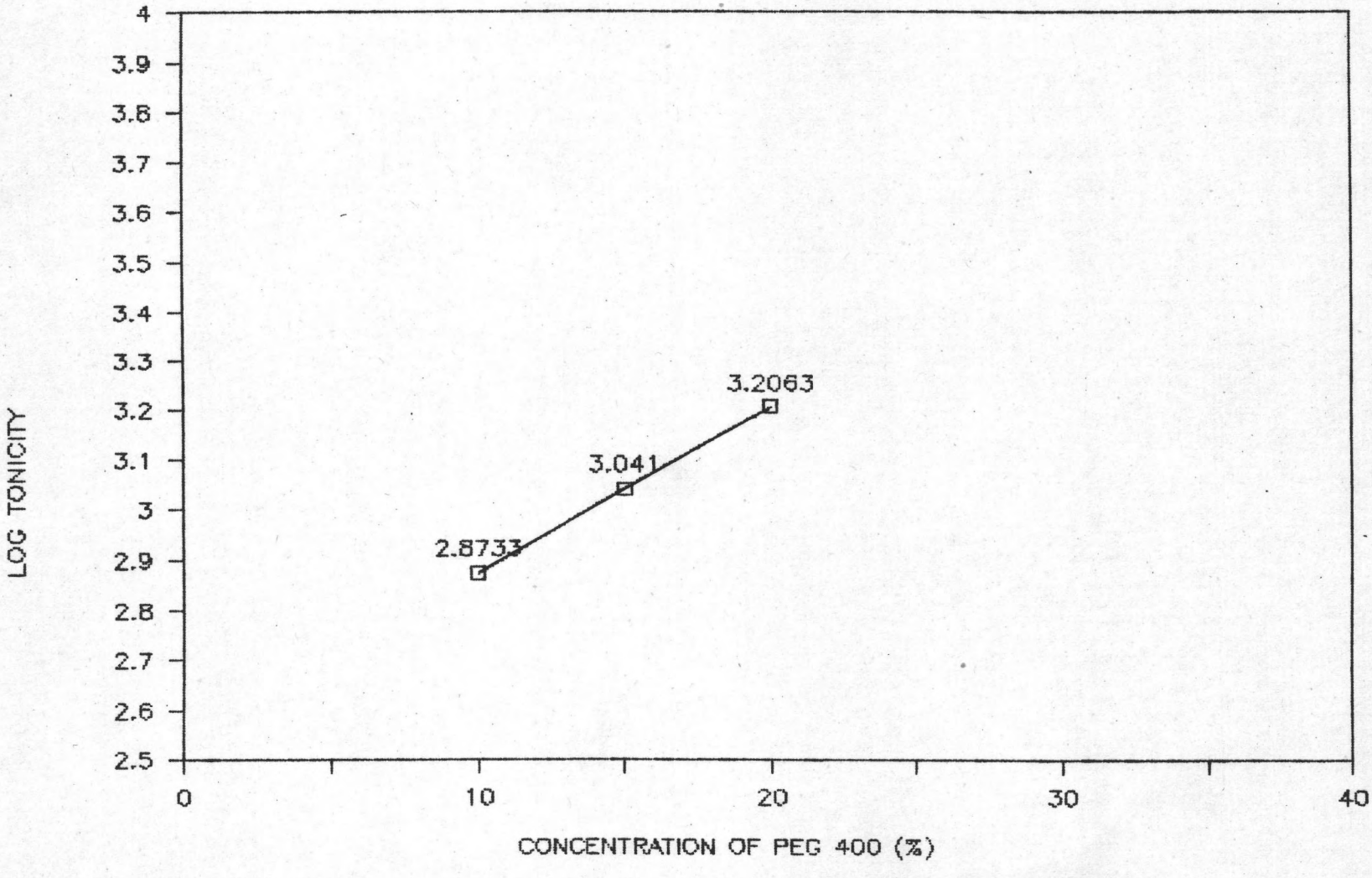

Figure 35 The logarithm of the tonicity against concentration of PEG_{400} in the formulations.

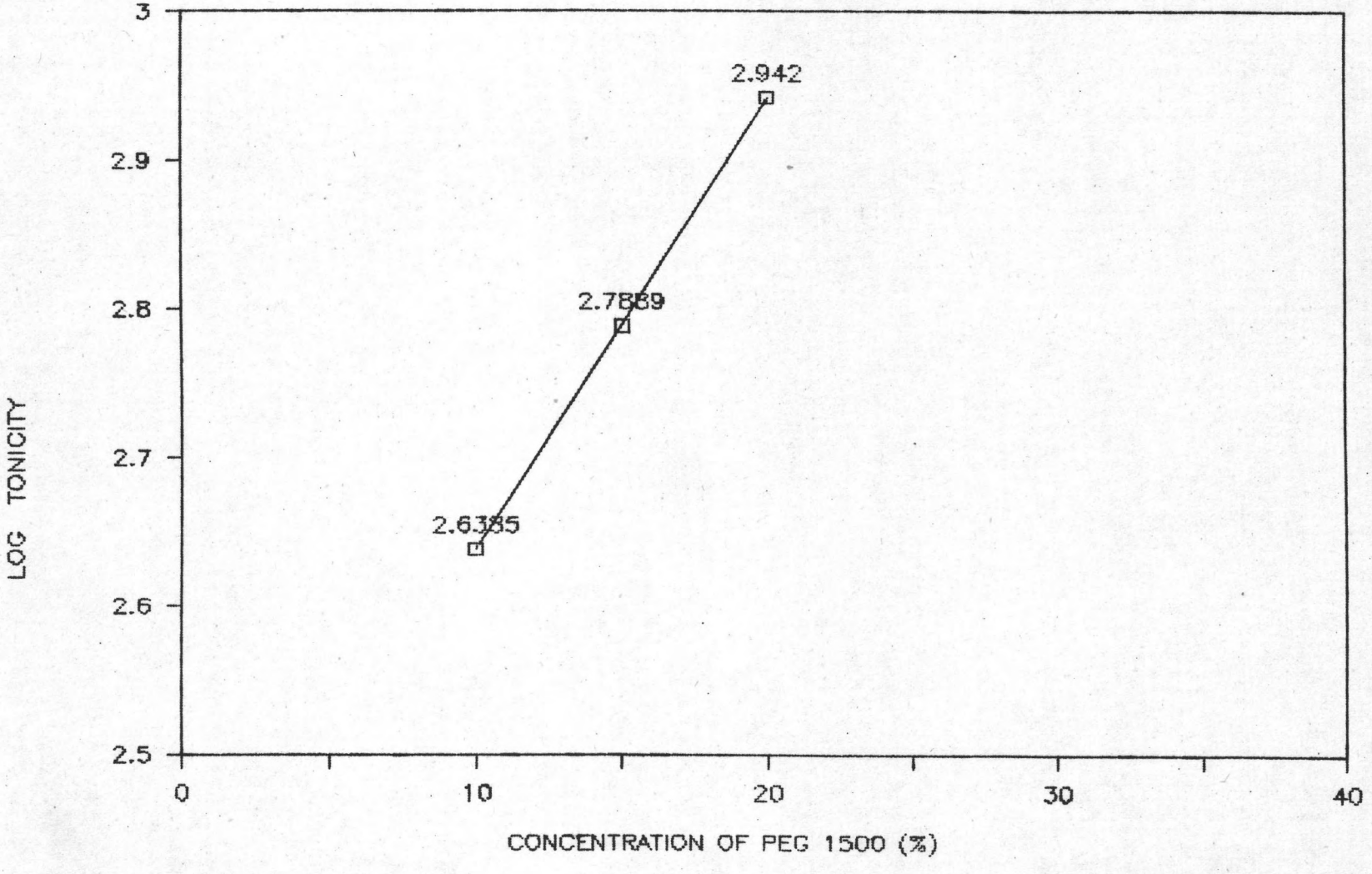

Figure 36 The logarithm of the tonicity against concentration of PEG_{1500} in the formulations.

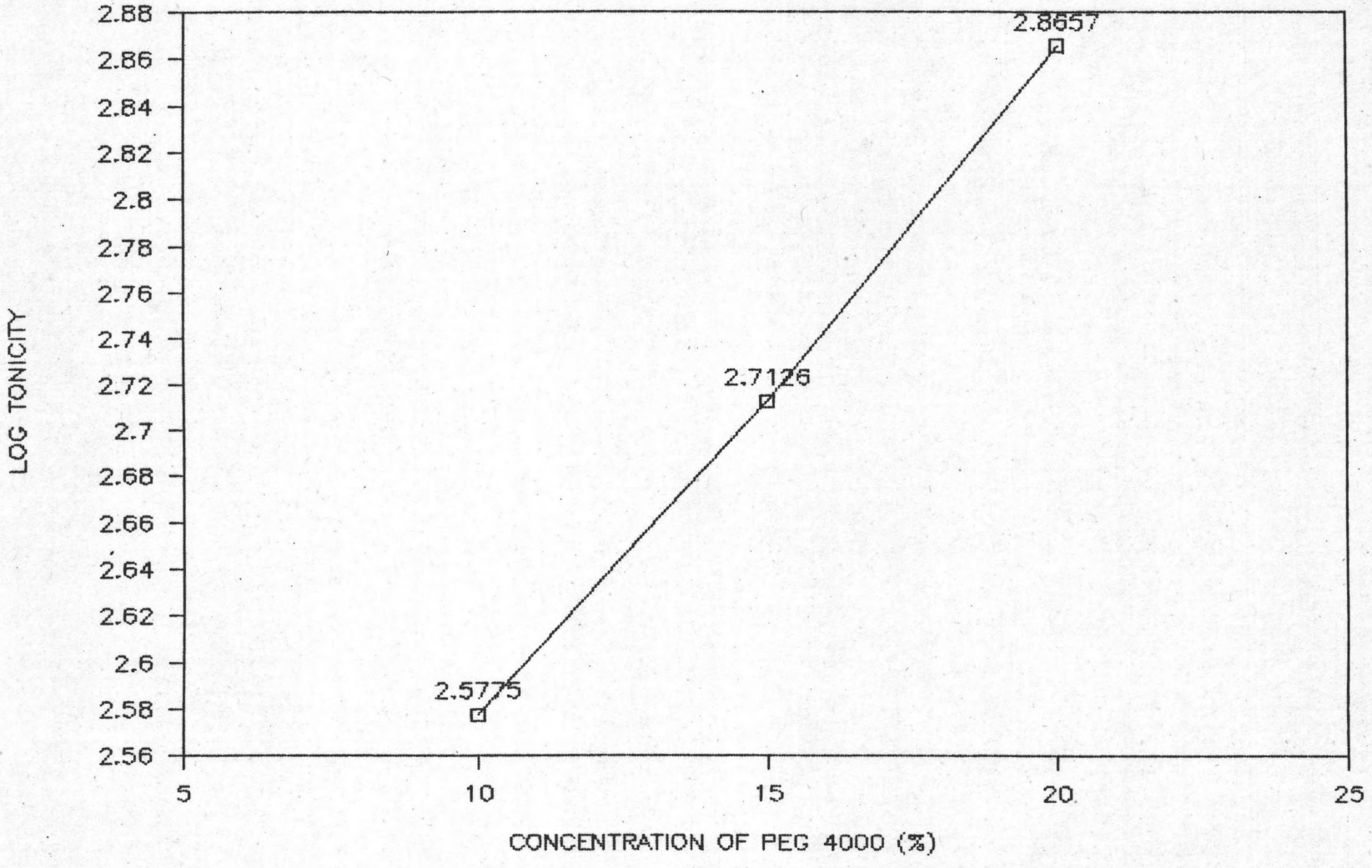

Figure 37 The logarithm of the tonicity against concentration of PEG_{4000} in the formulations.

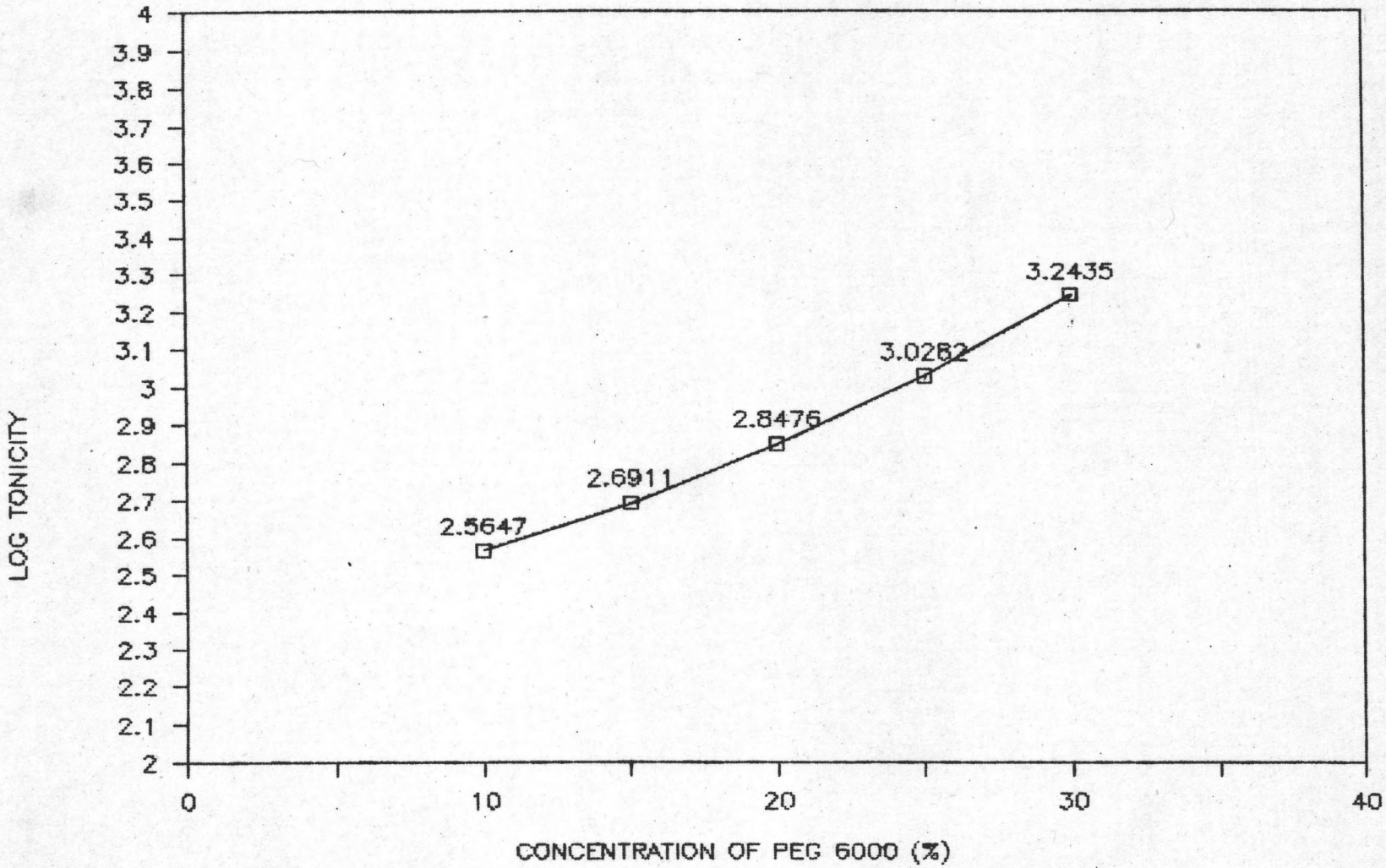

Figure 38 The logarithm of the tonicity against concentration of PEG_{6000} in the formulations.

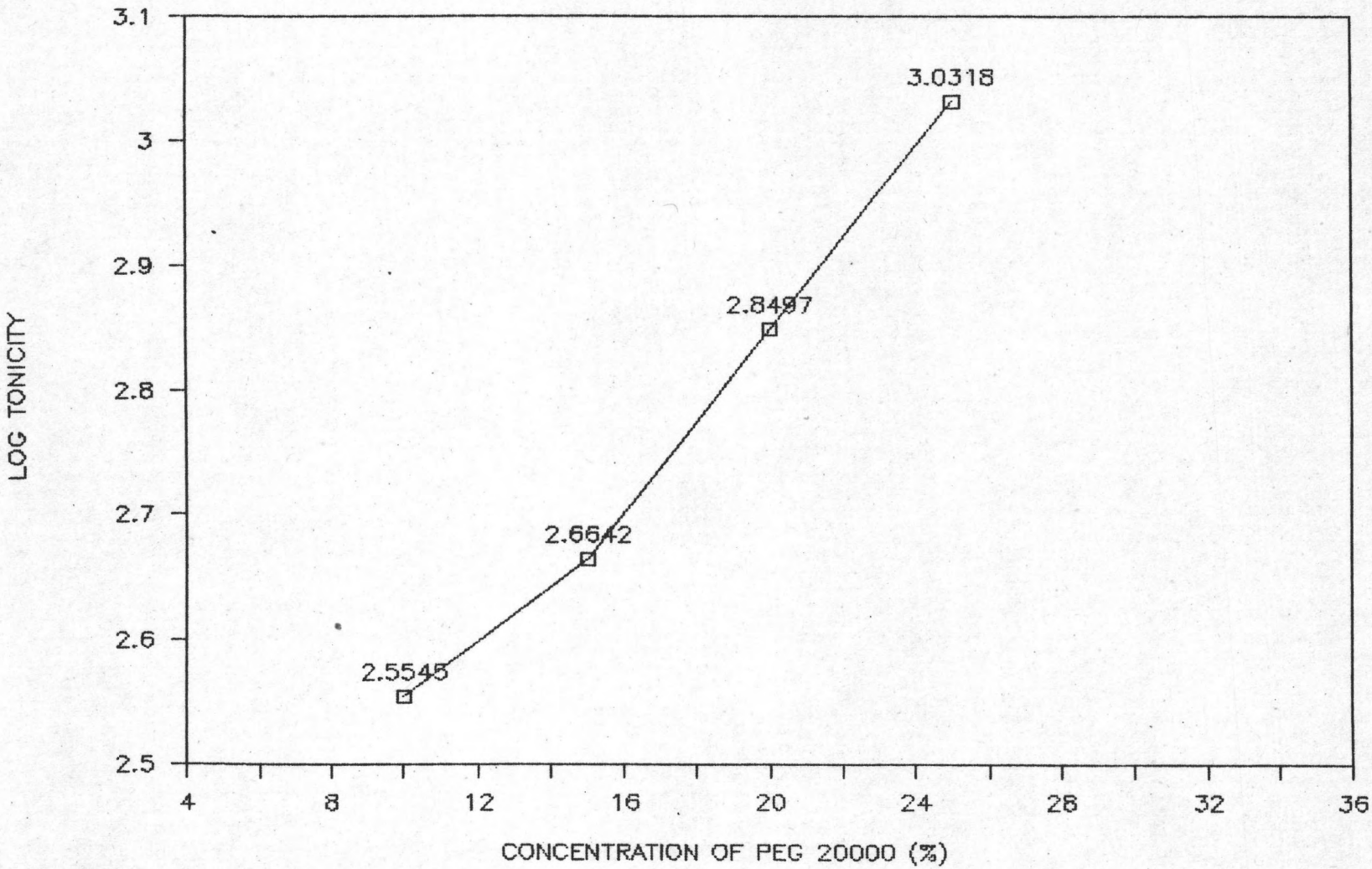

Figure 39 The logarithm of the tonicity against concentration of PEG_{20000} in the formulations.

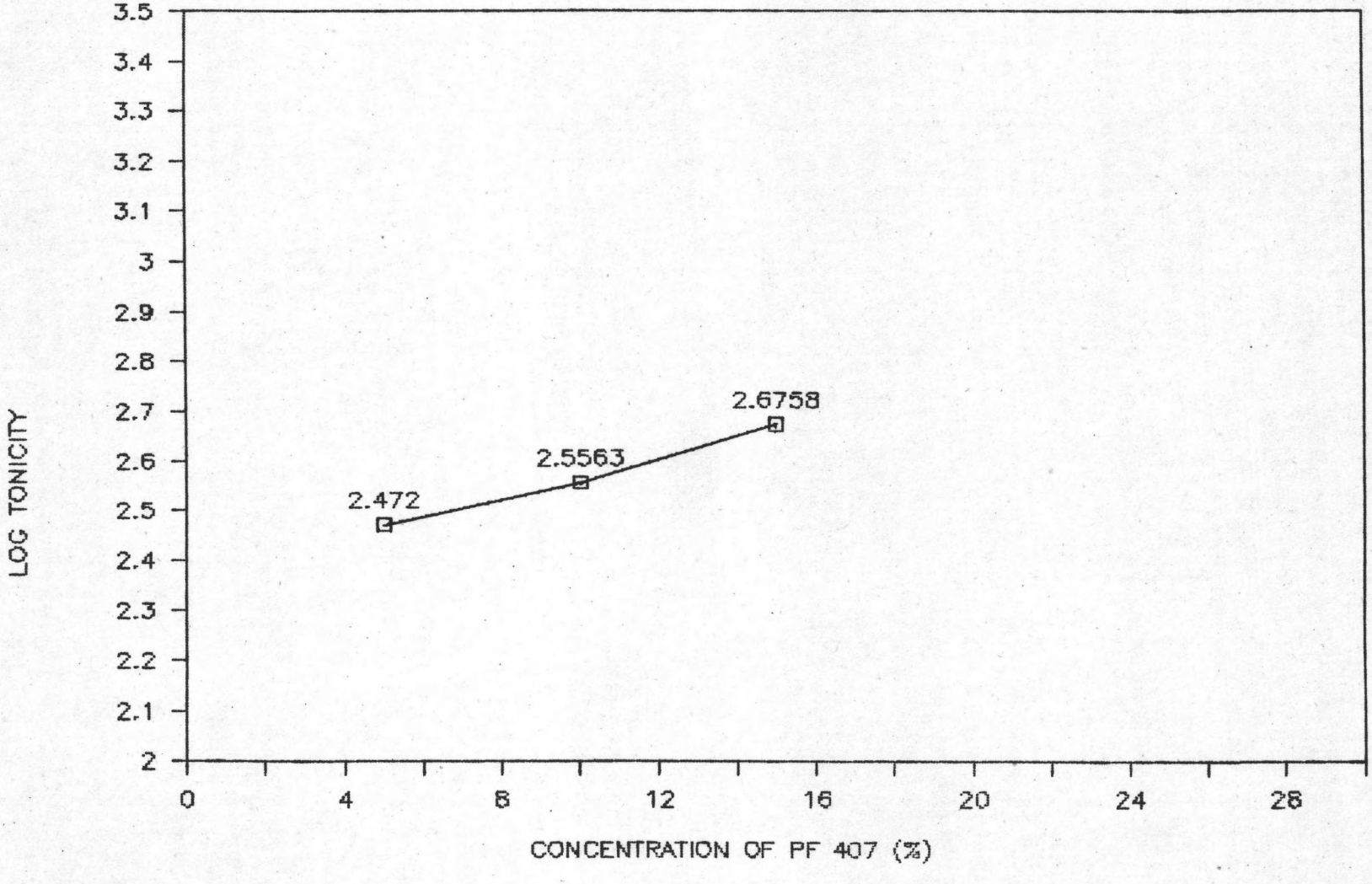

Figure 40 The logarithm of the tonicity against concentration of PF_{407} the formulations.

Table 38 The relationships between tonicity and logarithm of the tonicity of PEG and PF_{407} calculated by simple linear regression technique and the r^2 values were listed.

Formulation	r^2
PEG_{400}	0.9999
PEG_{1500}	0.9999
PEG_{6000}	0.9899
PEG_{4000}	0.9987
PEG_{20000}	0.9877
PF_{407}	0.9902

Table 39 Comparison of the tonicity of formulations containing the same concentration but different molecular weight of PEG.

Substance	Concentration					
	10%	15%	20%	25%	30%	40%
PEG_{400}	747	1099	1608	X	X	X
PEG_{1500}	435	615	875	X	X	X
PEG_{4000}	378	516	734	X	X	X
PEG_{6000}	367	491	704	1067	1752	X
PEG_{20000}	358.5	461.5	707.5	1076	X	X

X unmeasurable

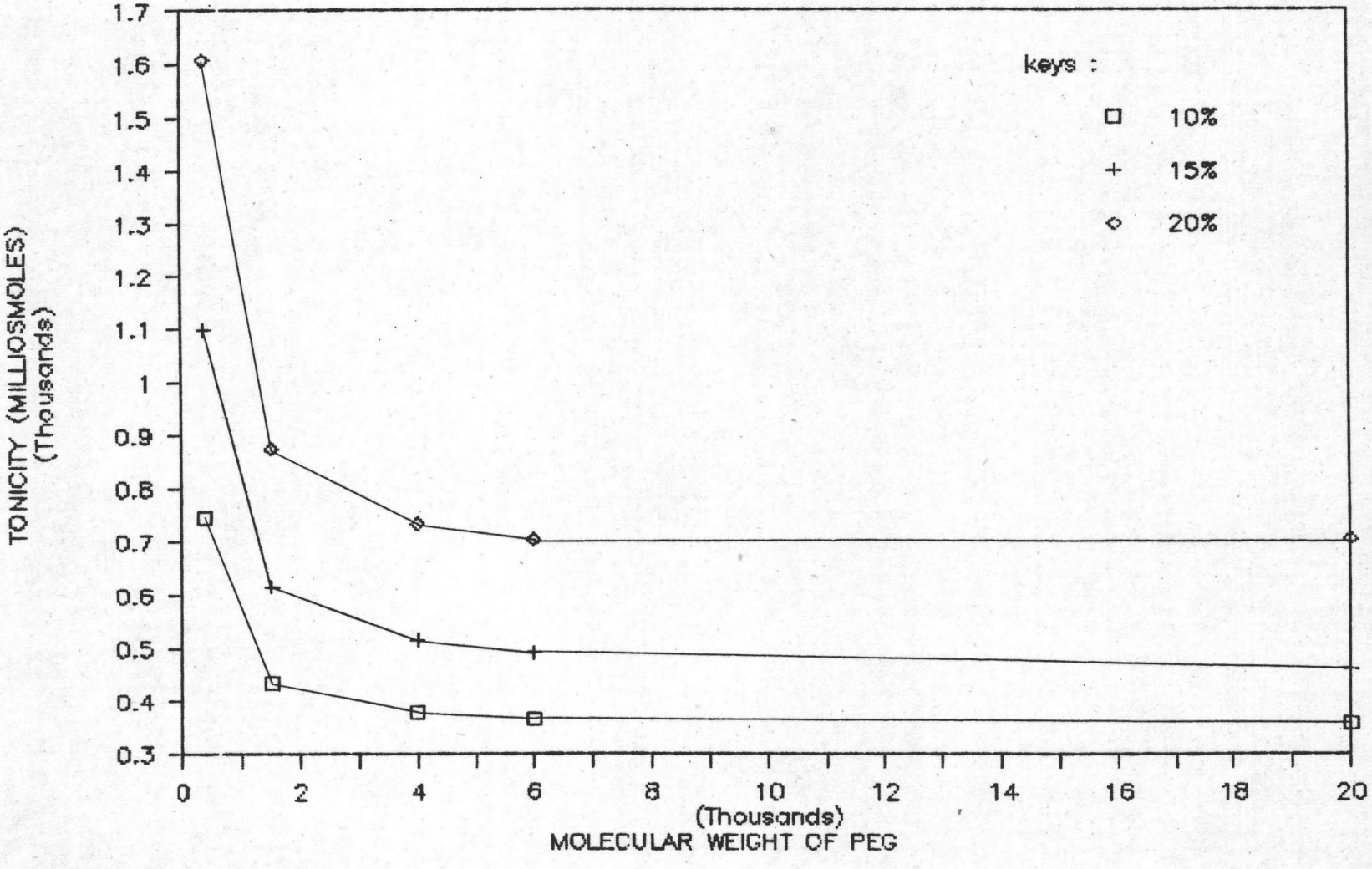

Figure 41 Comparison of the tonicity of formulations containing the same concentration but different molecular weight of PEG.

concentrations exhibited curve lines which seemed to be parallel. It was concluded that PEG of higher molecular weight were better than one with lower molecular weight because of lower tonicity which led to less eye irritation.

In addition, the tonicity exhibited additive property. If the tonicity of CPC eye drops BPC (263.5) plused the tonicity of PEG solution of various concentrations was compared to the actual tonicity of CPC eye drops BPC containing PEG, as shown in Figures 42-47 and Tables 40-45. The percent different of tonicity between actual and calculated was not more than 10%. It is concluded that tonicity of formulation exhibited additive property which would be useful to estimate the tonicity of formulation. If there were tables of toicity of added substances, the tonicity of formulation could be calculated. Moreover , the actual tonicity was always 10% higher than calculated tonicity which should be accounted.

There were some problems about tonicity of PEG. At high concentration, the tonicity of PEG were higher than tolerance limit especially 20% PEG_{6000} , 25% PEG_{6000} , 25%PEG_{20000} and 30% PEG_{20000} which showed good stability. These formulations may irritate the eyes unless the flow rate of tears was rapid enough to neutralize any excess tonicity.

High tonicity of PEG could be explained from Arrhenius observation (53). Compounds could be devided into two classes on the basis of the effects they produce on the properties of the solvent liquid: (a) those which gave the normal molecular effects on freezing

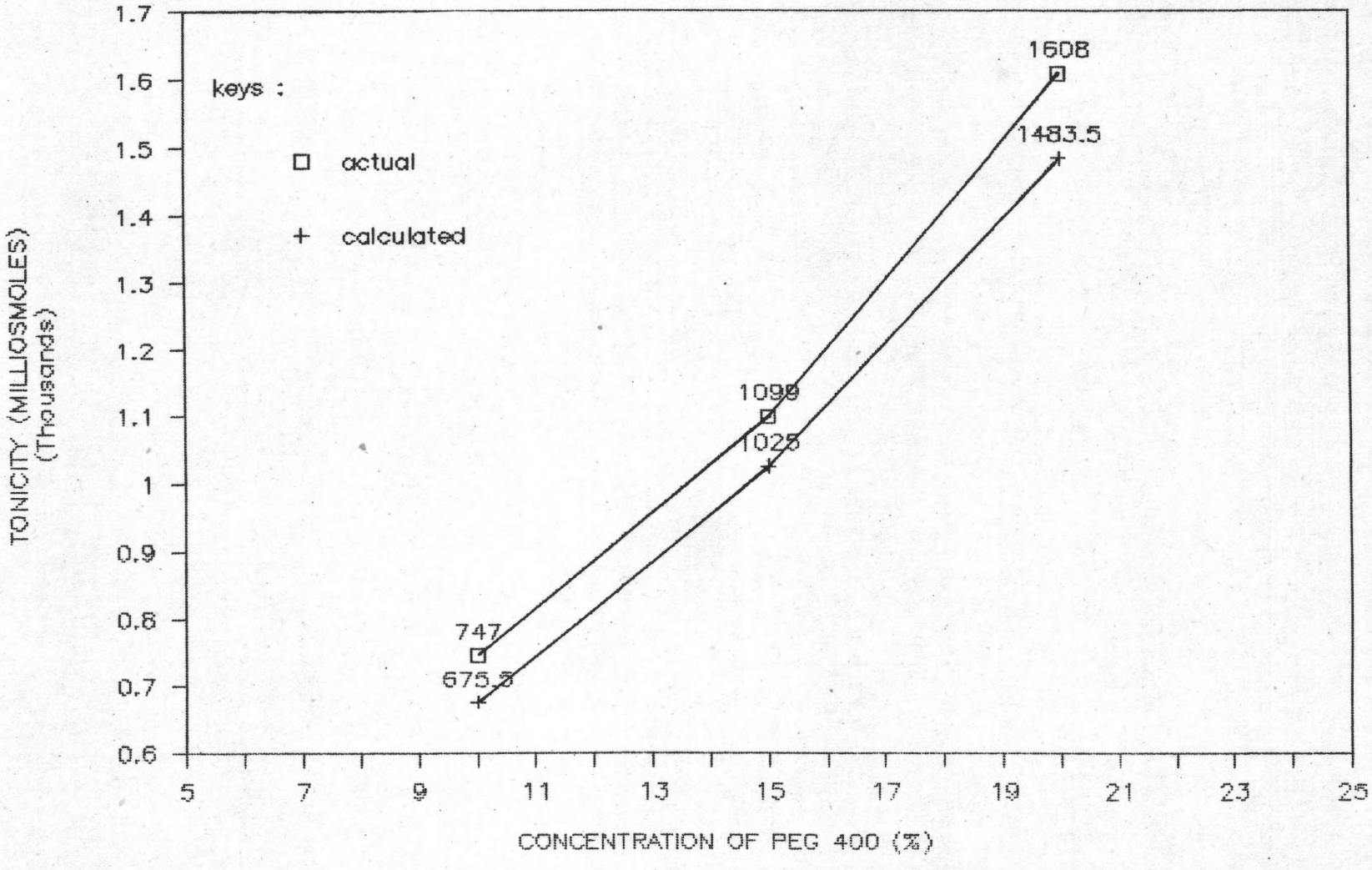

Figure 42 Additive property of the tonicity of CPC eye drops with various concentrations of PEG_{400}.

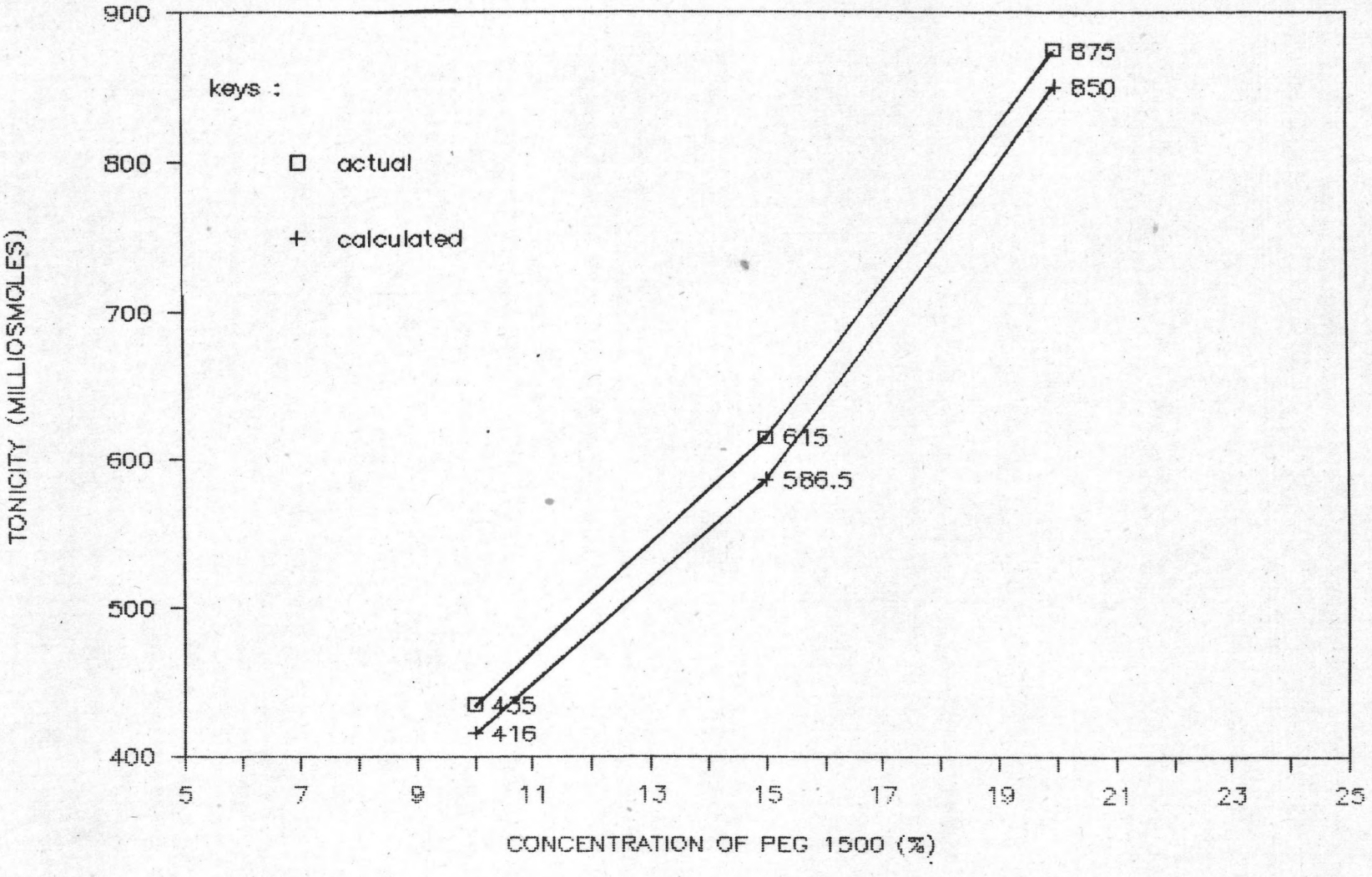

Figure 43 Additive property of the tonicity of CPC eye drops with various concentrations of PEG_{1500}.

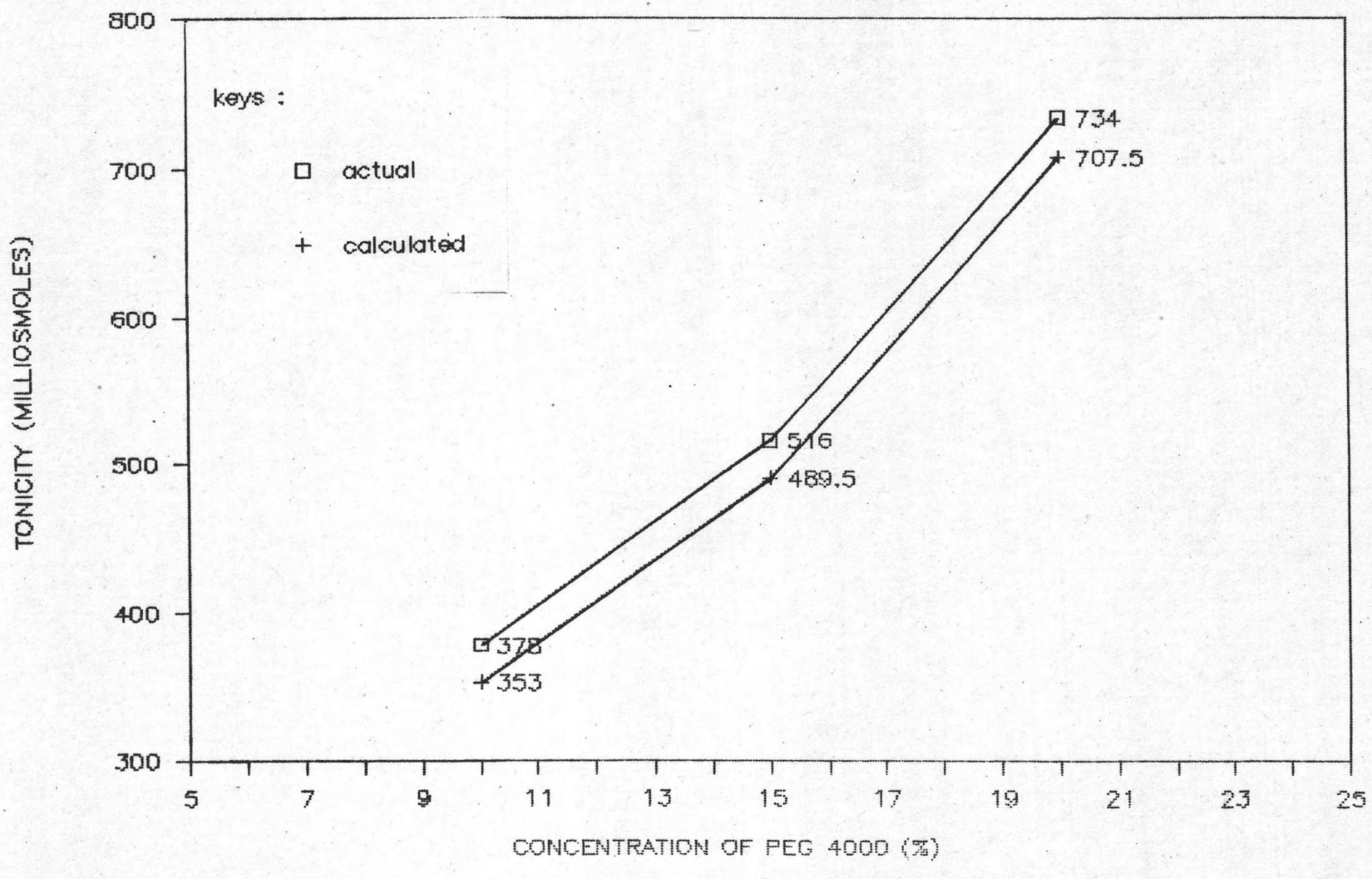

Figure 44 Additive property of the tonicity of CPC eye drops with various concentrations of PEG_{4000}.

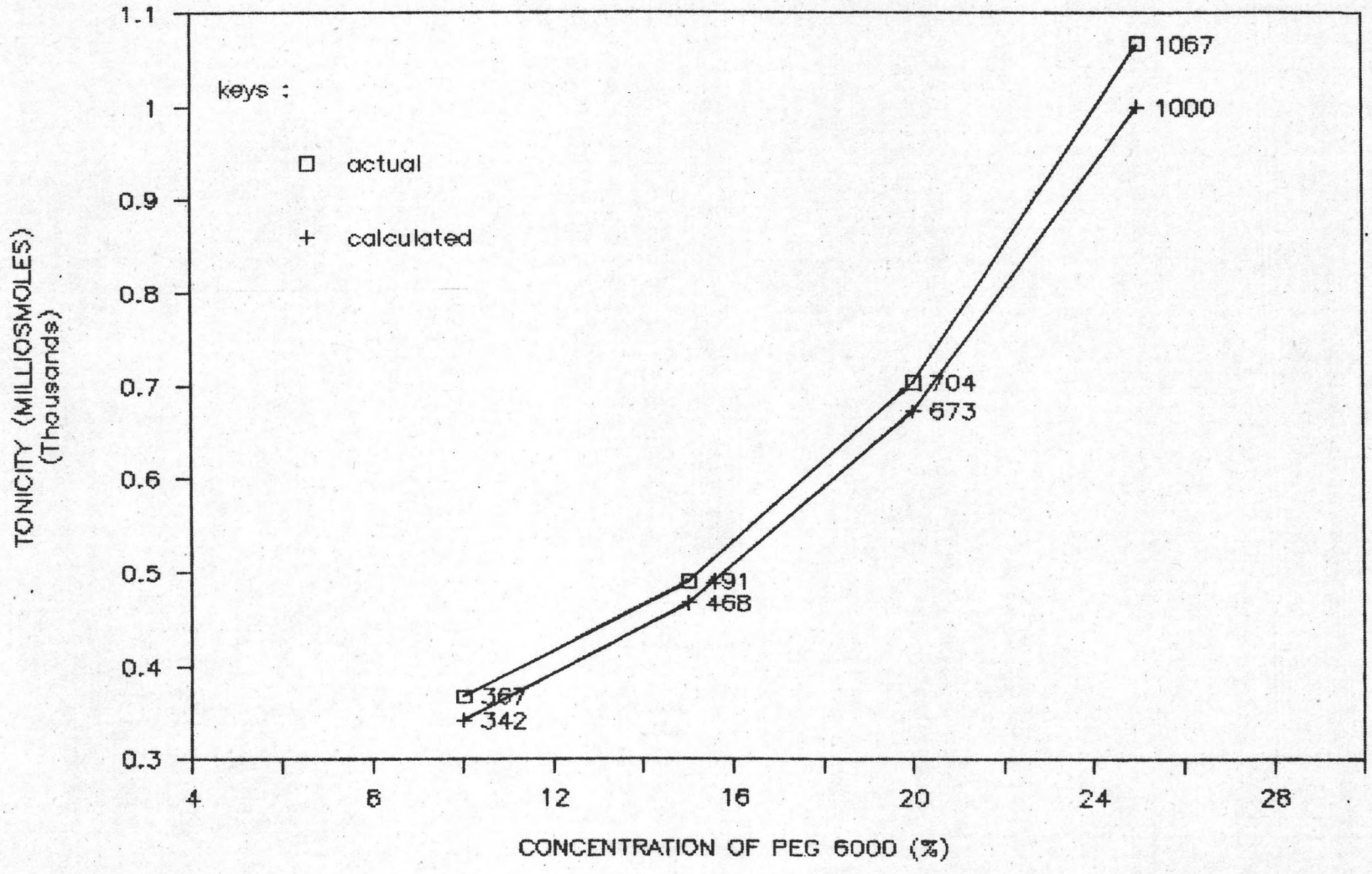

Figure 45 Additive property of the tonicity of CPC eye drops with various concentrations of PEG_{6000}.

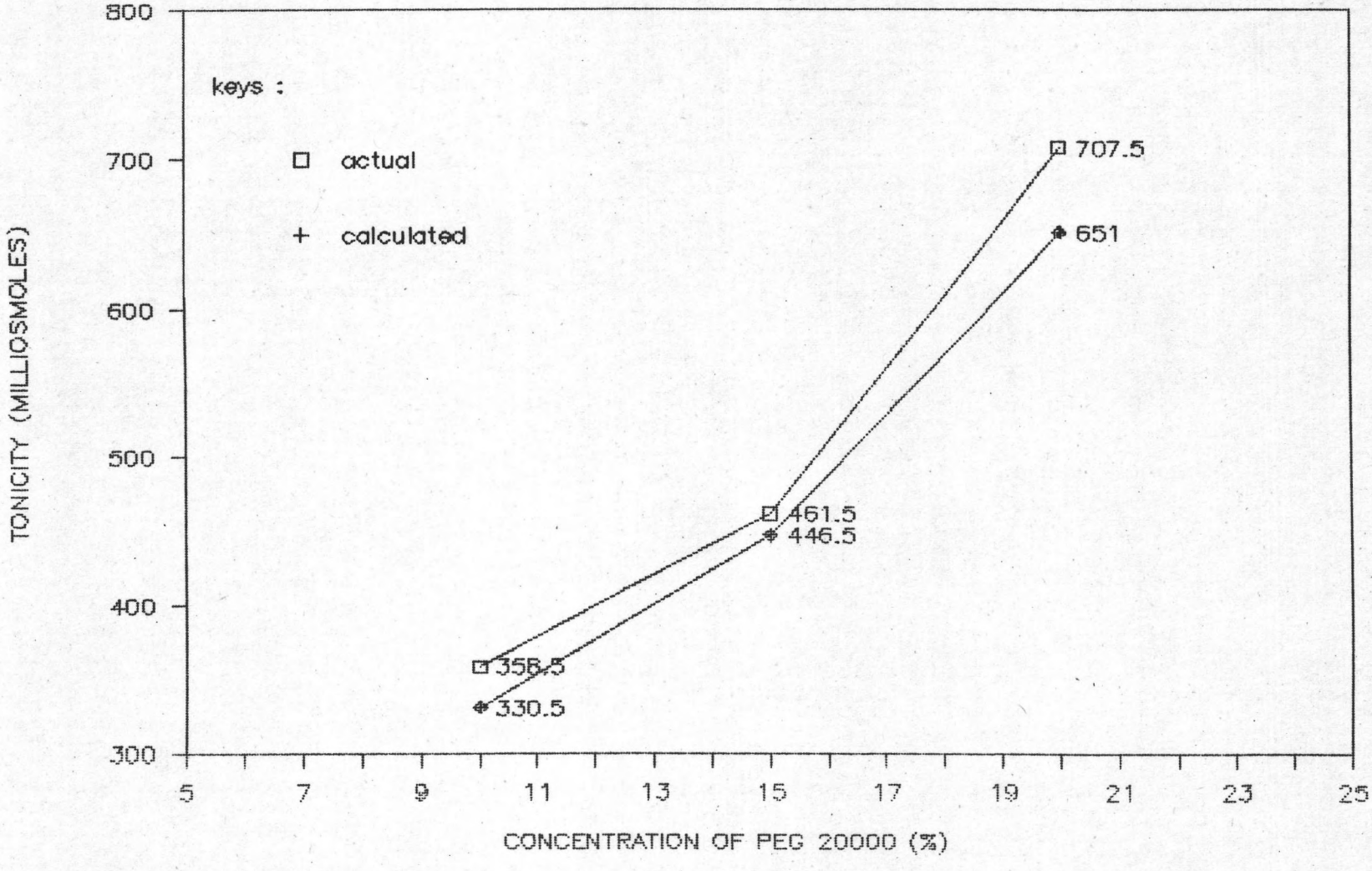

Figure 46 Additive property of the tonicity of CPC eye drops with various concentrations of PEG_{20000}.

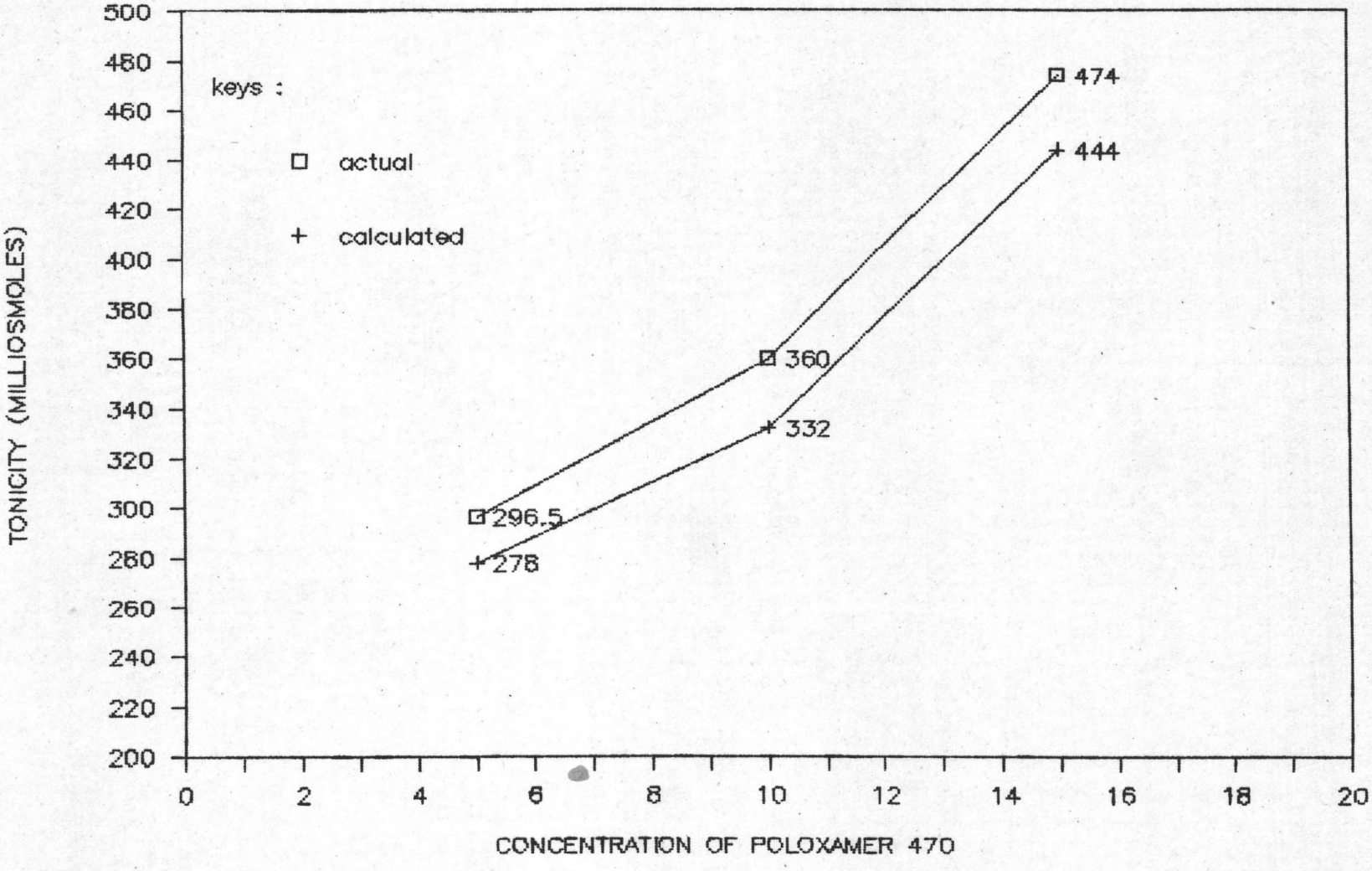

Figure 47 Additive property of the tonicity of CPC eye drops with various concentrations of PF_{407}.

Table 40 Additive property of the tonicity of CPC eye drops by PEG_{400}.

Concentration of PEG_{400}	(1) Tonicity of PEG_{400} solution	(2) (Actual) Actual tonicity of CPC eye drops BPC containing PEG_{400}	(3) (Calculated) The tonicity of PEG_{400} solution (1) + tonicity of CPC eye drops BPC(263.5)	(4) % different (2)−(3)
5%	166	-	-	-
10%	412	747	675.5	9.57%
15%	761.5	1099	1025	6.73%
20%	1220	1608	1483.5	7.74%

Table 41 Additive property of the tonicity of CPC eye drops by PEG_{1500}.

Concentration of PEG_{1500}	(1) Tonicity of PEG_{1500} solution	(2) (Actual) Actual tonicity of CPC eye drops BPC containing PEG_{1500}	(3) (Calculated) The tonicity of PEG_{1500} solution (1) + tonicity of CPC eye drops BPC(263.5)	(4) % different (2)−(3)
5%	50	-	-	-
10%	152.5	435	416	4.4%
15%	323	615	586.5	4.6%
20%	586.5	875	850	2.9%

Table 42 Additive property of the tonicity of CPC eye drops by PEG_{4000}.

Concentration of PEG_{4000}	(1) Tonicity of PEG_{4000} solution	(2) (Actual) Actual tonicity of CPC eye drops BPC containing PEG_{4000}	(3) (Calculated) The tonicity of PEG_{4000} solution (1) + tonicity of CPC eye drops BPC(263.5)	(4) % different (2)-(3)
10%	89.5	378.00	353	6.6%
15%	226.0	516.00	489.5	5.1%
20%	444.0	734.00	707.5	3.6%

Table 43 Additive property of the tonicity of CPC eye drops by PEG_{6000}

Concentration of PEG_{6000}	(1) Tonicity of PEG_{6000} solution	(2) (Actual) Actual tonicity of CPC eye drops BPC containing PEG_{6000}	(3) (Calculated) The tonicity of PEG_{6000} solution (1) + tonicity of CPC eye drops BPC(263.5)	(4) % different (2)-(3)
10%	78.5	367	342	6.8%
15%	204.5	491	468	4.7%
20%	409.5	704	673	4.4%
25%	736.5	1067	1000	6.3%

Table 44 Additive property of tonicity of CPC eye drops by PEG_{20000}.

Concentration of PEG_{20000}	(1) Tonicity of PEG_{20000} solution	(2) (Actual) Actual tonicity of CPC eye drops BPC containing PEG_{20000}	(3) (Calculated) The tonicity of PEG_{20000} solution(1) + tonicity of CPC eye drops BPC(263.5)	(4) % different (2)−(3)
10%	67	358.5	330.5	7.8%
15%	183	461.5	446.5	3.3%
20%	387.5	707.5	651	8.0%

Table 45 Additives property of the tonicity of CPC eye drops by PF_{407}.

Concentration of PF_{407}	(1) Tonicity of PF_{407} solution	(2) (Actual) Actual tonicity of CPC eye drops BPC containing PF_{407}	(3) (Calculated) The tonicity of PF_{407} solution (1) + tonicity of CPC eye drops BPC(263.5)	(4) % different (2)−(3)
5%	14.5	296.5	278	6.2%
10%	68.5	360	332	7.8%
15%	180.5	474	444	6.3%

point , vapor pressure , boiling point , and osmotic pressure and (b) those which produced excessive effects on these properties. Faraday's electrolytes included all compounds which solutions showed abnormal excessive effects in freezing-point depression and boiling point elevation. Also from Raoult's law, the freezing-point lowering was proportional to the number and not the kind of dissolved molecules. If each solute molecule separated , on dissolving , into two or more particles , each of which had the same effect on the freezing-point depression as that of the whole molecule, the observed excessive effect would appear. The assumption that substance dissociated into charged particles rather than neutral atoms gave the excessive effect on the lowering of the freezing-point (53).

The structure of PEG is $HOCH_2 (CH_2 OCH_2)_n CH_2OH$, where n represents the average number of oxyethylene groups (54). The "OH" groups are the polar groups , they were expected to be polar substance and give the excessive effect on high tonicity.

PEG of lower molecular weight showed higher tonicity than that of higher molecular weight may be explained by the equation 1 (55).

$$T_f = K_f \times m \qquad \text{eq (1)}$$

$$= K_f \times (W_2/M_2) \times (1000/W_1)$$

where T_f = freezing point depression

K_f = molar freezing point constant

m = molality of solution

M_2 = molecular weight of solute

W_2 = weight of solute that was dissolved in solvent

W_1 = weight of solvent

If molecular weight of solute (M_2) was high , freezing point depression (T_f) was low , thus the tonicity of high molecular weight PEG gave lower tonicity than the low molecular weight. Oppositely, if weight of solute (W_2) was high , freezing point depression (T_f) was high , thus the high concentration solution gave higher tonicity than low concentration.

The tonicity of solution showed additive property may be explained by the equation 1 (55).

$$T_f = K_f \times m \qquad \text{eq (1)}$$

where m is the molality of solute. If different kinds of solute were dissolved in the solution , m would be the sum of m of each solute , thus T_f or tonicity would be shown additive property.

4.2 Analysis of Chloramphenicol.

The contents of intact CPC were assayed by HPLC. The degradation products and the other ingredients in the formulations were reported not to interfere the assay of CPC (4,5). In this experiment, the peaks of HPMC, PVP, PEG, PF_{407}, boric acid, borax and PMA were not appeared, thus all additives (HPMC , PVP , PEG , PF_{407}) and the other ingredients (boric acid, borax, PMA) also did not interfere the analysis of CPC.

4.3 Stability Determination at 60° C

The comparison of the rate constants (k) was discussed according to the absolute value (do not interest minus value). If high

value was obtained, the degradation rate was fast. On the other hand, if the value was low, slow degradation rate occurred. Table 21 and Figure 17 showed the rate constants of all formulations at 60° C.

4.3.1 Effect of the pH

From previous stability study (11,24) maximum stability was at pH = 6.0. In this experiment, comparison between pH = 6.0 and pH = 7.0 in three different formulations was performed as listed in Table 46.

The k values at 60°C of the formulations prepared to pH = 6.0 were not lower than those prepared to pH = 7.0. However, the k values of formulations prepared to pH = 6.0 were within the confidence interval of those prepared to pH = 7.0. In addition the analysis of variance showed that the rate constants at pH = 6.0 and pH = 7.0 were not significantly different at 95% confidence.

The possible reason was due to less concentration of borax in the formulations of pH = 6.0 than those of pH = 7.0 (0.3% at pH = 7.0, 0.05% at pH = 6.0) James and leach (10 , 20) suggested that complexation between borate ion and CPC (1:2) was responsible for increased stability of CPC in borate buffer system. The concentration of borate ion at pH = 6.0 was too low to form complexation completely, thus the stability at pH = 6.0 was not greater than at pH = 7.0. Further study on this effect was suggested.

4.3.2 Effect of HPMC

The purpose for adding HPMC was to increase viscosity in order to improve stability. However, the result showed that the degradation rate was not better than the standard formulation.

Table 46 Effect of the pH on the rate constant.

Formulation	$k \times 10^{-2}$ (day^{-1})	Confidence interval of $k \times 10^{-2}$	pH	
			Before 60°C Incubation	After 60°C Incubation
BPC pH = 7.0	4.2770	4.02 - 4.53	7.06	6.84
BPC pH = 6.0	4.4300	4.19 - 4.67	5.95	5.54
20% PEG_{6000} pH=7.0	3.4784	3.24 - 3.71	7.27	6.40
20% PEG_{6000} pH=6.0	4.0167	4.02 - 4.02	5.82	3.70
25% PEG_{6000} pH=7.0	3.3615	3.12 - 3.59	7.27	6.35
25% PEG_{6000} pH=6.0	3.4882	3.25 - 3.72	6.06	3.85

To explain this result , there was an interesting report about stabilizing ascorbic acid (56). It was found that natural hydrophilic colloids (methylcellulose , carboxymethylcellulose , tragacanth and pectin) which were added to produce solutions of greater viscosity seemed to accelerated oxidation. This could have been due to trace metals or other impurities introduced by these gums. The specifications of trace metals in HPMC monograph in pharmacopeia were listed (57).

	USP	BPC
Asenic	< 3 ppm	< 2 ppm
Heavy metals	0.001%	-
Lead	-	< 5 ppm
Sulfated ash	-	< 1.0%

These trace elements in HPMC may accelerate hydrolysis and oxidation of CPC , thus degradation rate was not retarded.

In addition, at high temperature the solubility of HPMC was decreased and precipitation occurred so the viscosity at high temperature was approximately equal to water. Hence it may not increase the stability.

4.3.3 Effect of PVP.

PVP decreased the stability of CPC eventhough increasing the viscosity.

To explain this result , it was supposed that PVP may form complexation with CPC , the higher concentration the more complexation , thus less amount of free CPC. The analysis of CPC in

this experiment measured specifically free CPC , therefore the contrary result appeared.

If the supposition was true the chromatogram of CPC would show peak of complexation, however there was no such peak. Moreover, D. Horn and W.Ditter studied about this complexation in "Chromatographic Study of Interactions Between Polyvinylpyrrolidone and Drugs", they stated that the binding of CPC and PVP was not measurable by sorption method and chromatography method (HPLC)(58). Accordingly, the supposition was doubtful. On further study , Kassem et al. found that I.R. spectra data indicated occurrence of interaction between CPC and PVP via hydrogen bonding while chromatographed (TLC) did not show any sign of complexation (59). Further study was suggested.

4.3.4 Effect of PEG

The experimental data (Table 21,Figure 17) indicated that both PEG_{6000} and PEG_{20000} increased the stability of formulations. The degradation rate constants (k) were lower than that of the standard formulation.

The reason for the increasing stability by PEG was decreasing the rate of hydrolysis by replacing some part of water in the formulation with PEG. The more amount with PEG, the more decreasing of hydrolysis. As a result the experimental data indicated that when the concentration of PEG increased, the stability also increased.

The relationship between decreasing of k and concentration of PEG in the formulation was interesting. The percentage of decreasing the k value was calculated by comparison with the k of the standard

formulation.

$$\% \triangle k = \left[\frac{k_{sf} - k_f}{k_{sf}} \right] x\ 100$$

$$= \left[\frac{4.2770 - k_f}{4.2770} \right] 100$$

where $\% \triangle k$ = percentage of decreasing the k

k_{sf} = k of standard formulation

k_f = k of formulation

The results were tabulated in Table 47. It could be seen that the value of the percentage of decreasing the k value was approximately equal to the concentration of PEG that replaced water in the formulations. It may be meant that the percentage of replacing with PEG was the percentage of decreasing of k or the percentage of replacing with PEG was the percentage of decreasing of hydrolysis.

4.3.5 Effect of PF_{407}

From the experiment , 15% PF_{407} was the best to increase the stability (38.7%). The explainations were listed.

1. The concentration of PF_{407} was 15% , that was high enough to decrease hydrolysis by replacing some part of water.

Table 47 The relationship between the concentration of PEG in the CPC eye drops formulations and the percentage of decreasing the rate constant.

Formulation	Decreasing percentage of rate constant
20% PEG_{6000}	18.7%
25% PEG_{6000}	21.4%
20% PEG_{20000}	14.7%
25% PEG_{20000}	30.4%
30% PEG_{20000}	30.8%

2. The previous study showed that solubilization of a drug by surfactants in many cases protected againt hydrolysis (28). In aqueous solution , drug was solubilised in micelle. The magnitude of the effect depended on

2.1 Location of the drug within micelle. A solubilisate may be incorporated into the micelle in variety of locations.

It was generally accepted that non-polar solubilisates were dissolved in the hydrocarbon core. Water - insoluble compounds containing polar groups were orientated with the polar group at the surface of the ionic micelle amongst the micellar charged head groups and the hydrophobic group buried inside the hydrocarbon core of the micelle as shown in Figure 48 (28).

Because non-polar compounds were thought to be solubilised within the lipophilic core so they were likely to be more effectively removed from the attacking species than compounds that were located close to the micellar surface. Sheth and Parrott (28) noted a greater stabilization of benzocaine compared with homatropine when solubilised by non - ionic , and attributed this effect to a deeper penetration of the less-polar benzocaine into the micellar palisade layer , making it less susceptible to attack by hydroxyl ions.

2.2 The concentration of surfactant was an important factor on the rate of degradation of the drug. Swarbrick and Rhodes in a study on the oxidation of linoleic acid in aqueous solution of polyoxyethylene lauryl ether (Brij 35) found that increasing the

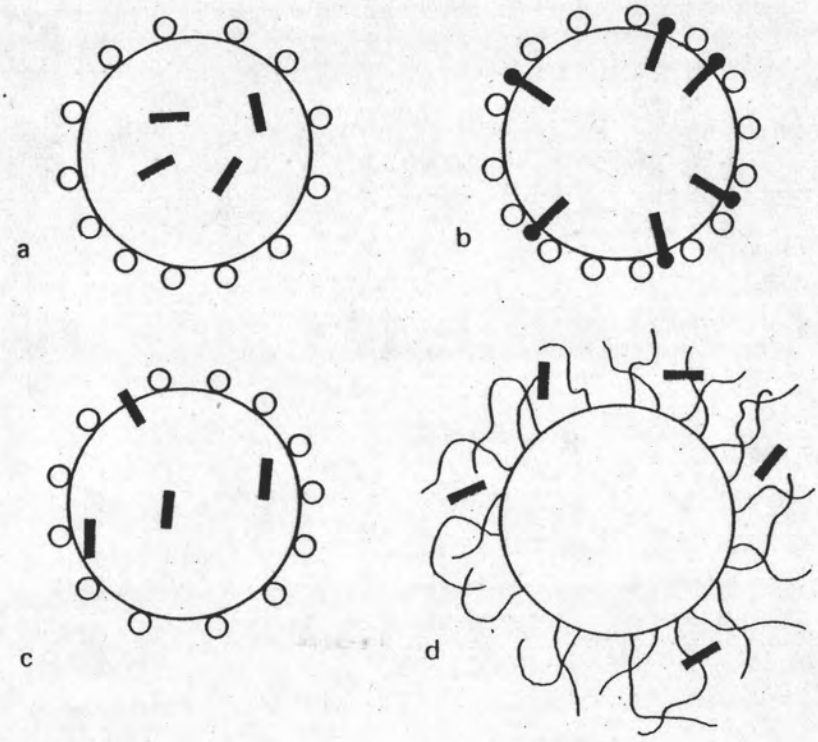

Figure 48 Schematic representation of sites of solubilization in ionic and nonionic micelles. (a) Non-polar solubilisate; (b) amphipathic solubilisate; (c) slightly polar solubilisate; and (d) polar solubilisate in polyoxyethylene shell of a nonionic micelle.

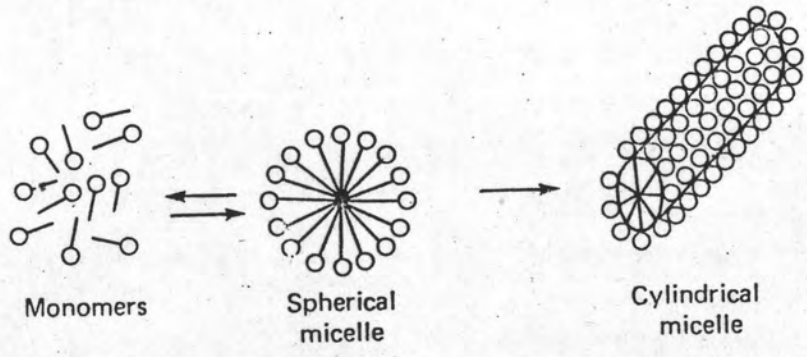

Figure 49 Schematic representation of possible changes in micellar structure with increase in concentration .

surfactant concentration significantly decreased the rate of oxidation (60). In a series of study , Carless et al. found that the rate of oxidation of certain aldehydes and oil was decreased by increasing the concentration of non-ionic surfactant used (cetomacrogol and betaine) (61-65). In a similar study, Mitchell and Wan also found that for a fixed concentration of aldehydes, an increase in surfactant concentration caused a decrease in the rate of oxidation (66).

Rolland I. Poust and John L. Colaizzi stated that oxidation of ascorbic acid in aqueous solutions of polysorbate 80 decreased as a function of surfactant concentration. It was explained that increasing surfactant concentration changed the shape of micelles from spherical or globular to larmellar structure (Figure 49) , and led to be more effectively removed form the attacking species (28).

PF_{407} was non-ionic surfactant and CPC was non-polar substance. CPC was thought to be solubilised within the lipophilic core so it was likely to be more effectively removed from the hydrolysis attacking species. For this reason, PF_{407} was shown a greater stabilization than the other additives.

The concentration of the non-ionic surfactant was 15% which was the maximum concentration of PF_{407} to be solution form at room temperature. If the concentration was more than 15% , it would form gel. This concentration of 15% was thought to be high enough to increase the number of micelles , the micellar aggregation number , the size of the micelles and to change the shape of micelles from spherical or globular to lamellar structure and led to be more

effectively removed from the attacking species.

3. PF_{407} was more soluble in cold water than in hot water. It had been reported to exhibit reverse thermal gelation behavior , gel upon heating and melt on cooling. At higher temperatures , PF_{407} desolved and enhanced entanglement may contribute to gel formulation (57). In the incubator , at 60° C , PF_{407} formed gel , increased viscosity thus hydrolysis was decreased.

4.4 Determination of Physical Properties

4.4.1 Viscosity

The formulation containing 30% PEG_{20000} showed maximum viscosity . The requirement of eye drops did not limit the maximum viscosity (35). Increasing viscosity increased the contact time to the eye. It may increase the bioavaibility (35,41).

The viscosity was affected by temperature. The viscosity data were determined at 25°C but the experimental stability temperature were 40° , 50° , 55° and 60°C. Hence the effect of viscosity to stability should be determined at the accelerated temperature. When the temperature increased , the viscosity of all formulations were decreased except PF_{407}. At high temperature the viscosity of PF_{407} increased and finally formed gel (50). On the contrary , at high temperature the solubility of HPMC was decreased and precipitation occurred so its viscosity at high temperature was approximately equal to water. Hence it may not increase the stability. In conclusion , the dependent of viscosity on the stability should be determined at the determination temperature and the viscosity was depended on the

property of the substances.

4.4.2 pH

The hydrolysis degradation products are dichloroacetic acid and hydrochloric acid , therefore pH of formulations was decreased after incubation.

For formulations which pH's were not initially adjusted to 6.0, after incubated at 60°C , the lowest pH appeared was 6.35 while the concentration was decreased to about two half-life (25%). This pH was within the tolerant limit of 5-9. Upon normal storage , the substandard formulations (concentration 80-90%) should have a pH of higher than 6.35. In conclusion , the degradation of CPC did not cause eye irritation by pH changing.

After incubation, the pH's of the formulations containing BPC, 20%PEG_{6000}, 25%PEG_{20000} , prepared to pH 6.0 were 5.54 , 3.85 and 3.70 respectively. The latter two pH's were lower than the lower tolerance limit that would cause eye irritation. The sharply decreasing of the latter two pH's was not only due to the degradation products of CPC but also the changing of the additive (PEG_{6000}). In addition , the changing of ratio of boric and borax may also affected the buffer capacity.

4.4.3 Color

Mubarak et al. reported that photodegradation products of CPC in water were yellow substances. The more degradation produced, the more yellow substances. The concentration of yellow color indicated the degree of degradation (23).

After incubation at 60° for about 1 month, color change in standard formulation was not occurred. On the contrary the more stable formulations containing PF_{407} or PEG showed the more yellow. Hence the determination of stability was not decided by the concentration of yellow color because some additives produced such color.

The formulation containing PVP exhibited the yellow color at the beginning of the preparation. The more concentration of PVP, the deeper color appeared. The literature of BASF Wyandotte corp , stated that "Aqueous solution of PVP have no buffering action. On standing or on heating they turn slightly yellow , particularly if they are somewhat acid. The discoloration can be prevented by means of reducing agents such as sodium hydrogen sulphite (0.5%-3.0% of the mass of PVP)". If the formulations were added with sodium hydrogen sulphite , the problem of discoloration would be disappeared.

The formulations containing HPMC did not show problem of discoloration.

After incubation , a yellow color appeared in formulation containing PEG. PEG_{20000} showed yellower than PEG_{6000} and the more concentration of the PEG, the yellower solution appeared. The yellow color may be caused by oxidation of PEG. Oxidation may occur if PEG was exposed for a long period to temperatures exceeding 50°C. Storage under nitrogen or suitable anti-oxidation would reduce the possibility of oxidation (54).

4.5 Stability Determination at 40°, 50°, 55°, 60°C

4.5.1 Order of Reaction Rate

The order of reaction rate was first order kinetic , similarly to previous reports (11,16,67). The major cause of CPC degradation in aqueous media was hydrolysis. , Both molecules of CPC and water affected the degradation rate. However , the water concentration was held constant by having a large excess, as in most solutions then the reaction was apparent first-order or pseudofirst-order (68).

4.5.2 Comparison of Rate Constant

The rate constants (k) of the six formulations at four temperatures were compared in Table 48. The k values were orderly ranked from the minimum to the maximum. In general , the stability of formulations at four temperatures were assumed orderly rank as BPC <20% PEG_{6000} <25% PEG_{6000} <25% PEG_{20000} <30%PEG_{20000} <15% PF_{407}.

It could be concluded that PF_{407} was the best additive for improving stability. In addition , PEG significantly improved stability. Moreover higher concentration of PEG led to higher stability because of higher replacing of water and the higher molecular weight led to higher stability because of lower di-electric constant of the formulation.

But there were some exceptions, the stability of 20% PEG_{6000} was very slightly better than 25% PEG_{6000} at 55°C. The stability of 30% PEG_{20000} at 50° , 55° and 60°C was also better than both 25%PEG_{6000} and 25% PEG_{20000} but at 40°C its stability was worse and the determination coefficient (r^2) was lesser than the others (0.9853)

Table 48 The rate constant (k) of the six formulations at four temperatures were compared and the k value were orderly ranked from minimum to maximum.

60°		55°		50°		40°	
Formulation	k X 10^{-2} (day^{-1})	Formrlation	k X 10^{-2} (day^{-1})	Formulation	k X 10^{-2} (day^{-1})	Formulation	k X 10^{-3} (day^{-1})
BPC	4.2770	BPC	2.6253	BPC	1.5695	BPC	5.0102
20% PEG_{6000}	3.4784	25% PEG_{6000}*	2.1941	20% PEG_{6000}	1.4181	20% PEG_{6000}	4.3245
25% PEG_{6000}	3.3615	20% PEG_{6000}*	2.1082	25% PEG_{6000}	1.3050	30% PEG_{20000}*	3.9889
25% PEG_{20000}	2.9756	25% PEG_{20000}	1.8933	25% PEG_{20000}	1.2769	25% PEG_{6000}*	3.6975
30% PEG_{20000}	2.9598	30% PEG_{20000}	1.7791	30% PEG_{20000}	0.9517	25% PEG_{20000}*	3.6872
15% PF	2.6221	15% PF	1.7530	15% PF	0.9372	15% PF	3.3150

(Table 27).

4.6 Arrhenius Plot and Arrhenius Equation

The influence of temperature on reaction velocity is the quantitative relation proposed by Arrhenius.

$$\ln k = \ln A - (Ea/R).(1/T) \qquad (\text{eq } 2)$$

The rate constants (k) of the four temperatures affected the Arrhenius correlation. The coefficient of determination (r^2) of Arrhenius equation of the six formulations were between 0.9924-o.9996. It stated that the correlation between temperature and rate constant was very good. It similared to previous reports (11,16,67).

From Figure 24 , the straight lines of Arrhenius plot of most formulations (except 25% PEG_{6000} , 30% PEG_{20000}) were rather parallel. It was due to similarly slope and led to similarly activation energy.

4.7 Heat of Activation (Ea)

Heat of activation was in the range of 20-23 k. cal/ mol (Table 35), by similar to Suwanna laungchonlatan ; 20-22 k.cal/mol , K.A. Connors ; 20 k.cal/mol ; T. Higuchi ; 23 k.cal/mol (5,68,17).

The heat of activation (Ea) represents the influence of temperature on reaction velocity. If heat of activation is in the range of 10 to 30 k.cal/mol , the reaction depends on temperature, thus the advantage is gained by accelerated temperature studies in prediction rate of reaction at low temperature (69). Ea of CPC was in the range of 10-30 k.cal/mol , thus accelerated temperature studies was advantage.

On the other hand , if diffusion or photolysis are the rate determining steps of the reaction , the heat of activation is only of the magnitude of 2 to 3 k.cal/mol , and little advantage is gained by accelerated temperature studies in prediction , since the temperature effect on rate is small. For reactions such as pyrolysis of polyhydroxylic materials ,in which the heat of activation can be of the magnitude of 50 to 70 k.cal/mol , the rate of degradation , which may be great at elevated temperatures , may not be of any practical significance at the temperature of marketing and storage of pharmaceutical preparation (69).

Ea of each reaction was different , it was reported that Ea of the halogenation of CPC was 30 k.cal/mol (16) but amide hydrolysis of CPC is 23 k.cal/mol (17) , thus major degradation of CPC in this experiment was a result of amide hydrolysis.

Comparison of Ea's of six formulations showed that there was a slightly difference. The Ea of 25% PEG_{6000} was the highest (23 k.cal/mol) and the Ea of 30% PEG_{20000} was the lowest (20 k.cal/mol). It was due to slightly non parallel of slope that affected to the estimation of degradation rate at 25° and 8°C and calculation of shelf-life.

4.8 Calculated Rate Constant and Shelf-Life at 25°C and 8°C

The confidence of calculation of rate constant at room or refrigeration temperature depended on (70)

a) the accuracy of the data

b) the number of data point collected

c) the extent of extrapolation

The more confidence based on the small standard deviation,the better linearity and the greater number of measurement.

The coefficients of determination (r^2) of the six formulations were in the range of 0.9924 - 0.9960 (Tables 29-34), thus the accuracy of the data and linearity was good. The number of data point collected were 4 points of 4 temperatures that obtained resonable accuracy in applying the Arrhenius treatment and making an extrapolation (71). The longer extrapolation, the larger the scatter of point or the further away the actual experiment points were from the extrapolation and the greater is the variation. This emphasized the inaccuracy that resulted from attempts to extrapolate over too wide a temperature range (71).

In the experiment the lowest temperature was 40°C. From 40°C it was calculated to 25°C that was not further away from 40°C ,thus the variation was not great. On the other hand, it was extrapolated to 8°C , that was further away, thus the interval of k_s was too wide that led to wide range of shelf-life.

At 25°C, the calculated shelf-life was orderly ranked from shortest to longest (Table 36). The result was BPC <20% PEG_{6000} <30% PEG_{20000} <25% PEG_{20000} <25% PEG_{6000} <15% PF_{407}.

The formulation containing 15% PF_{407} showed the longest shelf-life. According to the standard of BP 1980, the calculated shelf-life (t_{110-90}) was 11.80 months. Therefore the calculated shelf-life of best formulation was 3.81 months or 47.68% longer than the standard formulation (BPC 1973). According to standard of USP

XXI , the calculated shelf-life (t_{130-90}) was 21.62 months , thus 6.98 months or 47.68% longer than the standard formulation. The calculated shelf-life according to USP XXI was about 2 years that may be long enough for marketted production.

Normally, in order to ensure the quality of product , the shelf-life was calculated from the lowest value of the range. The shelf-life of formulation containing 15% PF_{407} calculated from lower limit and according to USP XXI was about 12.98 months at 25°C that may be possible for marketted production and much longer than BP 1980 as 4 months at room temperature (3).

The comparison of calculated shelf-life in PEG group showed that the formulation containing 25% PEG_{6000} produced the longest shelf-life (11.24 months).

The shelf-life of CPC eye drops according to BP 1980 was 4 months at 25°C after preparation (3) but the calculated shelf-life of BPC 1973 from the experimental was 7.99 months or about two times. It may be explained that the shelf-life of CPC eye drops according to BP 1980 was calculated the concentration from 100% to 90% labelled amount but the experiment was calculated from 110% to 90% labelled amount, thus the shelf-life was about two times. In addition, the shelf-life of CPC eye drops BPC 1973 in the experiment was calculated to 33°C in order to compare with Suwanna laungchonlatan's result. At 33°C the shelf-life of BPC 1973 was 2.99 months that was similar to the average shelf-life resulted from Suwanna as 2.52±0.85 (actual storage) (5). It may be expected that the calculated shelf-life at

25°C in this experiment would be similar to actual storage.

At 8°C the intervals of the calculated shelf-life of all formulations according to BP 1980 was and according to USP XXI were too wide because of the long extrapolation. To compared with the shelf-life specified by BP 1980 as 18 months from the date of preparation at 2° to 8°C (3) or 36 months for calculated the concentration decreased from 110% to 90% labelled amount ,it differed from the calculated shelf-life in this experiment (the lowest was 76.10) , thus the calculated shelf-life at 8°C may be different from actual storage.

When the calculated shelf-life at 8°C was orderly ranked from the shortest to the longest , it stated that 20% PEG_{6000} < BPC < 30% PEG_{20000} <25% PEG_{20000} <15% PF_{407} <25% PEG_{6000}. To compare with calculated shelf-life at 25°C , the order was different , BPC 1973 was changed to the second and 20% PEG_{6000} moved to the first, it was due to slope of BPC was more sharp than 20% PEG_{6000} when the extrapolation was long , ln k_s of BPC was lower than 20% PEG_{6000} . In the same reason that calculated shelf-life at 8°C of 25% PEG_{6000} was longer than 15% PF_{407}. Hence the value of shelf-life depended on intercept on Y axis , slope of Arrhenius equation and the location of extrapolation.

4.9 Conclusion

Improving stability of CPC eye drops via vehicle compositions by decreasing hydrolysis. The method was adjusting the vehicle to the optimum pH, increasing viscosity (HPMC and PVP), partial replacement

of water with cosolvent (PEG) and solubilization of a drug by surfactant (PF_{407}). The accelerated thermodegradation process was applied for stability testing at 40°, 50°, 55° and 60°C. The degradation of CPC in all formulations was found to be first ordered. The reaction rates at 40°, 50°, 55° and 60°C of each formulation were correlated to recipocal of temperature in Arrhenius relationship (r^2 = 0.9924-0.9996). The calculated degradation rates at 25°C and 8°C were obtained from Arrhenius plot. The heat of activation calculated from the slope of Arrhenius equation was between 20-23 k.cal/mol, similarly to Higuchi et al., and Suwanna laungchonlatan (17,5).

The contents of CPC were analysed by HPLC according to material and method in Charpter II. The degradation products and CPC remained were seperated by the system. Other additives did not interfere the analysis and propylparaben was a good internal standard.

The tonicity measurement was an indicator for eye irritation. It was measured by osmometer. The tolerance limit of the eyes was between 160-630 milliosmoles or 0.5 - 2.0% NaCl. The tonicity measurement by osmometer can apply for other eye drops or parenteral preparations in order to decrease pain and lysis of red blood cell.

The tolerance limit pH range was 5 - 9, If the formulations were not adjusted to pH = 6.0, the pH was slightly dropped but within the tolerance limit. The stability of formulations adjusted to pH = 6.0 according to the method in this experiment, were not

greater than those adjusted to pH = 7.0. However, further study on this topic was suggested.

Both HPMC and PVP were used as viscosity-increasing agents. At the experimental concentration they did not increase the stability. They markedly increased the viscosity but slightly affected the tonicity, and caused slightly pH changes. HPMC did not exhibit discoloration but PVP changed to yellow color at the beginning of preparation and increasing the concentration darkened the color of preparation.

PEG was used as cosolvent. Both PEG_{6000} and PEG_{20000} increased stability significantly because of partial replacement of water and lower dielectric constant. However, it was not concluded that the more increasing of concentration, the more stability occurred and the higher molecular weight of PEG stated more stability than the lower molecular weight of PEG. PEG extensively increased tonicity and viscosity. The tonicity of high molecular weight was lower than the low one. The tonicity of PEG and PF_{407} stated the additive property that could be applied for prediction of the tonicity of other formulations. They showed slightly change of pH , marked discoloration especially at both higher concentration and higher molecular weight.

The non-ionic surfactant, PF_{407} of 15% increased the maximum stability. The shelf-life was 47.68% longer than the standard formulation and shelf-life at 25°C according to BP 1980 was 11.80 months (7.09-19.65), according to USP XXI was 21.62 months

(12.98-35.99) that may be long enough for marketted production. In addition, if it was stored in the refrigerator,the shelf-life would be longer. For the physical properties, there was no problem about viscosity and pH change ,only slight discoloration appeared.

The shelf-life at 25°C according to BP 1980 and USP XXI were orderly ranked from the shortest to the longest as BPC <20% PEG_{6000} <30% PEG_{20000} <25% PEG_{20000} <25% PEG_{6000} <15% PF_{407}.

The shelf-life at 25°C was calculated from Arrhenius relationship that was plotted from four temperatures and the lowest temperature was 40°C that was not further away from 25°C. The r^2s of Arrhenius equations > 0.99. In addition, the calculated shelf-life of CPC eye drops BPC 1973 at 33°C was similar to actual shelf-life of marketted formulations from Suwanna laungchonlatan's research. It may be expected that the calculated shelf-life at 25°C in this experiment would be similar to actual storage.

The interval of calculated shelf-life at 8°C was too wide because of long extrapolation therefore the accuracy of prediction was doubted.

Finally, this experiment improved the stability of CPC eye drops via vehicle composition by adding 15% PF_{407} into CPC eye drops BPC 1973 that led to 47.68% improvement. The shelf-life according to BP 1980 was 11.80 months and according to USP XXI was 21.62 months. Its physical properties were accepted for eye drops.