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A STUDY ON THE SOLUBILITY AND STABILITY OF ETHINYL OESTRADIOL

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Atrial was made to prepare solutions of ethinyl oestradiol by series of selected surfactants. It was found that the cationic surfactant was the best solubilizer for this hormone followed by the anionic then the non-ionic ones. The solubilized systems were further investigated for their stability under different storage conditions. It was noticed that the solutions prepared by non-ionic surfactants gave rise to the most stable preparation for this hormone, especially in the presence of antioxidants and preservatives. Storage of this preparation in amber coloured ampoules at 5° was also desirable, as it can keep its potency for a period up to 6 months .

Introduction

The preparation of contraceptive hormones in liquid dosage forms is of interest, in order to counteract many of the problems arised during the tablet manufacture of this hormone. Many steroids were successfully prepared in solutions by using bile salts[1], anionic and cationic surfactants[2], ethoxylated cholesterols[3-5], as well as non-ionic surfactants[6-8]. The effect of variation in the steroid molecular structure on their solubility was also investigated[7-11]. In this work solutions of ethinyl oestradiol were prepared by different classes of surfactants, mainly anionic (sod. lauryl sulphate), cationic (cetrimide) and non-ionic (Tween 20 and Tween 80). Furthermore, the stability of solutions containing this hormone was investigated in the presence of different additives and under various storage conditions .

Experimental :

Materials :

Ethinyl oestradiol[a] Tween 20 and Tween 80[b], sodium lauryl sulphate, cetrimide, sodium metabisulphite, EDTA disodium, and sod. Benzoate[c].

Methods :

A- Solubility measurement :

Excess amount of the tested hormone was placed in 25 ml stopperd tubes together with 10 ml of the selected surfactant solutions of increasing concentration up to 10% w/v, and up to 2 Molar of urea. The solutions were equilibrated by shaking for 10 days at 25 ± 1° in a Thermostatically controlled water bath After equilibration, the solutions were filtered through seitz filter No. 3. The absorbance of the solutions were measured at 280 nm after appropriate dilutions with absolute ethyl alcohol for their ethinyl oestradiol contents, using the corresponding surfactant concentration in absolute ethyl alcohol as a blank.

B- Stability studies :

The solutions of this hormone were prepared using Tween 20, Tween 80, sodium lauryl sulphate as well as urea, and subjected to stability study under exaggerated storage conditions. They were placed in colourless and amber coloured ampoules and stored for aperiod up to 6 months at 5°, 35° and 45° + 2°.Then they were examined physically and chemically at different time intervals.

C- Physical stability:

The prepared solutions were examined for their colour change, clarity, and mould growth, visually, and PH values were measured using PH meter.

a - Schering Corp. Bloom Field, N.J. U.S.A.

b - Atlas Chemical Industries, Inc., Wilmington, Del, U.S.A.

c - B.D.H. Poole,England.

D- Chemical Stability:

During the period of 6 months, samples from the colourless and amber coloured ampoules stored at 5^{o} , 35^{o}, and45^{o} were analyzed spectrophotometrically for the their Ethinyl oestradiol contents.

Values for the apparent first order decomposition rate constant (K) was obtained from the plot of log percent concentation remained against time in months. On using the least square regression analysis, the value of K, half life time(t_{50}) and the correlation coefficent were obtained.

The effect of addition of the different antioxidants and preservatives on the stability of this hormone was also investigated. The solubilized systems containing 10% W/v Tween 20 and Tween 80 as solubilizing agent were selected for this study. The following materials were tried 0.1%W/v sodium metabisulphite (as antioxidant). 0.1% EDTA disodium salts(as chelating agent) and 0.2%W/v sodium benzoate as preservative. The solutions were stored at 35^{o} and 60^{o}, then they were examined physically and chemically as mentioned before.

RESULTS AND DISCUSSION

The solubilization of the tested hormone in aqueous solutions containing various concentrations of the tested surfactants and urea were conducted and their stability were investigated.

A- Solubility Studies:

From the solubility results, it was noticed that the amount of hormone solubilized increased by increasing the surfactant concentration in a linear manner. Similar relationshipwas reported on the solubilization of other steroids by sjoblom(7,8) and by Guttman et al (11). The solubility of ethinyl oestradiol was found to be greater in Tween 80 solution than Tween 20. This could be due to the bigger volume of the core (hydrocarbon part) of Tween 80 than Tween 20, consequently this hormone could be expected to be localized mainly in the micellar core rather than the capsule.

With regards to the different classes of surfactants, e.g cationic surfactants (cetrimide) which was found to be good solubilizer than the anionic one (sodium lauryl sulphate) above its CMC and both werebetter than the two non-ionic surfactants investigated . This may be due to stronger interaction between such ionic micelles and the hormone rather than the non-ionic micelles. The results obtained are shown in Fig.1. On the other hand, urea solution was the least effective solubilizer of this hormone.

. This may be due to the difference in the mechanism of solubilization(Clatherate formation).

It was evident from spectral studies that the presence of Tween 20, Tween 80, sodium lauryl sulphate, cetrimide and urea did not result in the appearance of new absorption maxima for ethinyl oestradiol. This indicated that no complexation takes place between the hormone and each of the tested surfactants in the concentration range used. Thus, the possible solubilization mechanism is micellar solubilization. This can occur by occlusion of the solute into the core of the surfactant micelles, or by incluslon into the palisade layer of the micelle, A combination of these mechanisms may also occur. The location of the solubilized molecule withinthe different regions can be expected from the balancebetween the polar and non-polar part of the molecule. It can be suggested that ethinyl oestradiol was localized mainly within the hydrocarbon core of the micelle due to its less polar nature. Furthermore its solubility was found to be greater in the micelles of bigger core volume than smaller ones.

B- Stability Studies:

(a) Physical stability

With regards to results of stability, the following physical observations were obtained in this study.

i- Colour change:

It was noticed that ethinyl oestradiol in Tween 20 or Tween 80 solutions developed a colour change in all the tested preparations, contained in either colourless or amber coloured ampoules stored at various storage temperatures.

After 6 monthsoof storagein colourless ampoules the colour change was faint yellow-yellow-yellowish brown-ligh brown at 5^{o}, 35^{o} and 45^{o}, while, in amber glass ampoules,the respectine solutions were faint yellow-yellow-yellowish brown this may be due to the autoxidation of the compounds by the presence of unlinked ethylene oxide chains normally present as impurities especially in commercial non ionic surfactants, or as a degradation product from the surfactants themselves. But with sodium lauryl sulphate and urea the preparations appeared colourless in either colourless or amber coloured ampoules.

On the addition of some preservatives as sodium benzoate and anti-oxidants as sodium metabisulphate, or EDTA, the colour of solutions in either Tween 20 or Tween 80 was yellow and no change in colour takes place at all the different temperatures used

ii- Clarity:

The solutions of ethinyl oestradiol deposited crystals in Tween 20, Tween 80 and in sodium lauryl sulphate solutions in both colourless and amber coloured ampoules at higher surfactant concentration or surfactan and at all the tested temperatures. This may be due to degradation product from the surfactants, viz., the presence of compounds with short chained ethylene oxide group which are normally insoluble in water. But, with urea solutions the crystals deposited in all preparations especially at 5^{o}.

On the otherhand no depositions occurred on the addition of antioxidant (sodium metabisulphite at 60), and preservative (sodium benzoate at 35^{o}), in case of solution in either Tween 20 or Tween 80

iii- Mould growth :

With regards to mould growth, all preparations without preservatives showed mould growth in amber coloured ampoules at 35^{o} but , not at either elevated or lowered temperatures and not in colourless ampoules at all temperatures but, on using sodium metabisulphite, EDTA and sodium benzoate, . mould growth did not occur at any temperature .

iv- pH Values :

It was noticed that solution of ethinyl oestradiol in Tween 20, reduced the pH form 5.6 to 4.2 and to 4.7 in colourless and amber coloured ampoules respectively. But in Tween 80 the pH was reduced from 6.4 to 6.2 and to 6.1 in colourless and amber coloured ampoules respectively. The pH varied from 5.99 to 5.65 on addition of mixture of the anti-oxidant and preservative to Tween 20 solutions. On the other hand the pH value varied from 6.04 to 5.9 on the addition of mixtures of anti-oxdants and preservatives to Tween 80 solutions .

The shift of pH may be due to incorporation of this hormone by surfactant micelles and the effect of the different additives on the process of micellization. Incorporation of solutes within the micellar interior may offer more protection of the solute molecules espcially against oxidation or hydrolytic degradation, therefore more stable solutions can be prepared

b- Chemical stability :

It was noticed that all surfactant preparations containing this hormone stored at 5^{o} and 35^{o} were more stable than those stored at 45^{o}. With urea, opposite results were obtained where the hormone was more stable when stored at 45^{o} and 35^{o} than at 5^{o} . This may be due to the break of clatherate formed at higher temperature when stored at 5^{o} due to crystallization of urea from clatherate. As shown in Fig. 2-5. The solution of this hormone in Tween 20 was found to be the most stable one especially in the presence of the following additives, 0.1% EDTA, or 0.2% sodium benzoate than the solubilized systems containing surfactant solutions alone. As shown in Table 1. The storage of the preparations at 35^{o} gave rise to more stable solution than that stored at 60^{o}.

The same result were obtained for the solutions prepared with Tween 80, although solutions containing 0.1% sodium metabisulphite were found to be more stable than in the case of Tween 20. Again, the solution prepared using a mixture of 0.1% sodium metabisulphite + 0.1% EDTA and 0.2% sodium benzoate was found to be superior and more stable preparation was obtained as shown in Table 2 .

This may be due to the synergestic effect of antioxidant and the difference in the mechanism by which sodium metabisulphite and EDTA can stabilize the solutions against oxidation.

With regards to t_{50}, it was noticed that the stability of ethenyl oestradiol solutions in Tween 20 and Tween 80 was increased by increasing the surfactant concentrations in both colourless and amber coloured ampoules as shown in Table 3,4 .

On the other hand, in the case of sodium lauryl sulphate, the t_{50} was increased by increasing the concentrations of the surfactants. On the other hand, in the case of urea solutions, the t_{50} decreased by increasing the concentration.of urea

From the above results solutions of this hormone prepared in Tween 20 and Tween 80 solutions were more stable than the solutions prepared by sodium lauryl sulphate or urea especially if stored at 5.

Thus, a suitable mixture can be prepared by solubilizing these hormones in Tween solutions containing 0.1% sodium metabisulphite, 0.1% sodium benzoate as indicated from the previous results.

The t_{50} values for solutions containing Tween 20 and Tween 80 were higher in the presence of a mixture of 0.1% sodium metabisulphite. 0.1% EDTA and 0.2% of sodium benzoate especially at 35^{o}.

In conclusion, it can be stated that the preparation of ethinyl oestradiol containing the required concentration in Tween 80 solutions in the presence of a mixture of 0.1% sodium metabisulphate, 0.1% EDTA disodium salts and 0.2% sodium benzoate may be recommended as stable solution suitable for oral administration of this contraceptive hormone. .

Table 1: Effect of Different Antioxidants and Preservatives on the Stability of Ethinyl Oestradiol Solutions Prepared Using Tween 20 and Stored in Amber Coloured Ampoules at Different Temperatures.

Additives	Conc. %w/v.	Temp. °C	Time in Month / Concentration % 0	1	2	3	4	5	6	Slope	K	t_{50}	C.V.
Sodium Meta-bisulphite	0.1	35	100	96.50	92.25	89.50	88.50	83.75	80.50	0.0156	0.036	19.25	0.9928
		60	100	94.50	89.75	84,25	79.00	75.00	70.75	0.0252	0.058	11.95	0.9987
EDTA	0.1	35	100	91.75	88.00	84.25	77.00	67.25	67.25	0.0287	0.066	10.50	0.9957
		60	100	94.5	82.75	81.51	76.50	71.75	66.75	0.0291	0.067	10.34	0.9980
Sod Benzoate	0.2	35	100	97.50	95.00	94.00	93.25	90.25	89.00	0.0083	0.019	36.47	0.9881
		60	100	96.00	92.25	88.00	82.00	81,75	78.00	0.0178	0.041	16.90	0.9989
Sod.metabis-ulphite,EDTA Sod. Benz-oate.	as above	35	100	98.75	97.50	96.25	96.25	95.5	93.75	0.0048	0.011	93.00	0.9801
		60	100	99.25	94.50	93.75	73.32	91.75	90.50	0.0074	0.017	40.76	0.9760

Table 2. Effect of Different Antioxidants and Preservatives on the Stability of Ethinyl Oestradiol Solutions Prepared Using Tween 80 and Stored in Amber Coloured Ampoules at Different Temperatures.

Additives	*Conc.* % W/V.	*Temp* °C	*Time in Month / Concentration %* 0	1	2	3	4	5	6	*Slope*	*K*	t_{50}	*C. V.*
Sodium Meta-bisulphite	0.1	35	100	96.50	90.25	89.00	87.00	82.75	79.50	0.0152	0.035	19.8	0.9743
		60	100	94.50	87.75	83.75	79.00	75.00	70.50	0.0252	0.059	11.75	0.9965
EDTA	0.1	35	100	97.00	95.50	92.25	91.75	89.00	87.00	0.0100	0.023	30.31	0.9929
		60	100	96.50	93.75	90.50	88.50	85.00	82.50	0.0132	0.032	21.66	0.9986
Sod Benzoate	0.2	35	100	97.75	95.25	95.5	92.75	90.50	89.00	0.0083	0.019	36.47	0.9872
		60	100	94.5	91.75	88.00	84.50	79.50	78.25	0.0178	0.041	16.90	0.9939
Sod. Metabis-ulphite, EDTA, Sod Benzoate	as above	35	100	98.75	98.75	98.50	97.75	97.00	96.5	0.0035	0.008	86.62	0.9744
		60	100	97.00	94.50	89.00	89.00	86.00	84.25	0.0126	0.024	23.90	0.9825

Table 3: Effect of Storage on the Stability of Ethinyl Oestradiol Solutions prepared Using Tween 20 and Stored in Amber Coloured Ampoules at Different Tempratures.

Surfactant % W/V.	Temp. °C	Time in Month / Concentration%							Slope	K	t_{50}	C.V.
		0	1	2	3	4	5	6				
2.5	5	100	97.5	93.90	91.46	84.14	84.14	80.48	0.016	0.036	19.14	0.985
	35	100	95.12	91.46	87.80	80.48	80.48	76.82	0.019	0.044	15.79	0.986
	45	100	93.90	90.24	85.36	78.04	78.04	75.60	0.020	0.047	14.87	0.979
5.0	5	100	97.72	95.45	92.04	86.36	86.36	84.09	0.013	0.029	23.98	0.982
	35	100	97.72	93.18	89.77	84.09	84.07	81.81	0.015	0.034	20.69	0.981
	45	100	97.72	94.31	89.77	81.81	81,81	79.54	0.017	0.038	18.14	0.977
7.5	5	100	97.22	94.44	93.05	90.27	86.11	84.72	0.012	0.029	25.11	0.994
	35	100	97.22	93.05	91.66	87.5	84.72	81.94	0.014	0.033	20.87	0.997
	45	100	95.83	91.66	87.5	8o.55	80.55	76.38	0.020	0.045	15.43	0.988
10.0	5	100	98.71	96.15	93.58	89.74	87.17	87.17	0.010	0.023	30.26	0.987
	35	100	97.43	94.87	91.02	85.89	85.89	83.33	0.013	0.030	22.80	0.985
	45	100	97.43	92.30	89.74	82.05	82.05	78.94	0.017	0.040	17.59	0.983

Table 4: Effect of Storage on the Stability of Ethinyl Oestradiol Solution Prepared Using Tween 80 and Stored in Amber Coloured Ampoules at Different Temperatures.

Surfactant	*Temp.* ^{o}C	*Time in Month / Concentration %*							*Slope*	*K*	t_{50}	*C.V.*
		0	*1*	*2*	*3*	*4*	*5*	*6*				
2.5	5	100	94.68	89.36	85.10	79.78	75.53	72.34	0.023	0.054	12.83	0.998
	35	100	94.68	87.23	87.97	77.65	73.40	69.14	0.027	0.062	11.27	0.996
	45	100	93.61	89.36	84.04	78.72	72.34	70.21	0.026	0.059	11.75	0.996
5.0	5	100	95.23	92.85	89.28	85.71	83.33	79.76	0.016	0.038	18.38	0.997
	35	100	96.42	94.04	90.47	86.90	84.52	80.95	0.022	0.051	13.70	0.999
	45	100	95.23	90.47	85.71	80.95	77.38	73.80	0.022	0.051	13.70	0.998
7.5	5	100	97.33	94.66	92.0	88.0	85.33	84.0	0.013	0.029	23.81	0.996
	35	100	97.33	93.33	88.0	86.66	84.00	81.33	0.015	0.034	20.15	0.390
	45	100	96.03	92.00	88.0	85.33	82.66	78.66	0.017	0.040	17.33	0.997
10.0	5	100	97.10	94.20	92.76	89.85	88.40	85.50	0.011	0.026	26.55	0.996
	35	100	97.10	94.20	91.30	88.40	85.50	84.05	0.012	0.029	23.98	0.997
	45	100	95.65	94.20	89.85	86.95	84.05	81.15	0.015	0.035	19.91	0.997

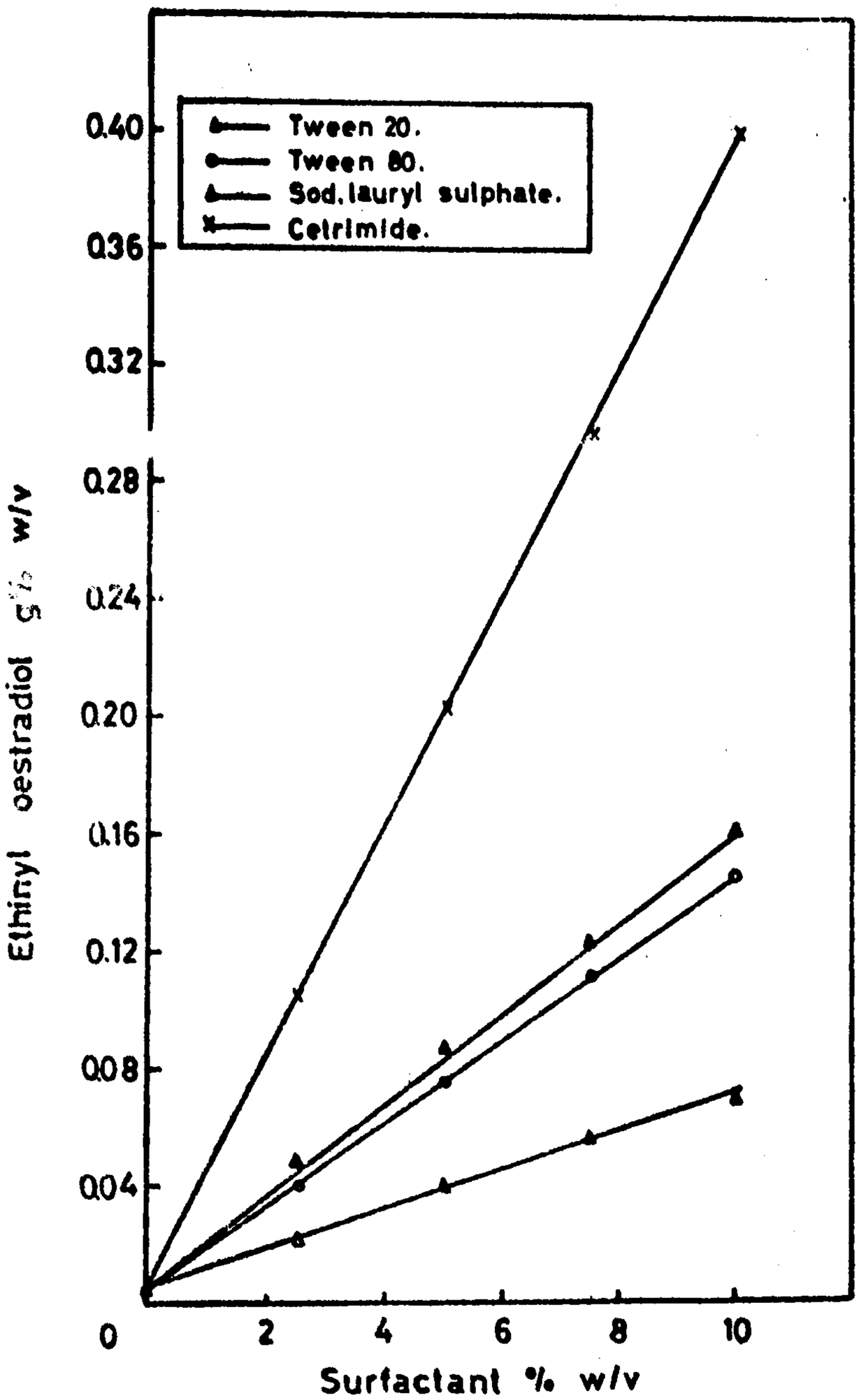

Fig.1 Effect of surfactant concentration on the solubility of Ethinyloestradiol at 25°.

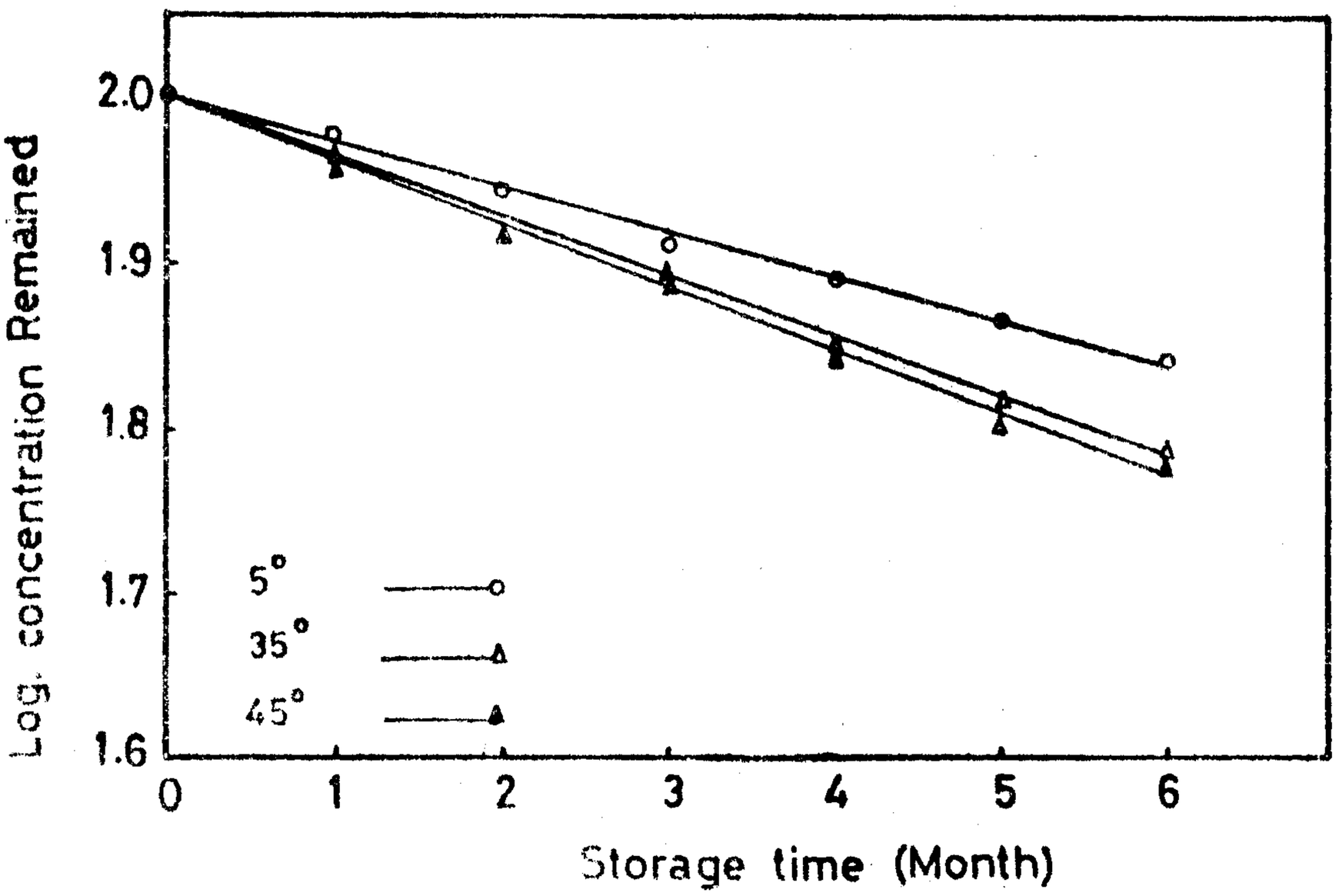

Fig 2 Effect of storage on the stability of Ethinyloes-
-tradiol in Tween 20 solution in colourless amp-
-oules at different temp.

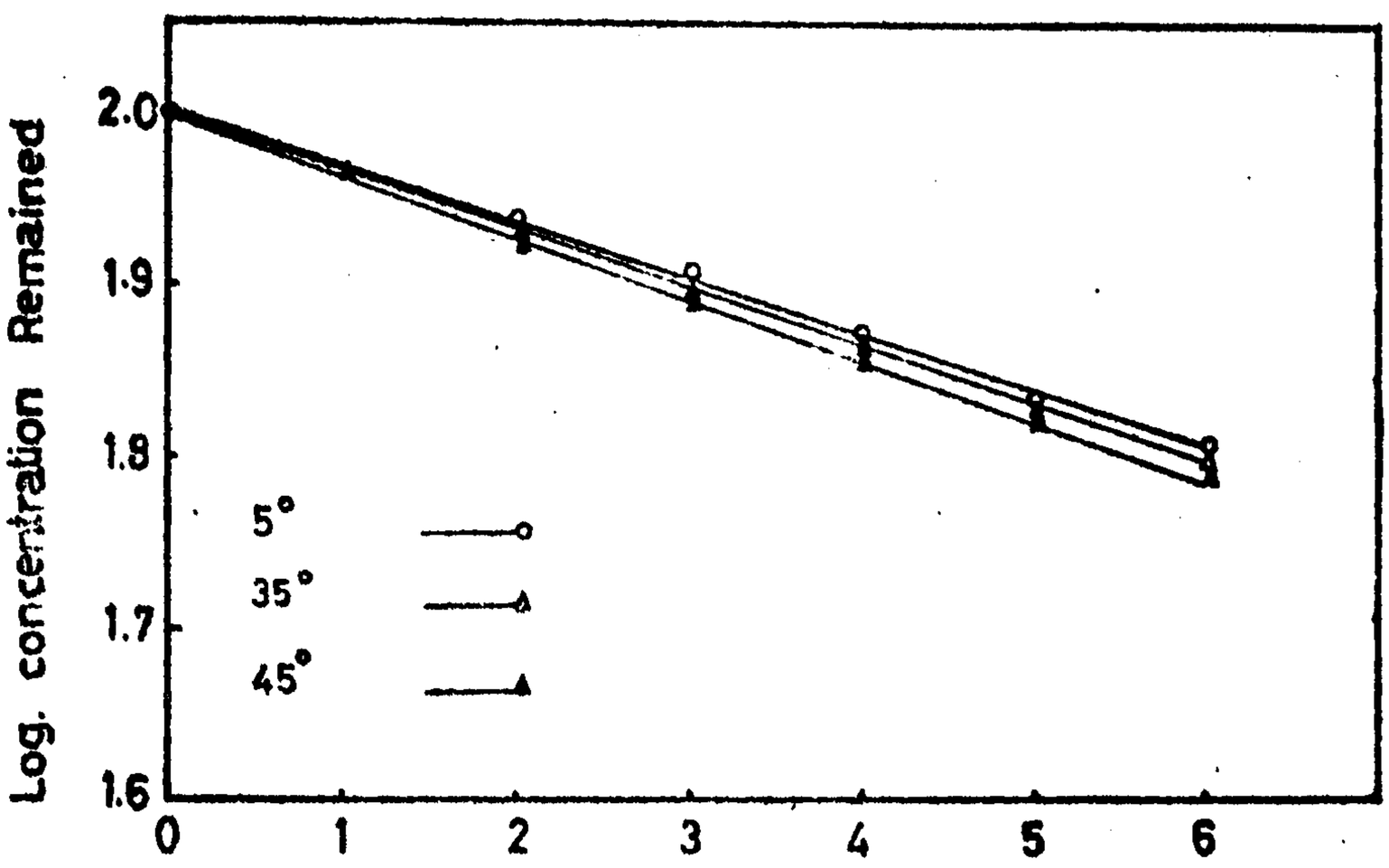

Fig. 3. Effect of storage on the stability of Ethinyloes--tradiol in Tween 80 solution in colourless amp--oules at different temp.

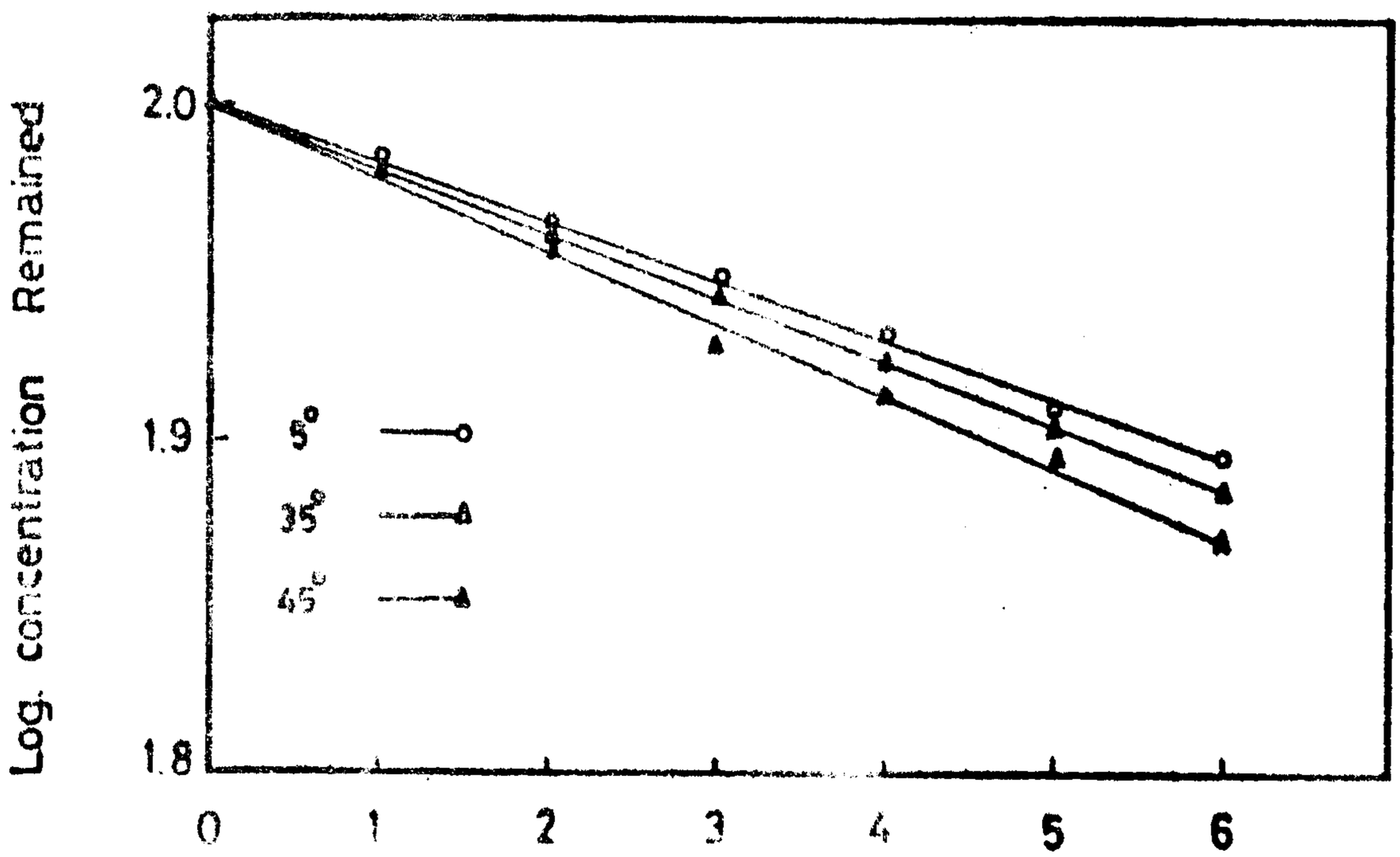

Fig. 4 Effect of storage on the stability of Ethinyl-oestradiol in sod.lauryl sulphate solution in colourless ampoules at different temp.

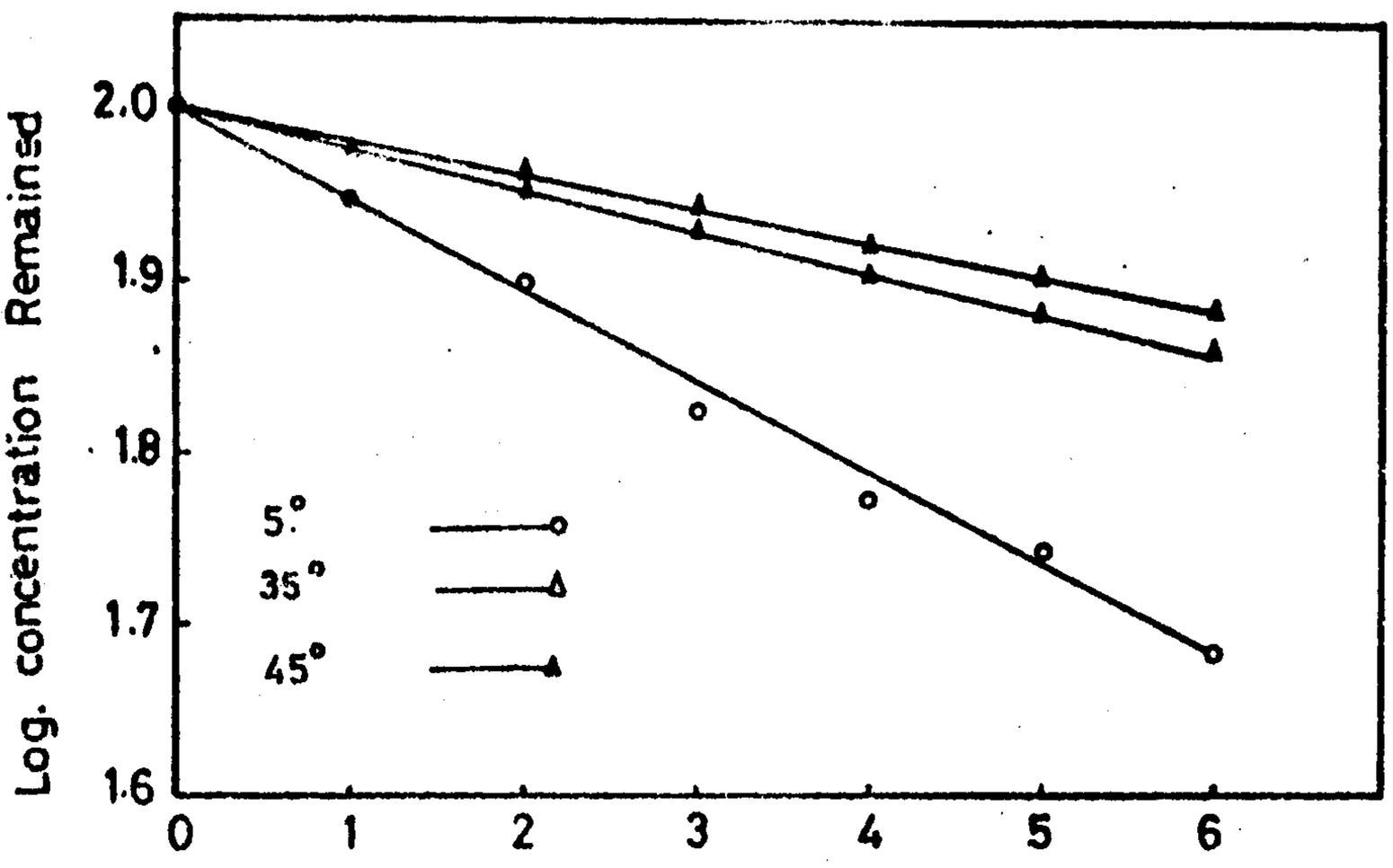

Fig. 5 Effect of storage on the stability of Ethinyl oestradiol in urea solution in colourless amp -oules at different temp.

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دراسة على اذابة وثبات اثينيل واستراديول

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اجريت محاولة لتحضير محاليل من اثينيل اوستراديول بواسطة مجموعة منتخبة من منشطات السطح •

وقد وجد ان منشطات السطح موجبة الشحنة لها تاثير اكثر فى الاذابة من سالبة الشحنة او المنشطات الغير متاينة •

تم فحص الانظمة المذابة فى المحاليل المحضرة تحت ظروف تخزين مختلفة • وقد لوحظ ان المحاليل المحضرة بواسطة المنشطات الغير متاينة تعطى مستحضرات اكثر ثباتا خاصة فى وجود بعض المواد المانعة للتاكسد والمواد الحافظة ، كذلك وجد انه من المستحب تخزين المستحضرات فى امبولات زجاجية عنبرية اللون عند د رجة ٥ د رجة مئوية حيث يحتفظ العقار بفاعليته لمدة ٦ شهور