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SOLUBILIZATION AND STABILITY OF CLONAZEPAM BY DIFFERENT CLASSES OF SURFACTANTS

A.P. Simonelli*, A.E. Aboutaleb** and A.A. Abdel-Rahman**
* School of Pharmacy, University of Connecticut, STORRS, CT 06268, U.S.A.,
** Department of Industrial Pharmacy, Faculty of Pharmacy, University of Assiut, Assiut, Egypt.

ABSTRACT

Clonazepam a 1,4-benzodiazepine derivative acting as tranquillizer and hypnotic is practically water insoluble. It was solubilized by three different classes of surfactant solutions at pH 5,6,7 and 8 and of ionic strength (U) of 0.2. Sodium dodecyl sulphate (SDS), cetyl trimethyl ammonium bromide (CTAB) and Brij 35 were chosen for this study, which was carried out at 25, 35 and 65°. Neither the presence of the surfactant solutions in the concentrations used, nor the presence of the degradation product (s) of clonazepam interfer in the spectrophotometric assay of the drug. At all the temperatures, and pH values investigated it was found that SDS solutions is the most efficient solubilizers for clonazepam followed by CTAB and Brij 35 solution respectively.

Accelerated stability study of clonazepam was carried out in different concentrations of the last mentioned solutions at 65°. The half life ($t_{\frac{1}{2}}$) of clonazepam was calculated in the prepared solutions and the longest $t_{\frac{1}{2}}$ for clonazepam is in SDS solution of pH 6.

The distribution coefficient (k_m) of clonazepam in the studied solutions was calculated from solubility and compared with the partition coefficient obtained from stabiltiy data ($P^@$).

INTRODUCTION

The formulation of parenteral drugs containing 1,4-benzodiazepines often involves problems concerning solubility and stability. 1,4-Benzodiazepines possess lipophillic characteristics and are almost insoluble in water; they also undergo hydrolytic degradation in aqueous solution[1-6]. Pain and thrombophlebitis occuring after interamuscular injection of benzodiazepine preparations are due to the high concentrations of organic co-solvents used in their formulation and/or precipitation of the drug at the injection site.[7-10] Detergents added to water in concentrations above the critical micelle concentration (cmc) form micelles in which lipophillic drugs can be considerably solubilized[11-16]. Solubilization often causes a change in the chemical reactivity of a drug and catalytic or inhibitory effects of detergents have been reported[14,17-23].

This study was undertaken to investigate the effect of detergents on the solubility of clonazepam in aqueous solutions of different pH values as well as their effect on stabilization of clonazepam against hydrolytic degradation.

EXPERIMENTAL

- Clonazepam was obtained in a pure grade[a]
- Surfactants: Polyoxyethylene 23 lauryl ether (Brij 35) obtained from a commercial source[b] was used without

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a- Hoffman-La Roche. New Jersey U.S.A.

b- ICI American Inc. Wilmington.

further purifieation. Cetyltrimethyl ammonium bromide[c] and sodium dodecyl sulphate[d] were purified by methods reported by Mysels[24] and Grunwald[25] respectively.

Reagents:

- Spectrograde N-Ndimethyl acetamide was obtained commercially[e].
- Sodium monobasic phosphate and sodium dibasic phosphate were supplied commercially also[f].
- Sodium chloride was used without further purification.
- Double distilled water was used throughout the entire study.

EXPERIMENTAL PROCEDURES

Solubility measurements:

Solutions containing varying concentrations of surfactant i.e., 2.5, 5, 7.5 and 10% w/v were adjusted to pH 5,6, 7, and 8 using phosphate buffer. The last solutions were adjusted also to ionic strength (μ) of 0.2 using sodium chloride. Stoppered tubes containing the solutions were equilibrated at 25, 35 and 65°C in a constant temperature water bath. After equilibration sufficient amount of solid clonazepam was added to each of the test tubes to ensure that an excess of solid of clonazepam will be present at equilibrium. The test tubes were shaken in an ultrasonifier at the desired constant temperature.

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c- Pflatz and Bauer Co., Connecticut.

d- Ruger Chemical Co., New Jersey.

e- Eastman Chemicals, New York.

f- Baker Analyzed Reagent, Phillipsburg, N.J.

After ultrasonification, the test tubes were incubated at the same temperature in the water bath for a fixed time period to allow the system to reach equilibrium which was checked by sampling at intervals. After equilibrium has been reached, a hypodermic adapter containing a millipore 3μ filter inside was first warmed to the temperature of the solubility study and used to obtain particles free samples of the solutions. It was then diluted with a solvent taking appropriate aliquot with microliter pippetts whose tips were warmed at the desired temperature to prevent precipitation.

The samples were analyzed spectrophotometrically in 304 nm.

It was noticed that the surfactants used in these dilutions used neither made any shift of the maximum absorbance of clonazepam nor they interfer with the spectrophotometric assay of the drug. The amount of the drug solubilized percent w/v was plotted versus surfactant concnetration to obtain solubility gram per gram from the slope. The distribution coefficients (K_m) of clonazepam between the micellar, phases and continuous aqueous phases were calculated.

Kinetic measurements:

- Preparation of solution:

Since clonazepam is relatively insoluble in water, stock solutions were prepared by dissolving the desired amount of the drug in spectrograde N,N-dimethylacetamide, kinetic studies were carried out over pH 5-8 using phosphate buffer.

Sodium chloride was added to maintain the ionic strength at 0.2. The pH values of the solutions were measured at room temperature with pH meter before and after the reaction.

For kinetic studies, reaction solutions of interest were mixed at different pH and incubated at 65° in a constant temperature water bath.

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Samples were withdrawn periodically and the extent of reaction determined by recording the U.V absorbance of these samples at 304 nm and determining the concentration of clonazepam using the previously determined Beer's law plot.

Sampling were taken until equilibrium was reached at a particular pH . It was determined that the degradation product of clonazepam did not interfer in the assay of the drug at different time intervals, nor any shift for maximum absorbance of clonazepam took place as seen from (Fig. 1)

Log concentration was plotted versus time for clonazepam hydrolysis study, and the first order rate constants were determined from the initial slope. $T_{\frac{1}{2}}$ and $P^{@}$ were calculated for clonazepam at each particular pH at 65^{o}.

RESULTS AND DISCUSSIONS

The solubility of clonazepam increases linearly with increasing surfactant concnentration (Fig. 2) indicating that micellar solubilization of clonazepam is the possible mechanism[11,13]. On comparing the solubility of clonazepam in different surfactant solutions of various pH values at different temperatures studies , (Table 1), it is obvious that a positive temperature effect was observed[11,12].

On comparing the solubilizing efficiency of all the studied surfactant solutions toward clonazepam at different temperatures and pH values, Table 1, it was found that the surfactants used was very efficient as solubilizers for clonazepam compared with the buffer solution alone. SDS has higher solubilizing efficiency toward clonazepam than CTAB and Brij 35 respectively except for CTAB solutions of pH 5 and 6 at 65^{o} which exceeds their analoges of SDS solutions.

From the study it is concluded that the solubilizers used succeed to solubilize the therapeutic dose of clonazepam

in the lowest concentration used in 5 ml of the solutions investigated. The distributions coefficient, Km, is the ratio between the quantity of clonazepam in the micellar phase to that present in the continuous phase. Comparing the K_m value for clonazepam in different surfactant solutions of different pH values, Table 3, it was observed that the k_m values have the same order as the solubilizing efficiency of the surfactant solutions used.

The hydrolysis of clonazepam in the prepared buffers as well as the surfactant solutions was found to be first order, the curvature appearing when plotting log concentration virsus time is related to the approach to equilibrium. Thus from the first order equation the hydrolysis rate constant and the half life ($t_{\frac{1}{2}}$) of clonazepam were calculated.

Table 2 and Fig. 3 illustrate the stability of clonazepam in buffer solutions adjusted to pH 5, 6,7 and 8 and having ionic strength of 0.2 at 65°. The $t_{\frac{1}{2}}$ values for clonazepam in different buffer solutions were calculated from the first order equation. It was found that the highest stability values for clonazepam in buffer solutions at 65° was obtained at pH 6 while the least stability was found at pH 8.

Fig. 4 and Table 2 illustrate the stability of clonazepam at SDS solutions of different pH values. It is clear that the longest $t_{\frac{1}{2}}$ for clonazepam at 65° was obtained by SDS solutions of pH 6 followed by that of pH 5, pH 7 and pH 8 respectively.

Comparing the $t_{\frac{1}{2}}$ of clonazepam in buffer solutions to that in SDS solutions of different pH values, it is obvious that solubilizing clonazepam in SDS solutions increases the stability of the drug because of the shielding of the drug inside the core of SDS micelles.

As for the effect of varying the concentration of SDS on the stability of clonazepam, it is concluded that, this depend upon the pH value of the studied solutions. 5% w/v SDS solution of pH 5 has the longest $t_{\frac{1}{2}}$ for clonazepam while

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7.5% w/v SDS solution of pH 6 and 7 have the longest $t_{\frac{1}{2}}$ for clonazepam comparatively. As for SDS solutions of pH 8, 10% w/v has the longest $t_{\frac{1}{2}}$ for clonazepam.

The stability of clonazepam in CTAB solutions of different pH values at 65° is shown in Table 2 and Fig. 5. Investigating the role of pH it is observed that CTAB solution of pH 5 has the longest $t_{\frac{1}{2}}$ for clonazepam among the other pH value investigated followed by those of pH 6,7 and 8 respectively. Comparing the stabilizing effect of CTAB solutions of different pH values to the buffer solutions of the same pH, it is clear that solubilizing the drug in CTAB solutions stabilize clonazepam than the buffer solutions alone. Comparing the stabilizing effect of CTAB to that of SDS, it is obvious that the latter causes more stabilizing effect than the former. This can be interpreted on the ionization theory[21,22].

Investigating the effect of varying the concentration of CTAB on the stability of clonazepam, it concluded that increasing the concentration of CTAB from 2.5 to 10% w/v leads to an increase in the $t_{\frac{1}{2}}$ for the drug in the different pH values except for solutions of pH 8 which show the longest $t_{\frac{1}{2}}$ in 5% w/v CTAB.

Table 2 and Fig 6 show the effect of Brij 35 solutions of different pH values on the stabilization of clonazepam at 65°.

Comparing the stabilizing effect of Brij 35 on clonazepam to the buffer solutions alone it is, obvious that Brij 35 stabilize the drug. As for the comparison between the surfactant solutions studied for the stability of clonazepam, it is concluded that SDS solutions have the highest stabilizing effect followed by Brij 35 and CTAB respectively . This may be interpreted on the drug ionisation bases, as at higher pH values 5,6,7 and 8, the drug may be protonated, repelled by the positively charged CTAB, strongly attracted by the negatively charged SDS, thus the drug would be entraped within SDS micelles and shielded from aqueous hydrolysis.

The effect of varying the pH of Brij 35 solutions for stabilizing clonazepam is illustrated in Table 2. It is observed that Brij 35 solution of pH 6 has more or less equal stabilizing effect to that of pH 5 followed by that of pH 7 and 8 respectively.

On studying the effect of varying the concentration of Brij 35 solution on the stability of clonazepam at different pH values, no regular pattern is concluded for the effect of concentration on the stability. This may be related to the nature of the surfactant as well as the complex reactions which may takes place between the components of the system. When k_w, the rate constant for clonazepam degradation in the buffer at 65°, is devided by K (obs), the rate constant for clonazepam degradation in surfactant solutions, and the resulting values plotted versus the surfactant concentration % w/v, the relation gives a linear relationships whose slope equals $P^{@}$ which is the partition coefficient of clonazepam in micellar and aqueous phases. $P^{@}$ calculated from kinetics has the same meaning as K_m, the distribution coefficient calculated from solubility Table 3 shows a comparison between $p^{@}$ and K_m values for the different surfactant solutions studied for their effects on stabilizing clonazepam. It is evident that always $k_m > p^{@}$ in all the investigated solutions that is because in K_m values the quantity of the drug in the core of the micelle as well as the capsule are effective, while in $P^{@}$ the quantity of the drug in the outer layer of the micelle, the capsule, may be hydrolyzed by time, leaving the other part in the core only effective in the measurements[21-23]

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Table 1: Solubility of clonazepam in various surfactants solutions of different pH values at different temperatures.

	Solubility of Clonazepam mg/g surfactant in											
PH	*Buffer alone*			*SDS*			*CTAB*			*Brij 35*		
	25°	*35°*	*65°*	*25°*	*35°*	*65°*	*25°*	*35°*	*65°*	*25°*	*35°*	*65°*
5	0.007	0.009	0.016	2.489	2.594	5.31	2.015	2.339	5.46	0.503	0.650	1.380
6	0.008	0.008	0.0166	2.368	2.465	3.58	1.982	2.398	4.33	0.556	0.656	1.42
7	0.006	0.009	0.0140	2.336	2.491	4.31	2.094	2.29	3.47	0.541	0.598	1.27
8	0.007	0.010	0.029	2.343	2.59	3.19	2.137	2.50	2.38	0.535	0.658	1.14

Table 2: Stability of clonazepam in different surfactants concentration of different pH values at 65°

	$t_{\frac{1}{2}}$ *(days) of clonazepam in different surfactant conc.%w/v*											
pH	*buffer*	*SDS*				*CTAB*				*Brij 35*			
	alone	*2.5*	*5.0*	*7.5*	*10*	*2.5*	*5.0*	*7.5*	*10*	*2.5*	*5.0*	*7.5*	*10*
5	14.4	51.7	66.0	38.9	54.5	22.6	32.8	30.5	45.0	66.0	37.2	31.7	24.9
6	19.3	73.2	53.7	74.1	40.8	8.3	12.6	13.5	14.7	41.2	38.2	30.5	52.9
7	4.3	20.5	16.5	39.1	39.1	6.6	7.0	7.3	9.0	18.7	9.2	9.3	8.1
8	4.5	8.5	6.5	8.0	10.7	5.7	6.3	5.0	4.0	4.8	4.6	4.1	4.3

Table 3: Comparison between distribution coefficient (K_m) obtained from solubility and parition coefficient ($P^@$) obtained from stability for Clonazepam at 65°

	K_m *and* $P^@$ *for Clonazepam in different surfactant solutions*					
pH	*SDS*		*CTAB*		*Brij 35*	
	K_m	$P^@$	K_m	$P^@$	K_m	$P^@$
5	3.23	0.43	3.32	0.20	0.83	--
6	2.15	0.42	2.60	0.04	0.85	0.46
7	3.07	0.57	2.47	0.07	0.907	0.014
8	1.10	0.18	0.82	0.05	0.39	0.02

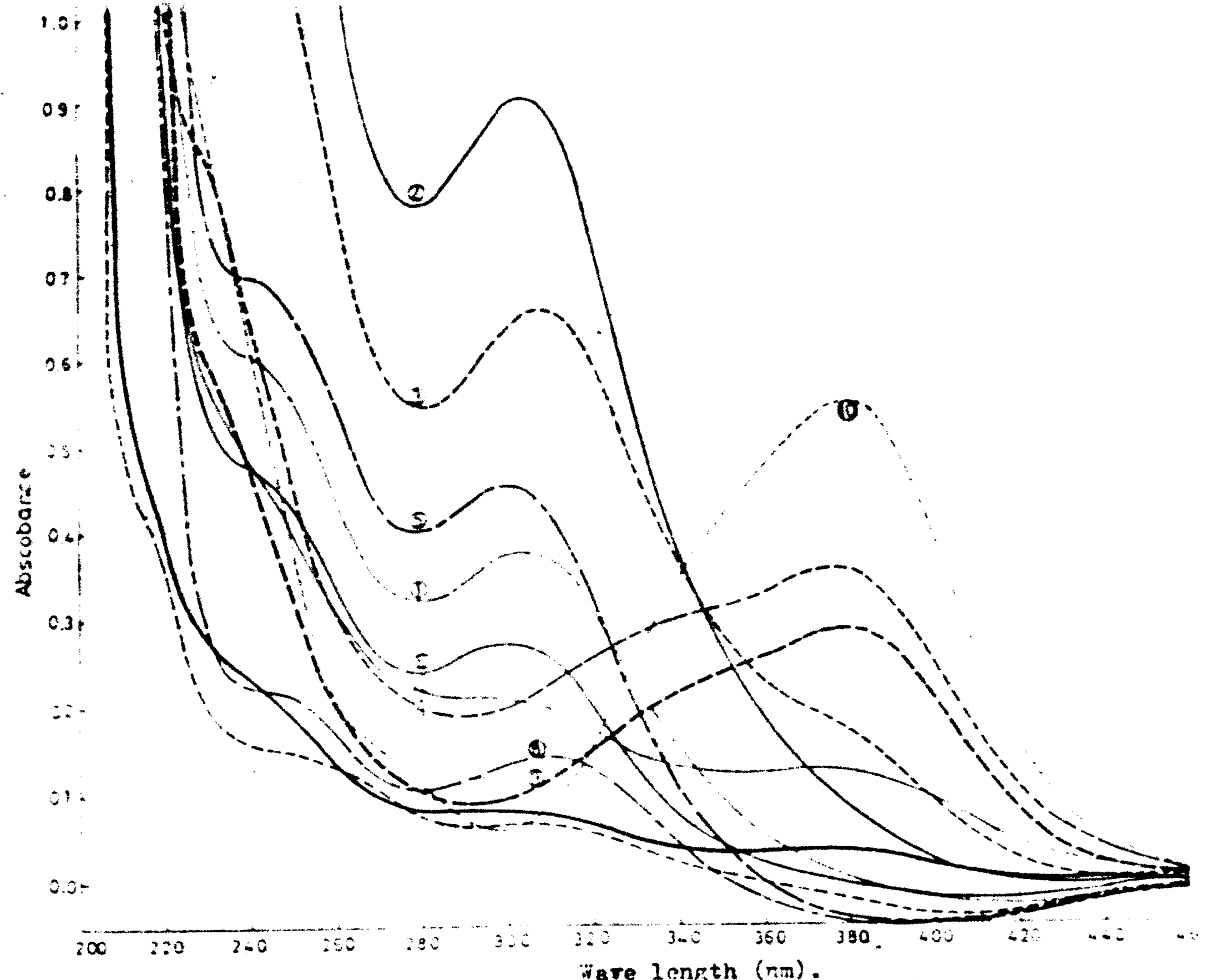

Fig. (1): Ultraviolet absorbance of Clonazepam and its degradation product(s) in different surfactant solutions of different pH values, stored for 4 days at 65C.

Key: (1) distilled water (2) Brij 35 solution of pH (3) Brij 35 solution of pH 7
(4) Brij 35 solution of pH 8 (5) SDS solution of pH 5 (6) SDS solution of pH 7
(7) SDS solution of pH 8 (8) CTAB solution of pH 5 (9) CTAB solution of pH 7
(10) CTAB solution of pH 8

Fig. 2: Solubilization of clonazepam in different surfactants concentrations of pH 5 at 25°C .

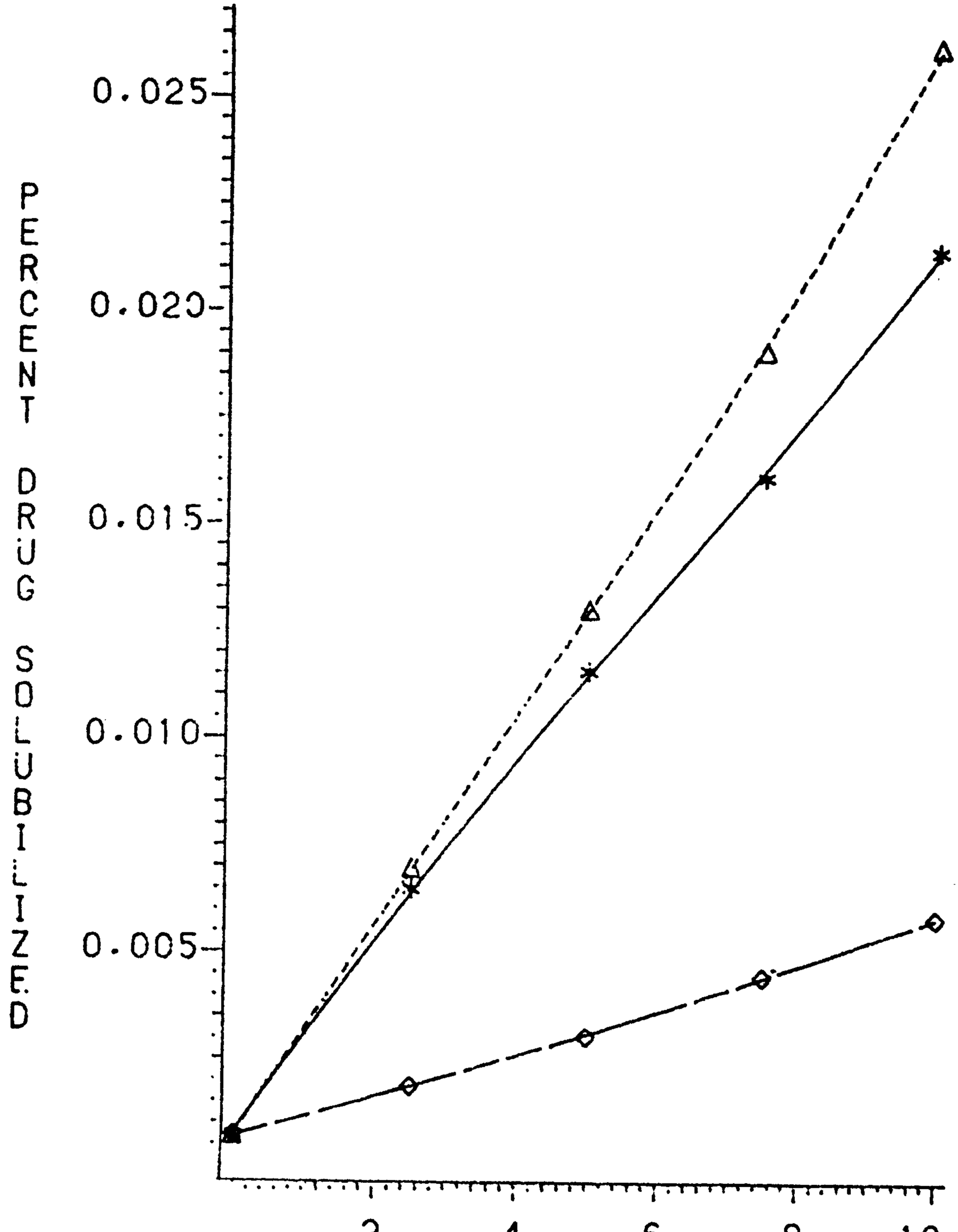

Fig. 3: Stability of clonazepam buffer solutions of pH 5.6,7 & 8 at 65°C

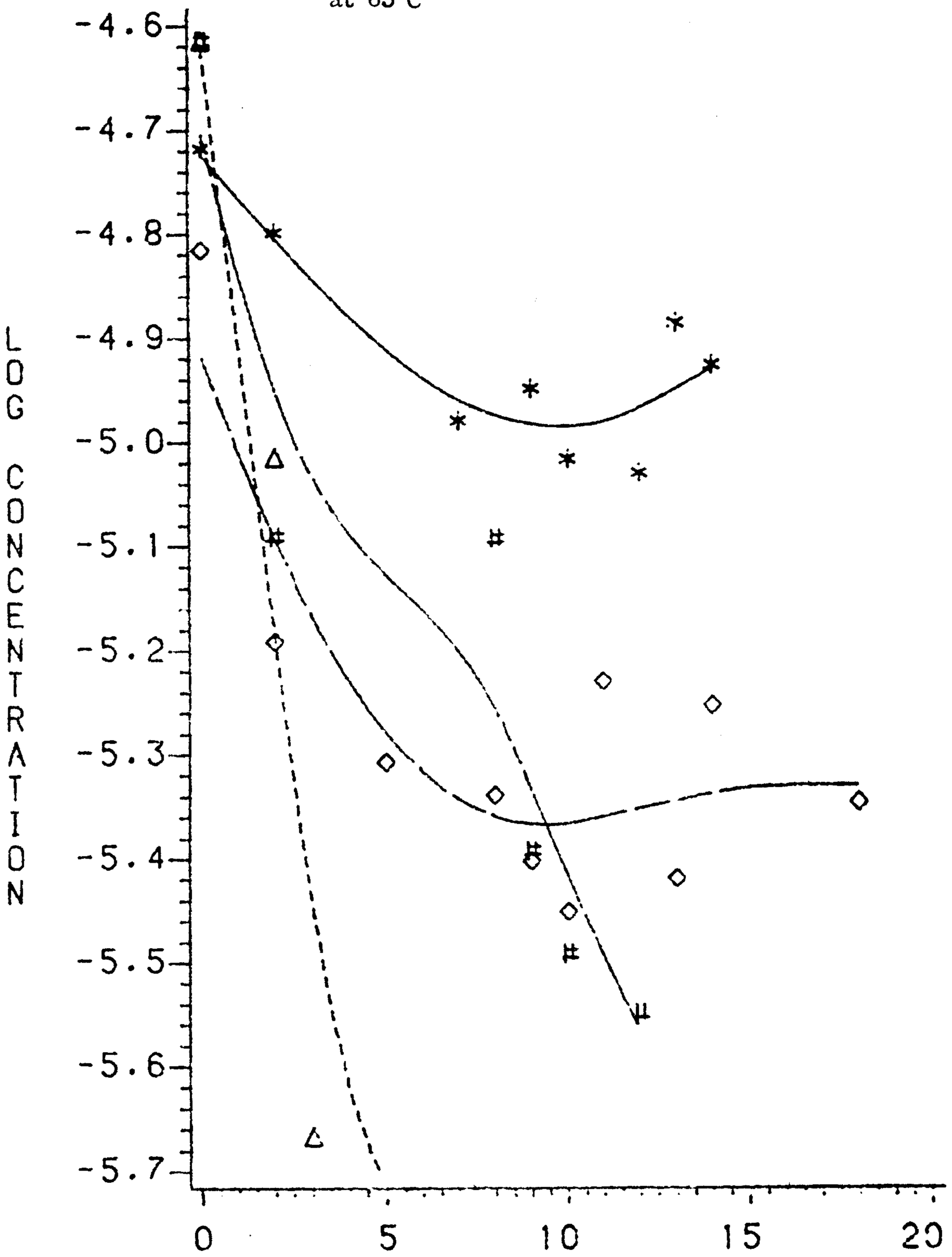

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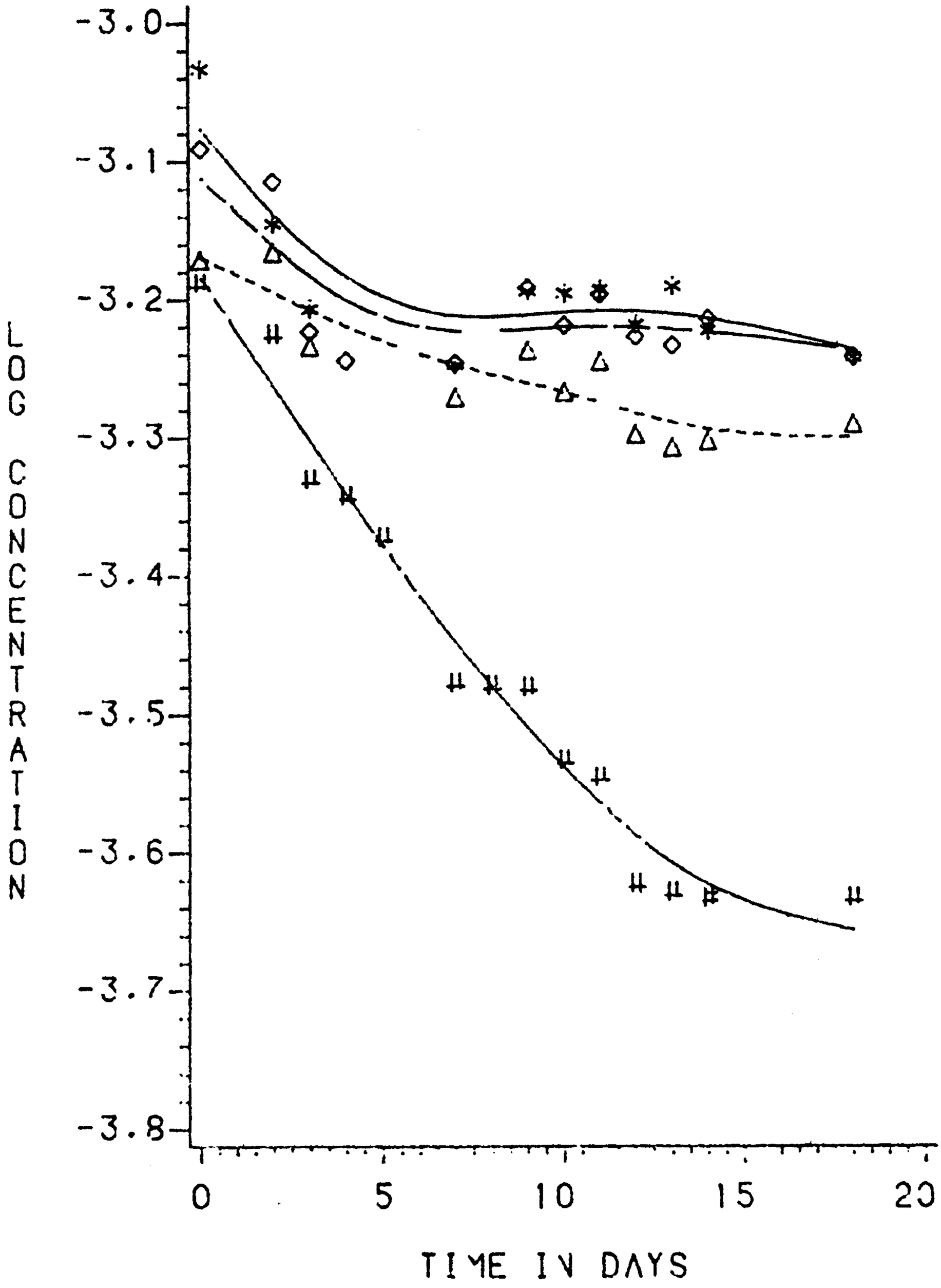
Fig

Fig. 4: Stability of clonazepam in 10% w/v SDS solutions of pH 5,6,7 & 8 at 65°C



CLONAZEPAM STABILITY

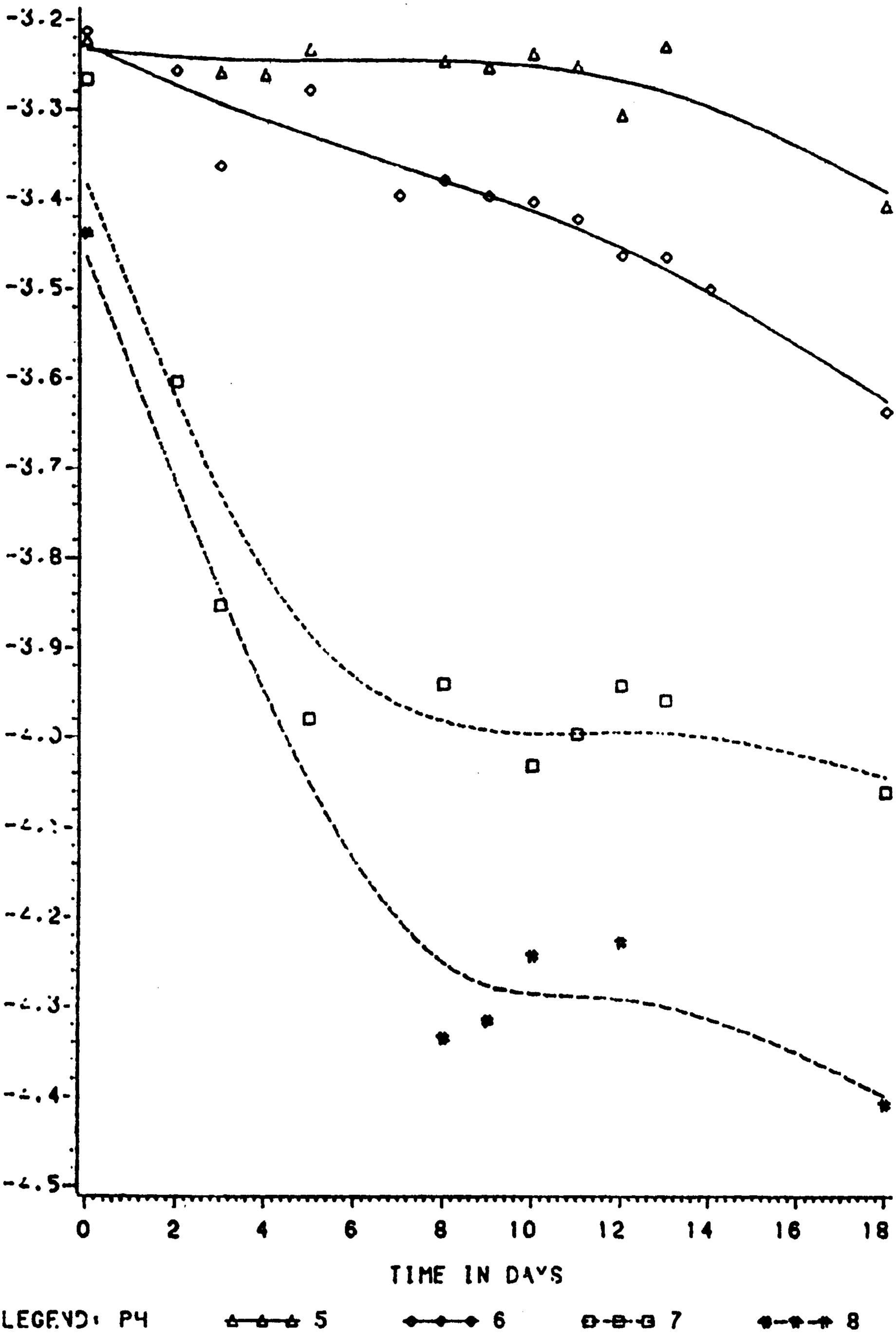
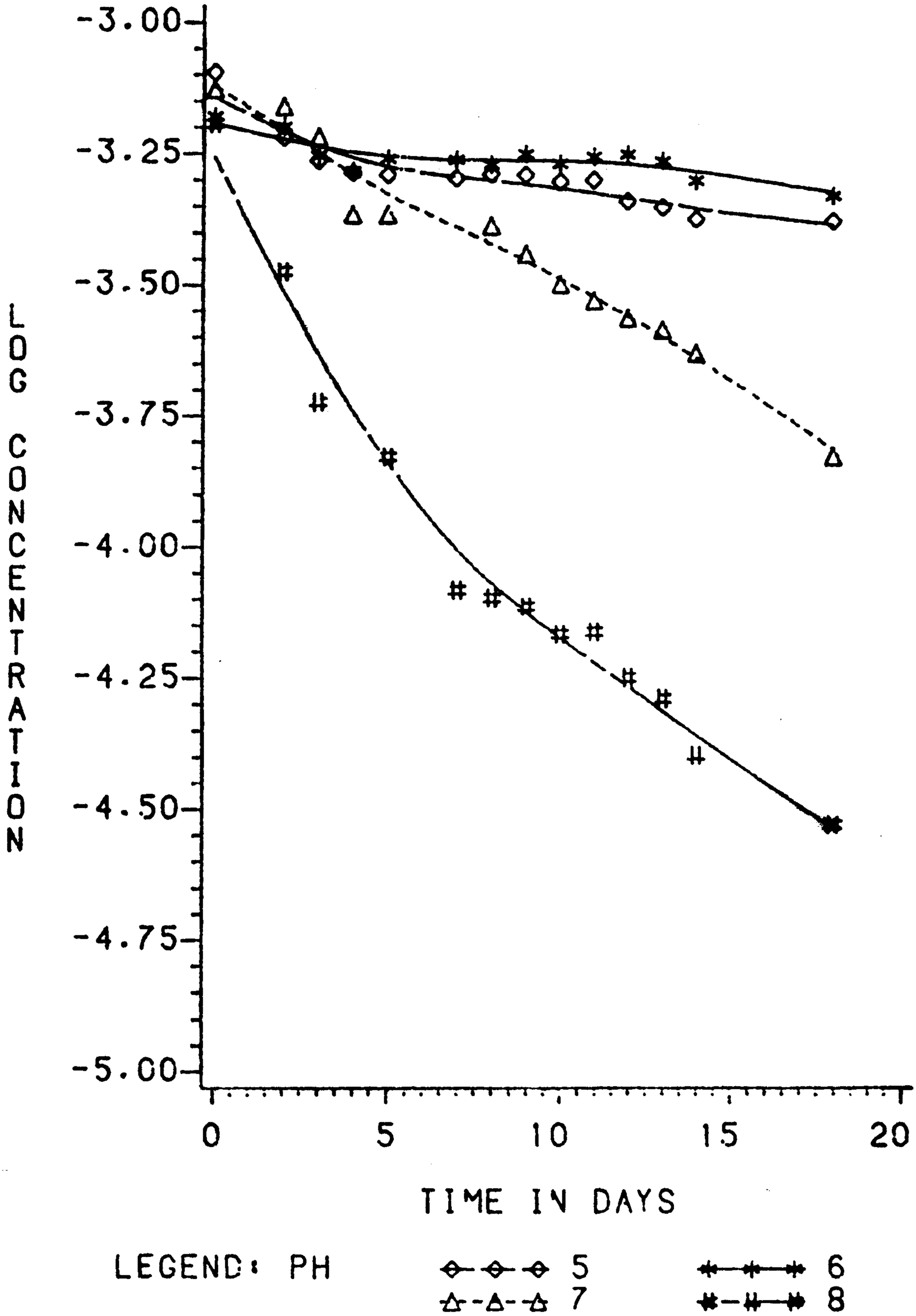


Fig. 5: Stability of clonazepam in 10% w/v CTAB solutions of pH 5,6,7 & 8 at 65°C.

Fig. 6: Stability of clonazepam in 10% w/v BRIJ35 solutions of pH 5,6,7 & 8 at 65°C.



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تذويب وثبات الكلونازيبام فى مختلف لوائف منشطات السطح

أنتونى سيمونيللى - أحمد السيد أبو طالب - على عبد الظاهر عبد الرحمن

كلية الصيدله جامعة كونكتيكت بالويلايات المتحده الأمريكيه

كلية الصيدله - جامعة أسيوط .. مصر

الكلونازيبام - أحد مشتقات ١ ،٤ - بنزوديبازيبنيات والذى له أثر طبى مطمئن وكذلك منوم لايذوب فى الماء .

ولقد أذيب هذا العقار بثلاث طوائف من منشطات السطح المختلفه فى أسى أيدروجينى مقداره ٥ ،٦ ، ٧ ، ٨ وكذلك تركيز أيونى مقداره ٠ر٠٢ . ولذلك أختير كل من دودكيل سلفات الصوديوم والستيل تراى ميثل أمونيوم بروميد والبرج ٣٥ لانجاز تلك الدراسه فى درجات حراره مقدارها ٢٥ ، ٣٥ ، ٦٥ درجه مئويه .

ولقد وجد أنه لاتداخل فى مقياس تركيز العقار بالأشعة فوق البنفسجيه بوجود منشطات السطح فى التركيزات المستعمله وكذلك أيضا فى وجود تحللات العقار نفسه لم يلاحظ أى تداخل بالمره . وفى كل درجات الحراره التى درست وأيضا فى كل قيم الأس الأيدروجينى وجد أن دودكيل سلفات الصوديوم هو أقوى مذيب للعقار متبوعا بالستيل تراى ميثل أمونيوم بروميد ويأتى فى المؤخره البرج ٣٥ .

ولقد طبقت دراسة الثبات بالطريقة السريعه لدراسة ثبات الكلونازيبام فى مختلف تركيزات منشطات السطح السابقه عند حراره ٦٥° مئوى .

ولقد حسب نصف العمر للعقار فى المحاليل المحضره ووجد أن أطول نصف عمر للعقار فى محلول دودكيل سلفات الصوديوم ذا أس أيدروجينى مقداره ٦

ولقد قورن معدل التوزيع المحسوب من الأذابه بمعدل التجزئ المحسوب من دراسة الثبات للكلونازيبام .

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