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# SOLUBILIZATION AND STABILITY OF PYRIDINE-3-CARBOXALDHYDE THIOSEMICARBAZONE BY DIFFERENT MICELLAR FORMING MATERIALS

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## ABSTRACT

*Pyridine-3-Carboxaldhyde thiosemicarbazone(P3CTZ) used in cancer therapy was solubilized by anionic cationic and nonionic surfactant solutions of pH 5,6, 7 , 8 and ionic strength (u) of 0.2 at 47. So sodium dodecyl sulphate (SDS), cetyl trimethyl ammonium bromide (CTAB) and Brij 35 were chosen to represent the last three classes respectively. It was found that SDS has the highest solubilizing efficiency toward P3CTZ followed by Brij 35 and CTAB respectively at all pH values studied.*

*The stability of P3CTZ was studied in different concentrations of surfactants at pH values of 5,6, 7 and8 and u of 0.2.*

*It was found that the longest $t_{\frac{1}{2}}$ observed was in Brij 35 solution of pH 5 at 47° (nearly $t_{\frac{1}{2}}$ equal one year). The shortest $t_{\frac{1}{2}}$ was found in CTAB solutions. It was found also that the partition coefficient from solubility slightly higher than that obtained from stability measurments.*

*It was concluded that the stability of P3CTZ is greater relatively at higher pH values, than at lower pH values as reported by other workers[1].*

## INTRODUCTION

Much of the reported work in the micellar systems has been performed on the hydrolysis of esters catalyzed by hydroxyl ions.

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There are very few reports on reactions involving two reactants and hydrogen ion catalyzed hydrolysis. The investigation of bimolecular reactions can be important as the micelles can inhibit or catalyze the reactions either by bringing the reactants intimately together by incorporating both into a small volume element, or keeping them apart by incorporating one reactant and rejecting the other.

Recently, a multi-plot approach was derived and successfully applied to data previously reported in the literature and to sulphonyl floride hydrolysis[2]. This approach permitted all parameters to be determined from kinetic data which were found to be more consistent and yielded micellar rate constants which were positive.

Ifudu and Simonelli[2] found that electrostatic theory was not applicable to the sulfonyl floride system which was explained on the basis that the substrates were submerged below the surface of the micelle.

Wong and Simonelli[3] found that the hydrolysis and formation of thiosemicarbazones[4-11] in acidic pH give the corresponding aldehyde and thiosemicarbazide as end products[1].

The micellar sits of an orthoester[12] solubilized is considered to be the stern layer of the micelle and, hence, the rate of acceleration in the acid catalyzed hydrolysis can be attributed to electrostatic stabilization of the developing carbonium ion. A considered amount of experimental evidence has been presented in support to this interpretation[13].

The effect of SDS on general acid catalyzed hydrolysis of para-substituted benzaldhyde diethyl acetals is similar in many respects to that of the hydrolysis of para-substituted methyl orthobenzoate[14,15]. The second order rate constants

for hydrolysis of these acetals were found to increase with increasing anionic surfactant concentrations up to approximately 0.036M and to decrease slightly at higher concentrations.

The effect of micelles on the hydrolysis of 2-methoxy-3-methyl benzoic acid is analogous to that observed for other acetals. At pH 2, SDS enhanced the rate markedly while the influence of cationic micellar CTAB and uncharged Igepal was significant[16].

Hydrolysis of para-substituted tetrahydropyrans were also affected in aqueous micelles[15,17]. Micellar SDS and hexadecyloxyethyl sulphate enhanced the hydrolysis rate 15 to 50 fold. In contrast, both non-ionic and zwitterionic surfactants inhibited the hydrolysis rate[4,18].

Studies on the micellar effects on reaction rates were extended recently by Ifudu[19].

The present work represents a trial to solubilize the water insoluble drug P3CTZ, then studying the effect of the solubilizers on the stability of this unstable drug in solutions of pH 5,6,7 and 8.

**Experimental Materials:**

Pyridine-3-carboxaldhyde thiosemicarbazone(a), Polyoxyethylene[23] lauryl ether (Brij 35)(b) obtained commercially. Cetyl trimethyl ammonium bromide(c) and sodium dodecyl sulphate(d) were further purified by methods reported by Mysels[20] and Grunwald[21] respectively.

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a) Aldrich Chemical Co., Wisconsin. U.S.A.
b) ICI American Inc.Wilmington.
c) Pflatz and Bauer Co., Connecticut U.S.A.
d) Ruger Chemical Co., New Jersey.

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**Reagents:**

Spectrograde N-Ndimethyl acetamide[e].
Sodium monobasic phosphate[f], sodium dibasic phosphate[f]. Sodium chloride, and Double distilled water were used throughout the entire study.

**Experimental Procedures:**

**Solubility measurements:**

Solution containing various concentrations of surfactant i.e., 2.5, 5 and 7.5 w/v were adjusted to pH 5,6,7 and 8 using phosphate buffer. The last solutions were then adjusted to ionic strength (u) of 0.2 using sodium chloride. Stoppered tubes containing the solutions were equilibrated at 47°C in a constant temperature water bath. After equilibration sufficient amount of solid P3CTZ was added to each of the test tubes to ensure that an excess of solid of P3CTZ will be present at equilibrium. The test tubes were shaken in an ultrasonifier at the desired constant temperature. 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 (3u) 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 pipetts whose tips were warmed at the desired temperature to prevent precipitation.

The samples were analyzed spectrophotometrically* at 304 nm. It was noticed that the surfactants solutions at the dilutions

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e) Estman Chemicals, New York.
f) Baker Analyzed Reagent, Phillipsburg,
*) Self-recording Pye-Unicam spectrophotometer.

used neither made any shift of the maximum absorbance of P3CTZ nor they interfer with the spectrophotometric assay of the drug[3-11]. The amount of the drug solubilized percent w/v was plotted virsus surfactant concentration to obtain solubility gram per gram from the slope. The distribution coefficient ($k_m$) of P3CTZ between the micellar phase and continuous aqueous phase was calculated.

**Kinetic measurements:**

- Preparation of solution:

Since P3CTZ 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 47°C in a constant temperature water bath.

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 $P_3$CTZ using the previously determined Beer's law plot.

Samples were analyzed after equilibrium was reached at a particular pH and temperature. It was determined that the degradation products of P3CTZ did not interfer in the assay of the drug at different time intervals, nor any shift for

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** Digital Pye Unicam pH meter

maximum absorbance of P3CTZ took place[3-11].

Log concentration was plotted versus time for P3CTZ hydrolysis study, and first order rate constants were determined from the slope. Also $t_{\frac{1}{2}}$ and $P^{@}$ were calculated for P3CTZ at each pH for each particular temperature.

## RESULTS AND DISCUSSION

The solubility of P3CTZ in the different surfactant solutions studied of pH 5,6,7, and 8 at 47°C increased linearly with increasing surfactant concentrations. The systems investigated were always one liquid plus solid which repesent true micellar solubilization of this drug[22]. Also this linearity indicates that the solubility of P3CTZ obeys the partition model of solubilization proposed by McBain and Hutchinson,[22] indicating that P3CTZ was distributed between continuous aqueous phase and a pseudo-organic phase which is the micellar phase[23,24]. From the linearity of the solubility isotherms, the slope, which represents the solubility of the drug gram per gram surfactant, was calculated.

From Table 1, it is concluded that those non-ionic surfactant solutions of pH 8 have the highest solubilizing efficiency toward P3CTZ followed by those of pH 5, pH 6 and pH 7 respectively. This may be attributed to the pka value of the drug at different pH values investigated. Among the surfactant solutions investigated, it was found (Table 1) that SDS has the highest solubilizing efficiency toward P3CTZ followed by Brij 35 and CTAB respectively , except for CTAB solution of pH 5 which deviates . The different affinities of those

differently charged micelles to incorporate P3CTZ may be attributed to the drug ionization properties at the different pH values investigated.

The $K_m$ value, the distribution coefficient, denotes the ratio of P3CTZ in the micellar phase to that in the aqueous phase. From Table 1, it is evident that the $K_m$ values for P3CTZ in the different micellar forming materials has the same order as the solubilizing efficiency , that is because nearly the solubility of P3CTZ in the studied buffer solutions is the same, thus causing no change in the $K_m$ values.

The hydrolysis of P3CTZ at the solutions studied was found to be first order (Fig. 1-3 are included as a model). Thus from the first order equation the hydrolysis rate constant and the half life ($t_{\frac{1}{2}}$) of P3CTZ in each studied solution were calculated.

Table 2 illustrates the stability of P3CTZ, expressed as $t_{\frac{1}{2}}$ in days, in all the studied solutions of pH 5,6,7 and 8 at 47°C. Buffer solution of pH 5 has the highest $t_{\frac{1}{2}}$ for P3CTZ followed by pH 7, pH 6 and pH 8 respectively. Comparing the effect of pH of CTAB solutions on the stability of P3CTZ as seen from Table 2 and Figs. 1-3 it is obvious that CTAB solutions of pH 5 has the highest $t_{\frac{1}{2}}$ value for P3CTZ followed by pH 6, pH 7 and pH 8 respectively

Comparing the effect of CTAB solutions at different pH values on the stability of P3CTZ with buffer solutions alone, it is clear that at pH 5, pH7 and pH 8 P3CTZ is more stable in buffer solution alone while the contrary was found at pH 7. The effect of different concentrations of CTAB solutions on the stability of P3CTZ at pH 5,6,7 and 8 is shown in

Table 2. Generally, increasing CTAB concentration at pH 5 and pH 6 leads to P3CTZ stabilization, while at pH 7 increasing CTAB concentration results in destabilization of P3CTZ. In CTAB solutions of pH 8 no significant effect of varying the cocnentration is observed.

The effect of SDS solutions of pH 5,6,7 and 8 on the stability of P3CTZ is shown in Table 2. At each individual pH, SDS solutions have higher stabilizing effect for P3CTZ than the buffer solutions of the same pH. Comparing the stability of P3CTZ in SDS solutions at each pH value, it is clear that SDS solution of pH 5 has the highest stabilizing effect for P3CTZ followed by that of pH 6, pH 7 and pH 8 respectively. Except for 2.5% w/v SDS solution of pH 6 which deviates. Comparing the stabilizing effect of SDS and CTAB solutions for P3CTZ at each individual pH, generally SDS has more stabilizing effect for P3CTZ specially at pH 7 and pH 8. Increasing the SDS concentration from 2.5% w/v to 7.5% w/v has an effect on the stability of P3CTZ at each individual pH. Increasing concentraion of SDS solutions of pH 5 leads to decreased stability of P3CTZ, and the opposite situation was found at pH 6. As for SDS solution of pH 7 and 8 increasing the concentration from 2.5 to 5% w/v leads to increased stability of P3CTZ then the stability falls at 7.5% w/v[16].

The effect of Brij 35 solutions on the stability of P3CTZ is shown in Table 2. It is obvious that Brij 35 has the highest stabilizing effect for P3CTZ at all the pH values studied. The half life ($t_{\frac{1}{2}}$) of P3CTZ in Brij 35 solutions of PH 5 nearly equals one year at 47°C. Investigating the role of pH on the stability of P3CTZ at Brij 35 solutions it

is obvious that the solutions of pH 5 has the highest stabilizing effect followed by those of pH 7, pH 6 and pH 8 respectively. No regular pattern is observed for the effect of concentration of Brij 35 solutions on the stability of P3CTZ

When $K_w$, the hydrolysis constant for P3CTZ in water is divided by K (obs), the hydrolysis constant for P3CTZ in surfactant solution, and the resulting value plotted virsus surfactnat concentration % w/v, the relation gives a linear plot whose slope equals $P^@$ which is the partition coefficient of P3CTZ between micellar and aqueous phases. $P^@$ calculated from kinetics has the same meaning as $K_m$ calculated from solubility.

Table 3 shows a comparison between $P^@$ and $K_m$ at different pH values at $47^oC$ . In all surfactant solutions studied of different pH values $K_m$ value is higher than $P^@$, that is because in solubility there are two loci of P3CTZ solubilization, while in stability, the relatively stable P3CTZ would be only inside the core of the micelle.

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Table 1: Solubilization of P3CTZ in different surfactant solutions of pH 5, 6, 7, and 8 and ionic strength 0.2 at 47°C.

| pH | Buffer alone Solubility g/g* | SDS Solubility g/g* | $K_m$ | CTAB Solubility g/g* | $K_m$ | Brij 35 Solubility g/g* | $K_m$ |
|---|---|---|---|---|---|---|---|
| 5 | 0.001 | 0.0251 | 0.237 | 0.0134 | 0.126 | 0.0128 | 0.120 |
| 6 | 0.0009 | 0.0237 | 0.263 | 0.0129 | 0.143 | 0.0144 | 0.159 |
| 7 | 0.0009 | 0.0230 | 0.248 | 0.0133 | 0.143 | 0.0142 | 0.153 |
| 8 | 0.0009 | 0.0262 | 0.279 | 0.0146 | 0.155 | 0.0157 | 0.167 |

* g/g: gram/gram.

Table 2: Stability of P3CTZ by surfactant solutions of pH 5,6,7 and 8 and ionic strength of 0.2 at 47°C.

| | $t_{\frac{1}{2}}$ (days) of P3CTZ in diff. Conc. of Surfactant solutions w/v | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| pH | Buffer Solutions Alone | SDS 2.5 | SDS 5.0 | SDS 7.5 | SDS 2.5 | SDS 5.0 | SDS 7.5 | Brij 35 2.5 | Brij 35 5.0 | Brij 35 7.5 |
| 5 | 126.4 | 105.0 | 133.2 | 105.0 | 157.5 | 11.7 | 108.2 | 364.7 | 135.8 | 364.7 |
| 6 | 71.6 | 105.0 | 97.6 | 100.4 | 70.0 | 161.1 | 177.6 | 121.5 | 161.1 | 126.0 |
| 7 | 75.3 | 26.4 | 22.0 | 18.9 | 88.8 | 117.4 | 105.0 | 161.1 | 178.6 | 161.1 |
| 8 | 52.9 | 7.2 | 7.9 | 7.0 | 58.2 | 81.5 | 77.8 | 73.7 | 63.0 | 73.7 |

Table 3: Comparison between distribution coefficient ($K_m$) obtained from solubility and partition coefficient ($P^@$) obtained from stability for P3CTZ at different pH values at 47°C.

| Surfactant | $K_m$ $pH_5$ | $K_m$ $pH_6$ | $K_m$ $pH_7$ | $K_m$ $pH_8$ | $P^@$ $pH_5$ | $P^@$ $pH_6$ | $P^@$ $pH_7$ | $P^@$ $pH_8$ |
|---|---|---|---|---|---|---|---|---|
| SDS | 0.237 | 0.263 | 0.248 | 0.279 | 0.089 | 0.092 | 0.094 | 0.176 |
| CTAB | 0.126 | 0.143 | 0.143 | 0.155 | 0.088 | 0.016 | — | 0.0048 |
| Brij 35 | 0.120 | 0.159 | 0.153 | 0.167 | 0.115 | 0.120 | 0.130 | 0.097 |

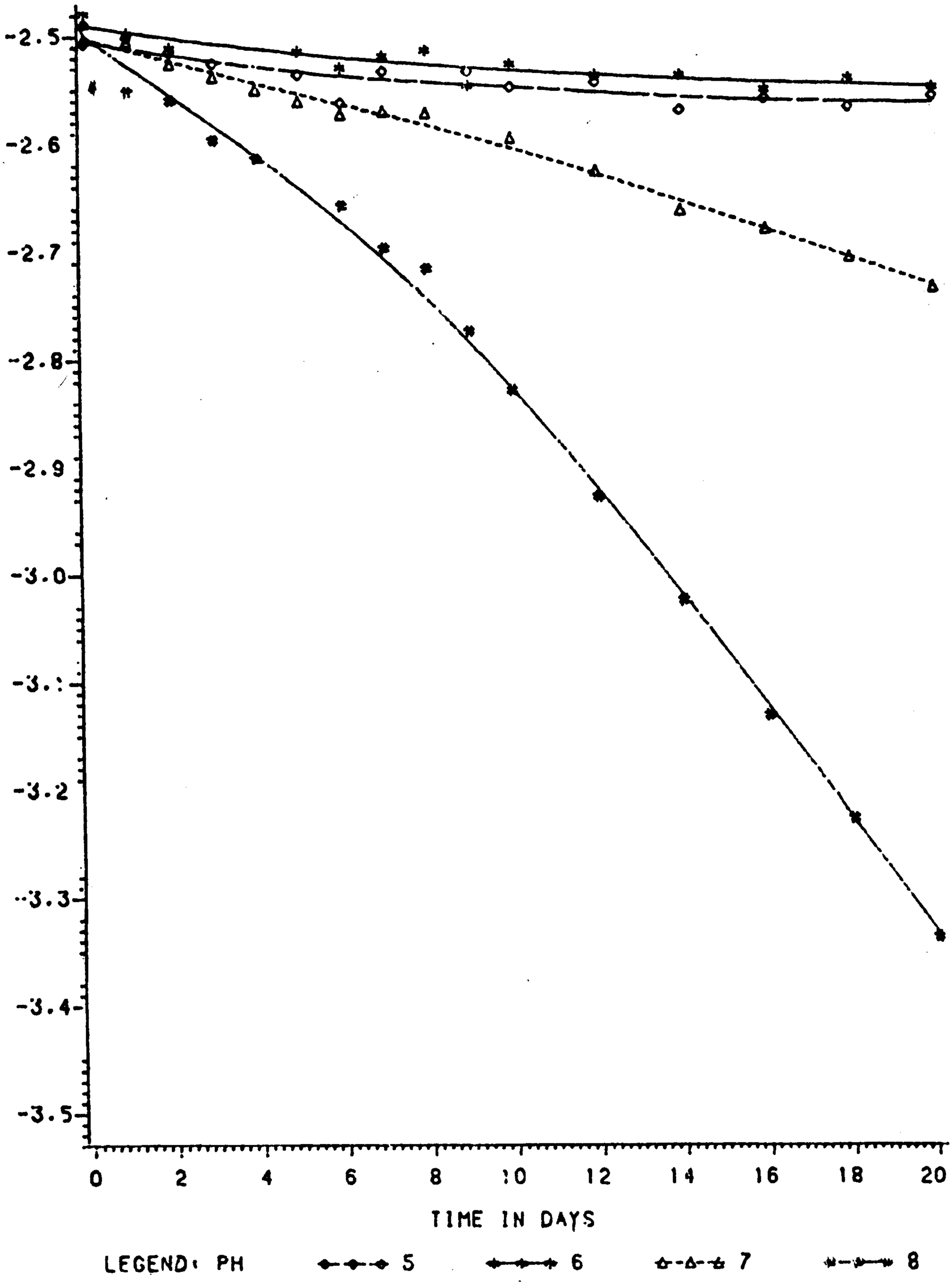


Fig. 1: Stability of P3CTZ in 2.5 w/v CTAB solutions of pH 5,6,7 and 8 at 47°C.

# P3CTZ STABILITY

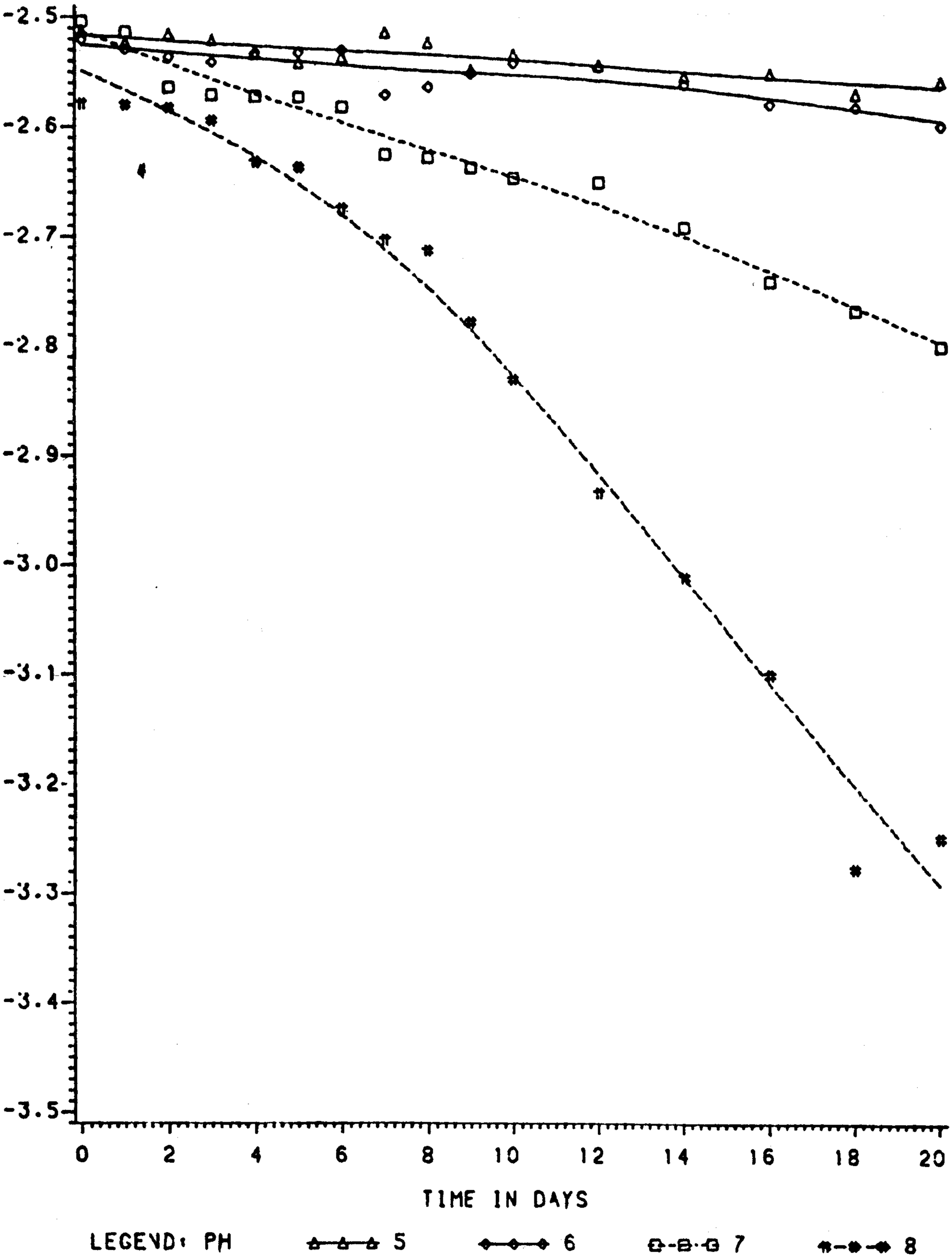


Fig. 2: Satbility of P3CTZ in 5 w/v CTAB solutions of pH 5,6,7 and 8 at 47°C.

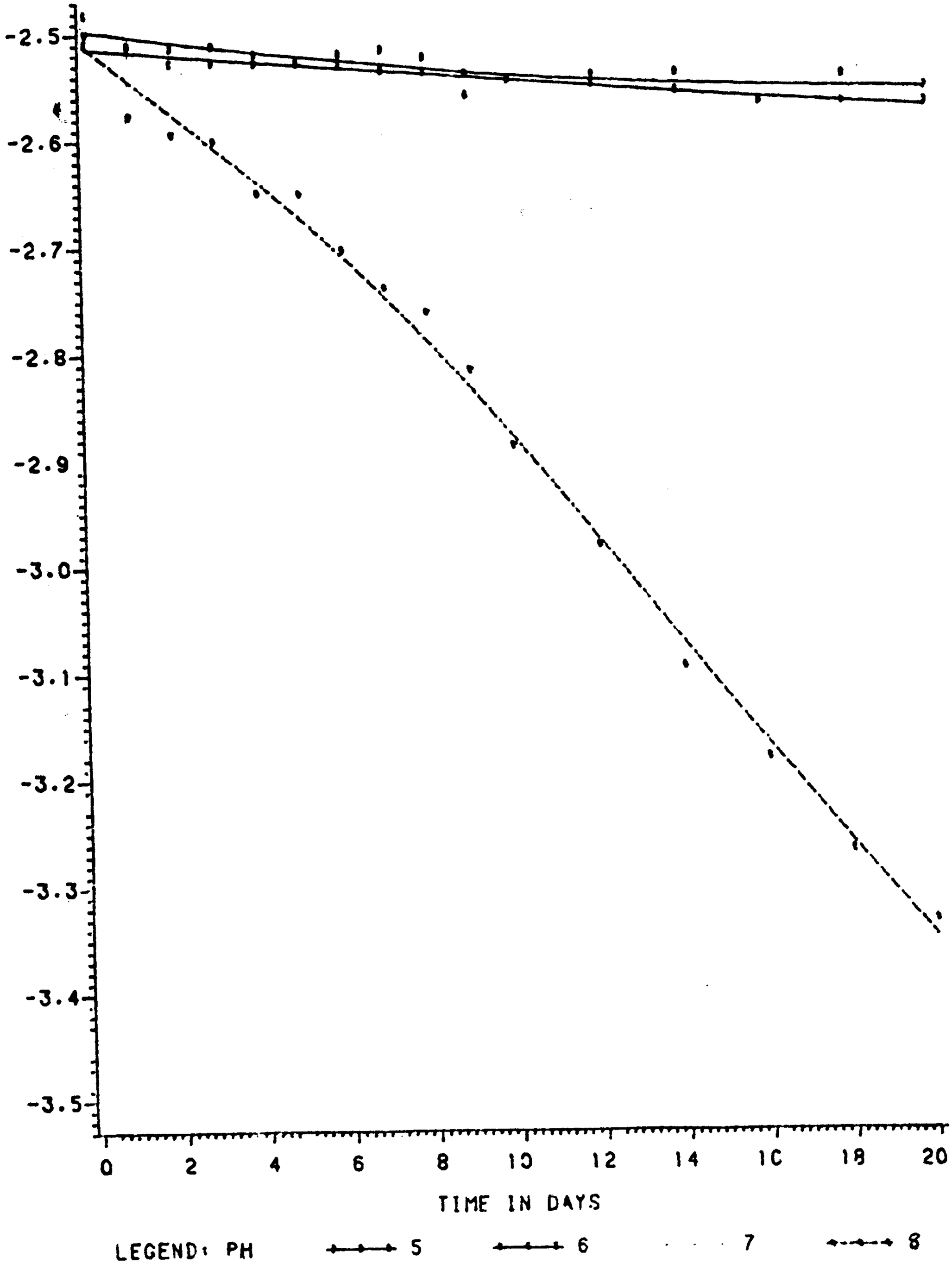


Fig. 3: Stability of P3CTZ in 7.5 w/v CTAB solutions of pH 5,6,7 and 8 at 47°C.

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# تذويـــــــب وثبـات مادة البيريدين ٣ ـ كربوكسالدهيد ثيوسيميكاربـازون بواسطة مختلف المـــواد الصانعـــه للشـــــــــــــــــــــباك

أنتونى سيمونيللى ـ أحمد السيد أبو طالـــــب ـ على عبد الظاهـــــــــــر
كلية الصيدله ـ جامعة كونكتيت بالولايات المتحده الأمريكيه

البيريدين ٣ ـ كاربوكسالدهيد ثيوسيميكاربـازون ( ب ٣ س ت ز ) المستعمل فى علاج السرطان ـ أذيب بمختلف الأنواع من منشطات السطح ـ موجب التـايـــــن. وسالبه التـاين وغير المتاينه فى أسس أيدروجينيـــه مقدارها ٥ ، ٦ ، ٧ ، ٨ وتركيز أيونى ( ى ) قدره ٢و ٠ فى درجتى حراره ٤٧ ، ٥٧ درجة مئويـــــــــه. ولهذا أختـــــير الستريميد ، الصوديوم دوديكيل سلفات ، البرج ٣٥ لتمثيـــل الأنواع الثلاثة فى درجتى حراره ٤٧ ، ٥٧ درجه مئويه وقد وجد أن الصوديـــــوم دوديكيل سلفات له أعلى معدل تذويب تجاه ( ب ٣ س ت ز ) يليـــه البرج ٣٥ ثـم الستريميد عـــند الأسس الأيدروجينيه المدروســـــــه .

ولقد درس ثبات ( ب ٣ س ت ز ) فى مختلف تركيزات محاليل منشطات السطـــح سالفة الذكر فى أس أيدروجينى ٥ ، ٦ ، ٧ ، ٨ وفى تركيز أيونى (ى) قدره ٠٢و فى درجات حراره ٤٧ ، ٥٧ درجه مئويـــه .

وقد حسب معدل الثبات لدواء ( ب ٣ س ت ز ) فى مختلف التركيزات لمنشطات السطح المختلفه مقارنه بمعدل الثبات فى الأنظمه المنظمـــــه .

وقد حسب نصف الحياه لدواء ( ب ٣ س ت ز)فى المحاليل المحضره . ولقـــد وجد أن البرج ٣٥ أس أيدروجينى ( ٥ ) فى درجه ٤٧ مئويه يحقق أطول نصف حيـــاه لدواء ( ب ٣ س ت ز ) . تقريبا عـام كامل . ولقد وحد أن الستريميد يحقـــق أقصر نصف حياه لـــــدواء(ب ٣ س ت ز ) .

ولقد حسب معـــــدل التجزىء لدواء ( ب ٣ س ت ز ) ومعدل التوزيـــــــع أيضـــــــا من كلا من دراســـة الثبات ـ دراسة التذويب ولقد وحـــــــد أن معدل التوزيع أكبر من معدل التجـــــــزىء .

ولقد ثبت أن ( ب ٣ س ت ز ) يحقق ثبات أعلى فى محاليل منشطـــــــات السطح المختلفه فى أس أيدروجـــــينى مرتفع عنه فى أس أيدروجـــــــينى منخفـــــــض .

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