Stability Studies of Gabapentin in Aqueous Solutions

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Gabapentin is a γ-aminobutyric acid analogue, which has been shown to be an effective antiepileptic. The solution stability of gabapentin in buffered systems was studied in order to facilitate the formulation of a liquid product. The degradation of the drug was followed as a function of pH, buffer concentration, ionic strength, and temperature. The results indicated that the rate of degradation was proportional to the buffer concentration and temperature. The pH–rate profile of gabapentin degradation showed that the rate of degradation was minimum at an approximate pH of 6.0. Further, the data suggested a slower solvent-catalyzed degradation rate for the zwitterionic species compared to the cationic or anionic species in the pH range of 4.5 to 7.0. There was no influence of ionic strength on the rate of degradation. Arrhenius plots of the data indicated that a shelf life of 2 years or more at room temperature may be obtained in an aqueous solution at a pH value of 6.0.

KEY WORDS: antiepileptic; gabapentin; 3,3-pentamethylene-4-butyrolactam (lactam); solution stability; degradation.

INTRODUCTION

Gabapentin [1-(aminomethyl)cyclohexaneacetic acid] is a γ-aminobutyric acid (GABA) analogue which penetrates the blood–brain barrier (1). The compound has been shown to be an effective anticonvulsant in various animal seizure models (2). Its efficacy has now been demonstrated in humans in the treatment of epilepsy (3). Being an antiepileptic, the drug has the potential of being used in pediatric patients. Therefore, the development of a palatable liquid pediatric dosage form was considered essential.

Initial studies in aqueous solutions have shown that gabapentin undergoes degradation via intramolecular cyclization to 3,3-pentamethylene-4-butyrolactam ("lactam"). The lactam is the only degradation product known thus far and the specification for the maximum allowable limit for lactam is 0.5% in any dosage form. The development of a liquid pediatric formulation of gabapentin would require not only the stabilization of the drug, but also the masking of the extremely bitter taste of the substance. Preliminary studies on the kinetics of lactamization of gabapentin in a 5% unbuffered aqueous solution (under acidic, basic, and neutral conditions) indicated that the drug was most stable around neutral pH. At this pH, an aqueous shelf life of 4 to 5 months at room temperature (25°C), with a lactam limit of 0.5%, was predicted. Since the kinetics of an unbuffered aqueous solution were examined, the design of a stable liquid formulation of gabapentin would require a more elaborate study of the stability of gabapentin in a buffered aqueous solution.

The main objective of this study was therefore to determine the degradation of gabapentin in aqueous solution in the pH range of maximal stability (4.5 to 7.0).

MATERIALS AND METHODS

Materials

Gabapentin was obtained from Parke–Davis Chemical Manufacturing (Holland, MI) and 3,3-pentamethylene-γ-butyrolactam was obtained from Gödecke AG (Freiburg, Germany). High-performance liquid chromatography (HPLC)-grade methanol and acetonitrile and other reagents were obtained from Fisher Scientific (Fairlawn, NJ).

Analytical Method

All the samples in these studies were assayed for lactam, by an HPLC procedure which was specific and accurate for gabapentin and the lactam. The HPLC system used was a Hewlett Packard 1090 series L fitted with a diode array detector (1090L; Hewlett Packard Company, Valley Forge, PA). The samples were assayed on a reversed-phase Beckman C_{18} Ultrasphere ODS 5-μm, 4.6 mm × 25-cm, column. The mobile phase consisted of a water–methanol–acetonitrile (55:35:10) mixture and the flow rate was 1.0 ml/min. The detection was carried out at 210 nm. All samples assayed were diluted 10-fold and then 50 μl of the sample was injected into the HPLC system. The retention times of gabapentin and the lactam were found to be 3.1 and 13.3 min, respectively. The assay was found to be linear between 0.1 and 6.32 mg/ml gabapentin (r^2 = 0.9999) and 2 and 50 μg/ml lactam (r^2 = 0.9999). The system suitability was determined every day by six replicate injections of a standard solution containing gabapentin and lactam and the relative standard deviation was consistently found to be less than 1.0%.

The assay could detect the lactam reproducibly at 12.5 μg/ml, which was used as a standard concentration in the subsequent analysis of the samples. Due to the volume of samples analyzed, the lactam assay values were calculated from the peak heights of lactam in the samples and in standards containing 6.25 mg/ml gabapentin and 12.5 μg/ml lactam, which were interspersed with the samples. The latter represents a 0.2% (w/w) concentration of degradation product relative to the concentration of gabapentin.

Kinetic Studies

Since earlier studies had indicated a high rate of degradation of gabapentin at extremes of pH values, the present experiments were limited to a study of the degradation of gabapentin in the pH range of 4.5 to 7.0. For all the studies, a weighed quantity of gabapentin was dissolved in the buffer to give a final concentration of about 20 mg/ml. Two types of buffer systems (acetate and phosphate), at different concentrations (0.025, 0.05, and 0.1 *M*) but at a constant ionic strength of 0.5 (adjusted with KCl), were used. The acetate buffers were used in the pH range of 4.5 to 5.5, whereas the phosphate buffers were used at pH values between 6.0 to

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7.0. The temperature dependence of the degradation rate of gabapentin was studied at 45, 60, 70, and 80°C. Screw-capped vials containing the solutions were placed at these temperatures and aliquots of samples were drawn at appropriate time intervals. The influence of different ionic strengths (0.1, 0.25, and 0.5) on the degradation of gabapentin was studied at pH 6.0 and 80°C in 0.025 *M* phosphate buffer.

RESULTS AND DISCUSSION

Degradation Kinetics

The apparent first-order rate constants were determined by following the initial rate of appearance of lactam for less than 5% of the reaction (4). The formation rate constants for lactam were obtained from the slopes of these plots, and the corresponding degradation rate constants for gabapentin are listed in Tables I through IV.

Effect of Buffer Concentration

Figures 1 and 2 display the effect of increasing buffer concentrations on the degradation rate constant for gabapentin at 45°C in acetate and phosphate buffers, respectively. These data show that the degradation rate of gabapentin increases with increasing buffer concentration. The *y*-intercept of these plots represents the degradation rate constant at zero buffer concentration. The degradation rate constants for zero buffer concentrations at 60, 70, and 80°C were similarly estimated and are listed in Tables I through IV.

Table I. Apparent First-Order Rate Constant for the Degradation of Gabapentin in Aqueous Solutions at 45°C and at an Ionic Strength of $\mu = 0.5$

Buffer	pH	Buffer conc. (*M*)	k_{obs} (hr^{-1})	*r**
Acetate	4.5	0.025	3.634×10^{-5}	0.9941
		0.05	5.760×10^{-5}	0.9881
		0.1	7.795×10^{-5}	0.9760
			2.616×10^{-5a}	
Acetate	5.0	0.025	2.203×10^{-5}	0.9848
		0.05	2.910×10^{-5}	0.9959
		0.1	3.872×10^{-5}	0.9964
			1.722×10^{-5a}	
Acetate	5.5	0.025	9.723×10^{-6}	0.9887
		0.05	1.310×10^{-5}	0.9888
		0.1	1.624×10^{-5}	0.9961
			8.153×10^{-6a}	
Phosphate	6.0	0.025	1.044×10^{-5}	0.9904
		0.05	1.480×10^{-5}	0.9890
		0.1	2.282×10^{-5}	0.9996
			6.430×10^{-6a}	
Phosphate	6.5	0.025	9.301×10^{-6}	0.9895
		0.05	1.346×10^{-5}	0.9917
		0.1	2.101×10^{-5}	0.9921
			5.526×10^{-6a}	
Phosphate	7.0	0.025	1.472×10^{-5}	0.9911
		0.05	1.686×10^{-5}	0.9916
		0.1	1.899×10^{-5}	0.9981
			1.365×10^{-5a}	

[a] Estimated rate constant at zero buffer concentration.
* Correlation coefficient was significant at $P < 0.001$.

Table II. Apparent First-Order Rate Constant for the Degradation of Gabapentin in Aqueous Solutions at 80°C and at an Ionic Strength of $\mu = 0.5$

Buffer	pH	Buffer conc. (*M*)	k_{obs} (hr^{-1})	*r**
Acetate	4.5	0.025	3.844×10^{-3}	0.9948
		0.05	7.537×10^{-3}	0.9944
		0.1	9.606×10^{-3}	0.9888
			2.819×10^{-3a}	
Acetate	5.0	0.025	4.920×10^{-3}	0.9920
		0.05	6.347×10^{-3}	0.9890
		0.1	6.726×10^{-3}	0.9860
			4.730×10^{-3a}	
Acetate	5.5	0.025	2.026×10^{-3}	0.9893
		0.05	2.660×10^{-3}	0.9905
		0.1	4.779×10^{-3}	0.9896
			9.660×10^{-4a}	
Phosphate	6.0	0.025	3.335×10^{-3}	0.9839
		0.05	3.873×10^{-3}	0.9854
		0.1	8.740×10^{-3}	0.9860
			9.015×10^{-4a}	
Phosphate	6.5	0.025	5.934×10^{-3}	0.9897
		0.05	—[b]	—[b]
		0.1	7.733×10^{-3}	0.9882
			5.334×10^{-3c}	
Phosphate	7.0	0.025	1.310×10^{-2}	0.9840
		0.05	1.446×10^{-2}	0.9830
		0.1	1.479×10^{-2}	0.9800
			1.288×10^{-2a}	

[a] Estimated rate constant at zero buffer concentration.
[b] Missing data.
[c] Estimated rate constant from two buffer concentrations.
* Correlation coefficient was significant at $P < 0.001$.

The buffer catalysis rate constants k_{cat} for acetate and phosphate buffers were calculated from the slopes of the plots of k_{obs} versus the buffer concentration at 45°C (Figs. 1 and 2). The influence of buffer species was evaluated by

Table III. Apparent First-Order Rate Constant for the Degradation of Gabapentin in Aqueous Solutions at 70°C and at an Ionic Strength of $\mu = 0.5$

Buffer	pH	Buffer conc. (*M*)	k_{obs} (hr^{-1})	*r**
Acetate	4.5[a]	0.025	7.651×10^{-4}	0.9916
Acetate	5.0	0.025	5.388×10^{-4}	0.9961
		0.05	6.605×10^{-4}	0.9962
		0.1	1.405×10^{-3}	0.9964
			1.665×10^{-4b}	
Acetate	5.5[a]	0.025	3.839×10^{-4}	0.9870
Phosphate	6.0	0.025	4.885×10^{-4}	0.9864
		0.05	5.155×10^{-4}	0.9954
		0.1	1.094×10^{-3}	0.9971
			1.990×10^{-4b}	
Phosphate	6.5[a]	0.025	6.373×10^{-4}	0.9966
Phosphate	7.0	0.025	1.162×10^{-3}	0.9939
		0.05	1.249×10^{-3}	0.9967
		0.1	1.347×10^{-3}	0.9974
			1.113×10^{-3b}	

[a] Only 0.025 *M* solutions were examined at these pH values.
[b] Estimated rate constant at zero buffer concentration.
* Correlation coefficient significant at $P < 0.001$.

Table IV. Apparent First-Order Rate Constant for the Degradation of Gabapentin in Aqueous Solutions at 60°C and at an Ionic Strength of $\mu = 0.5$

Buffer	pH	Buffer conc. (M)	k_{obs} (hr^{-1})	r*
Acetate	5.0	0.025	6.797×10^{-5}	0.9932
		0.05	8.848×10^{-5}	0.9739
		0.1	1.164×10^{-4}	0.9964
			5.401×10^{-5}[a]	
Phosphate	6.0	0.025	3.703×10^{-5}	0.9943
		0.05	5.158×10^{-5}	0.9956
		0.1	7.660×10^{-5}	0.9947
			2.452×10^{-5}[a]	
Phosphate	7.0	0.025	6.320×10^{-5}	0.9918
		0.05	7.147×10^{-5}	0.9959
		0.1	8.513×10^{-5}	0.9962
			5.640×10^{-5}[a]	

[a] Estimated rate constant at zero buffer concentration.
* Correlation coefficient was significant at $P < 0.001$.

plotting the buffer catalysis rate constant against the fraction of acetate (CH_3COO^-) (Fig. 3) and phosphate in its dianionic form (HPO_4^{2-}) (Fig. 4) species. The fractions of acetate and phosphate species were calculated from the pK_a of acetic acid (4.76) and pK_{a2} of phosphoric acid (7.21), since these are the active pK_a's in the pH range studied. The plots support a general acid-catalyzed degradation of gabapentin as evidenced by the decreasing buffer catalysis rate constant with an increase in the acetate and phosphate species.

Effect of Ionic Strength

Figure 5 shows the effect of ionic strength on the degradation kinetics of gabapentin in 0.025 M phosphate buffer at 80°C. Since the slope of this line was zero, the kinetic salt effect was interpreted to be negligible on the degradation kinetics of gabapentin. The higher value for the degradation rate constant in the solution adjusted to 0.5 with KCl may be due to the day-to-day variation in the assay and the longer duration of study (7 days vs 30 hr) for this particular sample.

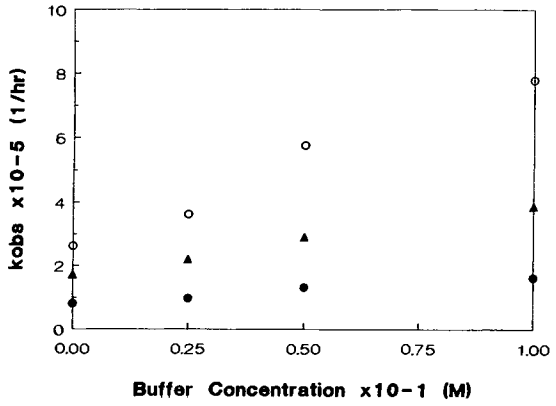


Fig. 1. Effect of increasing acetate buffer concentration on the degradation rate constant of gabapentin at 45°C and pH 4.5 (○), pH 5.0 (▲), and pH 5.5 (●). $\mu = 0.5$ M.

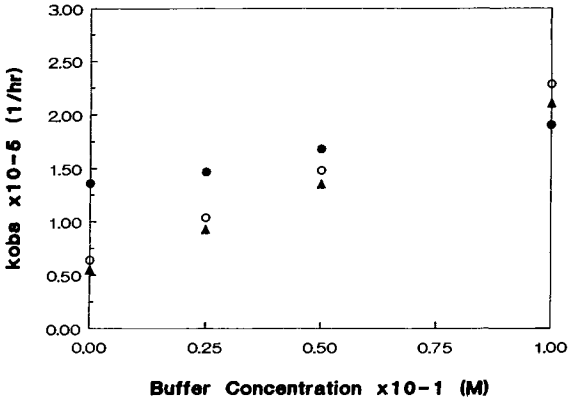


Fig. 2. Effect of increasing phosphate buffer concentration on the degradation rate constant of gabapentin at 45°C and pH 6.0 (○), pH 6.5 (▲), and pH 7.0 (●). $\mu = 0.5$ M.

pH–Rate Profile

The ionization of gabapentin is presented in Scheme I. Three major forms of gabapentin can exist in solution: the cation (a), the zwitterion (b), and the anion (c), reflecting the pK_{a1} (3.7) and pK_{a2} (10.7) for the drug. The influence of the pH on the degradation kinetics of gabapentin is demonstrated in Fig. 6, in which the logarithms of the k_{obs} for zero buffer concentrations at 45 and 80°C were plotted as a function of the pH. The rate profiles reached a plateau at pH values between 5.5 and 6.5 at these temperatures. Although other kinetically equivalent systems are possible, the shape of these pH–rate profiles was explained on the basis of the following proposed kinetic system:

$$k_{obs} = k_H{}^+[H^+](f_a) + k_o(f_b) + k_o'(f_c) + k_o''(f_a) + k_{OH}{}^-[OH^-](f_c) \quad (1)$$

where $k_H{}^+$ is the acid-catalyzed degradation rate constant, $k_{OH}{}^-$ is the base-catalyzed degradation rate constant, k_o is the uncatalyzed rate constant for the zwitterionic species, k_o' is the uncatalyzed rate constant for the anionic species, k_o'' is the uncatalyzed rate constant for the cationic species (5), and f_a, f_b, and f_c represent the fractions of the three species existing in the solution at any given time. The different fractions were determined using the relationship between K_{a1} and K_{a2} and the different ionic species:

$$f_a = [H^+]^2/([H^+]^2 + K_{a1}[H^+] + K_{a1} \cdot K_{a2}) \quad (2)$$

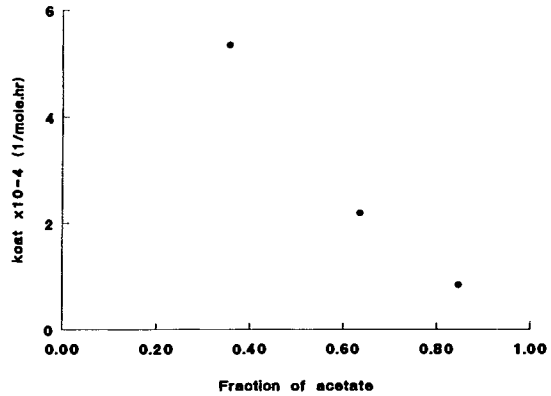


Fig. 3. Effect of the fraction of acetate (CH_3COO^-) species on the acetate buffer catalysis rate constant at 45°C.

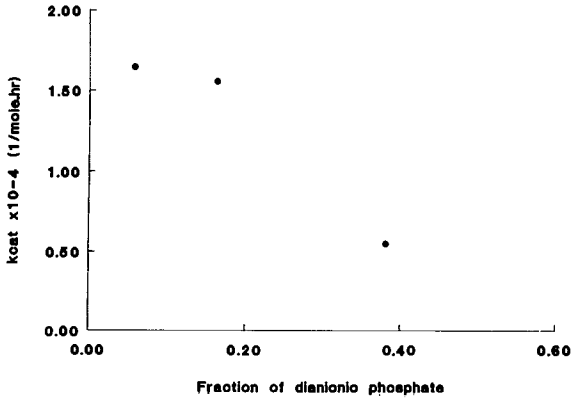


Fig. 4. Effect of the fraction of dianionic phosphate (HPO_4^{2-}) species on the phosphate buffer catalysis rate constant at 45°C.

$$f_b = [H^+] \cdot K_{a1}/([H^+]^2 + K_{a1}[H^+] + K_{a1} \cdot K_{a2}) \quad (3)$$

$$f_c = K_{a1} \cdot K_{a2}/([H^+]^2 + K_{a1}[H^+] + K_{a1} \cdot K_{a2}) \quad (4)$$

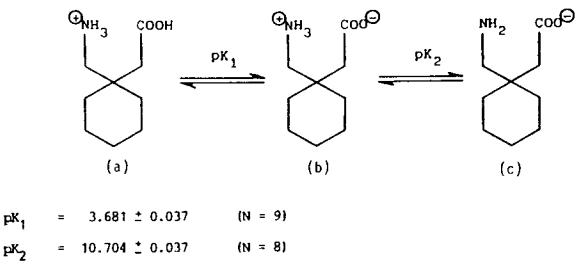
The values for the various rate constants were determined using Eq. (1), K_{a1} and K_{a2} values, and a nonlinear regression program (NLIN-SAS). It was observed that the inclusion of only the second, third, and fourth terms in Eq. (1), corresponding to the uncatalyzed degradation of gabapentin, for the generation of pH–rate profiles, yielded good fits for the observed data in the pH range of 4.5 to 7.0 (Fig. 6). The shape of the pH–rate profile in Fig. 6 was therefore explained on the basis of the following equation:

$$k_{obs} = k_o(f_b) + k_o'(f_c) + k_o''(f_a) \quad (5)$$

The values for the uncatalyzed rate constants were determined and are listed in Table V.

Although the exact mechanism of the intramolecular cyclization of gabapentin is unknown, we have attempted to provide possible mechanistic routes for the different ionic species of gabapentin to undergo cyclization in Scheme II. These routes do not imply that the kinetic studies described in this report have confirmed each of the possible mechanisms listed.

According to Scheme I, the majority of the species in the pH range of 4.5 to 7.0 should be the zwitterionic species,



Scheme I. The ionization of gabapentin.

along with some cationic and negligible anionic species. The degradation rate constant for the zwitterionic species, in the pH range of 4.5 to 7.0, was found to be the smallest, implying that these species are probably the most stable of the three species under neutral conditions. At first glance the zwitterion would seem to be the one to cyclize most rapidly because the oppositely charged ends of the molecule would be attracted to each other. However, the fact that the rate constants for the cyclization of the cationic and anionic species are much larger than the rate constant for the zwitterion indicates that there are other factors which more than compensate for this phenomenon.

In order for the molecule to form the lactam, an unprotonated amino group must attack an unionized (protonated) carboxyl group. In the zwitterion, the amino group is protonated and the carboxyl group is negatively charged (unprotonated). Neither of these situations is favorable for reactivity. In addition, both ends of the molecule would be highly solvated because they are charged, thereby, perhaps preventing the amino group from effectively approaching the carboxyl group. Although the amino group is protonated in the case of the cationic species, the cyclization reaction is favored because the carboxyl group is also protonated. The amino group can attack the protonated carboxyl group if it can transfer its proton to the solvent, etc. In the case of the anionic species, the cyclization reaction is favored despite the carboxyl group being unprotonated. This is because the amino group is also unprotonated and it can use its free pair of electrons to attack the carboxyl group leading to the lactam formation.

Although the inherent reactivity of the anionic and cat-

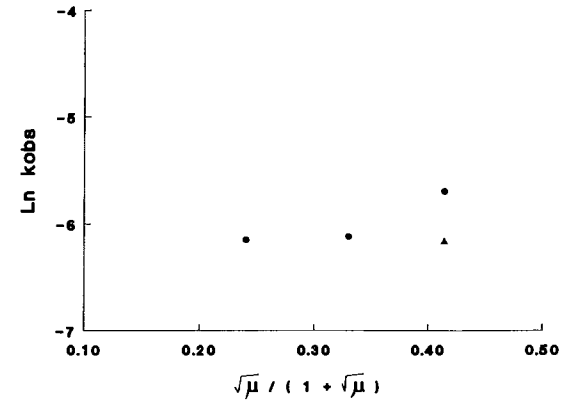


Fig. 5. Effect of ionic strength on the degradation rate constant of gabapentin in 0.025 *M* phosphate buffer at 80°C and pH 6.0. Ionic strengths were adjusted with KCl (●) and NaCl (▲).

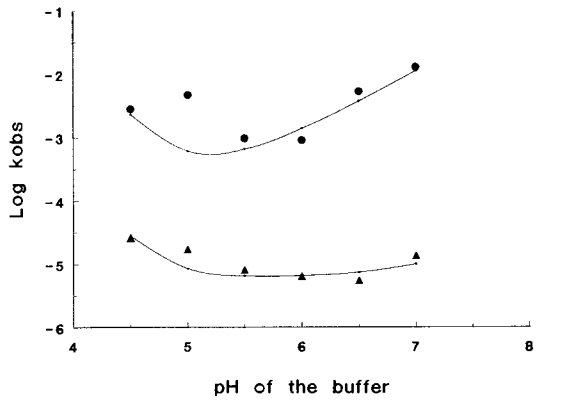


Fig. 6. pH–rate profile for the degradation of gabapentin at 45°C (▲) and 80°C (●) with an ionic strength of 0.5 *M*. The solid lines represent the nonlinear fits obtained from Eq. (1).

Table V. Effect of Temperature on the Apparent Rate Constants for the Degradation of Gabapentin in Aqueous Solution[a]

Temperature (°C)	Rate constant (hr^{-1})[b]		
	k_0[c]	k_0'[d]	k_0''[e]
80	2.995 (2.97–3.01) × 10^{-4}	5.52 (5.5–5.53) × 10^{1}	4.85 (0.51–9.18) × 10^{2}
45	6.277 (6.0–6.6) × 10^{-6}	1.89 (1.88–1.9) × 10^{-2}	5.41 (3.74–7.08) × 10^{0}

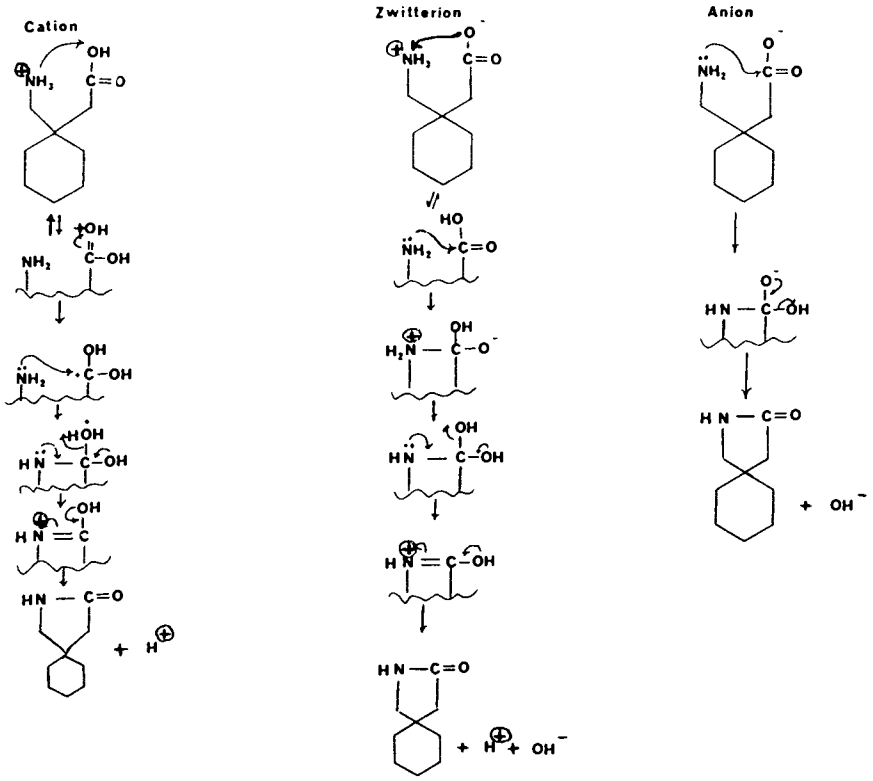
[a] Obtained from a nonlinear least-squares fit of the pH–rate profile (Fig. 6) to Eq. (5).
[b] Estimated for the rate constants extrapolated to zero buffer concentrations. Values in parentheses are the 95% confidence intervals.
[c] The uncatalyzed rate constant for the zwitterionic species.
[d] The uncatalyzed rate constant for the anionic species.
[e] The uncatalyzed rate constant for the cationic species.

ionic species is greater than that of the zwitterion, their negligible concentration in the solution accounts for their lack of contribution to the overall observed degradation rate constant for gabapentin. Thus, in the pH range of 4.5 to 7.0, the contribution of the uncatalyzed degradation rate constant (k_o) of zwitterionic species to the observed degradation rate constant (k_{obs}) would be the highest, since the zwitterions are the predominant species in the solution in the aforementioned pH range.

Effect of Temperature

Figure 7 shows the Arrhenius plot for the degradation of gabapentin at pH 6.0. The logarithm of the rate constant at the lowest buffer concentration studied was plotted against the reciprocal of the temperature in degrees Kelvin. The data from the lowest buffer concentration were used in all the estimations, in order to predict a shelf life in the presence of a weak buffer. A linear regression was performed on the data for the 0.025 *M* buffer concentration and the energy of activation was calculated from the slopes. The value of the observed degradation rate constant was extrapolated to 25°C, and a shelf life of 2 years or more was predicted by these estimates, at a pH value of 6.0. All calculations were made based upon the assumption that the energy of activation does not change with temperature. The raw data for the observed concentration versus time at any given temperature were also analyzed for the effect of temperature by the one-step nonlinear approach described by King *et al.* (6). The only difference in this study was that the shelf life was determined as $t_{99.5\%}$.

The parameters estimated by NLIN-SAS and the classical approach are listed in Table VI. The estimates were found to be in good agreement. The energy of activation in the pH range of 4.5 to 7.0 was calculated to be between 34



Scheme II. The proposed mechanisms for the intramolecular cyclization of the three ionic species of gabapentin.

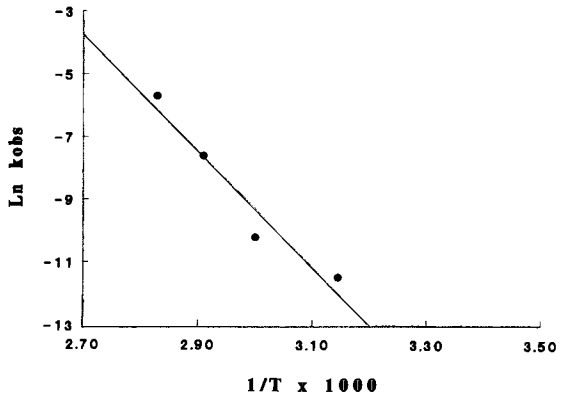


Fig. 7. Arrhenius plot for the degradation of gabapentin in 0.025 *M* phosphate buffer (●) at pH 6.0.

and 40 kcal/mol. The high energy of activation implies that gabapentin is inherently quite stable in this pH range. However, the limit of 0.5% on the lactam in the finished product demanded a detailed investigation of various approaches to stabilize the drug.

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Table VI. Parameter Estimates for the Degradation of Gabapentin

	Parameter[a]					
	Energy of activation (E_a, kcal/mol)		$k_{25°C} \times 10^7$ (hr^{-1})		$t_{99.5\%}$ (months)[b]	
pH	NLIN	Classical	NLIN	Classical	NLIN[c]	Classical
5.0	33.8	34.0	3.9	3.6	17	19
6.0	39.8	37.6	1.1	1.2	63	58
7.0	35.8	35.9	4.7	2.4	14	29

[a] Estimated by a nonlinear approach using raw data by NLIN-SAS and by the classical approach of construction of Arrhenius plots.
[b] Estimated as $\ln(100/99.5)/k_{25°C}$.
[c] Estimated from the mean $k_{25°C}$ obtained from the NLIN-SAS.

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