pK_a Determination of Verapamil by Liquid–Liquid Partition

JIRO HASEGAWA *x, TAKESHI FUJITA *, YAYOI HAYASHI ‡, KIKUO IWAMOTO ‡, and JUN WATANABE ‡

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Abstract □ The pK_a of verapamil was determined by measuring the partition coefficient of verapamil between *n*-heptane and aqueous buffer solution at various pH values. The magnitude of the effects of ionic strength or temperature on the pK_a of verapamil was in agreement with those reported previously. The estimated pK_a of verapamil in human plasma was 8.75. The pK_a of 2,4,6-trimethylpyridine measured by means of the present partition method was in good agreement with that determined by UV spectrophotometry.

Keyphrases □ Verapamil—pK_a determination, liquid–liquid partition coefficients, effects of ionic strength and temperature □ Partition coefficients—liquid–liquid, use in determining the pK_a of verapamil, effects of ionic strength and temperature □ Dissociation constants—verapamil, determination using liquid–liquid partition coefficients, effects of ionic strength and temperature

Verapamil, an effective antiarrhythmic agent, exerts its effect through selective inhibition of slow inward transport of calcium across cell membranes. Although the drug was first introduced in 1962 in Germany, no report on the pK_a value of verapamil, a weakly basic compound, has yet appeared. We required this information to analyze our results on the mechanism of salivary excretion of verapamil in humans and dogs.

Potentiometric or UV spectrophotometric methods are generally most useful for the accurate and reproducible determination of aqueous dissociation constants of organic compounds. However, these methods are not applicable for the determination of the pK_a of verapamil because the drug is insufficiently soluble in aqueous media to give a reliable titration curve and also because of the lack of a significant pH-dependent change in the absorption spectra. Several alternative methods exist for the determination of pK_a values of such compounds (1). For instance, mixed aqueous solvents can be used for the titration of sparingly soluble compounds.

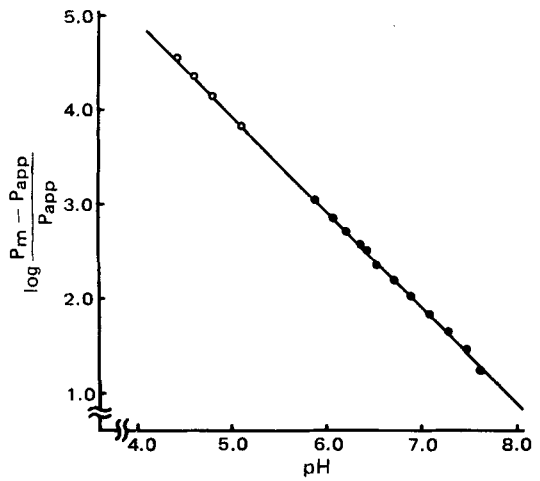

Figure 1—*Plot of log* $[(P_m - P_{app})/P_{app}]$ versus *pH. Initial concentration of verapamil in* n*-heptane, 0.003 M; ionic strength, 0.0315; temperature, 25°C. Key: (○) in phosphate buffer; (●) in acetate buffer.*

However, there is no completely satisfactory method for converting the results to the aqueous pK_a scale (2). The solubility (3, 4) and modified titration (5) methods are also unsuitable for verapamil, since it is difficult to determine the aqueous solubility of the un-ionized form of the compound with sufficient accuracy because of the extremely low solubility.

In the present study, we attempted to determine the pK_a of verapamil by a partition method based on measurement of the partition coefficient between water and an immiscible organic solvent at various pH values of the aqueous phase. Although this method was first proposed several decades ago (6, 7), it has not been generally recognized as a useful method for the pK_a determination of sparingly soluble compounds. Recently, Ezumi and Kubota extended this method to determine pK_a values of weak organic bifunctional acids and bases (8), but their proposed equation cannot be applied to the pK_a determination of verapamil because of the extremely high partition coefficient of this drug. To test the general applicability of the partition method, the effects of organic solvent, ionic strength, temperature, and concentration of verapamil on the pK_a value of verapamil were also studied. Furthermore, to confirm the validity of this method, the pK_a value of 2,4,6-trimethylpyridine was measured by means of both the partition and the UV spectrophotometric methods.

EXPERIMENTAL

Materials—Verapamil hydrochloride[1] and 2,4,6-trimethylpyridine were used as received. Organic solutions of the free base of verapamil or 2,4,6-trimethylpyridine were prepared by extracting alkaline solutions of these compounds (pH 11.0) with the appropriate organic solvent, followed by adjustment of the volume to 100 mL. *n*-Heptane[2], *n*-hexane[2], and carbon tetrachloride[2] were of spectroscopic grade, and other reagents[3] were of analytical grade.

Equipment—The pH values of aqueous buffer solutions were measured with a pH meter[4] with an accuracy of 0.01 pH unit. UV absorption measurements relevant to the determination of partition coefficients and the pK_a determination of 2,4,6-trimethylpyridine by the UV method were made using a spectrophotometer[5]. A spectrofluorophotometer[6] was used for the determination of verapamil in the low concentration range.

Determination of Partition Coefficient—Five milliliters of organic solvent containing verapamil or 2,4,6-trimethylpyridine and 5 mL of phosphate buffer or sodium hydroxide solution were added to a screw-capped vial and incubated for 2 h in a temperature-controlled incubator (±0.1°C) with occasional vigorous shaking by hand. The tube was centrifuged for 5 min at 3000 rpm, then immersed in the incubator again and allowed to stand for 30 min. Aliquots of both phases were withdrawn and subjected to analyses, and the pH of the aqueous phase was measured immediately.

Analytical Procedure—For verapamil, the concentration in the aqueous phase was determined either spectrophotometrically (λ_{max} = 277 nm) or spectrofluorophotometrically (excitation at 275 nm, emission at 339 nm) after appropriate dilution of the solution with 0.5 M HCl depending on the con-

[1] Knoll A. G., Ludwigshafen, West Germany.
[2] Dojin Chemical Co., Ltd., Tokyo, Japan.
[3] Wako Chemicals, Co., Ltd., Osaka, Japan.
[4] Model HM-5B with a type GST 155C glass electrode; Toa Electric Co., Ltd.
[5] UV 300; Shimadzu Co., Ltd., Kyoto, Japan.
[6] Model RF 502; Shimadzu Co., Ltd., Kyoto, Japan.

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Table I—Effect of pH on P_m [a]

Conc. of NaOH[b]	pH	P_m[c]
0.01 M	11.85	487 ± 12
0.03 M	12.38	523 ± 18
0.10 M	12.89	492 ± 15

[a] Organic solvent, *n*-heptane; initial concentration of verapamil in *n*-heptane, 0.001 M; temperature, 25°C. [b] Ionic strength was adjusted to 0.1 with sodium chloride. [c] Mean ± *SD* of three experiments.

Table II—Effect of Ion Species on P_m [a]

Salt[b]	pH[c]	P_m[d]
KCl	11.90	434 ± 21
NaCl	11.95	467 ± 6
Na_2SO_4	11.92	426 ± 6

[a] Organic solvent, *n*-heptane; initial concentration of verapamil in *n*-heptane, 0.001 M; temperature, 25°C. [b] Ionic strength was adjusted to 0.03 with the indicated salt. [c] Concentration of sodium hydroxide was 0.01 M. [d] Mean ± *SD* of three experiments.

centration of verapamil present. The organic phase was evaporated to dryness under a nitrogen stream at 40°C, and the residue was dissolved in 0.1 M HCl for spectrophotometric measurement. For 2,4,6-trimethylpyridine, concentrations in the aqueous and *n*-heptane phases were determined spectrophotometrically (λ_{max} = 266 nm and 263 nm, respectively) after appropriate dilution with 0.5 M HCl and *n*-heptane, respectively.

Preparation of Buffer Solution—All buffer solutions were prepared using monobasic potassium phosphate and dibasic sodium phosphate. The ionic strength of the aqueous phase after the partition experiment was calculated by means of the following:

$$I = \frac{1}{2}\left\{\frac{4[P]}{1 + 10^{7.21\text{-pH}}} + [P]\left(1 - \frac{1}{1 + 10^{7.21\text{-pH}}}\right) + [K] + [Na] + [V]\frac{1}{1 + P_{app}}\right\} \quad \text{(Eq. 1)}$$

where I is the ionic strength of the aqueous phase; [P], [V], [K], and [Na] are the molar concentrations of total phosphate, verapamil, potassium, and sodium, respectively; and P_{app} is the apparent partition coefficient of verapamil. The calculated ionic strength in the aqueous phase after partition varied appreciably with pH because of the differences in the concentration of verapamil in the aqueous solution. However, since deviations in ionic strength of this magnitude were estimated to change the pK_a value by no more than 0.01 pK_a unit, the variation of ionic strength with pH was disregarded and the ionic strength was expressed as the mean value.

Calculation of pK_a from Apparent Partition Coefficient—During partitioning of a weak base between organic and aqueous phases, the distribution of each species can be described by Scheme I: where, K_a and K_a' are the stoichiometric ionization constants of BH^+ in the aqueous and organic phases, respectively; P_m and P_i are the partition coefficients of un-ionized and ionized molecules, respectively; $[B]_o$ and $[B]_w$ are the molar concentrations of un-ionized molecules in the organic and aqueous phases, respectively; and $[BH^+]_o$ and $[BH^+]_w$ are the molar concentrations of ionized molecules in the organic and aqueous phases, respectively.

If the concentration of the ionized molecule is far less than that of the un-ionized molecule in the organic phase, that is $[BH^+]_o \ll [B]_o$, the apparent partition coefficient can be expressed by:

$$P_{app} = \frac{[B]_o}{[B]_w + [BH^+]_w} \quad \text{(Eq. 2)}$$

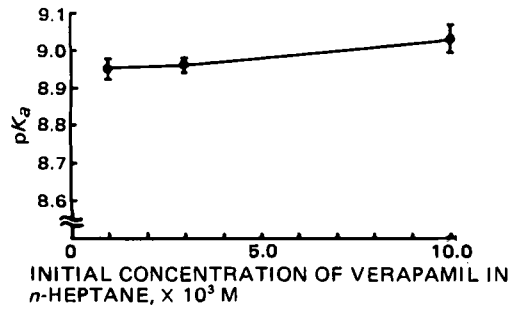
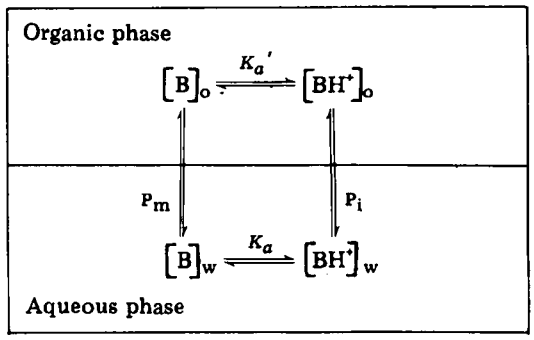


Figure 2—*Effect of concentration of verapamil on* pK_a. *Each point represents the mean* ± SD *of 10 experiments. Ionic strength, 0.1050–0.1005; temperature, 25°C.*



Scheme I

From the definition:

$$P_m = \frac{[B]_o}{[B]_w} \quad \text{(Eq. 3)}$$

$$K_a = \frac{[B]_w[H^+]}{[BH^+]_w} \quad \text{(Eq. 4)}$$

$$pK_a = pH + \log\frac{[BH^+]_w}{[B]_w} \quad \text{(Eq. 5)}$$

From Eqs. 2, 3, and 5:

$$pK_a = \log\frac{P_m - P_{app}}{P_{app}} + pH \quad \text{(Eq. 6)}$$

Equation 6 can be rearranged to:

$$P_{app} = P_m - \frac{1}{K_a}[H^+]\,P_{app} \quad \text{(Eq. 7)}$$

By plotting P_{app} *versus* $[H^+]\,P_{app}$, K_a, and P_m can be obtained simultaneously from the slope and the intercept of the plot, respectively. However, for a compound with a large P_m value, such as verapamil, this plot gives inaccurate results. Instead, by measuring P_m at a pH which is higher than the pK_a value of the compound by 2.0 pH units and P_{app} at a pH where the P_{app} value is ~1.0, the pK_a value can be calculated from Eq. 6.

Measurement of pK_a of 2,4,6-Trimethylpyridine by UV Spectrophotometry—The UV absorbances of 1×10^{-4} M 2,4,6-trimethylpyridine in phosphate buffer (from pH 6.98 to pH 7.57, I = 0.03), and in 0.1 M HCl and 0.1 M NaOH were measured at 266 nm and at 25°C. The pK_a value was calculated by using:

$$pK_a = pH + \log\frac{d - d_M}{d_I - d} \quad \text{(Eq. 8)}$$

where d, d_I, and d_M are the absorbances in buffer solution, 0.1 M HCl solution (where 2,4,6-trimethylpyridine exists predominantly as the ionized form), and 0.1 M NaOH solution (where it exists predominantly as the un-ionized form), respectively.

RESULTS AND DISCUSSION

Estimation of P_m—The present method inherently requires the measurement of the P_m value. Since the ion composition of the solution used for the measurement of P_m is inevitably different from that of the buffer solution used for the measurement of P_{app}, it is necessary to estimate the effect of ion species on the P_m value. As shown in Tables I and II, the observed P_m values change appreciably with change of pH or of the kind of neutral salts added to the solution. This discrepancy in P_m value results in a variation in the calculated

Table III—Effect of Organic Solvents on pK_a [a]

Solvent	pK_a[b]	P_m[c]
n-Heptane	8.93 ± 0.001	479 ± 5
	8.92 ± 0.001	448 ± 5
	8.90 ± 0.003	460 ± 2
Mean ± *SD*	8.92 ± 0.01	
n-Hexane	8.89 ± 0.02	561 ± 17
	8.88 ± 0.01	632 ± 18
Mean ± *SD*	8.89 ± 0.01	
n-Pentane	8.93 ± 0.02	486 ± 4
Carbon tetrachloride	8.54 ± 0.02	$3.63 \times 10^4 \pm 0.13 \times 10^4$

[a] Initial concentration of verapamil in organic solvent, 0.003 M; ionic strength, 0.0315; temperature, 25°C. [b] Mean ± *SD* of seven experiments; different values for the same solvent show results obtained on different days. [c] Mean ± *SD* of three experiments; different values for the same solvent show results obtained on different days.

Table IV—pK_a Values of 2,4,6-Trimethylpyridine Determined by the UV Spectrophotometric Method [a]

pH	Absorbance[b]	pK_a[c]
6.98	0.694	7.50
7.09	0.678	7.52
7.19	0.653	7.49
7.28	0.634	7.49
7.38	0.610	7.48
7.48	0.592	7.51
7.57	0.574	7.51
Mean ± *SD*		7.50 ± 0.01
0.1 M HCl	0.788	
0.1 M KOH	0.384	

[a] Concentration of 2,4,6-trimethylpyridine, 1×10^{-4} M; ionic strength, 0.03; temperature, 25°C. [b] Absorbance was measured at 266 nm. [c] $pK_a = pH + \log (d - d_M)/(d_I - d)$, where d, d_I, and d_M are the absorbances in solutions of the indicated pH, in 0.1 M HCl, and in 0.1 M KOH, respectively.

pK_a of up to 0.03 pK_a unit depending on the salt used; this possible deviation of the pK_a values obtained by the present method must be borne in mind. P_m measurements in the following experiments were carried out in 0.01 M NaOH solution whose ionic strength had been adjusted to 0.03 with sodium chloride.

Validity of Equation 6—To test the validity of Eq. 6 for the pK_a determination of verapamil, the apparent partition coefficients of verapamil between *n*-heptane and aqueous buffer solution at pH from 4.42 to 7.64 were obtained. The resultant log $[(P_m - P_{app})/P_{app}]$ *versus* pH plot was linear with a slope of −1.016 (r = 0.9999) (Fig. 1). This means that Eq. 6 is applicable to the calculation of pK_a from partition coefficients obtained under the proposed experimental conditions. There should be no appreciable effect of buffer composition on the resultant pK_a value, since the results obtained in acetate and phosphate buffers as the aqueous phase are essentially on the same regression line.

Effect of Verapamil Concentration—To determine the effect of verapamil concentration, pK_a values were measured at three different initial verapamil concentrations. As shown in Fig. 2, the obtained pK_a values increased slightly with increase of the verapamil concentration; in particular, the results at 0.01 M gave a significantly higher pK_a value than those at the lower concentrations.

One possible reason for this finding might be the self-association of verapamil in the solution, although extensive studies would be necessary to elucidate the actual reason. At the initial concentrations of 0.001 and 0.003 M, the obtained pK_a values were 8.95 and 8.96, respectively. These values are essentially identical, especially when the experimental error is taken into account. In the following experiment, the initial concentration of verapamil in *n*-heptane was fixed at 0.003 M.

Effect of Organic Solvent—If the theories and assumptions in the proposed method are valid, the pK_a values obtained by this method should be independent of the kind of organic solvent used for the determination of the partition coefficient. One possible source of error in determining the true pK_a value would be partial miscibility of the organic solvent with the aqueous phase during the partition experiment, which might interfere with the dissociation reaction in the aqueous phase. To minimize the extent of contamination of the aqueous phase with the organic solvent, paraffinic hydrocarbons were chosen as organic solvents for the partition experiment in view of their low water solubility. The pK_a values obtained with various organic solvents are presented in Table III. Three highly nonpolar solvents gave comparable pK_a values (*n*-heptane, 8.92; *n*-hexane, 8.89; and *n*-pentane, 8.93) even though they differ in water solubility by a factor of four (9, 10).

On the other hand, carbon tetrachloride gave a fairly low pK_a value compared with those obtained with paraffins. This result is consistent with those reported by Irving and Bell (7), who showed that the pK_a value of dithizone as determined by the partition method using carbon tetrachloride was smaller than that determined using cyclohexane by 0.4 pK_a unit. *n*-Heptane was chosen in the following experiments to minimize organic solvent contamination in the aqueous layer as well as to minimize evaporation, which may lead to volume loss of the organic phase during the partition experiment.

Table V—pK_a Values of 2,4,6-Trimethylpyridine Determined by the Partition Method

pH	P_{app}[a]	pK_a
5.88	0.276	7.53
6.08	0.433	7.53
6.20	0.579	7.51
6.38	0.835	7.53
6.42	0.929	7.52
6.55	1.165	7.54
6.71	1.718	7.51
Mean ± *SD*		7.52 ± 0.01
0.01 M NaOH	12.5[b]	

[a] Initial concentration of 2,4,6-trimethylpyridine in *n*-heptane, 0.003 M; ionic strength, 0.0315; temperature, 25°C. [b] Mean ± *SD* of three experiments.

Table VI—Temperature Dependency of pK_a^T of Verapamil

Temp.	Observed pK_a[a]	pK_a^T	
25°C	8.92[b]	8.84[c]	
31°C	8.79	8.71[c]	8.73[d]
37°C	8.68	8.60[c]	8.61[d]

[a] Initial concentration of verapamil in *n*-heptane, 0.003 M. [b] Mean of three experiments carried out on different days. [c] Calculated using Eq. 10 and the observed pK_a. [d] Calculated by the method of Albert and Sergent (14).

Comparison of the Partition Method with the UV Spectrophotometric Method—To confirm the reliability of the partition method, the pK_a value of 2,4,6-trimethylpyridine was determined by means of both the partition and UV spectrophotometric methods. As shown in Tables IV and V, the results obtained by the two methods were almost identical (7.52 and 7.50, respectively); the thermodynamic pK_a value (pK_a^T), calculated by subtracting the value of $-\log \gamma^{\pm}$ (*cf.*, Eq. 10) from the mean pK_a value to correct for the activity coefficient of the aqueous solvent, was 7.43, identical with the reported value (11). This result strongly supports the reliability of this method.

Effect of Ionic Strength—Methods to correct for ionic strength and obtain the pK_a^T value are well documented (1, 2). One method is to subtract the following log $\gamma^{\pm}$ value from the experimentally determined pK_a value:

$$-\log \gamma^{\pm} = \frac{AZ^+Z^-\sqrt{I}}{1 + Ba_i\sqrt{I}} \qquad \text{(Eq. 9)}$$

where I is the ionic strength of the solvent, $\gamma^{\pm}$ is the ionic activity coefficient, A and B are constants dependent on the dielectric constant of the solvent and the temperature, Z^+ and Z^- are the charge of the ion ($Z^+ = Z^- = 1$ for verapamil), and a_i is the ion size parameter. Since a_i for most electrolytes used in this experiment is equal to $3\text{–}4.5 \times 10^{-8}$ (12) and B changes only from 0.330×10^8 to 0.333×10^8 with the change of temperature from 25°C to 40°C, Eq. 9 can be simplified (13)[7]:

$$-\log \gamma^{\pm} = \frac{A\sqrt{I}}{1 + \sqrt{I}} \qquad \text{(Eq. 10)}$$

An alternative method is to determine pK_a at several different ionic strengths and then to plot pK_a *versus* $\sqrt{I}/(1 + \sqrt{I})$ and extrapolate the plot to infinite dilution (2). Figure 3 is the obtained pK_a *versus* $\sqrt{I}/(1 + \sqrt{I})$ plot for verapamil at 25°C. The intercept was 8.85 and is very close to the calculated value obtained from Eq. 10 (8.84). Although accurate and theoretically sound, this method is tedious. Therefore, since the two methods gave almost identical pK_a^T values at 25°C, Eq. 10 seems to be satisfactory for the determination of the pK_a^T of verapamil.

Temperature Dependency of pK_a^T—Determination of the pK_a is usually performed at 25°C. However, to interpret the absorption, distribution, metabolism, and excretion of drugs in terms of pK_a, one must obtain the pK_a value at 37°C experimentally or by calculation. Several equations have been proposed to express the variation of pK_a with temperature (1). Albert and Sergent proposed a temperature coefficient of pK_a of organic nitrogenous bases in the range of 0–40°C (14), but it is generally recognized that compounds vary somewhat in the sensitivity of their acidity constants to temperature changes. Therefore, the pK_a^T values of verapamil of 25°C, 31°C, and

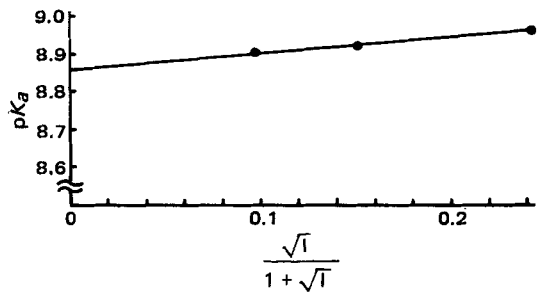


Figure 3—*Plot of* pK_a versus $\sqrt{I}/(1 + \sqrt{I})$. *Initial concentration of verapamil in organic solvent, 0.003 M; temperature, 25°C.*

[7] A = 0.488 at 0°C, 0.500 at 15°C, 0.509 at 25°C, 0.524 at 45°C.

Table VII—pK_a of Verapamil Under Various Conditions

Temp.	pK_a[a]				
	I = 0.00	I = 0.01	I = 0.03	I = 0.05	I = 0.10
5°C	9.28	9.32	9.35	9.37	9.40
10°C	9.16	9.21	9.23	9.25	9.28
15°C	9.05	9.10	9.12	9.14	9.17
20°C	8.94	8.99	9.01	9.03	9.06
25°C	8.84	8.89	8.92	8.93	8.96
30°C	8.74	8.79	8.82	8.83	8.86
37°C	8.60	8.65	8.68	8.70	8.73

[a] These values were calculated using Eqs. 10 and 12.

37°C were calculated, using Eq. 10, from the experimentally determined pK_a values at these temperatures. Experimentally obtained pK_a and pK_a^T values as well as pK_a^T values estimated using the temperature coefficients reported by Albert and Sergent are shown in Table VI.

Since the pK_a^T values calculated by the method of Albert and Sergent were close to the experimentally obtained values, their method seems to be applicable for the rough estimation of the pK_a^T of verapamil at various temperatures. On the other hand, the variation of pK_a^T with temperature can be expressed by (1):

$$pK_a^T = \frac{\Delta H^\circ}{2.303RT} - \frac{\Delta S^\circ}{2.303R} \qquad \text{(Eq. 11)}$$

where ΔH° is the enthalpy change under standard conditions, ΔS° is the entropy change under standard conditions, R is the gas constant, and T is the absolute temperature. As shown in Fig. 4, a plot of experimentally obtained pK_a^T values *versus* $1/T$ gave a straight line and could be expressed by Eq. 12. The pK_a^T values at arbitrary temperatures can be calculated by using:

$$pK_a^T = 1.833 \times 1/T \times 10^3 + 2.696 \qquad \text{(Eq. 12)}$$

CONCLUSIONS

The pK_a values of verapamil at various temperatures and ionic strengths can be estimated by means of Eqs. 10 and 12. The resultant values are presented in Table VII. On the assumption that sodium chloride is the main ionized component in plasma, the pK_a of verapamil in human plasma at 37°C can be calculated to be ~8.75.

Subject to the following restrictions, the present method should be applicable to the determination of pK_a of weak organic bases or acids[8], whose aqueous solubility is too low to give concentrations adequate for the determination of pK_a by the titration method:

1. A paraffinic hydrocarbon should be used as the organic solvent for the partition experiment to prevent the contamination of the aqueous phase with organic solvent.

[8] For acidic compounds, use $pK_a = \log [P_{app}/(P_m - P_{app})] + pH$ in place of Eq. 6 and add the $-\log \gamma^{\pm}$ value to the obtained pK_a in order to get pK_a^T.

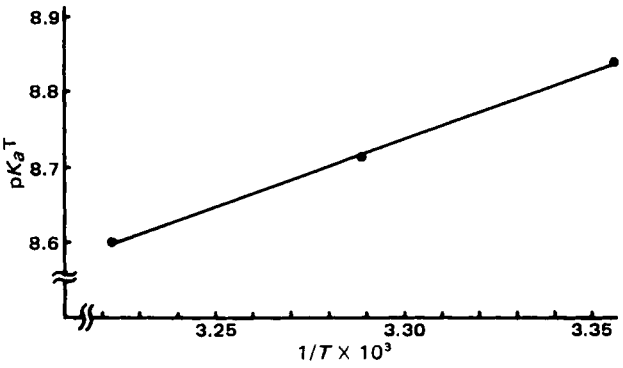


Figure 4—*Plot of* pK_a^T versus 1/T. *Initial concentration of verapamil in organic solvent, 0.003 M; temperature, 25°C; ionic strength, 0.0315.*

2. A P_m value of 1.0–1000 is advisable from the standpoint of accuracy.

3. The concentration of the drug in the partition experiment should be as low as possible.

4. Low buffer concentration is advisable to make the correction for the activity coefficient of ions simple. A swamping electrolyte such as sodium chloride should be added to adjust the ionic strength to a certain value (such as 0.05) to minimize the contribution of drug concentration change in the aqueous phase to the ionic strength.

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