The positive CD band assumes the stable structure at a faster rate than that of the negative band (as reflected from measurements of the total amplitude) in the case of the epinephrine–poly A system (Table I). In the norepinephrine–poly A system, however, both bands of poly A attain their stable features at approximately the same rate. The activation energy calculated from the changes in the total amplitude and the positive band is very similar in the epinephrine–poly A system, while a slight difference appears to exist in the norepinephrine–poly A (Table I). In comparing both systems, one may observe the high temperature dependence of the rate constants; a change from 4° to 25° can bring about an 8- to 10-fold increase in the rate constant.

The significance of the contributions of electrostatic interactions to the total binding energy was verified by changing the ionic strength of the solutions. It was found that increasing the ionic strength to 0.2 *M* by the addition of sodium chloride had the effect of restoring almost entirely the initial CD spectra of poly A in the three systems (Fig. 1). This can be explained on the basis of competition occurring between sodium ions and the positively charged amino groups existing at pH 7 in the negatively charged phosphate residues. The contribution of other weaker forces to the intercalative binding cannot be neglected, however, since it has been demonstrated (11–13) that adenine can form charge transfer complexes with catechol, epinephrine, and isoproterenol.

The exact geometry of the complexes formed between the catecholamines and poly A in its single- and double-stranded conformations cannot be determined from the CD investigation alone. On the other hand, it should be emphasized that the conformational changes occurring in the poly A molecule cannot be attributed solely to the electrostatic interactions, due to the fact that isopropylamine does not change the CD spectrum of poly A (10). Finally, catecholamines may exert a control mechanism through induction of the coil → helix transition on the regulatory role in genetic code translation which has been hypothesized for the poly A segments present in most mRNA molecules.

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Stability of Triamcinolone Acetonide Solutions as Determined by High-Performance Liquid Chromatography

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Received July 6, 1982, from the *Department of Pharmaceutics, University of Houston, Houston, TX 77030.* Accepted for publication October 14, 1982.

Abstract □ A stability-indicating assay method based on reverse-phase high-performance liquid chromatography has been developed for the quantitation of triamcinolone acetonide. The method was used to study the stability of triamcinolone acetonide in water–ethanol solutions of varying pH, buffer concentration, and ionic strength. The decomposition of triamcinolone followed pseudo-first-order law and was minimal at pH ~ 3.4. Above pH 5.5, the decomposition increased rapidly and was directly related to phosphate buffer concentration. The decomposition decreased with increasing ionic strength when the pH of the solution was >7. Two new peaks corresponding to decomposition products were noted in the chromatogram; their ratio varied significantly with the composition of the vehicle.

Keyphrases □ Triamcinolone acetonide—decomposition in solution, effect of pH, buffer concentration, and ionic strength, high-performance liquid chromatography □ High-performance liquid chromatography—stability indicating, triamcinolone acetonide and its decomposition products, effect of pH, buffer concentration, and ionic strength □ Stability—triamcinolone in solutions, effect of pH, buffer concentration, and ionic strength, high-performance liquid chromatography

Triamcinolone acetonide (I) is available in different dosage forms such as creams, ointments, and suspensions. Despite its extensive use, little is known about the stability of I in aqueous systems and water-washable ointment bases such as polyethylene glycol ointment base USP (1). In general, corticosteroid decomposition is first order (2) with two parallel routes of decomposition. One route (attack on ring A) produces neutral product(s) and the other (attack on the C-17 side chain) produces acidic product(s).

This study evaluates the stability of triamcinolone acetonide (*a*) in water–ethanol solutions of varying pH with different buffer concentrations and ionic strengths and (*b*) in polyethylene glycol ointment base USP (1) using a stability-indicating reverse-phase high-performance liquid chromatographic (HPLC) assay method developed in our laboratory.

EXPERIMENTAL

Reagents and Chemicals—All reagents and chemicals were either ACS, USP, or NF grade and were used without further purification. Triamcinolone acetonide[1] was used as received.

Chromatographic Conditions—Two columns (30 cm × 4-mm i.d.) were used. One contained a semipolar material[2], the other a nonpolar

[1] E. R. Squibb & Sons, Princeton, N.J.

[2] μBondapak CN; Waters Associates, Milford, Mass.

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Table I—Composition of 0.025% Solutions of Triamcinolone Acetonide Prepared in Ethanol-Water and the Estimated K_{obs} Values

Solution Number	Concentration of Buffer	Buffer	pH of the Buffer Solution[a]	Ionic Strength[b]	Final pH (±0.1)	K_{obs} Value at 25° (Per Day)[c]
1	0.1	HCl	1.5	0.1	1.6	0.0068
2	0.01	HCl	2.0	0.1	2.1	—[d]
3	0.05	Phosphate	3.1	0.1	3.4	—[d]
4	0.05	Phosphate	4.1	0.1	4.5	—[d]
5	0.05	Phosphate	5.1	0.1	5.5	—[d]
6	0.05	Phosphate	6.1	0.1	6.6	0.0095
7	0.05	Phosphate	7.1	0.1	7.5	0.0345
8	0.01	NaOH	11.7	0.1	11.8	3.6
9	0.1	NaOH	12.5	0.1	12.6	18.2
10	0.05	Phosphate	7.2	0.1	7.6	—[e]
11	0.05	Phosphate	7.2	0.2	7.6	—[e]
12	0.05	Phosphate	7.2	0.25	7.6	—[e]
13	0.05	Phosphate	7.2	0.3	7.6	—[e]
14	.075	Phosphate	7.2	0.2	7.6	—[e]
15	0.1	Phosphate	7.2	0.2	7.6	—[e]
16	.05	Phosphate	6.1	0.2	6.6	—[e]
17	0.075	Phosphate	6.1	0.2	6.6	—[e]
18	0.1	Phosphate	6.1	0.2	6.6	—[e]
19	0.2	Phosphate	7.1	—[f]	7.5	0.078
20	0.2	Phosphate[g]	7.1	—[f]	7.4	0.090
21	0.2	Phosphate[g]	7.1	—[f]	7.5	0.108
22	—	—	—	—[f]	6.8	—[e]
23	—	—	—	—[f]	6.9	—[e]
24	—	—	—	—[f]	7.0	—[e]
25	—	—	—	—[f]	7.0	—[e]
26	—	—[g]	—	—[f]	6.2	—[e]
27	—	—[g]	—	—[f]	5.8	—[e]

[a] Each solution contained 25% v/v ethanol except solutions 20 and 21 (10%), 23 (50%), 24 (75%), and 25 (100%). [b] Adjusted with potassium chloride based on the pH of the buffer solutions. [c] The data at 50° are presented in Fig. 4. [d] It was not possible to estimate K_{obs} at 25° since these solutions did not decompose significantly in 8 weeks. [e] Not determined at this temperature. [f] Ionic strength not adjusted. [g] Solutions 20 and 26 contained 15% v/v and 25% v/v glycerin, respectively. Solutions 21 and 27 had 15% v/v and 25% v/v propylene glycol, respectively.

material[3]. The chromatograph[4] was connected to a multiple-wavelength detector[5], a recorder[6], and an integrator[7]. All pH values were determined using a pH meter[8]. Four mobile phases were used:

1. A 0.02 *M* aqueous solution of monobasic potassium phosphate containing 16% v/v of acetonitrile (pH ~4.2).
2. Same as 1 except that the buffering agent was 0.02 *M* ammonium acetate (pH ~7).
3. Same as 1 except that the pH was adjusted to ~2.5 with an 85% aqueous solution of phosphoric acid.
4. Same as 1 except that the acetonitrile concentration was 32% v/v (pH ~4.2).

The flow rate was 2.5 ml/min (3.0 ml/min with mobile phase 4), and the sensitivity was set at 0.04 AUFS (254 nm). The temperature was ambient, and the chart speed was 30.5 cm/hr.

Preparation of Samples—All solutions for stability studies were prepared as presented in Table I using a simple solution method. After the initial data was obtained (pH values and the assays), the solutions were stored at 50 ± 1° (some also at 25 ± 1°) in 60-ml amber-colored bottles[9].

Forty milligrams of I was thoroughly mixed with 19.96 g of polyethylene glycol ointment base USP (1) using the process of trituration. After the initial data was obtained (assay and the pH value of a 1% aqueous solution), the ointment was stored at 50 ± 1° in an opaque white ointment jar[9].

Assay Method—*Preparation of Standard Solutions*—A stock solution of triamcinolone acetonide (I) was prepared by dissolving 100.0 mg of the powder in enough ethanol to make 100 ml. A stock solution of hydrocortisone (the internal standard) of identical concentration was also prepared in ethanol. The standard solution was prepared by mixing 8.0 ml of the stock solution of I and 3.2 ml of hydrocortisone solution with 20 ml of methanol and then bringing the volume to 100 ml with water. The solutions of other concentrations were prepared as needed. Each solution contained identical concentrations of methanol and hydrocortisone.

Assay Solutions—An 8.0-ml quantity of each solution was mixed with 0.8 ml of the stock solution of hydrocortisone and 5 ml of methanol, and the mixture was brought to volume (25 ml) with water. One gram of the ointment was mixed with 5 ml of methanol, 5 ml of water, and 0.8 ml of the hydrocortisone solution. The mixture was stirred until a clear solution was formed and then brought to volume (25 ml) with water.

HPLC Assay Procedure—A 20-μl aliquot of the assay solution was injected into the chomatograph using the described conditions (nonpolar column[3] and mobile phase 4). An identical volume of the appropriate standard solution was injected for comparison after the assay solution eluted. The standard solution contained exactly the same quantity of I as the label claim of the assay solution.

Calculations—Since preliminary investigations indicated that ratios of peak heights of I and the internal standard were directly related to concentrations of I, the results were calculated using:

$$\frac{(R_{ph})_a}{(R_{ph})_s} \times 100 = \text{percent contained in the assay solution} \quad \text{(Eq. 1)}$$

where $(R_{ph})_a$ is the ratio of peak heights of I to the internal standard (hydrocortisone) in the assay solution and $(R_{ph})_s$ is the ratio of the triamcinolone acetonide peak to the internal standard in the standard solution. If the standard solution does not contain an identical concentration of I, the results can be corrected by multiplying the percent found by: Factor = [actual concentration of I (mg/ml) in the standard solution] ÷ [label claim of I (mg/ml) in the assay solution].

When the triamcinolone acetonide peak was too small (<25% of the standard) to quantify accurately, a new assay solution containing a higher concentration of I was injected. Appropriate calculations were then made to determine the exact concentration of I in the assay solution.

RESULTS AND DISCUSSION

Assay Method—The assay method using a nonpolar column and mobile phase 4, containing 0.02 *M* KH_2PO_4 and 32% v/v acetonitrile in water, separated the decomposition products from the intact triamcinolone acetonide and the internal standard (Figs. 1 and 2). The results were reproducible, accurate, and precise with relative standard deviations ranging from 1.6 to 1.9% based on six readings. Before these chromatographic conditions were finalized, mobile phase 1 containing 16% v/v

[3] μBondapak C18; Waters Associates, Milford, Mass.
[4] Model ALC 202 equipped with a U6K universal injector; Waters Associates, Milford, Mass.
[5] Spectroflow monitor SF770; Schoeffel Instruments Corp., Westwood, N.J.
[6] Omniscribe 5213-12; Houston Instruments, Austin, Tex.
[7] Autolab minigrator; Spectra-Physics, Santa Clara, Calif.
[8] Beckman SS-3 Zeromatic; Beckman Instruments, Irvine, Calif.
[9] Brockway Glass Co., Brockway, Pa.

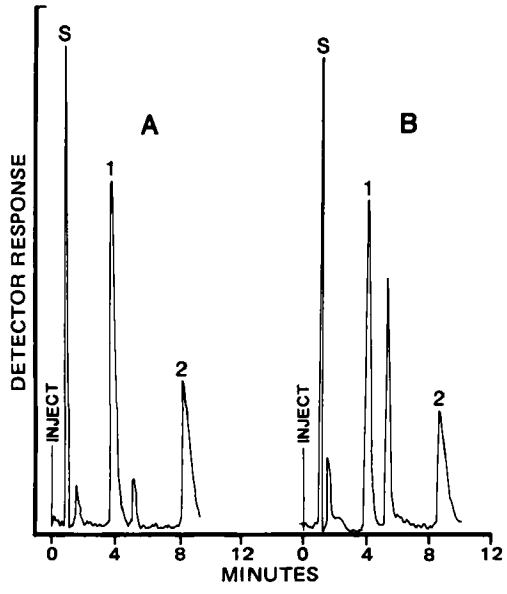


Figure 1—*Typical chromatograms of (A) a standard solution and (B) an 11-day-old solution (solution 19) using a nonpolar column and mobile phase 4. Key: (1) hydrocortisone (internal standard); (2) triamcinolone acetonide; (S) solvent. The two unlabeled peaks are decomposition products.*

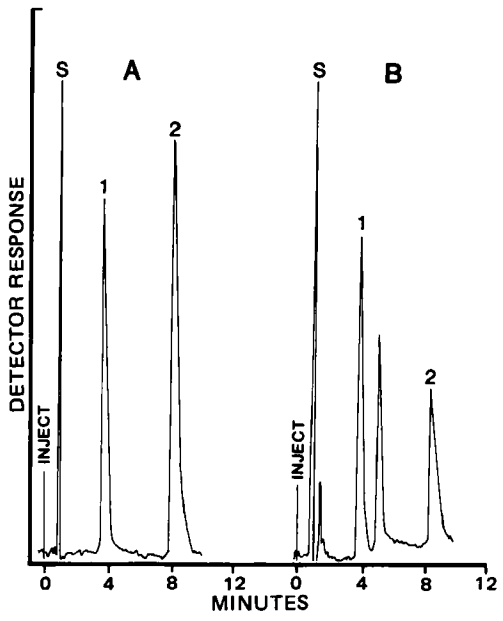
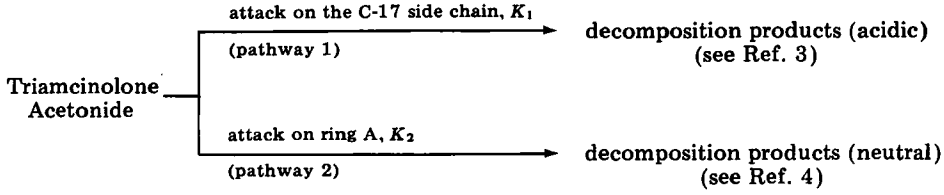


Figure 2—*Typical chromatograms using a nonpolar column and mobile phase 4 of two 11-day-old solutions stored at room temperature. Key: (A) solution 20; (B) solution 21; (1) hydrocortisone (internal standard); (2) triamcinolone acetonide; (S) solvent. All other peaks are decomposition products.*

acetonitrile was tried with a semipolar column. With these chromatographic conditions, the second product of decomposition (the peak between peaks 1 and 2 in Fig. 1B) did not separate from peak 2. Therefore, the results obtained using a semipolar column were erroneous and did not follow first-order kinetics.

Decomposition Products—Corticosteroids usually decompose with two parallel routes of decomposition (2), producing either acidic or neutral products:



By changing the pH of the mobile phase (mobile phases 1–3), it was possible to decrease (with a mobile phase of higher pH) or increase (with a mobile phase of lower pH) the retention time of the first decomposition product (peak appearing after the solvent peak in Fig. 2). The retention time of the second peak (between peaks 1 and 2 in Fig. 2) was not affected. It appears, therefore, that the first decomposition product peak is from an acidic compound and the second is from a neutral substance.

In an ethanolic solution (solution 19), the peak from the neutral product was higher (Fig. 1B) than the peak from the acidic product. In the presence of glycerin (solution 20), these peaks were smaller and of approximately the same size (Fig. 2A). In propylene glycol (solution 21), the chromatogram (Fig. 2B) was similar to the ethanolic solution chromatogram (Fig. 1B). It is obvious that the ratio of K_1 and K_2 would be different in ethyl alcohol–propylene glycol than in glycerin.

Order of Reaction and Effect of pH—As expected, first-order kinetics were followed (Fig. 3). The rate constants varied with the pH of the buffer solution (Fig. 4) and an optimum pH of stability was estimated to be ~3.4.

Effect of Phosphate Buffer—It is apparent (Fig. 5) that the K_{obs} is directly related to the phosphate concentration. Therefore, triamcinolone acetonide is subject to general acid–base catalysis.

The decomposition of triamcinolone acetonide may be represented by:

$$K_{obs} = K_0 + K_1[H^+] + K_2[OH^-] + K_3[H_2PO_4^-] + K_4[HPO_4^{2-}] \quad \text{(Eq. 2)}$$

At constant pH, $K_1[H^+]$ and $K_2[OH^-]$ are constant and Eq. 2 may be rewritten as:

$$K_{obs} = K + K_3[H_2PO_4^-] + K_4[HPO_4^{2-}] \quad \text{(Eq. 3)}$$

where $K = K_0 + K_1[H^+] + K_2[OH^-]$.

Since the decomposition is first order (Fig. 3), the K_{obs} values were determined using first-order plots, and Eq. 3 may be rearranged as follows:

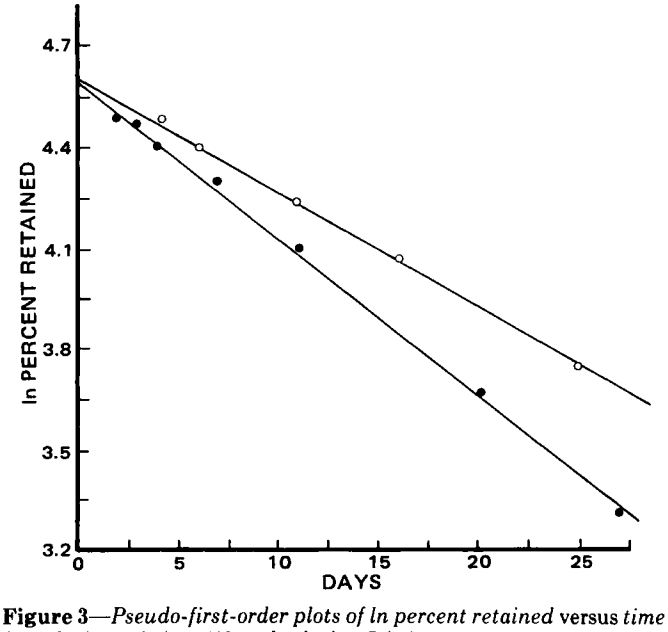


Figure 3—*Pseudo-first-order plots of ln percent retained* versus *time for solution 1 (●) at 50° and solution 7 (○) at 25°.*

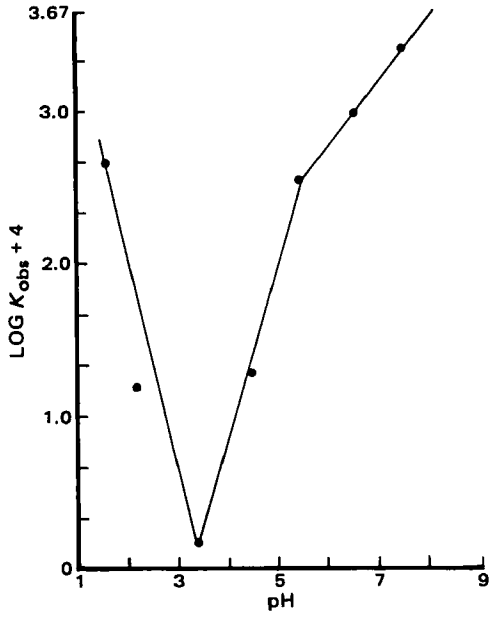


Figure 4—*pH-rate profile curve from* K_{obs} *values at 50°.*

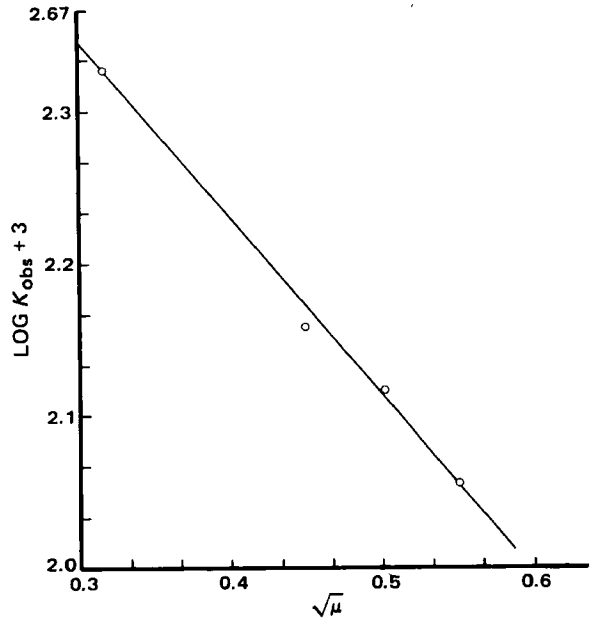


Figure 6—*Plot of log* K_{obs} versus $\sqrt{\mu}$ *at pH 7.6 (solutions 10–13).*

$$K_{obs} = K + [H_2PO_4^-]\left(K_3 + \frac{K_4}{q}\right) \quad \text{(Eq. 4)}$$

where $q = [H_2PO_4^-]/[HPO_4^{2-}]$. On plotting K_{obs} *versus* concentrations of $H_2PO_4^-$, straight lines were obtained (Fig. 5). From the slopes and q values of 3.98 and 0.398 at pH values of 6.6 and 7.6, respectively, the two simultaneous equations were solved for K_3 and K_4. The K_3 and K_4 values were estimated to be 0.575 and 3.79 M^{-1}·day^{-1}. Furthermore, by substituting these values in Eq. 3, K was determined to be negligible.

Effect of Hydrochloric Acid Buffer—In the presence of hydrochloric acid buffer (solution 1), it appears that the major reaction is hydrolysis of triamcinolone acetonide to triamcinolone and acetone. The triamcinolone peak was identified using pure powder and quantified by comparing the peak heights.

Effect of Ionic Strength—From Fig. 6, it is obvious that K_{obs} decreases with increasing ionic strength. Therefore, the reacting ions are probably the protonated form of I and $H_2PO_4^-/HPO_4^{2-}$. However, the slope of the line was ~1.16 *versus* the expected value of ~2.04. This may be due to high ionic strengths, since the equation log K = log K_0 + 1.02 $(Z_A Z_B)\sqrt{\mu}$ was deduced by assuming a value of ≤0.01 (5). In these studies, it was necessary to keep the concentration of buffering agent high in order to maintain constant pH values. Therefore, the ionic strengths were high. The equation cannot be expected to hold at salt concentrations much beyond the range of validity of the Debye–Huckel theory (5).

The other explanation may be that one route of decomposition involves reaction between the undissociated molecule of I and $H_2PO_4^-/HPO_4^{2-}$. If so, this route will not be affected by ionic strength and, hence, the slope will be reduced. Triamcinolone acetonide does decompose at lower pH (~1.6) and higher pH (~12.6) without the presence of phosphate ion. Therefore, H^+ and OH^- also catalyze the reaction only when present in high concentrations. The K_{obs} value at 25° and pH 1.6 was 0.0068 day^{-1} *versus* 18.2 day^{-1} at pH 12.6.

Later, it was determined that at lower pH (solution 1), an increase in ionic strength slightly increased the rate constant in hydrochloric acid buffer. This slight increase may be an experimental error or the difference could be due to a different reaction mechanism in hydrochloric acid buffer *versus* phosphate buffer, as explained above.

Effect of Temperature—As expected, the solutions were much more stable when stored at 25° than at 50°. From the estimated K values, the solutions were stable up to 9 times longer at 25° than at 50°.

Effect of Solvent—The data (solutions 19–21) indicate that ethanol is better than glycerin/propylene glycol for stability of I as long as the same pH is maintained. However, without pH or ionic strength adjustments (solutions 23, 26, 27), glycerin proved better than ethanol/propylene glycol. The estimated K values at 50° were 0.003, 0.004, and 0.007 day^{-1} for solutions 26, 23, and 27, respectively.

In polyethylene glycol ointment base USP (1), K_{obs} was estimated to be 0.008 day^{-1}, which is similar to solution 27 (Table I) which contained 25% v/v propylene glycol. The pH of a 1% aqueous solution of the ointment was 4.2.

In another experiment, an aqueous solution of I (40.0 μg/ml) containing 20% v/v methanol and 0.3% v/v hydrogen peroxide was stored at 50° in a 50-ml volumetric flask. After 5 days, it had decomposed 11.5%.

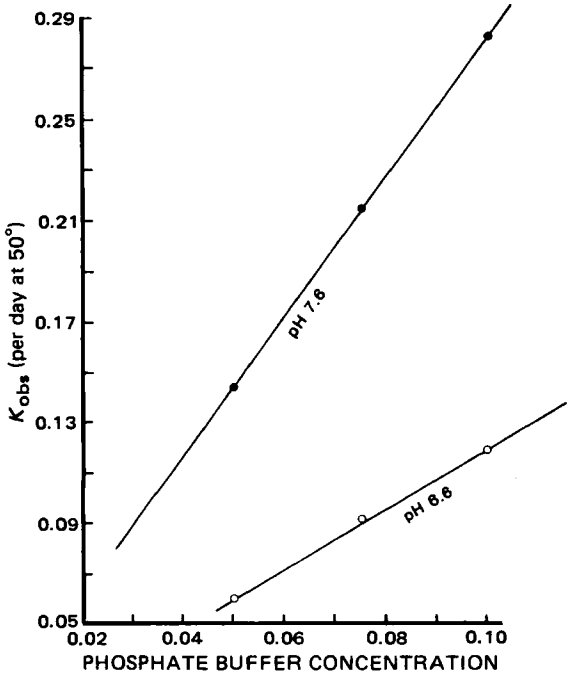


Figure 5—*Plots of* K_{obs} *values* versus *concentrations of phosphate buffer at pH 6.6 (○) and 7.6 (●).*

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