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Stability of Pilocarpine Ophthalmic Formulations

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The stability of various dosage forms of pilocarpine ophthalmic formulations available on the Japanese market was studied. For ophthalmic solutions, hydrolysis was found to be a main route of degradation, and the stability depended largely on the pH and the concentration of general bases. Nonlinear regression analysis allowed estimation of the rate constants of degradation, including reversible hydrolysis to pilocarpic acid and reversible epimerization to isopilocarpine. It was also found that an ointment formulation was stable, while a conjunctival insert preparation was susceptible to epimerization and hydrolysis. The factors affecting the stability of pilocarpine preparations are discussed.

Keywords——pilocarpine; stability; degradation kinetics; hydrolysis; epimerization

Pilocarpine, a miotic used topically in the treatment of glaucoma, is generally administered in dosage forms such as eye drops, ointments and conjunctival inserts. It is well known that pilocarpine degrades *via* two reaction pathways, hydrolysis and epimerization, resulting in loss of pharmacological activity. Many studies have been reported on the kinetics and mechanisms of degradation in acidic or basic aqueous solution.[1–7] Only a few papers, however, have dealt with the kinetics of the degradation in neutral solution (eye drops)[8] or in the dry state (conjunctival insert preparations).

In this paper we present stability data on various dosage forms of pilocarpine ophthalmic preparations available on the Japanese market, and discuss the factors affecting the stability of pilocarpine preparations.

Experimental

Materials——Pilocarpine hydrochloride and isopilocarpine nitrate were obtained from Wako Pure Chemical Industries, Ltd. and Sigma Chemical Co. (St. Louis), respectively.

Determination of Pilocarpine and Its Degradation Products by High-Performance Liquid Chromatography (HPLC)——The liquid chromatographic separation method reported by Kennedy and McNamara[9] and Van Ackeren *et al.*[10] was modified and utilized to analyze pilocarpine and its degradation products in commercial preparations and to follow the degradation kinetics. The HPLC system comprised a chromatograph equipped with a multiple-wavelength detector (model 655, Hitachi, Ltd.), a sample injector fitted with a 10 μl loop (Rheodyne), an integrator (model C-R3A, Shimadzu Seisakusho, Ltd.), and a μBondapack phenyl column (30 cm × 3.9 mm) maintained at 25 °C in a column oven (model 655A-52, Hitachi, Ltd.). The mobile phase was 5% KH_2PO_4 solution adjusted to pH 2.5 with phosphoric acid, which was delivered at a rate of 1 ml/min. The column eluate (Fig. 1) was monitored at 215 nm.

The sample solutions of pilocarpine and isopilocarpine were diluted with the same solvent as the mobile phase for HPLC to give a concentration of about 0.02 mg/ml, and subjected to HPLC. Pilocarpine and isopilocarpine were found to be stable in the solvent (no change in the peak height was observed up to 1 h). A small amount of isopilocarpine coexisting with pilocarpine was determined on the basis of the calibration curve prepared each time in the presence of a predetermined amount of pilocarpine. Figure 2 shows the calibration curve for isopilocarpine nitrate coexisting with 0.5 mg/ml of pilocarpine hydrochloride.

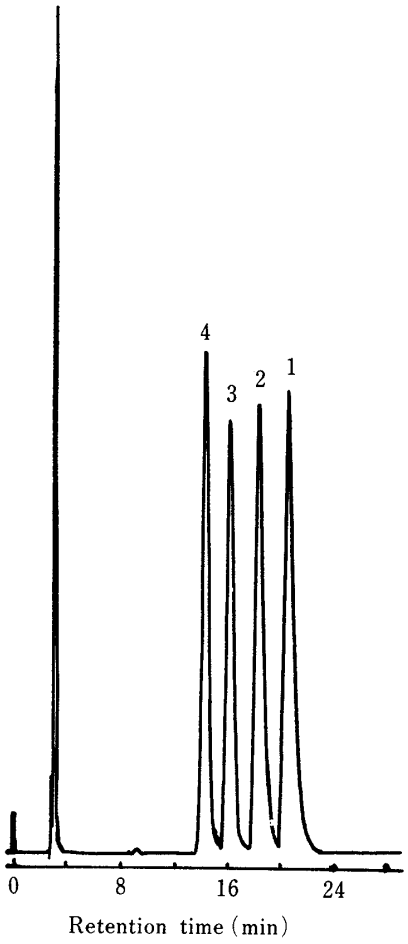

Fig. 1. High-Performance Liquid Chromatogram Showing the Separation of Standard Pilocarpine (1), Isopilocarpine (2), Isopilocarpic Acid (3) and Pilocarpic Acid (4)

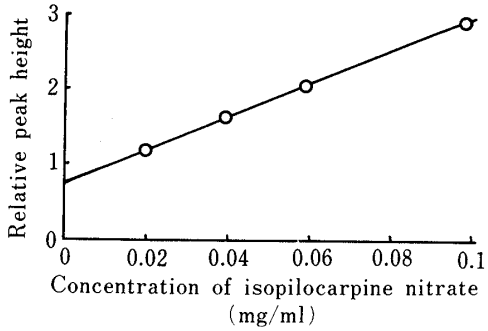

Fig. 2. Calibration Plots for Isopilocarpine Coexisting with a Large Amount of Pilocarpine

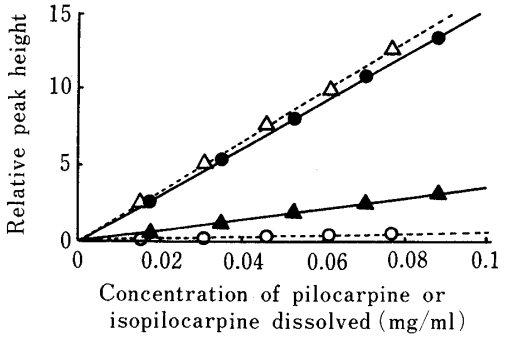

Fig. 3. Calibration Plots for Pilocarpic Acid and Isopilocarpic Acid

——, peak heights of pilocarpic acid (●) and isopilocarpic acid (▲) formed by dissolving pilocarpine in 0.1 N NaOH.

– – – –, peak heights of pilocarpic acid (○) and isopilocarpic acid (△) formed by dissolving isopilocarpine in 0.1 N NaOH.

Pilocarpic acid, for which a reference standard was not available, was determined by comparing the peak height to that of pilocarpic acid formed by the hydrolysis of pilocarpine in an alkaline solution. Pilocarpine hydrochloride and isopilocarpine nitrate were separately dissolved in 0.1 N NaOH to give concentrations of 0.02—0.12 mg/ml, and after 5 min standing, the solutions were diluted ten-fold with the mobile phase solvent, and subjected to HPLC immediately. Both pilocarpine and isopilocarpine were found to be hydrolyzed completely into pilocarpic acid and isopilocarpic acid in 0.1 N NaOH within 5 min at 25 °C, and the acids formed were found to be stable in 0.1 N NaOH (no change in the peak heights was observed up to 1 h). When the 0.1 N NaOH solution was diluted with the mobile phase solvent, that is an acidic solvent, pilocarpic acid and isopilocarpic acid were converted slowly to pilocarpine and isopilocarpine, respectively, and the peak heights of these compounds decreased with time as shown in Table I. Thus, the sample solutions were subjected to HPLC immediately after dilution. As shown in Fig. 3, the ratio of pilocarpic acid to isopilocarpic acid formed by the hydrolysis of pilocarpine differed from that formed by the hydrolysis of isopilocarpine. The percent of pilocarpic acid formed when a certain amount of pilocarpine is dissolved in 0.1 N NaOH, x, could be calculated by means of the following equation:

$$(A_2/A_1)x+(B_2/B_1)(100-x)=100$$

where A_1 and B_1 are the peak heights of pilocarpic acid and isopilocarpic acid formed upon dissolving a certain amount of pilocarpine in 0.1 N NaOH, respectively. A_2 and B_2 are those upon dissolving the same amount of isopilocarpine. Since x was estimated to be 78.8%, pilocarpic acid in the sample solutions was determined by comparing the peak height with that of pilocarpic acid formed as the standard, corresponding to 0.788 times of the amount of pilocarpine dissolved.

Pilocarpine and its degradation products in ointment and conjunctival insert preparations were determined by HPLC after the following pretreatment; a unit of conjunctival insert preparation and 500 mg of the ointment were dissolved in 50 and 100 ml of $CHCl_3$, respectively. Five milliliters of the solution was shaken vigorously with 5 ml of

Table I. Stability of Pilocarpic Acid and Isopilocarpic Acid in the Mobile Phase

	Temperature	Relative peak height (%)		
		0 min[a]	15 min[a]	30 min[a]
Pilocarpic acid	r.t.	100	99.6	98.7
	4 °C	100	99.9	100
Isopilocarpic acid	r.t.	100	92.6	86.4
	4 °C	100	99.0	97.9

a) Time after dilution of 0.1 N NaOH solution containing pilocarpic acid and isopilocarpic acid with the mobile phase solvent. r.t. = room temperature.

5% KH_2PO_4 solution adjusted to pH 2.5 with phosphoric acid, and 10 μl of the solution was immediately subjected to HPLC.

Kinetic Studies——The kinetic study to clarify the effects of the pH and concentration of buffer solution on the degradation rate of pilocarpine in aqueous solutions was carried out at 80 °C with 0.1% pilocarpine solutions buffered at pH 4.0, 5.0 and 6.0. The most concentrated buffer solution at each pH was prepared by mixing 0.4 M Na_2HPO_4 and 0.2 M citric acid, and diluted to prepare lower concentrations of buffer solutions. The ionic strength of the buffer solutions was adjusted to be 0.76 with KCl.

One milliliter of the solution was withdrawn at appropriate intervals, diluted ten-fold with the mobile phase solvent, and subjected to HPLC.

Stability Test of Commercial Products——One of the commercial eye drops (denoted as solution B in Table II) was stored in a water bath thermostated at 80 ± 0.2 °C. An ointment sample and a conjunctival insert preparation were stored in a desiccator containing a saturated solution of NaCl placed in a thermostated chamber; temperature control was ± 1 °C.

Results and Discussion

Contents of Degradation Products in Commercial Pilocarpine Formulations

Pilocarpine is known to degrade to isopilocarpine, pilocarpic acid and isopilocarpic acid through epimerization and hydrolysis as represented in Chart 1. The contents of pilocarpine and its degradation products in various dosage forms of pilocarpine ophthalmic preparations were determined and the results are shown in Table II. The conjunctival insert preparation is a diffusion-control unit, in which pilocarpine free base is put between two polymer membranes.

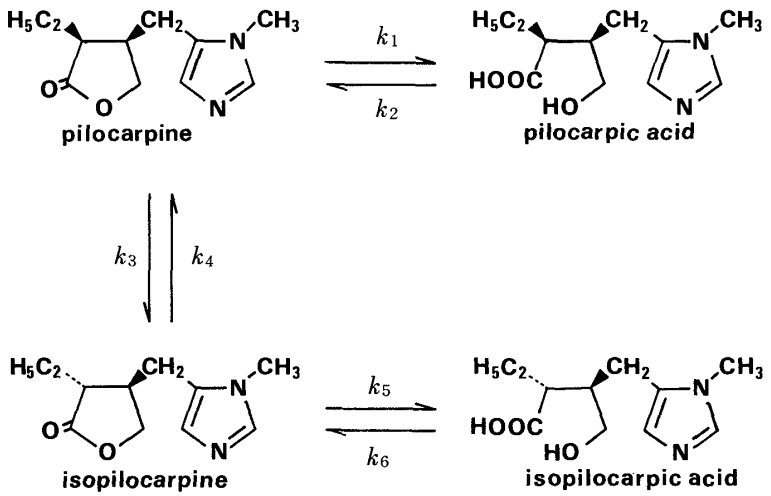

Chart 1

TABLE II. The Contents of Pilocarpine and Degradation Products in Commercial Ophthalmic Preparations

Preparation	Label claim	pH	Percent label claim determined[a]		
			Pilocarpine	Isopilocarpine[b]	Pilocarpic acid[b]
Solution A	0.5%	5.21	96.3 (1.4)	0.89 (0.07)	2.35 (0.06)
Solution B	0.5%	5.29	100.8 (0.8)	1.89 (0.06)	5.97 (0.03)
Solution C	0.5%	5.52	99.4 (1.2)	1.29 (0.03)	9.71 (0.08)
Solution D	0.1%	4.78	92.3 (2.7)	0.52 (0.03)	1.83 (0.03)
Ointment	1.0%		100.4 (2.7)	nd	nd
Conjunctival insert	11 mg/unit		95.2 (4.3)	nd	nd
	5 mg/unit		90.4 (4.5)	1.3 (0.3)	0.8 (0.8)

a) ( ): standard deviation. Three units of each preparation were tested. *b*) Percent of pilocarpine degraded to isopilocarpine or pilocarpic acid with respect to the label claim of pilocarpine content. nd: not detected.

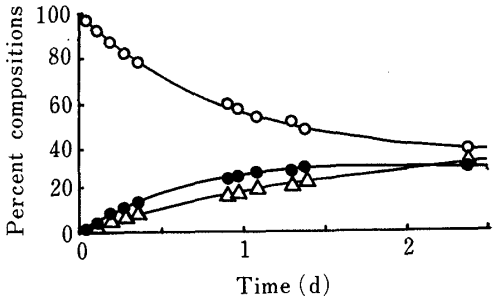

Fig. 4. Degradation of Pilocarpine and Formation of Isopilocarpine and Pilocarpic Acid in Buffer Solution (pH 6.0) at 80 °C

○, pilocarpine; ●, pilocarpic acid; △, isopilocarpine. Concentration of phosphate: 0.25 M.

Isopilocarpic acid was not detected in any of the preparations. The eye drops and conjunctival insert preparations tested were found to contain a considerable amount of isopilocarpine and pilocarpic acid as degradation products, while no product was detected in the ointment, in which pilocarpine hydrochloride was mixed with an oil base. This suggests that pilocarpine is stable in a hydrophobic environment, assuming that the impurities found in eye drops and the conjunctival insert preparation arise from the degradation of pilocarpine. Comparison of the contents of degradation products indicates that hydrolysis to pilocarpic acid is the predominant pathway in the degradation of eye solutions, especially at higher pH. In the case of the conjunctival insert preparation, however, isomerization to isopilocarpine seems to prevail over hydrolysis.

Degradation in Aqueous Solution

Degradation of pilocarpine in aqueous solution is known to include hydrolysis and epimerization.[1-8] The present studies showed that hydrolysis of isopilocarpine to isopilocarpic acid was not involved in the degradation of pilocarpine in the pH range of 4.0 to 6.0, except for the final stage of the degradation at pH 6.0. A typical degradation pattern is shown in Fig. 4. A relatively high concentration of buffer solution was needed to maintain a constant pH, and a marked drop in the pH was observed in diluted phosphate buffer solution such as that used by Porst and Kny.[8]

The rate equations for the degradation of pilocarpine in aqueous solution of pH 4.0 to 6.0 are expressed by Eq. 1,

$$dC_1/dt = k_4C_2 - k_3C_1 - k_1C_1 + k_2C_3$$

$$dC_2/dt = k_3C_1 - k_4C_2$$

$$dC_3/dt = k_1C_1 - k_2C_3$$

$$C_1 + C_2 + C_3 = 1 \qquad (1)$$

where C_1, C_2 and C_3 are the concentrations of pilocarpine, isopilocarpine and pilocarpic acid, respectively. Equation 1 can be integrated, employing the conditions that $C_1=1$ and $C_2=C_3=0$ at $t=0$, to give Eq. 2.

$$C_2=A_1e^{r_1t}+A_2e^{r_2t}+k_3k_2/\beta$$
$$C_3=A_3e^{r_1t}+A_4e^{r_2t}+k_4k_1/\beta$$
$$C_1=1-C_2-C_3 \qquad (2)$$

where

$$A_1=k_3(\beta+k_2r_2)/(r_1-r_2)\beta$$
$$A_2=k_3(\beta+k_2r_1)/(r_2-r_1)\beta$$
$$A_3=k_1(\beta+k_4r_2)/(r_1-r_2)\beta$$
$$A_4=k_1(\beta+k_4r_1)/(r_2-r_1)\beta$$
$$r_1=-\alpha+\sqrt{\alpha^2-\beta}$$
$$r_2=-\alpha-\sqrt{\alpha^2-\beta}$$
$$\alpha=(k_1+k_2+k_3+k_4)/2$$
$$\beta=k_4k_1+k_3k_2+k_4k_2$$

Based on the changes in concentrations of pilocarpine and its degradation products as a function of time, observed at pH 4.0 to 6.0, the rate constants, k_1, k_2, k_3 and k_4, were estimated by nonlinear regression analysis using the NONLIN computer program.[11] The estimated values of the rate constants for each pH were found to depend on the oncentration of buffer solution used. Figure 5 shows the plots of the rate constants estimated for the degradation at pH 6.0 against the concentration of the buffer solution. Extrapolation of the k values to the y-axis provided estimates of k_1, k_2, k_3 and k_4 without the effect of buffer

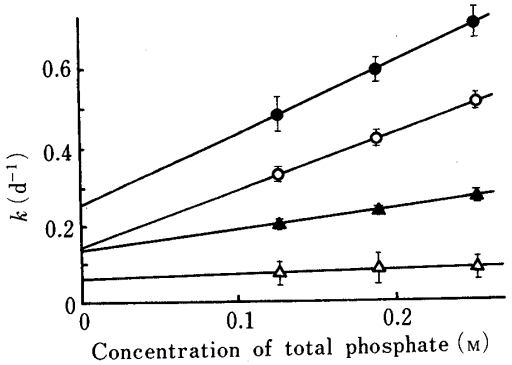

Fig. 5. Effect of General Base Concentration on the Degradation of Pilocarpine in Buffer Solution (pH 6.0) at 80 °C

○, k_1; ●, k_2; ▲, k_3; △, k_4.

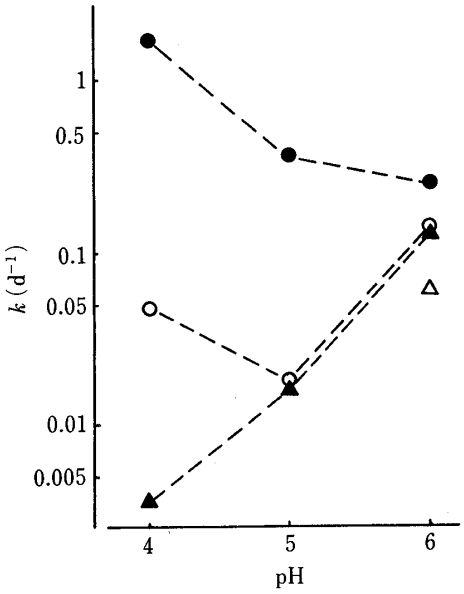

Fig. 6. Effect of pH on the Degradation of Pilocarpine

○, k_1; ●, k_2; ▲, k_3; △, k_4.

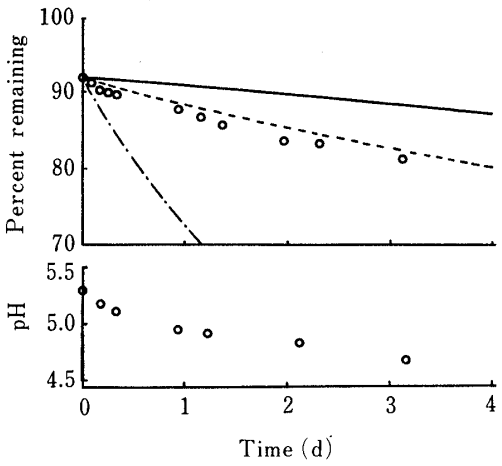

Fig. 7. Degradation Curves of Pilocarpine in a Commercial Ophthalmic Solution (0.5%, pH 5.29)

The lines are simulated for pH 6 (—·—) pH 5.29 (------) and pH 5 (——) with the k values estimated in Fig. 6.

TABLE III. Stability of Pilocarpine in Ointment and Conjunctival Insert Preparations

	Storage conditions	Storage time (month)	Percent lable claim determined[a]		
			Pilocarpine	Isopilocarpine[b]	Pilocarpic acid[b]
Ointment	40 °C, 75%RH	0	100.4 (2.7)	nd	nd
		2	102.2 (1.2)	nd	nd
Conjunctival insert	40 °C, 75%RH	0	90.4 (0.5)	1.3 (0.3)	0.8 (0.8)
		1	80.8 (1.0)	3.9 (1.2)	1.8 (0.5)
		2	76.0 (2.8)	6.5 (1.2)	4.1 (1.4)
	25 °C, 75%RH	1	89.7 (3.8)	2.3 (0.6)	2.1 (0.3)
		2	86.2 (3.6)	3.0 (1.3)	2.3 (1.3)

a) ( ): standard deviation. *b*) Percent of pilocarpine degraded to isopilocarpine or pilocarpic acid with respect to the label claim of pilocarpine content. nd: not detected. RH: relative humidity.

components, and the resulting values are plotted against pH in Fig. 6.

Porst and Kny recently determined the rate constants in a similar pH range, assuming that reverse formation of pilocarpine from isopilocarpine was negligible ($k_4=0$).[8] In the present study, however, a significant value of k_4 was estimated at pH 6.0 (Fig. 5), which suggests that it is unreasonable to neglect k_4 in calculation of the rate constants. Nonlinear regression analysis allowed estimation of the rate constants including k_4.

Factors Affecting the Stability of Pilocarpine Preparations

As shown in Fig. 6, the degradation rate in aqueous solution depends largely on pH, so pH is one of the main factors affecting the stability of eye drops. Degradation of pilocarpine in a solution formulation (solution B) was followed to clarify further the factors affecting the stability and the results are shown in Fig. 7, where the change in pH is also plotted. The lines drawn in Fig. 7 represent the degradation time course simulated with the values of rate constants estimated for pH 5.00, 5.29 (the original pH value of the preparation) and 6.00. It was found that pilocarpine in the preparation degraded faster than expected from the pH value, though the degradation was accompanied with a decrease in pH. This suggests that additives in the preparation, such as general bases, may enhance the degradation.

Table III shows the stability of pilocarpine ointment and conjunctival insert preparations. As expected from the impurity data on the preparations, pilocarpine was found to be stable in the ointment preparation, while it was susceptible to epimerization and hydrolysis in the conjunctival insert preparation. It is suggested that pilocarpine in ointment is protected from degradation because of the hydrophobic environment. On the other hand, pilocarpine in the

conjunctival insert preparation, which exists as a film in the solid state, is suggested to be relatively unstable. As for the degradation pathway, epimerization prevailed over hydrolysis, in accordance with the observation by Hill and Barcza[1] that pilocarpine undergoes epimerization in the dry state.

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