Application of Microcalorimetry to Stability Testing of Meclofenoxate Hydrochloride and *dl*-α-Tocopherol

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Utility of the microcalorimetric technique as a method of predicting drug stability was investigated. The hydrolysis of meclofenoxate hydrochloride and the oxidation of *dl*-α-tocopherol were chosen as model reactions. The hydrolysis rate was calculated from the time profile of the heat production. The rate was also calculated from the time profile of the degradation obtained by HPLC. The agreement in the rates confirms the applicability of microcalorimetry in determining rates of chemical reactions. The oxidation of *dl*-α-tocopherol was slow and a constant heat production rate was observed. The oxidation rate was calculated from the constant heat production rate and the enthalpy change for the reaction. To determine the rate constant required only a day by microcalorimetric method, whereas from the time profile of the degradation obtained by HPLC it would require almost one year. The saving in time to determine the degradation rate of *dl*-α-tocopherol demonstrates the value of this method.

Keywords microcalorimetry; stability; meclofenoxate hydrochloride; *dl*-α-tocopherol

Accelerated stability testing is usually carried out to predict drug stability. However, this method suffers from the uncertainty of extrapolation of results to a temperature of interest. Since heat is a by-product of most chemical reactions, measurement of the rate of heat production or consumption accompanied by drug degradation can be used to evaluate the degradation kinetics at a temperature of interest. These rates can be rapidly and easily measured by microcalorimetry. Though the utility of microcalorimetry for the stability studies of drugs has been documented,[1-8] the applicability of the method is still obscure.

The purpose of this paper is to evaluate the utility of the microcalorimetric method as a method of predicting drug stability. Hydrolysis and oxidation are common mechanisms of drug degradation. Meclofenoxate hydrochloride (MF) is hydrolyzed at its ester bond in aqueous solution and *dl*-α-tocopherol (TP) is oxidized in the air. These reactions were chosen as model reactions, and the degradation rates were determined by microcalorimetry to examine the applicability and the usefulness of the method.

Theory

The quantity directly measured by microcalorimetry is the rate of heat production, q (J/s). The rate q is the time derivative of the total heat production and is expressed as

$$q = \Delta H(dm/dt) \quad (1)$$

where ΔH is the enthalpy change for the reaction (J/mol), and m is the amount of drug (mol) to be reacted.[2,3] Exothermic heat flow signals are given positive signs in this paper. If degradation follows first-order kinetics, q is related to the first-order rate constant k (s^{-1}) by the following equation

$$q = -\Delta H k m_0 \exp(-kt) \quad (2)$$

where m_0 is the initial amount of drug (mol). The rate constant k can be obtained from q *vs.* t plot.

If the degradation rate is slow, the heat flow curve is obtained as a flat line during the first stage of the reaction. The value of the plateau of the heat flow curve, in other words, the initial rate of heat production, q_0, is used to obtain the rate constant. At the first stage of the reaction, Eq. 1 can be rewritten as

$$q_0 = -\Delta H k m_0 \quad (3)$$

The value of ΔH is calculated from the heat flow curve of the same sample at a higher temperature by using Eq. 2. If we assume that ΔH is constant over the temperature range studied, then the rate constant is calculated using Eq. 3.

Materials and Methods

Materials MF was supplied by Dainippon Pharmaceutical Co., Ltd., and TP was purchased from Wako Pure Chemical Industries, Ltd. All other chemicals were of reagent grade.

Microcalorimetry The isothermal microcalorimeter system used in this study was a Thermometric 2277 thermal activity monitor (TAM) equipped with four independent differential heat-conduction microcalorimeters. The sample was placed in a rubber-stoppered glass vessel (3 ml). A sealed sample and a reference vessel were lowered to a thermal equilibration position for 30 min, and then inserted into the measurement position.

MF was dissolved in pH 6.4 or pH 2.9 citrate–Na_2HPO_4 buffer solution. A sample vessel was loaded with 2 ml of MF solution immediately after solution preparation and a reference vessel was loaded with the same amount of the solvent. The initial amount of MF was 6.9×10^{-6} mol (pH 6.4) or 1.4×10^{-4} mol (pH 2.9).

For studies with TP, which is a slightly viscous liquid, a sample vessel was loaded with about 430 mg (10^{-3} mol) of the drug and a reference vessel was left empty. In order to attain the steady state of oxygen in TP liquid, the sample vessel with TP was put in a 100 ml sample tube and stored in a thermostat at the same temperature as the microcalorimetric experiment. After 1 d (50 °C), 1 week (40 °C), 2 weeks (30 °C) or 1 month (23 °C), the vessel was sealed and placed in the microcalorimeter immediately.

Determination of Degradation Rates by HPLC A certain amount of the MF solution stored at 25 °C was taken at suitable intervals for HPLC analysis. The amount of *p*-chlorophenoxyacetic acid, the decomposition product of MF, was determined by HPLC. The HPLC equipment consisted of a Hitachi Model 655 system, a Tosoh Model AS-8000 autosampler and a Shimadzu Model C-R3A computing integrator. Samples were injected through a 20-μl loop to the column (Inertsil ODS-2, 150 mm × 4.6 mm, Gasukuro Kogyo) maintained at 35 °C. The mobile

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phase was a mixture of methanol and 50 mM phosphate buffer (pH 2.5) (1 : 1) and the elute was monitored at 230 nm.

For measurement of the degradation rate of TP by HPLC, samples were prepared in a similar way as for microcalorimetric measurements, except that the vessels were not sealed. A microcalorimetric vessel containing 430 mg of TP was covered with aluminum foil to protect it from light, put in a 100 ml (50, 60 °C), 200 ml (70 °C) or 300 ml (80 °C) sealed flask and stored at the desired temperature. The flasks were sealed and were opened periodically to supply air. The vessels were taken at certain intervals and all the drug in the vessel was dissolved with methanol for the assay. The amount of TP remaining was determined by HPLC. The mobile phase was a mixture of methanol and water (49 : 1) and the elute was monitored at 292 nm.

Results and Discussion

Hydrolysis of MF MF is known to be hydrolyzed in aqueous solution.[9] Figure 1 shows the time course of its decomposition (MF: 1.0 mg/ml) in pH 6.4 buffer at 25 °C followed by HPLC, as well as the time course of the heat production for the same solution measured by microcalorimetry. The decomposition and the heat production both followed first-order kinetics. Agreement in the slopes of the lines indicates that the heat production was caused by the hydrolysis of MF. The degradation rate constant was calculated to be $1.29 \times 10^{-4}\,s^{-1}$ by the HPLC method and $1.14 \times 10^{-4}\,s^{-1}$ by the microcalorimetric method. The coincidence of these rate constants suggests that microcalorimetry can be used to determine the rates of chemical reactions.

Figure 1 also shows the time course of the heat production for MF (20 mg/ml) degradation in pH 2.9 buffer at 25 °C. The flat heat flow curve indicates that the hydrolysis at pH 2.9 was much slower than that at pH 6.4, and this observation agrees with the published data.[9] The initial rate of heat production was estimated to be 1 μJ/s, which was the lower limit of detecting heat production in our experiments. The rate constant was calculated to be $9.7 \times 10^{-7}\,s^{-1}$ using Eq. 3. The value of ΔH had been obtained as -7.4 kJ/mol from the time profile of the heat production for the same solution at 45 °C using Eq. 2. The HPLC method required 3 d to determine the rate constant as $9 \times 10^{-7}\,s^{-1}$. The agreement in the rates confirms the applicability of the microcalorimetric method in determining slow degradation rates.

Oxidation of TP TP is known to be oxidized in the air.[10] The time courses of TP degradation followed by HPLC are shown in Fig. 2. The solid lines in the figure were calculated by nonlinear regression analysis according to a first-order kinetic expression. The typical time courses of the heat production are shown in Fig. 3. Each heat flow curve had a plateau which was the initial rate of heat production, q_0. The value of ΔH was calculated to be -241 kJ/mol from the apparent rate constant obtained by the HPLC method and the initial rate of heat production at 50 °C using Eq. 3. The apparent rate constant at each temperature was obtained from each q_0 value and ΔH value also by using Eq. 3. The initial heat production rates and the apparent rate constants of TP degradation are listed in Table I.

The Arrhenius plot of the apparent first-order rate constant of TP degradation obtained by the HPLC and the microcalorimetric method is shown in Fig. 4. The solid line was calculated by least-squares fitting of the data. No

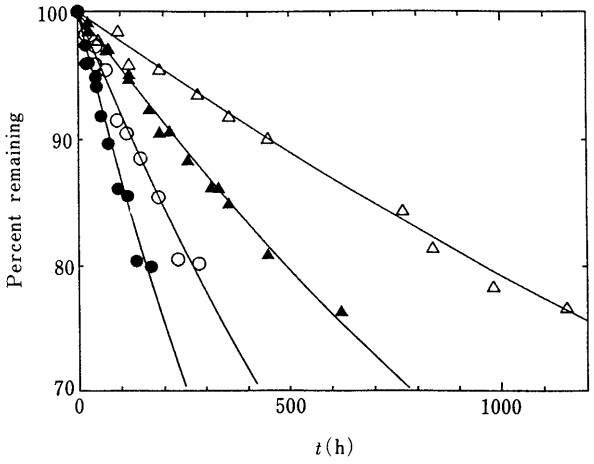

Fig. 2. Time Courses of the Degradation of TP at Different Temperatures Followed by HPLC

●, 80 °C; ○, 70 °C; ▲, 60 °C; △, 50 °C.

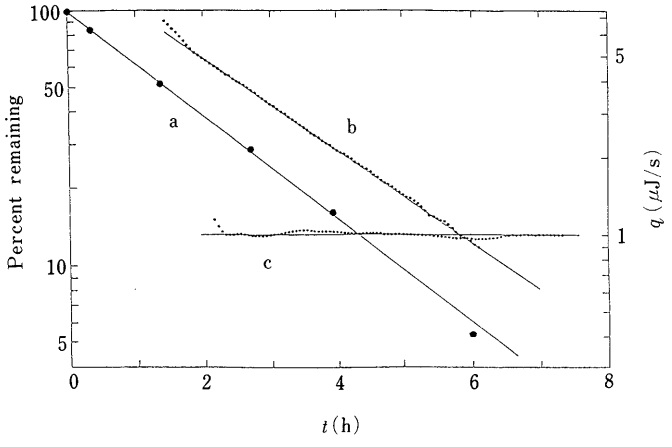

Fig. 1. Time Courses of Decomposition of MF and Heat Production for MF Degradation in Buffer at 25 °C

(a), decomposition (pH 6.4, 1 mg/ml) followed by HPLC; (b), heat production (pH 6.4, 1 mg/ml) measured by microcalorimetry ($m_0 = 6.9 \times 10^{-6}$ mol); (c), heat production (pH 2.9, 20 mg/ml) measured by microcalorimetry ($m_0 = 1.4 \times 10^{-4}$ mol).

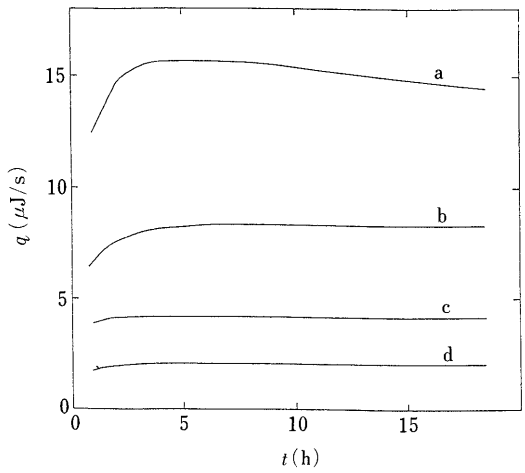

Fig. 3. Time Courses of Heat Production for TP Degradation at Different Temperatures Measured by Microcalorimetry

(a), 50° C; (b), 40 °C; (c), 30 °C; (d), 23 °C.

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TABLE I. Initial Heat Production Rates (q_0) and Apparent Rate Constants (k) of TP Degradation

Temp. (°C)	Microcalorimetry		HPLC
	q_0 (μJ/s)	k (h^{-1})	k (h^{-1})
80			1.41×10^{-3}
70			8.32×10^{-4}
60			4.54×10^{-4}
50	15.6±0.5		2.33×10^{-4}
40	7.9±0.3	1.2×10^{-4}	
30	4.2±0.2	6.3×10^{-5}	
23	2.0±0.3	3.0×10^{-5}	

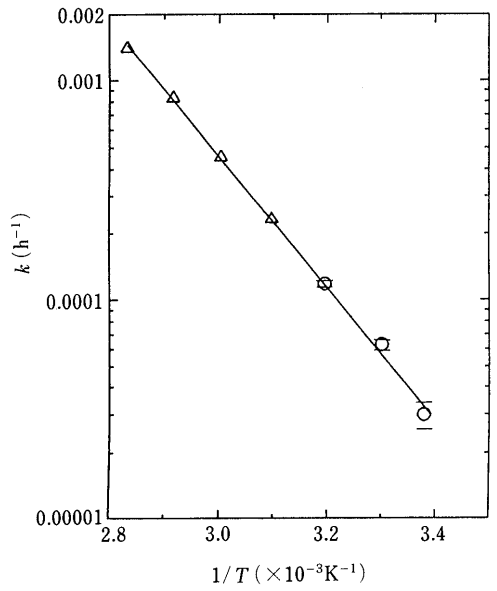

Fig. 4. Arrhenius Plot of the Apparent First Order Rate Constant of TP Degradation

△, data obtained by the HPLC method; ○, data obtained by the microcalorimetric method. Error bars represent standard deviation for $n=4$—8.

marked discrepancy was observed between the data obtained by the two methods, which strongly suggests that the heat production was caused by TP oxidation. This assumption was also supported by the fact that the heat flow curves were found to fall to near zero when the experiments were performed after replacement of the space of the vessels with N_2. As shown in Fig. 3, the heat production at 50 °C decreased gradually after about 8 h, which can be ascribed to the depletion of oxygen.

The apparent rate constant at 23 °C obtained by the microcalorimetric method was $3.0 \times 10^{-5}\ h^{-1}$. The HPLC method would require almost one year to determine the rate constant which could be easily and rapidly obtained by the microcalorimetric method. The considerable reduction in time and labor demonstrates the usefulness of microcalorimetry.

The value of enthalpy change for the TP oxidation was found to be greater than that for the MF hydrolysis. The fact that the smaller rate constant could be determined for the TP oxidation than for MF hydrolysis by the microcalorimetric method was ascribed mainly to the large enthalpy change for the TP oxidation, since heat production is proportional to an enthalpy change for a reaction. The microcalorimetric method is suggested to be advantageous to determine the rates of reactions with large enthalpy changes, as indicated in the oxidation of TP.

Conclusion

The agreement in the rate constants of MF hydrolysis obtained by the microcalorimetric and by the HPLC methods confirms the applicability of microcalorimetry in determining rates of chemical reactions. The rate of TP degradation at room temperature was rapidly and easily obtained by the microcalorimetric method, and this technique is suggested to be advantageous to determine the rates of the reactions with large enthalpy changes such as the oxidation of TP. The results in this paper offer information on suitable drugs for use in the microcalorimetric method to estimate drug stability.

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