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Utility of microcalorimetry in the characterization of the browning reaction

Rodney D. Vickery *, Michael B. Maurin

Pharmacy R&D, DuPont Pharmaceuticals Company, Experimental Station, PO Box 80400 Wilmington, DE 19880-0400, USA

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Abstract

An isothermal microcalorimeter was utilized to characterize a model solid-state interaction. The degradation of the HIV protease inhibitor, DMP 450, in a binary mixture with hydrous lactose was followed in the presence of 5% additional water. Heat produced in the microcalorimeter sample vessel from either chemical or physical change is channeled through extremely sensitive thermopile blankets and is measured as it flows into infinite heat sinks. Solid-state 1:1 mixtures of DMP 450 and hydrous lactose each with 5% water added were analyzed in the microcalorimeter at 50, 60 and 65°C. The resulting heat flow profiles were consistent with an autocatalytic rate law. An activation energy of 26.12 kcal mol^{-1} for the DMP 450:lactose mixture was determined from the slope of the Arrhenius plot of the microcalorimetry heat flow maximum value versus the reciprocal of the absolute temperature. The activation energy determined by the traditional method with HPLC analysis was found to be in excellent agreement with the microcalorimetry value at 26.38 kcal mol^{-1}.

Keywords: Isothermal microcalorimetry; DMP 450; Cyclic urea; HIV protease inhibitor; Lactose

1. Introduction

An isothermal microcalorimeter was utilized to characterize a model solid-state interaction [1,2]. The degradation of the HIV protease inhibitor, DMP 450, a primary amine, in a binary mixture with hydrous lactose was followed in the presence of 5% (w/w) additional water. A brown discoloration was observed in the post experiment mixtures. This 'browning reaction' resulted from a Maillard type condensation reaction between the lactose and the primary amine functionality of the DMP 450 [3,4]. The microcalorimeter measured accurately the heat generated in the sample vessel with temperature changes of less than 10^{-6}°C detectable. The data was interpreted assuming that any given reaction exchanges heat with its surroundings, that heat exchange rate is a function of the reaction rate. Heat produced in the microcalorimeter sample vessel from either chemical or physical change is channeled through extremely sensitive thermopile blankets and is measured as it flows into infinite heat sinks. By making measurements at several different temperatures, the relevant activation energy can be determined.

* Corresponding author. Tel.: +1-302-6958336.

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2. Experimental

2.1. Materials and methods

The isothermal microcalorimeter used was a ThermoMetric model LKB2277 Thermal Activity Monitor which was equipped with a VWR Scientific model 1156 refrigerated circulator bath. The 1156 circulator is a heat sink for the 25 l water thermostat which surrounds the reaction vessel of the microcalorimeter. The tandem systems maintained the measuring vessel temperature constant to $\pm 2 \times 10^{-4}$°C. The microcalorimetry data acquisition was completed with the ThermoMetric Digitam 3 software package. DMP 450 (Fig. 1) was synthesized by the DuPont Pharmaceuticals Company, Chambers Works, Deepwater, NJ and the hydrous lactose was USP/NF grade. The water added to the DMP 450:lactose mixtures was deionized by a Millipore Milli-Q plus system.

2.2. Isothermal microcalorimeter method

Samples were prepared for analysis by the microcalorimeter by blending geometrically the 1:1 mixture of DMP 450 and lactose in a mortar and pestle and then weighing 200 mg of the binary mixture into 4 ml glass ampules. To eliminate the thermal interference caused by the heat of dissolution, the addition of 5% water to the samples was accomplished by filling the appropriate amount of water into a 250 μl polypropylene vial insert. A reference vial was prepared with a vial insert and an amount of water equal to the sample vial. Both vials were stoppered with Teflon lined stoppers and crimped closed with aluminum seals. The microcalorimeter water bath temperature was equilibrated for at least 18 h, the amplifier adjusted to zero and a 300 μwatt electronic amplifier calibration was performed before each analysis. After the calibration step, the sample and reference vials were loaded on to the ampule lifter rods, lowered into the microcalorimeter measuring cylinder temperature equilibration position for 10 min, then lowered into the measuring position. The Digitam software provided a real-time heat flow profile. Samples were analyzed at 50, 60 and 65°C.

2.3. Chromatographic method

Samples were prepared for analysis using HPLC by blending geometrically the 1:1 mixture of DMP 450 and lactose in a mortar and pestle and then weighing 20 mg of the binary mixture into 4 ml glass vials. The 5% water was added directly to the mixture in the vials which were then stoppered with Teflon lined stoppers and crimped closed with aluminum seals. The storage conditions were 40, 50 and 60°C. Samples were taken at appropriate time points and prepared for HPLC analysis by extracting the DMP 450 from the coke-like degradation product using sonication in dimethyl sulfoxide. A reverse phase 4.6 × 15 cm Zorbax® SB-C8 separation column (Mac Mod Chromatography) was employed with the column oven temperature maintained at 55°C (Column Heater Module and Temperature Control Module, Waters Corporation). The mobile phase was composed of 0.025 M phosphate buffer pH 3.6:acetonitrile (55:45). An isocratic flow rate of 1.0 ml min^{-1} was utilized (Controller, Model 680 and HPLC pump, Model 510, Waters Corporation). Ultraviolet detection was employed at 220 nm (HP 1050 Variable Wavelength Detector, Hewlett-Packard). The retention time for DMP 450 was 5.1 min. Data acquisition was completed with a VAX-based program that calculated the sample concentrations from a standard curve using peak area (Multichrom® software, Fisons Instruments).

3. Results and discussion

The solid-state excipient compatibility study that utilized the HPLC assay followed the initial rate of loss of DMP 450 assuming a first order

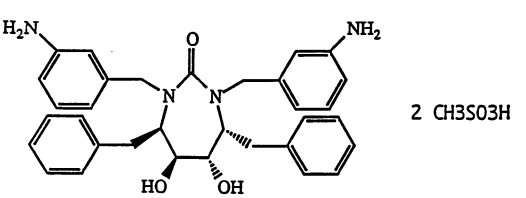

Fig. 1. Chemical structure of DMP 450.

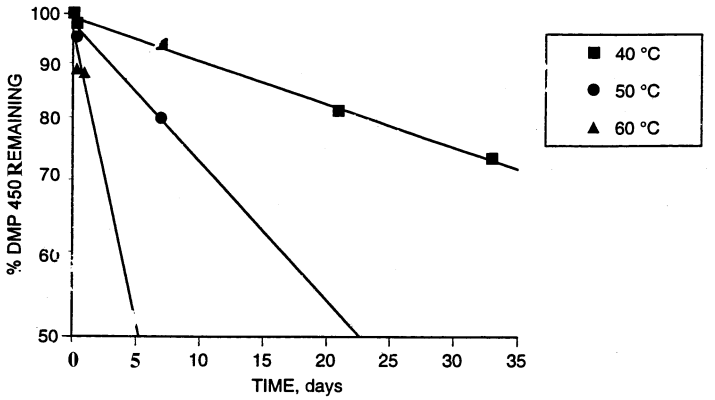

Fig. 2. The percent DMP 450 remaining vs. time for the DMP 450:lactose with 5% water added at 40, 50 and 60°C as determined by HPLC analysis.

degradation. The initial rate was utilized because the reaction progress could not be quantified beyond approximately 30% DMP 450 degradation due to the difficulties associated with extracting the DMP 450 from the insoluble coke-like degradation product. The degradation rate constants were 9.78×10^{-3}, 3.14×10^{-2} and 1.25×10^{-1} day^{-1} at 40, 50 and 60°C, respectively (Fig. 2). An activation energy value for the reaction of 26.38 kcal mol^{-1} resulted from the Arrhenius plot of the HPLC data (Fig. 4).

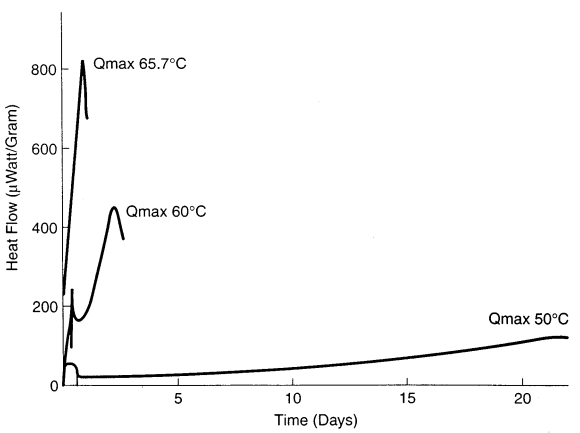

Fig. 3. Microcalorimetry heat flow profiles of 1:1 mixtures of DMP 450:hydrous lactose with 5% water added at 50, 60 and 65.7°C.

The resulting heat flow profiles from the microcalorimeter for the DMP 450:lactose mixtures with 5% water added were consistent with an autocatalytic rate law [1,2] and produced a heat flow maximum value (Q_{max}) in µwatt g^{-1} (Fig. 3). The Q_{max} value corresponds to the point in the reaction at which 50% of the reactant has been consumed. The Q_{max} value at 50°C was extrapolated from the HPLC analysis data to be at the time point equivalent to 51% unreacted DMP 450 remaining. The line resulting from plotting Q_{max} values versus the reciprocal of the absolute temperature (Fig. 4) has the equation,

$$\ln Q_{max} = \ln A - E/RT$$

where A is a constant, R is the gas constant and E is the activation energy. An activation energy value of 26.12 kcal mol^{-1} for the DMP 450:lactose with 5% water resulted from the microcalorimetry experiments and is in close agreement with the value which was determined by the traditional method with HPLC analysis.

Microcalorimetry has been found to be a reliable and accurate instrument which can be utilized for solid-state excipient compatibility studies. Microcalorimetry has the obvious time advantage over traditional methods involving the preparation of granulations, weighing several dozen individual samples, sample extraction/preparation and HPLC analysis. Utilization of

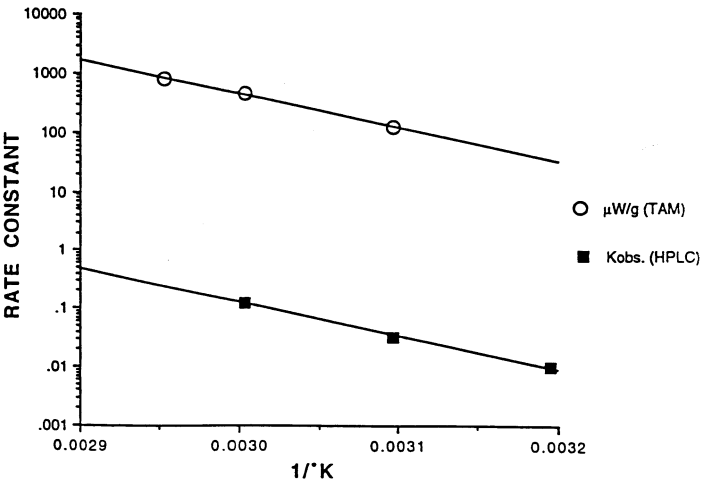

Fig. 4. Arrhenius plot comparing microcalorimetry and HPLC results for the degradation of DMP 450:lactose with 5% water added.

microcalorimetry for rapid screening of drug and excipient interactions can provide the same information in 1 week that takes 8–12 weeks by traditional methods.

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