An Experiment in Physical Chemistry: Polymorphism and Phase Stability in Acetaminophen (Paracetamol)

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Many active pharmaceutical ingredients exhibit multiple crystal forms, a phenomenon known as polymorphism. Polymorphs of biologically active compounds have different relative thermodynamic stabilities, but their conversion from one form to another can be very slow. As a result, it is possible to create, either purposely or accidentally, metastable crystal forms that might suddenly convert to a more stable form years after a medicinal formulation has been developed: this was the situation with ritonavir, an AIDS-cocktail drug that developed formulation problems and was temporarily withdrawn from the marketplace *(1)*. Because of the thermodynamic differences between crystal forms, more stable forms always have lower solubility than less stable forms *(2)*. Lower solubility may threaten the bioavailability of an active ingredient. Unfortunately, the kinetics of crystallization favor metastable forms *(3, 4)*.

Polymorphs also have economic value by affecting patent protection for solid-dosage forms. In one well-known example, the patents for two forms of the active pharmaceutical ingredient of Zantac, ranitidine hydrochloride, were held by GlaxoSmithKline. One patent expired in 1995, but the other patent on the second form remained in force. This enabled GlaxoSmithKline to extend their control of the product for several years at a time when the market exceeded $3.5 billion/year *(4)*.

A set of thermodynamically based rules for interpreting density, calorimetry, and spectroscopy of crystal forms has been developed that permit chemists to determine which form among several is probably the most stable at a given temperature and whether it remains the most stable at all temperatures (monotropism) or not (enantiotropism). The Burger—Ramberger rules *(5, 6)* and Yu's method *(7)* are simple but elegant approaches to analyzing thermal and other types of data for polymorphic crystal forms to determine whether a system is monotropic or enantiotropic and which form is the most stable at a given temperature.

The experiment presented here examines the differential scanning calorimetry of two acetaminophen (paracetamol) polymorphs *(5, 7, 8)* and asks students to apply the thermodynamics-based Burger—Ramberger rules *(5)* and calculations of the type used by Yu *(7)* to determine the relative stabilities of the forms. The two polymorphic forms of acetaminophen are, fortuitously, readily available and require no extensive recrystallization efforts. This experiment connects physical chemistry with an important career skill for chemists working with solid forms, including pharmaceuticals, foods, and the dye industry.

This experiment fits into the physical chemistry laboratory sequence as an experiment in thermodynamics. A differential scanning calorimeter (DSC) is required for this experiment. Our instruments have always been power-compensation-type instruments, such as the Perkin-Elmer Diamond DSC, but a heat-flux DSC will also work. Data collection for this experiment requires approximately 2 h.

Experiment

The commercially available acetaminophen is form I. The acetaminophen (Sigma-Aldrich) was dried in a vacuum oven at 70 °C for 90 min in an open DSC pan, then covered, crimped, and inserted into the DSC. Form I was scanned over 100—200 °C at a rate of 20 °C/min. After heating, it was cooled at a rate of 50 °C/min to 50 °C. To produce form II, the sample was ramped 50—140 °C at a rate of 20 °C/min and held at 140 °C for 5 min. This sample was then cooled and heated to 100—200 at 20 °C/min to analyze form II.

Hazards

This laboratory uses acetaminophen (proper IUPAC name: *N*-(4-hydroxyphenyl)acetamide), also known as paracetamol or 4-acetamidophenol. It should be stored, handled, and disposed of properly. The quantity used in each of these experiments is small compared to a typical recommended dose of acetaminophen in over-the-counter medications. Nevertheless, consumption of large quantities should be avoided, and students should avoid getting the powder in their eyes or inhaling it.

Results

Typical DSC traces for form I and form II acetaminophen are shown in Figure 1. From these traces, the temperature of transition and the enthalpy of transition are readily determined to be 172.3 °C and 30.57 kJ/mol for form I and 160.1 °C and 29.56 kJ/mol for form II. Whereas form I is the commercially available form, form II is created directly in the DSC pan during the experiment by an annealing step after the first melting run.

In 13 repeats of the experiment, the melting point observed via DSC for form 1 occurred in the range 172.3—176.3 °C, with an average of 174.2 °C. The observed melting point for form II ranged between 160.1 and 164.3 °C, with an average of 161.9 °C.

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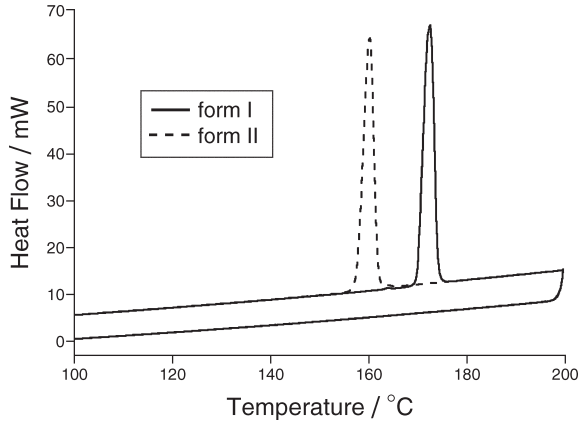

Figure 1. DSC traces for acetaminophen forms I and II. These data are for a 4.2 mg sample at a scan rate of 20 °C/min with data recorded at a rate of 1 Hz.

The fairly wide ranges in which these melting points occur make it appear that the determination of which form has the higher melting point is not as precise as one would like. However, the reported temperature depends somewhat on the ambient conditions, which can vary from one laboratory group to another. The difference between the melting points is reported more accurately than the melting points themselves. Thus, the difference in melting points recorded for forms I and II was 12.26 ± 0.29 °C, with form I always being higher.

The estimated enthalpies of fusion for forms I and II occurred in the range 27.71−30.57 kJ/mol and 26.60−29.56 kJ/mol, respectively, with average values of 29.3 ± 0.5 and 28.1 ± 0.5 kJ/mol (95% confidence interval, $n = 13$), respectively. Again these error limits make the uncertainties look relatively large. However, these errors are dominated by uncertainty in weighing the small DSC samples, which leads to errors in the magnitude of the enthalpy change. The ratio of the enthalpy changes is independent of this error because the same sample is recorded in both DSC traces. Thus, we find that the enthalpy of fusion for form II is 96.2 ± 0.5% (95% confidence interval, $n = 13$) of the value for form I.

Sacchetti *(8)* and Berger and Ramberger *(5)* also report data for acetaminophen. Both references and our own data show that form I has a higher melting point and a higher enthalpy of fusion than form II. The Berger−Ramberger heat of fusion rule states that if the higher melting form has the lower enthalpy of fusion, the forms are usually enantiotropes; otherwise, they are monotropes. Employing this rule leads to the conclusion that form I and form II are monotropically related forms.

A more quantitative approach is offered by Yu *(7)*. His method requires the four values calculated above, as well as the difference in heat capacities between form I and the melt at temperatures between the two melting points. Although the difference in heat capacities can be determined from a very careful DSC experiment *(9)*, that method is beyond the scope of our typical laboratory experiment. Yu offers the approximation, based on measurements of a number of polymorphic systems, that the difference in heat capacities (ΔC_p) is given by $\Delta C_p = 0.003/\mathrm{K} \times \Delta_{\mathrm{trans}}H$, where $\Delta_{\mathrm{trans}}H$ is the enthalpy of fusion for the higher-melting form. This gives a value of 90 J/(mol K) for ΔC_p in the present case. An alternative method used by Yu for estimating the change in heat capacity from the heating rate difference in the DSC traces leads to an estimate of 95.0 J/(mol K).

The goal of Yu's method is to identify the temperature at which two forms are in equilibrium. If the temperature is above the melting point of the lower melting form or is calculated to be below absolute zero, the forms are monotropic. If the equilibrium temperature is a real temperature at which both forms are solids, the two forms are enantiotropic. In Yu's method, we first calculate the enthalpy and entropy for a transition from the lower-melting to the higher-melting form at the lower melting point. Yu gives the following expressions for these quantities,

$$\Delta_{\mathrm{trans}}H = \Delta_{\mathrm{fus}}H_{\mathrm{II}} - \Delta_{\mathrm{fus}}H_{\mathrm{I}} + \Delta C_p(T_{\mathrm{fus,I}} - T_{\mathrm{fus,II}})$$

$$\Delta_{\mathrm{trans}}S = \frac{\Delta_{\mathrm{fus}}H_{\mathrm{II}}}{T_{\mathrm{fus,II}}} - \frac{\Delta_{\mathrm{fus}}H_{\mathrm{I}}}{T_{\mathrm{fus,I}}} + \Delta C_p \ln \frac{T_{\mathrm{fus,I}}}{T_{\mathrm{fus,II}}} \qquad (1)$$

$$\Delta C_p \equiv C_{p,\mathrm{L}} - C_{p,\mathrm{I}}$$

where the symbol Δ_{trans} is used on quantities for the transition from form II to form I at the melting point of form II, subscript fus means a fusion quantity for either form I or form II, T is a transition temperature in absolute degrees, H is enthalpy, C_p is the heat capacity at constant pressure, and L represents the liquid melt. If we make an approximation that the Gibbs energy of conversion has a linear dependence on temperature, the equilibrium temperature of the two solid forms is

$$T_{\mathrm{trans}} = \frac{\Delta_{\mathrm{trans}}H}{\Delta_{\mathrm{trans}}S} \qquad (2)$$

The use of Yu's method has a strong dependence on the difference in heat capacities that appears twice in eq 1, and these values tend to have higher uncertainty than any of the other four quantities of importance. Further, the method as described by eq 2 represents a linear approximation that is suitable if the transition temperature is close to the melting points of the polymorphic forms but becomes increasingly poor for more extreme estimates.

The quantity differences estimated from the DSC trace in Figure 1 are $\Delta_{\mathrm{trans}}H = -31.5$ J/mol, $\Delta_{\mathrm{trans}}S = +1.739$ J/(mol K), and $T_{\mathrm{trans}} = -18$ K, respectively. Under this approximation, one would conclude the system is monotropic, in agreement with the rule of Burger and Ramberger. Nevertheless, the transition temperature is not expected to lie below absolute zero, and it has been extrapolated far from the conditions where the enthalpy and entropy were measured. Under these conditions, the Burger−Ramberger rules and Yu's method become somewhat suspect because small errors due to less-than-completely accurate assumptions could play an important role in this conclusion. One of the most important factors omitted in our application of Yu's method, and in the reasoning of the Berger−Ramberger rules, is that we do not consider the details of how the heat capacities of the crystals change with temperature. Sacchetti *(8)* reports these data and makes what is probably a better estimate that ours: forms I and II of acetaminophen are enantiotropes, but with an extremely low equilibrium transition temperature near 100 K.

Summary

This experiment offers much that is desired in a physical chemistry laboratory. Detailed physical reasoning, which is explained in the supporting information for this report, leads

to the simple Burger−Ramberger rules. More quantitative approaches are available using different levels of approximation. The experiment is simple and quick using the protocol described in the supporting information.

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Supporting Information Available

Instructor notes; directions for the students. This material is available via the Internet at http://pubs.acs.org.

