JOURNAL OF CHEMICAL EDUCATION

Laboratory Experiment

pubs.acs.org/jchemeduc

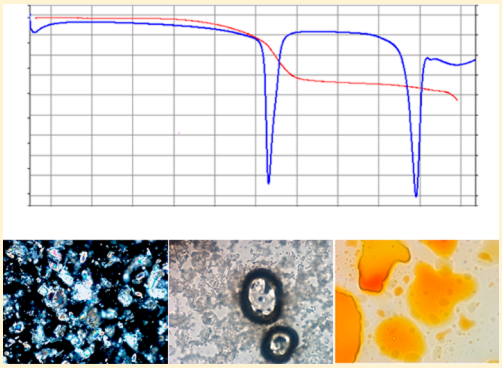
An Integrated Approach to Thermal Analysis of Pharmaceutical Solids

Shelley R. Rabel Riley*

Department of Natural Sciences, Northwest Missouri State University, Maryville, Missouri 64468 United States

Supporting Information

ABSTRACT: A three-tiered experiment for undergraduate Instrumental Analysis students is presented in which students characterize the solid-state thermal behavior of an active pharmaceutical ingredient (acetaminophen) and excipient (α-lactose hydrate) using differential scanning calorimetry, thermogravimetric analysis, and thermal microscopy. Students are required to perform qualitative and quantitative analysis, incorporating data from the three thermal analysis techniques to successfully interpret melting, decomposition, and dehydration thermal transitions. Students use thermal analysis software to determine transition temperatures and enthalpies of transitions, and use stoichiometric calculations to calculate the water of hydration.



KEYWORDS: *Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Hands-On Learning/Manipulatives, Drugs/Pharmaceuticals, Qualitative Analysis, Quantitative Analysis, Solid State Chemistry, Thermal Analysis, Instrumental Methods*

Thermal analysis plays a critical role in solid-state characterization in a wide range of disciplines such as pharmaceuticals,[1−3] nanotechnology,[4] polymers,[5,6] textiles,[7] and the food industry.[8] The pharmaceutical industry relies heavily on thermal analysis, along with spectroscopic and X-ray crystallographic methods to perform thorough physical chemical characterizations of active pharmaceutical ingredients (API) as well as inactive ingredients (excipients) that go into pharmaceutical products. Characterization of the solid state lays the foundation for formulation development and aids in science-driven rational decisions in the development of a stable and manufacturable drug product with a reasonable shelf life.

Thermal analysis spans a range of techniques that allow one to determine physical chemical properties as a function of temperature. Three of the most widely utilized thermal analytical methods used in pharmaceuticals are differential scanning calorimetry (DSC), thermogravimetric analysis (TGA), and thermal (hot stage) microscopy.[9] Using one or more of these techniques allows for the determination of the melting points for crystalline solids, the study of polymorphism, dehydration/desolvation behavior and stoichiometry, thermal stability, purity, amorphous content, glass transitions, and excipient compatibility.[10] In the majority of cases, compounds under investigation are new chemical entities, which have not been previously characterized. To gain a thorough understanding of the nature of thermal transitions most often requires not just a single analytical method to draw conclusions, but rather the collection and correlation of data from complementary techniques.

The experiment described herein is a three-part experiment in which Instrumental Analysis students are given two pharmaceutical compounds to characterize, one of which is an anhydrous crystalline API and the other an excipient that exists as a crystalline hydrate. Previous experiments reported in the literature for Instrumental Analysis students involved utilizing DSC and TGA to identify compounds based on properties that have known transitions that have already been well-characterized.[11,12] The current experiment requires students to perform some "investigative work" to determine the nature of the thermal transitions that they originally observe by DSC, and also incorporates thermal microscopy as a tool to aid in the identification of thermal events.[13] The exercise teaches students that real world problem-solving most often requires an analytical chemist to take a multifaceted approach using more than one technique to gain a complete understanding of solid state thermal properties.

EXPERIMENTAL SECTION

Overview

This multiday experiment has been performed in Instrumental Analysis during three different semesters with class sizes ranging from 10 to 12 students. The activity requires three laboratory periods (3 h/period) in which each group of 3−4 students will characterize an active pharmaceutical ingredient and an excipient. Students were provided the molecular weights and formulas for the two compounds, told one is anhydrous and the other hydrated, and charged with determining the nature of thermal events observed by DSC. Qualitative information such as whether the transitions are endothermic/exothermic, as well

Published: November 20, 2014

dx.doi.org/10.1021/ed500640d | J. Chem. Educ. 2015, 92, 932−935

Table 1. General Guidelines for Interpretation of DSC Data

Endotherms (High Energy, Well-Defined)	Endotherms (Low Energy, Broad/Shallow)
Melting of pure crystalline Substance	Sublimation
	Moisture loss (surface bound)
Dehydration/desolvation	Residual solvent loss
Decomposition	
Exotherms (High Energy, Well-Defined)	**Exotherms (Low Energy, Shallow)**
Crystallization	Decomposition/oxidation
Recrystallization	Curing (cross-linking) of polymers
Decomposition (highly unstable material)	

as the energy/shape of the transition, can be used initially to narrow down the possibilities (Table 1).[10]

Additional information gained by DSC with respect to the "reversibility" of events can also be used to determine the nature of transitions (e.g., melting is reversible, while dehydration is not). If one has access to modulated-DSC, in which the total heat flow may be separated into the reversible (heat capacity) and nonreversible (kinetic) heat flows, information on reversibility of thermal events may easily be obtained.[14] Alternatively, students may opt to use heat/cool/reheat cycling to probe the reversibility of an event using conventional DSC. Quantitative information from DSC included the temperatures of the transitions and the associated enthalpy. After DSC thermograms were collected and analyzed, students then performed TGA and overlaid the DSC/TGA thermograms to correlate thermal events. Both qualitative and quantitative information from TGA were analyzed. Students observed the shape of the transitions in the TGA thermogram (e.g., gradual weight loss might suggest loss of residual surface moisture/solvent, or a step-weight loss is indicative of dehydration/desolvation from the crystal lattice), and also took note of the visual appearance of the sample postanalysis. While typically there are no significant gravimetric effects upon the melting of a sample, there may be a perturbation in the TGA curve due to an increase in the vapor pressure, or if melting occurs along with or followed by decomposition. In the quantitative evaluation of the TGA curves, students may determine the temperature at which transitions occurred, total weight loss, and also the weight loss associated with step transitions in order to determine the stoichiometry of the hydrated material.[10]

After DSC and TGA, students generally have hypotheses regarding the nature of the thermal behavior observed, but to make final conclusions in the interpretation of events, the final experiment uses thermal microcopy. The use of a transmitting light microscope furnished with a polarizing attachment allowed students to determine particle characteristics such as shape and size, and also gain insight into the crystallinity of the material. Crystalline material will exhibit birefringence under polarized light in which, for an anisotropic crystal mounted under mineral oil, the polarized light will be separated into two different polarized beams that travel through the crystal at unequal speeds and thus are unequally refracted. The use of thermal microscopy augments the DSC/TGA by allowing students to visually observe phase transitions, decomposition (if discoloration occurs, or gases are evolved), crystallization/recrystallization, or dehydration/desolvation in which bubbles emanate from the crystals submerged in mineral oil. Thermal microscopy was used to visualize the thermal events observed by DSC/TGA and relate the behavior of crystals to that of the bulk material.

Procedure

USP grade acetaminophen (98.0−101.0%) and α-lactose monohydrate (≥99% total lactose) were used as received from Sigma-Aldrich. Thermograms were generated using a PerkinElmer DSC 6000 equipped with an Intracooler SP and a PerkinElmer TGA 4000 equipped with a Polyscience chiller unit, and nitrogen purge gas at 20 mL/min. Pyris Software was utilized for data analysis. Sample sizes ranged from 2 to 5 mg and 5 to 15 mg for DSC and TGA, respectively. Hermetically sealed aluminum pans, with or without a pinhole, were used for DSC, while ceramic crucibles were used for TGA samples. Thermal microscopy was performed using an Olympus BX60 polarizing light microscope equipped with a Linkam LS350 Hot Stage/Linksys Software and Paxcam 2+ digital camera/Pax-It! Software and a 20× objective. A small amount of compound was placed on a microscope slide and covered with type A immersion oil (Cargille). Students collected photomicrographs at temperatures corresponding to any changes in the sample visually observed as a function of temperature. Heating programs for all thermal methods consisted of a heating rate of 10 °C/min from 30 to 220 °C for acetaminophen and 30 to 250 °C for α-lactose.

■ HAZARDS

Acetaminophen and α-lactose are FDA approved ingredients in pharmaceutical products and present minimal safety concerns, particularly in the low milligram quantities used for experiments. General laboratory safety protocols are sufficient in handling these materials and compressed gases. Care should be exercised in the operation of thermal analysis instruments as surfaces may be hot during and after analysis.

■ RESULTS AND DISCUSSION

The overlays of the DSC and TGA thermograms for API (acetaminophen) and excipient (α-lactose hydrate) are shown in Figures 1 and 2, respectively. Students observed a single,

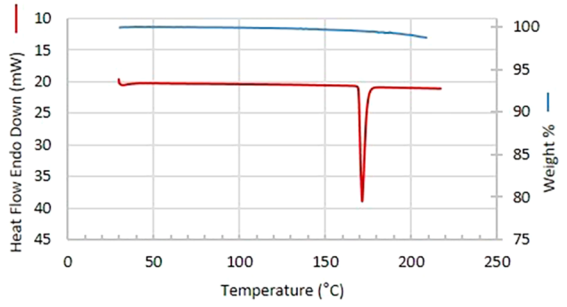


Figure 1. Overlay of DSC and TGA thermograms for API (acetaminophen). The samples were heated at 10 °C/min from 30 to 220 °C, and a closed pan configuration was used for DSC.

sharp endothermic transition for the API with no significant weight loss associated with the transition, suggesting that there was no dehydration or desolvation. Decomposition could not be ruled out; however, the appearance of the postanalysis sample showed a glassy film suggestive of melting and no substantial recrystallization of the API. The onset and peak temperatures and the enthalpy of the endothermic event were determined, and the compound was analyzed by hot stage microscopy to confirm suspicions that the endotherm was indeed due to melting of the compound as seen in Figure 3.

dx.doi.org/10.1021/ed500640d | J. Chem. Educ. 2015, 92, 932−935

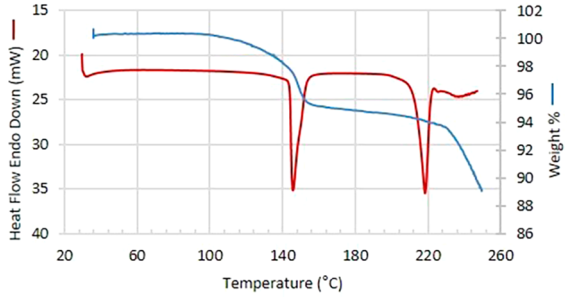


Figure 2. Overlay of DSC and TGA thermograms for excipient (α-lactose hydrate). The samples were heated at 10 °C/min from 30 to 250 °C, and a pinhole pan configuration was used for DSC.

The photomicrograph of the compound under polarized light shows birefringence, which suggests that it is crystalline and not amorphous, and thus also supports the presence of a distinct melting point. The melting temperature and enthalpy of fusion for acetaminophen were in good agreement with that reported in the literature. The thermograms for α-lactose were a bit more complex with two endothermic transitions with onset temperatures of 144.2 and 213.4 °C, respectively. A 5.0% step weight loss was correlated with the endothermic transition at 144.2 °C, while there appears to be the start of additional weight loss following the endothermic transition at 213.4 °C. Observations of the sample postanalysis revealed a brown-colored material suggestive of decomposition. The hot stage microscopy of α-lactose shown in Figure 4 was very helpful in confirming the nature of events observed in DSC and TGA. The material was crystalline under polarized light, and as the compound was heated, bubbles were evolved at approximately 147 °C, which corresponded to the temperature of the first endotherm and also the step weight loss seen in TGA. Based on these observations and stoichiometric calculations, the first transition was determined to be dehydration of the monohydrate of α-lactose. Students were posed with the question as to why the dehydration occurred nearly 50 °C higher than the boiling point of water, which pointed to the fact that water molecules are tightly held within the crystal lattice structure due to intermolecular forces such as hydrogen bonding, and that additional energy is required to overcome these forces. Additional heating led to melting with discoloration of the sample suggestive of melting with simultaneous decomposition resulting in the second endotherm at 213 °C, which is also in agreement with literature information on α-lactose monohydrate.[15] While there may not be time for students to perform multiple DSC analyses of the two samples, instructors may wish to provide students with supplemental DSC results from heat/cool/reheat cycles to demonstrate the reversibility or nonreversibility of the observed DSC endotherms, which aids further in the identification of thermal events prior to hot stage microscopy. The instructor may also choose to provide a comparison thermogram of the α-lactose analyzed in a closed pan to show the impact of pan configuration, particularly for hydrates in which pressure buildup of volatilized solvent can lead to additional "artifacts" in the DSC thermogram (see Supporting Information).

CONCLUSIONS

Students met the objective of gaining hands-on practical experience (including sample preparation, instrumental setup, and data analysis using software) for three different thermal analysis techniques. The experiment required qualitative and quantitative interpretation of thermal transitions and the use of an integrated approach using multiple analytical techniques to perform the thermal characterization of the solid state. In particular students enjoyed the visualization of thermal

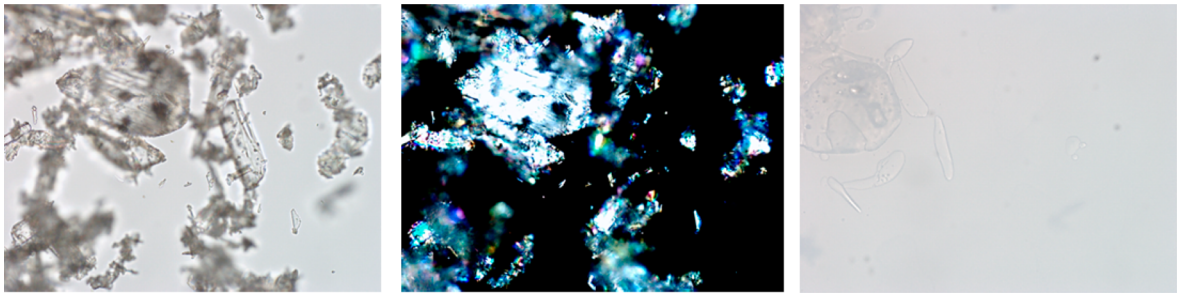


Figure 3. Photomicrographs of acetaminophen at room temperature (left), room temperature under polarized light (middle), and melting of particles corresponding to the endothermic transition at ~170 °C (right).

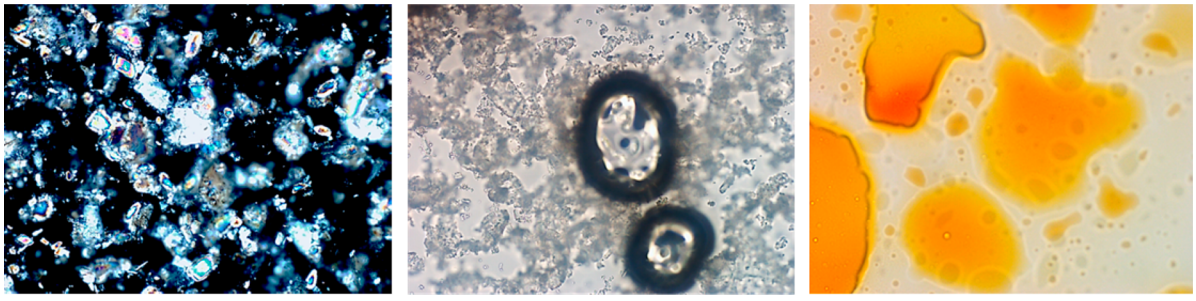


Figure 4. Photomicrographs of α-lactose at room temperature under polarized light (left), showing the evolution of bubbles at 147 °C corresponding to the temperature of the first DSC endotherm (middle), and melting with decomposition corresponding to the second endothermic DSC transition (right).

dx.doi.org/10.1021/ed500640d | J. Chem. Educ. 2015, 92, 932−935

transitions to confirm their hypotheses. The thermodynamic behavior of the crystalline pharmaceutical compounds can be related to concepts from other courses such as physical chemistry. An extension of the thermal analysis experiments would be to perform X-ray powder diffraction on the α-lactose monohydrate before and after dehydration to show differences in the crystal structure in the absence of water in order to prove that it is truly part of the crystal lattice rather than surface water. Over the course of the three part experiment, students documented all work in laboratory notebooks, which were graded each week. Once all experimentation was complete, a formal laboratory report was submitted by each group of students with conclusions rendered based on data for all three thermal techniques. This exercise gives upper level students "real-world" experience in problem-solving, thermal analysis instrumentation, documentation of work, and communication of results. Chemistry alumni have indicated their appreciation for this type of experience gained in an upper level chemistry course.

■ ASSOCIATED CONTENT

Supporting Information

Supplemental DSC data and thermal microscopy video clips; instructional and student notes; suggestions for additional APIs and excipients that could be analyzed by student groups. This material is available via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: sriley@nwmissouri.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The author is grateful to Jolaine Zweifel and Stephanie Crabtree for editing and annotation of the video clips, as well as Heemoon Jeong and Jae Hyun Park for their assistance in the thermal microscopy work.

■ REFERENCES

(1) Byrn, S. R.; Pfeiffer, R. R.; Stowell, J. G. *Solid-State Chemistry of Drugs*, 2nd ed.; SSCI, Inc.: West Layfayette, IN, 1999; pp 3−40, 69−75, 81−87.

(2) Howard, K. S.; Nagy, Z. K.; Saha, B.; Robertson, A. L.; Steele, G. Combined PAT-Solid State Analytical Approach for the Detection and Study of Sodium Benzoate Hydrate. *Org. Process Res. Dev.* **2009**, *13*, 590−597.

(3) Shevchenko, A.; Belle, D. D.; Tittanen, S.; Karjalainen, A.; Tolvanen, A.; Tanninen, V. P.; Haarala, J.; Mäkelä, M.; Yliruusi, J.; Miroshnyk, A. Coupling Polymorphism/Solvatomorphism and Physical Stability Evaluation with Early Salt Synthesis Optimization of an Investigational New Drug. *Org. Process Res. Dev.* **2011**, *15*, 666−672.

(4) Chakraborty, M.; Hsiao, F. W.; Naskar, B.; Chang, C. H. Surfactant-Assisted Synthesis and Characterization of Stable Silver Bromide Nanoparticles in Aqueous Media. *Langmuir* **2012**, *28*, 7782−7290.

(5) Ren, L.; Hardy, C. G.; Tang, S.; Doxie, D. B.; Hamidi, N.; Tang, C. Preparation of Side-Chain 18-e Cobaltocenium-Containing Acrylate Monomers and Polymers. *Macromolecules* **2010**, *43*, 9304−9310.

(6) Kuhtz, H.; Cheng, F.; Schwedler, S.; Böhling, L.; Brockhinke, A.; Weber, L.; Parab, K.; Jäkle, F. Luminescent Diazaborolyl-Functionalized Polystyrene. *Macro Lett.* **2012**, *1*, 555−559.

(7) Das, A. M. Kinetic Study and Reaction Mechanism of Vinyl Monomer Modified *Antheraea assama* Silk Composites. *Ind. Chem. Eng. Res.* **2011**, *50*, 1548−1557.

(8) Marcolino, V. A.; Zanin, G. M.; Durrant, L. R.; Benassi, M. D. T.; Matioli, G. Interaction of Curcumin and Bixin with β-Cyclodextrin; Complexation Methods, Stability and Applications in Food. *J. Agric. Food Chem.* **2011**, *59*, 3348−3357.

(9) *Thermal Analysis of Pharmaceuticals*; Craig, D. Q. M., Reading, M., Eds.; CRC Press: Boca Raton, FL, 2007.

(10) *Principles and Applications of Thermal Analysis*; Gabbott, P., Blackwell Publishing: Oxford, 2008; pp *88−118*, 286−322.

(11) Gray, F. M.; Smith, M. J.; Silva, M. B. Identification and Characterization of Textile Fibers by Thermal Analysis. *J. Chem. Educ.* **2011**, *88* (4), 476−479.

(12) Harris, J. D.; Rusch, A. W. Identifying Hydrated Salts Using Simultaneous Thermogravimetric Analysis and Differential Scanning Calorimetry. *J. Chem. Educ.* **2012**, *90*, 235−238.

(13) Vitez, I. M.; Newman, A. W.; Davidovich, M.; Kiesnowski, C. The Evolution of Hot-Stage Microscopy to Aid Solid-State Characterizations of Pharmaceutical Solids. *Thermochim. Acta* **1998**, *324*, 187−196.

(14) Reading, M.; Craig, D. Q. M.; Murphy, J. R.; Kett, V. L. Modulated Temperature Differential Scanning Calorimetry. In *Thermal Analysis of Pharmaceuticals*; Reading, M., Craig, D. Q. M., Eds.; CRC Press: Boca Raton, FL, 2007; pp 101−138.

(15) Listiohadi, Y.; Hourigan, J. A.; Sleigh, R. W.; Steele, R. J. Thermal analysis of amorphous lactose and α-lactose monohydrate. *Dairy Sci. Technol.* **2009**, *89*, 43−67.