Observing the transient assembly and disassembly of carboxylic anhydrides in the organic chemistry laboratory

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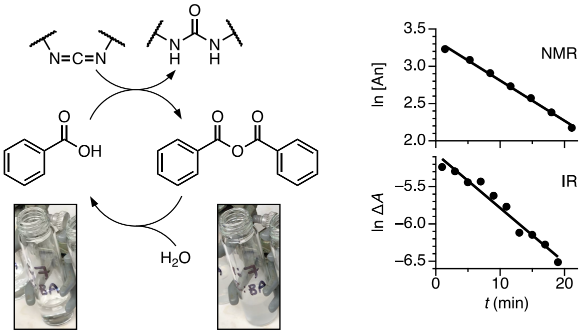


Abstract

Chemical reactions that mimic the function of ATP hydrolysis in biochemistry are of current interest in nonequilibrium systems chemistry. The formation of transient bonds from these reactions can drive molecular machines or generate materials with time-dependent properties. While the behavior of these systems can be complicated, the underlying chemistry is often simple: they are therefore potentially interesting topics for undergraduate introductory organic chemistry students, combining state of the art advances in systems chemistry with straightforward reactions. Here, a teaching experiment has been developed that explores the transient assembly of benzoic acid derivatives driven by carbodiimide hydration. Working in teams, students examine the formation and decomposition of anhydrides from two benzoic acids using a carbodiimide "fuel". The students examine classic reaction kinetics of anhydride hydrolysis using two independent methods, NMR and IR spectroscopies. They then explore how the amount of carbodiimide affects the lifetimes of precipitates of benzoic anhydride as a simple example of out-of-equilibrium self-assembly.

1 Introduction

Many biochemical systems operate or maintain their states using a continuous supply of energy. That is, they typically use an exergonic reaction to drive a reaction network out of equilibrium.[1–5] For example, myosin motor proteins use the hydrolysis of ATP to effect muscle contraction. Inspired by the emergent properties of such out-of-equilibrium chemical systems, non-biological chemically "fueled" systems have recently received significant attention.[1,4,6–8] Functional systems have been developed ranging from transient materials with unusual properties[9–12] to molecular machines.[13,14]

Student exploration of nonequilibrium reaction networks is uncommon, although some examples of complex behavior, especially from oscillating and related reactions,[15–20] have been presented, generally as activities for physical chemistry courses. Of particular relevance to this work, a recent report described a multi-week project for high school students on transient assembly, making use of the enzymatic hydrolysis of ATP to control the aggregation of gold nanoparticles.[21] To our knowledge, however, single-session laboratory activities for undergraduates that focus on demonstrating the concepts of nonequilibrium assembly using simple, nonbiological reactions have not yet been described, despite their significant pedagogical potential. These concepts are important to an emerging and exciting area of systems chemistry, but the core chemistry is well-established and suitable for students in their first college-level organic chemistry courses.

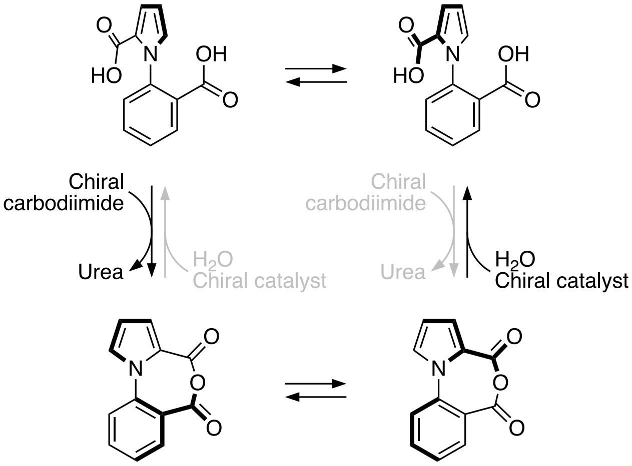
A laboratory activity in this area can therefore serve two purposes: as an introduction to a new field (nonequilibrium systems chemistry) and as an investigation of techniques for chemical kinetics. The experiment presented here covers both areas. The students compare rate constants determined for a simple reaction using two independent experimental techniques (NMR and IR spectroscopies). The students also connect the abstract concept of transient assembly to observable behavior by examining how the concentration of fuel affects the lifetime of a transient aggregate (precipitate) generated out of equilibrium. The experiment fits into the introductory organic chemistry lab curriculum, uses readily available materials and characterization tools, and is designed as a student-centered, guided-inquiry investigation.[22–25]

2 Background

At the outset, we should note that there is some controversy in the field about terminology, especially the terms "fuel", describing the reagents that are used to drive reaction networks out of equilibrium, and "dissipative", referring to the dissipation of energy throughout a process that is not at equilibrium (and effectively synonymous with "out-of-equilibrium").[26,27] The "fuel" metaphor in particular can be problematic because it invokes properties of macroscopic machines that are not transferable to chemical systems (please refer to a recent article from Goldup and Aprahamian[26] for a more-thorough discussion). Nevertheless, the term is widely used in the field to refer to ATP and reagents that play the same role in non-biological nonequilibrium systems, and will be used here with this caveat.

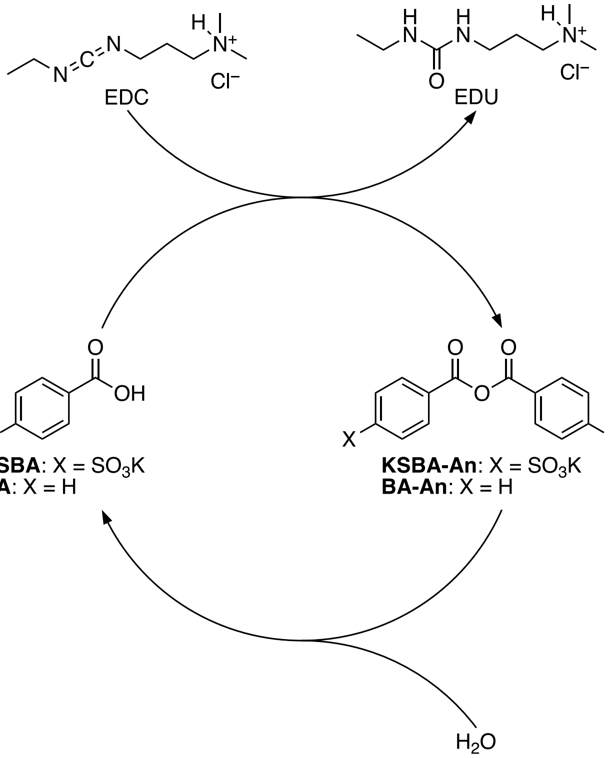
The basic technology underlying many current chemically fueled systems is straightforward: the decomposition of the fuel causes the formation of a bond between two species that subsequently breaks, returning the system to its resting state.[6] While there are many examples of fuel reactions, here we focus on the hydration of carbodiimides, which are commonly used as reagents for the formation of amides and esters (and introduced as such in many introductory organic chemistry courses).[28,29] The use of carbodiimides in nonequilibrium chemical systems was first reported by Boekhoven and coworkers[30] followed by us.[31] The Boekhoven group demonstrated that treatment of aqueous dicarboxylate precursors with carbodiimides gives cyclic anhydrides that assemble into gels before decomposing back to the original (soluble) materials.

Carbodiimide hydration has become arguably the most popular fuel reaction for simple nonequilibrium chemical systems. It has been used, for example, to generate transient supramolecular assemblies and gels,[32,33] droplets,[34] microgels,[35] supramolecular hosts,[36] and polymer networks,[37,38] and to operate various molecular machines.[13,39] Scheme 1 shows an example of a carbodiimide-driven molecular motor developed by Leigh.[13] In this system, both anhydride formation and hydrolysis are enantioselective through the use of a chiral carbodiimide and chiral catalyst, respectively. Consequently, if viewed from above, the (top) pyrrole ring undergoes preferential clockwise rotation with respect to the (bottom) benzene.



Scheme 1: Leigh's carbodiimide-driven artificial molecular motor. When treated with a chiral carbodiimide and chiral hydrolysis catalyst, the top unit undergoes preferential directional rotation with respect to the bottom.

The reaction network explored in this activity, shown in Scheme 2, represents the simplest version of a carbodiimide-driven nonequilibrium process. An aqueous solution of a carboxylic acid is treated with a water-soluble carbodiimide, EDC (*N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride). The network incorporates two independent reactions. In the "activation" step, the carboxylic acid catalyzes the hydration of the carbodiimide to the corresponding urea (EDU from EDC), simultaneously being converted to the analogous anhydride.[40,41] The anhydride persists for a short time (typically minutes to hours) before undergoing "deactivation": hydrolysis to regenerate the original acid.[42,43]



Scheme 2: Treatment of a carboxylic acid with EDC gives the transient formation of an anhydride.

Synthetically this system is trivial and accomplishes nothing other than the decomposition of the carbodiimide. The formation of the anhydride must be coupled another process, similar to how transient phosphorylation by ATP is used in biology. This can be as conceptually complex as the operation of the molecular motor in Scheme 1, or as simple as assembly of the anhydride state into an aggregate. Regardless of the behavior, the properties of these systems are fundamentally governed by kinetic control, which can lead to responsive behavior not possible in systems at equilibrium.

In this activity, the students explore this chemistry from two perspectives using two different benzoic acids, potassium 4-sulfobenzoic acid (**KSBA**, Scheme 2) and benzoic acid itself (**BA**). The difference is that the anhydride of **KSBA**, **KSBA-An**, remains soluble throughout the experiment, whereas **BA-An** precipitates at typical experimental concentrations.[44]

KSBA and **KSBA-An** have been previously used as a model system for ^{1}H NMR spectroscopy investigations of carbodiimide-driven anhydride formation.[31] Thus, they are well suited to investigation of anhydride hydrolysis kinetics; the concentration of **KSBA-An** can be conveniently followed using either IR or ^{1}H NMR spectroscopies. Here, the students use both methods, allowing the techniques to be compared (the experiment could also be modified to use one method alone). Conversely, when **BA** is used in place of **KSBA**, the formation of the **BA-An** precipitate represents a simple example of transient self-assembly, paralleling other (more-sophisticated) examples of transient crystallization.[45–47] Precipitation is easily observed by the naked eye and, importantly, the effect of varying the amount of fuel can be determined. The students observe how changing the amount of EDC affects both the activation rate (precipitation) and the deactivation rate (redissolution). The outcome is counterintuitive because of the kinetically controlled nature of the system.

3 Experimental Procedure

The activity consists of three independent parts, which can be done in a rotation to make best use of instrumentation.

In part I, the students observe anhydride formation and decomposition using ^{1}H NMR spectroscopy. An NMR tube is charged with **KSBA** and NaOH (0.5 equiv) in D_2O; dimethylacetamide (DMA) is also included as an internal standard for the determination of absolute concentrations of **KSBA-An**. The reaction mixture is then treated with EDC (0.3 equiv) immediately before acquisition of spectra is started. Generation of **KSBA-An** is typically fast enough that it is already formed by the time the first spectrum is acquired (we usually find it convenient to shim the instrument on a blank sample first so that acquisition can be started without delay after the EDC is added). The anhydride concentrations are easily

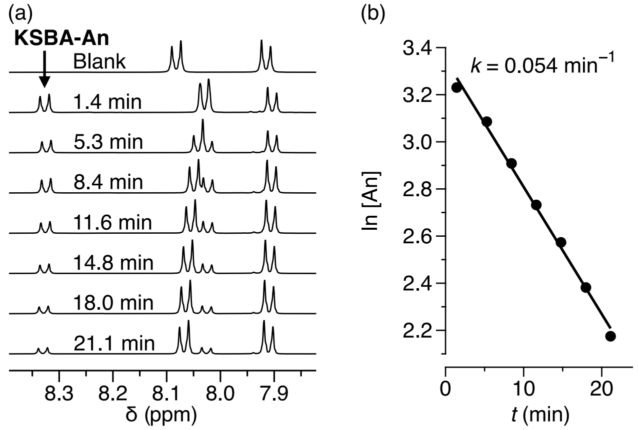


Figure 1: (a) NMR spectra of the **KSBA** reaction as a function of time (D_2O, rt). (b) **KSBA-An** concentration determined by NMR spectroscopy vs time ($R^2 = 0.995$).

determined from the integration of new signals appearing in the ^{1}H NMR spectra. The full ^{1}H NMR spectroscopy experiment can be conveniently carried out in about 20 min.

Representative ^{1}H NMR spectra are shown in Figure 1a. Signals associated with the **KSBA-An** are well-separated from those for the **KSBA** and can be easily integrated. There is a small drift of the chemical shifts of the signals from the **KSBA**, presumably because the pD of the reaction mixture changes slightly as the carboxylic acid is initially consumed and then regenerated.[31] A plot of ln[**KSBA-An**] against time, shown in Figure 1b, can be used to determine the rate constant k for the (pseudo-first-order) hydrolysis from a linear fit.

In part II, the students observe the same anhydride formation and decomposition of **KSBA-An** using IR spectroscopy. In our facility, these experiments were easily carried out using a standard attenuated total reflectance (ATR) IR spectrometer by running the reaction in a water droplet over the ATR cell surface (see Supporting Information). Deuterated water was used for this experiment to facilitate comparison to the NMR results. The students begin by scanning a D_2O droplet on the ATR cell surface as background. A droplet of the reaction mixture can then be scanned beginning immediately after the **KSBA** solution is treated with EDC. A single droplet is sufficiently stable on the ATR cell to be used throughout the roughly 20 min experiment.

Representative IR spectra are shown in Figure 2a. The anhydride is detected through

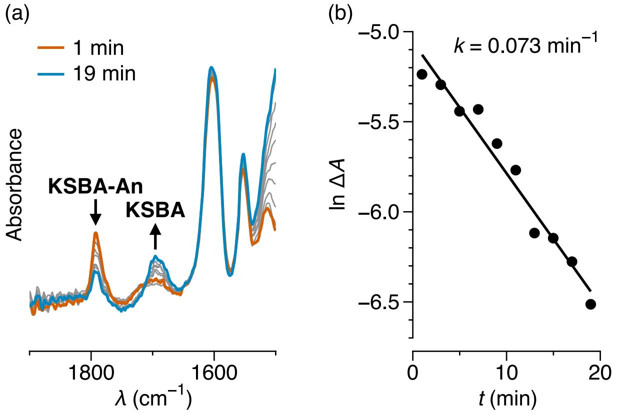


Figure 2: (a) IR spectra of the **KSBA** reaction as a function of time in (D_2O, rt). (b) **KSBA-An** IR absorbance ($\Delta A = A_{1793} - A_{1650}$) vs time ($R^2 = 0.964$).

the absorbance (A) of the peak at 1793 cm^{-1}, associated with the symmetric stretch of the anhydride and well-separated from the rest of the spectrum. On our instruments, there is significant drift in the baseline throughout the experiment. We found it helpful to reference the absorbance data to the absorbance at 1650 cm^{-1}, where the spectrum is unchanging (i.e., $\Delta A = A_{1793} - A_{1650}$). Plotting $\ln \Delta A$ against time allows k for the hydrolysis to be determined. For us, the data tends to be a bit noisier than that from the NMR experiments, but still yields a good linear fit.

The results of the two experiments can then be compared to assess whether the two techniques are in agreement. In the example data, the anhydride decomposition rate constants were determined to be $k = 5.4 \times 10^{-2}$ min^{-1} from NMR spectroscopy and 7.3×10^{-2} min^{-1} from IR spectroscopy. These values are in good agreement, with the discrepancy likely resulting from differences in ambient temperature between the NMR and IR spectrometers.

In part III, the students observe the transient precipitation of **BA-An** and NaOH (1 equiv) in H_2O with the naked eye, examining how the "assembly" depends on the amount of EDC that has been added. Each group of students prepares three vials charged with identical aqueous solutions of **BA**. The students then add different amounts of EDC (0.25, 0.14, and 0.10 eq) to the three vials at the same time and stir, noting the times at which changes are observed. The reaction mixtures first become cloudy as the **BA-An** precipitates, as shown in

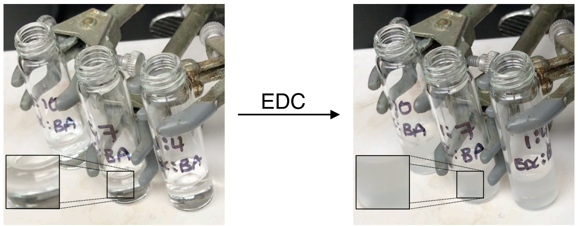


Figure 3: Benzoic acid anhydride formation in presence of EDC in water (298 K).

Figure 3. The **BA-An** then eventually hydrolyzes back to the **BA** and the mixtures become clear.

Both the activation (precipitation) and deactivation (dissolution) times of the system are dependent on the amount of fuel (EDC) added. In typical results, after adding the EDC to the vials, the mixture with the higher concentration of EDC gives a precipitate first, in about 80 s, as would be expected from the faster generation of more **BA-An**. The vial with the least EDC gives precipitate last, after about 110 s. Perhaps counterintuitively, the vial with the higher amount of EDC also goes clear first, after about 30 minutes, suggesting that the anhydride decomposes more quickly despite more having been produced. We expect the anhydride to be "protected" in the solid state, only undergoing hydrolysis once it dissolves.[48] According to the Ostwald ripening theory, solubility depends on the crystal size.[44] Thus, we believe the earlier dissolution with more fuel is because the more rapid formation of **BA-An** gives smaller particles, which then dissolve more quickly as the system returns to the resting state. The result may be unexpected, but underscores the kinetically controlled nature of the system.

4 Hazards

Potassium 4-sulfobenzoic acid and benzoic acid cause skin irritation and eye damage. *N*-(3-Dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) causes skin irritation and eye irritation. NaOH causes severe skin burns and eye damage. All chemicals in the experiment should be handled with goggles and protective gloves.

Table 1: Achievement of learning goals.

Learning goal	Does not meet[a]	Meets	Exceeds
1. Use the NMR spectra acquired at different times to determine the rate constants of anhydride hydrolysis.	38%	62%	n.a.[b]
2. Use the IR spectra acquired at different times to determine the rate constants of anhydride hydrolysis.	33%	67%	n.a.[b]
3. Compare rate constants derived from the two independent techniques.	49%	28%	24%
4. Interpret the (macroscopic) formation of the benzoic anhydride precipitate in terms of the amount of chemical ”fuel” added.	22%	72%	6%
5. Interpret the behavior or the KSBA and BA systems in terms of structural effects on solubility.	65%	25%	10%

[a] $N = 72$. [b] Criteria for exceeding this learning goal were not set.

5 Results and Discussion

Following a pilot study with one graduate student and one undergraduate, the experiment was performed by 77 chemistry and biochemistry majors (two sections) enrolled in the first semester of an introductory organic chemistry lab course during fall 2021. The activity was completed in one 4 h session (and can be easily completed in 2–3 h). The students worked in groups of four for the IR spectroscopy and **BA/BA-An** precipitation activities, with two groups working together for the NMR spectroscopy because of instrument time restrictions. Post-lab questions were done individually.

In general, the NMR results from group-to-group were very consistent; pooling the data of the student groups gave a mean k of 4.5×10^{-2} min^{-1} with a standard deviation of 3×10^{-3} min^{-1}. There was more variability in the IR results, with a mean k of 5×10^{-2} min^{-1} and a standard deviation of 2×10^{-2} min^{-1}.[49]

5.1 Student learning outcomes

Learning goals and student success in achieving these goals are presented in Table 1. Student responses to post-lab questions (see Supporting Information) were used for this analysis. In general, incorrect or blank responses were marked as not meeting the learning goal and correct responses were marked as meeting the learning goal; in some cases, especially detailed or thoughtful answers were marked as exceeding the learning goal (specific criteria by question are noted below).

A majority of students met most of the learning goals. Most of the students were able to interpret the NMR and IR spectroscopy results to correctly extract rate constants for anhydride hydrolysis (learning goals 1 and 2). A smaller majority was then able to reasonably compare the rate constants (learning goal 3). Responses were judged to meet this learning goal if they concluded that the two methods were in agreement. Responses were judged to exceed this learning goal if they provided a reasonable explanation based on experimental uncertainty (10% of the students), or if they concluded that the techniques did not agree but provided a thoughtful explanation, such as variations in temperature (14%). For example, the following answer was judged to exceed the learning objectives:

> The rate constants determined from the NMR and IR kinetics experiments are very close in value, but are not identical. The NMR determined rate constant was 0.0007831 1/s, which was slightly smaller/slower than the IR determined rate constant of 0.001245 1/s. While these two k values are meant be identical, their differences from lab can be attributed to specific factors as well. For example, temperature is a factor that can affect the rate constant. A reason for the NMR experiment is giving a smaller/slower rate constant could be due to the colder temperature in the basement of Hughes, which may have decreased the rate of the reaction and therefore produced a smaller k value.

Whereas the following explanation was not considered to meet the learning objective:

> [Disagreement between the rate constants] could be due to my lack of points for IR . . . or because IR might not be sensitive enough to [detect] the decomposition of such small concentrations over a period of time.

Gratifyingly, a strong majority of the students were able to recognize the effect that the amount of chemical fuel has on the lifetime of the **BA-An** precipitate. Students were judged to exceed this learning goal if they also provided a reasonable explanation. For example:

> The amount of EDC affects the benzoic acid system and the time it takes for crystallization to occur. Increasing the amount of EDC causes the crystallization to occur and dissapate [sic] faster. For example, 0.25 mL of EDC stock solution was added it took 52 seconds for onset cloudiness to occur. However, when 0.10 mL of EDC solution was added it took 118 seconds for onset cloudiness to occur. Therefore, increasing EDC allowed crystallization to occur faster.

Responses deemed not to meet this objective tended to be vague.

Curiously, a majority of the students failed to recognize the importance of the sulfonate group in **KSBA** in imparting solubility to its anhydride. The question, as posed, was deliberately open ended: "Why was KSBA used in the kinetics experiments and BA used in the crystallization experiment? Explain specifically how their molecular structures give appropriate properties for each type of experiment." Responses judged correct related the choice to the water solubility of **KSBA-An** and exceeded the learning goal if they supported this with specific insight into the charged/polar nature of the sulfonate group, as in:

> KSBA and BA were used in respective parts due to solubility and polarity. The BA created a nonpolar, crystalline anhydride which came out of solution to appear cloudy and be observable with naked eye. The KSBA had 2 polar $-SO_3$ group[s] which made it polar so that it would stay more in solution.

Incorrect responses tended to instead focus on reaction rates, often asserting (incorrectly) that the sulfonate group slows down the anhydride hydrolysis as in "The induction of the SO_3K causes [the] slower decomposition". These responses may reflect the emphasis on problems involving reactivity in the introductory organic chemistry curriculum, especially early on. Structure–property effects on even simple macroscopic properties like solubility, while generally covered, are not typically the focus.

6 Summary

A guided-inquiry experiment to distinguish the microscopic and macroscopic behavior of benzoic acid compounds and their anhydride formation and decomposition using a chemical fuel was designed and successfully implemented in an undergraduate organic chemistry course. The students observe transient assembly of carboxylic anhydrides using NMR and IR spectroscopies and then a macroscopic manifestation of transient assembly, precipitation, with the naked eye. They successfully calculated and compared anhydride decomposition rate constants from NMR and IR spectroscopy techniques. They also observed the precipitate lifetime of benzoic acid anhydride with the naked eye and examined how the behavior of the system depends on the amount of fuel added.

Acknowledgements

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Supporting Information

Instructor guide, including preparation instructions, solutions to student questions, and supplemental figures (DOCX)

Student handout (DOCX)

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(49) The IR data excludes two groups out of 16 that were clear outliers: one had a negative rate constant, and the other's was an order of magnitude too large.