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A Laboratory Experiment for Illustrating the Kinetic Isotope Effect

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Abstract:

This paper describes the study of the kinetic isotope effect for the reaction between cyclohexanone and iodine under acidic conditions that has been adapted for an advanced level undergraduate organic chemistry laboratory class. The kinetics of the reaction of iodine with cyclohexanone can be readily followed with a UV-Vis spectrophotometer or even a home-built colorimeter. The results are compared with the reaction of iodine with cyclohexanone- d_4 , a deuterated analog which students prepare by a simple and cheap deuteration. The deuterated cyclohexanone reacts more slowly than the non-deuterated cyclohexanone by a factor of 3.5 ± 1.2 , which confirms that a C-H bond is broken in the rate-determining step and illustrates a primary kinetic isotope effect.

Keywords

Laboratories and Demonstrations, Physical Organic Chemistry, Kinetic Isotope Effect, Halogenation of Ketones, Active Learning

Introduction

Reaction mechanisms are a key component of Organic Chemistry lecture courses at all levels. Not only are students expected to learn how to draw curved arrow mechanisms, they also need to appreciate the various techniques and studies chemists have carried out in the past, and still do, to obtain evidence for a proposed reaction mechanism. One important mechanistic tool in the chemist's repertory is to check whether or not a reaction shows a kinetic isotope effect.

A C-D bond is slightly stronger than a C-H bond. When a C-H bond is broken in the rate-determining step of a reaction, a deuterated substrate will react more slowly than its non-deuterated analog [1]. This difference in reactivity is called the primary kinetic isotope effect (PKIE). For a reaction that shows a PKIE, the rate constant for the deuterated compound, k_D , can be up to about 5 – 8 times smaller than the rate constant for the hydrogenated compound, k_H . If a reaction does not show a PKIE, the k_H/k_D ratio will be close to 1. The nitration of benzene is an example of a reaction with no PKIE; in this case the loss of a proton happens fast, leaving the attack of the electrophile as the rate-determining step. The bromination of acetone has a k_H/k_D ratio of about 7 and is a textbook case of a reaction with a PKIE.

Apart from its application in mechanistic studies, the kinetic isotope effect has been exploited recently in the development of partially deuterated pharmaceutical drugs, such as iloprost, nifedipine and a thalidomide analog [2,3,4,5]. Incorporation of deuterium at key positions can slow down the metabolism of a drug, thus allowing the dose to be reduced. In some cases the metabolic route is sufficiently altered to reduce side effects and improve the safety of the drug [5].

A number of teaching experiments have been reported in recent years which illustrate the kinetic isotope effect to undergraduate students. The bromination of acetone [6] is simple and easy to implement since deuterated acetone is a commercially available NMR solvent, but its disadvantages are high cost and volatility. Atkinson and Chechik described a synthetic procedure for deuterating butanone in a D₂O/DCl mixture and students then used the deuterated product for a kinetics study of the bromination of butanone [7]. However, the kinetics are complex due to differences in the rate constants for the bromination of the methyl and methylene groups. Kinetic isotope effects are also observed in deuterated solvents, for example, the hydrolysis of acetic anhydride proceeds slower in D₂O than in H₂O [8,9]. Although this looks simple to do at a cursory glance, the solvent kinetic isotope effect is usually small ($k_H/k_D < 2$) and its study requires great care.

Visual color changes in demonstrations of the kinetic isotope effect are easy to follow with the help of a UV-Vis spectrophotometer. The chromic acid oxidation of secondary alcohols, such as benzhydrol, exhibits a large PKIE and has been studied by GC-MS analysis [10] as well as adapted as a lecture demonstration [11]. The downside is the use of a carcinogenic chromium(VI) oxidant, the formation of heavy metal waste, and the need to make the deuterated alcohol by reduction of benzophenone with LiAlD₄, an expensive and hazardous reagent best handled by a technician. Oxidation of a secondary alcohol such as 1-phenylethanol can also be achieved with potassium permanganate which is easily followed photometrically until precipitation of MnO₂ begins to interfere with the measurement [12]. A more recent paper described the reaction of (deuterated) resorcinol with iodine in water which shows a sizeable PKIE of about 3 – 4 but the kinetics are second order or pseudo-first order and the reaction reaches completion only slowly [13].

Despite their importance in mechanistic studies, experiments about the kinetic isotope effect are not routinely included in Organic Chemistry undergraduate laboratories. At Heriot-

Watt University students learn about kinetic isotope effects as part of a lecture series on Physical Organic Chemistry during their third year. There was therefore an incentive to reinforce this topic by an appropriate laboratory experiment. However, none of the published examples was suitable for one reason or another, and we decided to design a new experiment. Since the experiment was supposed to be part of an Organic Chemistry laboratory and not concentrate solely on the kinetics measurements, we wanted students to synthesize their own deuterated substrate, ideally using a cheap deuteration agent (D_2O). Furthermore, the experiment should avoid toxic chemicals, create minimum waste, and be affordable even on a tight teaching budget. We opted for the deuteration of cyclohexanone since the reaction of cyclohexanone with iodine under acidic conditions has been thoroughly studied in the past and has a known PKIE of about 5 [14]. Both the synthetic procedure and kinetics analysis are described in this paper. We also looked at the feasibility of using a homemade colorimeter to study the kinetics of a reaction and determine a PKIE. Details of the construction and the results obtained with a homemade colorimeter are presented.

Experimental

All chemicals were purchased and used without further purification. 1H NMR spectra were recorded in $CDCl_3$ on a Bruker AVIII 300 spectrometer. Changes in absorption with time were followed with a PerkinElmer Lambda XLS spectrophotometer in the kinetics mode or with a home-made colorimeter.

Preparation of deuterated cyclohexanone: Cyclohexanone (1.0 g), D_2O (2 mL) and anhydrous potassium carbonate (0.1 g) are combined in a small glass vial, which is closed tightly, placed in a sand bath, and left to react at $80\text{ }^\circ\text{C}$ for 3 – 4 days. After cooling to room temperature, the organic layer (consisting of wet deuterated cyclohexanone) separates and is

removed with a pipette. The sample of the crude product is analyzed by ^1H NMR spectroscopy. A detailed description of the experimental procedure is provided in the Supporting Information.

Acid-catalyzed iodination of cyclohexanone: A 0.04 M stock solution of cyclohexanone in 1 molar aqueous HCl and a 0.002 M aqueous solution of iodine are prepared ahead of time for student use. Exactly 5.0 mL of the cyclohexanone solution and 5.0 mL of the iodine solution are combined in a beaker. A quartz cuvette is filled with the mixture and placed into the cell compartment of a UV-Vis spectrophotometer. Students then record the decrease in absorption of iodine at 460 nm over 12 minutes. After this, students make up their own 0.04 M stock solution of deuterated cyclohexanone in 1 M aqueous HCl and repeat the kinetics run under identical conditions.

Results and Discussion

The deuteration of cyclohexanone in D_2O can be conducted in a sealed vial and takes 3 – 4 days at 80 °C in the presence of potassium carbonate (Scheme 1). Although students do not need much time to set up the reaction and isolate the deuterated cyclohexanone, the experiment will require two laboratory sessions and some planning ahead. The procedure described in the literature recommends that cyclohexanone should be treated twice with D_2O at 100 °C for 1 – 4 days [15,16]. On adapting this procedure as an undergraduate laboratory experiment, we ask students to carry out the deuteration only once, using a sealed glass vial containing 1 g of cyclohexanone, 2 mL of D_2O and a weak base (K_2CO_3). The reaction mixture is left to react at 80 °C from one laboratory session to the next.

Whereas cyclohexanone and water are miscible at elevated temperature, they phase-separate on cooling which allows the deuterated cyclohexanone to be removed with the help of

a Pasteur pipette. Student yields range typically from 30 to 50%, and the deuterated cyclohexanone is not purified any further or dried to avoid accidental losses.

Students record a ^1H NMR spectrum of the product to confirm that deuteration has indeed taken place (Figure 1). A comparison of the integrals of the $\alpha\text{-CH}_2$ signal at δ_{H} 2.3 with the integral of the signals of the remaining CH_2 groups allows students to determine the extent of deuteration (see SI). The degree of deuteration reproducibly reaches values of 80 – 85% and is sufficient to exemplify the PKIE.

Students combine equal amounts of a ready-made stock solution of cyclohexanone (0.04 M) in 1 M aqueous HCl with a 0.002 M I_2/KI stock solution in a beaker, swirl or stir briefly, then fill a cuvette and start recording the change in absorbance at 460 nm as soon as feasible (Figure 2). The kinetics of iodination of cyclohexanone under acidic conditions is first order with respect to the ketone and acid concentration, and zero order with respect to the halogen concentration [14]. The consumption of iodine during the reaction is easily followed by recording the decrease in absorbance of iodine at 460 nm with time. Most students will realize that the observed linear decrease indicates a zero-order reaction. The analysis is simple as the rate constant is proportional to the slope of the absorbance vs. time curve.

The kinetics experiment is then repeated with a sample of deuterated cyclohexanone at the same concentration as the non-deuterated reference solution. Because samples of deuterated cyclohexanone are usually “wet” and the amount of cyclohexanone can no longer be weighed accurately, making a solution of defined concentration (0.04 M) requires a different approach. The molar absorptivity of cyclohexanone at 280 nm is 20 [17], and therefore a 0.04 M solution of deuterated cyclohexanone will have an absorbance of 0.8 at 280 nm. We ask students to make up a slightly more concentrated solution of their deuterated cyclohexanone, then check the carbonyl absorbance, and calculate by how much the solution needs to be diluted in order to achieve the required absorbance of 0.8. This procedure usually works well.

It is immediately evident that the reaction of the deuterated cyclohexanone with iodine takes considerably longer than the non-deuterated sample (Figure 3). The majority of students was able to determine a k_H/k_D ratio of 3.5 ± 1.2 . This PKIE value tends to be smaller than the k_H/k_D ratio of 5.2 reported in the literature [14] which is readily explained by the fact that the cyclohexanone is not completely deuterated at the α -CH₂ position. Occasionally some students have reported a PKIE <2 or even that their deuterated sample reacted faster than the non-deuterated reference. Closer investigation revealed that students had made up a sample solution incorrectly or had washed the cuvette with acetone, which would have easily doubled the concentration of ketone in their sample as both cyclohexanone and acetone will react with iodine.

Over the last 6 years, this experiment has been conducted by almost 100 students. Most students obtained more than enough deuterated cyclohexanone for a ¹H NMR analysis, as well as a subsequent kinetics experiment, which requires only 0.1 g. However, the experiment has its challenges and is more appropriate for senior years. It is the first time students are asked to analyze the NMR spectra of a deuterated compound, and students are frequently unsure how to determine the percentage of deuteration.

The measurement of the kinetic isotope effect requires solutions of cyclohexanone and deuterated cyclohexanone at identical concentration, with the same amount of iodine added to each solution. We provide students with a 0.04 M solution of cyclohexanone – partly due to time constrictions but mostly to ensure consistency between student data. Because the slope of the absorbance vs. time curve should be the same for all students, it is more obvious for an instructor to spot if a student has done a mistake. Care is required in making up the solution of the deuterated cyclohexanone, and recommendations on how to do this properly are included in the lab manual. When the experiment was first introduced into the laboratory, the results for the PKIE tended to vary more widely due to huge discrepancies in the concentration of deuterated

cyclohexanone solutions. We now advise students to measure the absorbance of the cyclohexanone stock solutions at 280 nm (the carbonyl $n \rightarrow \pi^*$ transition) and then adjust the concentration so that both the cyclohexanone and deuterated cyclohexanone solutions show comparable absorbance readings. This extra step has improved the quality of the results, and most students now have no difficulties in finding that the deuterated sample takes longer to react.

In adapting the experiment for the teaching lab, the final concentrations of the cyclohexanone, acid and iodine were chosen so that the initial absorbance starts slightly below 1 and the reaction finishes within under 10 minutes. The rate constants for the iodination of cyclohexanone under acidic conditions are known [14] which makes it possible to estimate how long it will take for the iodine color to fade (see SI). We have adjusted concentrations so that the reaction of non-deuterated cyclohexanone takes about 6 – 8 minutes and that of deuterated cyclohexanone is over within less than half an hour. The zero-order kinetics provides the advantage that the change in iodine concentration with time is linear which allows students to obtain enough data to analyze their kinetics experiment even if they have to stop their measurement before all the iodine is consumed. This has the benefit of preventing long queues at the UV-Vis spectrophotometer.

In recent years, a number of educators have reported on the design of simple homemade colorimeters from cheap and readily available components [18,19,20,21,22,23,24]. The emphasis of most of these colorimeters has been on analytical chemistry, usually involving absorbance measurements to determine unknown concentrations of a dye based on calibration curves with known standards. Only two reports used a homemade colorimeter for following the kinetics of a reaction [25,26].

We were interested in finding out whether a home-built colorimeter could be used to follow the kinetics of a chemical reaction and even to confirm a primary kinetic isotope effect. The set

up was optimized by several undergraduate students who extensively checked the results obtained. The colorimeter was constructed from a light-emitting diode (LED) as a light source, a digital light sensor, a push-button to zero absorbance readings, an Arduino microprocessor, and a few Lego™ bricks to hold everything in place (Figure 4). The overall design was similar to colorimeters reported by several authors who placed the cuvette in between an LED as the light source and the detector, often a second LED [23,24].

Our colorimeter used a blue LED, which emitted light at 455 – 465 nm, and a 16-bit digital light sensor which was sensitive over a wide range of light intensities to allow absorbance values up to about 1.5 to be measured quite accurately. A small wall of Lego™ bricks blocked out most ambient light from reaching the sensor which helped to reduce fluctuations in the absorbance readings. An Arduino program took light intensity readings every second, calculated the absorbance, and checked whether someone has pressed a push button in which case the absorbance reading was zeroed. The Arduino was connected to a desktop PC which collected the absorbance readings as a function of time.

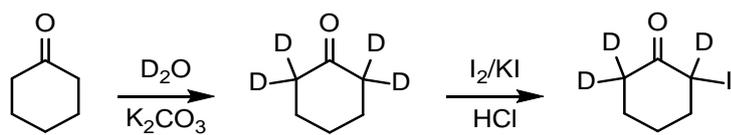
Figure 5 shows a photo of the colorimeter used for measuring the kinetic isotope effect. The kinetics data recorded with it was very similar to what is obtained with a commercial instrument (Figure 6). The change in iodine absorbance with time followed the same linear trend as previously observed with a commercial spectrophotometer and confirmed the zero-order kinetics for the reaction of cyclohexanone with iodine. It also nicely illustrated the validity of Beer's law over the entire range of absorbance.

Conclusions

The H–D exchange of cyclohexanone is accomplished by heating a mixture of cyclohexanone, D₂O and K₂CO₃ in a sealed vial for 3 – 4 days. After cooling to room temperature, the deuterated cyclohexanone phase-separates and can be removed with a Pasteur pipette without the need for further purification. Using this procedure, cyclohexanone is deuterated to about 80 – 85% at the α -CH₂ position, which is sufficient for the purpose of detecting a kinetic isotope effect. When cyclohexanone reacts with iodine under acidic conditions, the consumption of iodine during the reaction is easily followed photometrically by recording the decrease in absorbance at 460 nm with time. The reaction follows zero-order kinetics, and it is immediately evident that the deuterated sample takes considerably longer to react with iodine. The experiment has been carried out successfully by about 100 students over the last 6 years. The majority of students have no difficulty in identifying a k_H/k_D ratio of 3.5 with a standard deviation of 1.2. The experiment works successfully with a home-made colorimeter, and it is our intention to combine the assembly of a colorimeter with the measurement of a PKIE in future.

Acknowledgements. The authors thank Dr. Ai-Lan Lee for helpful discussions, and all the students who carried out the experiment in 2011 – 2017.

Supporting Materials. Detailed reagent list, student handout, instructor notes, analytical data for deuterated cyclohexanone, a summary of student results, a calculation to estimate the reaction time, instructions for making a colorimeter, Arduino code for following the kinetics, and typical results.



Scheme 1. Deuteration of cyclohexanone and subsequent iodination.

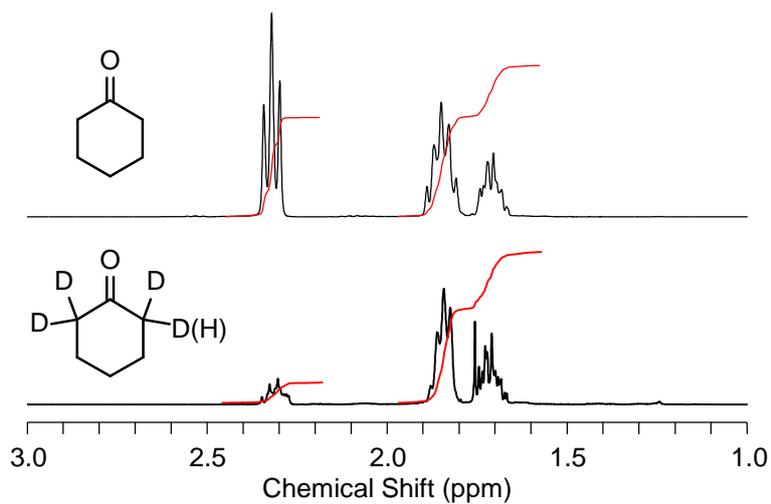


Figure 1. ¹H NMR spectra (300 MHz, CDCl₃) of cyclohexanone starting material and a partially deuterated cyclohexanone. The triplet at δ_{H} 2.3 is reduced to 15 – 20 % of its original intensity after deuteration of the α -methylene groups.

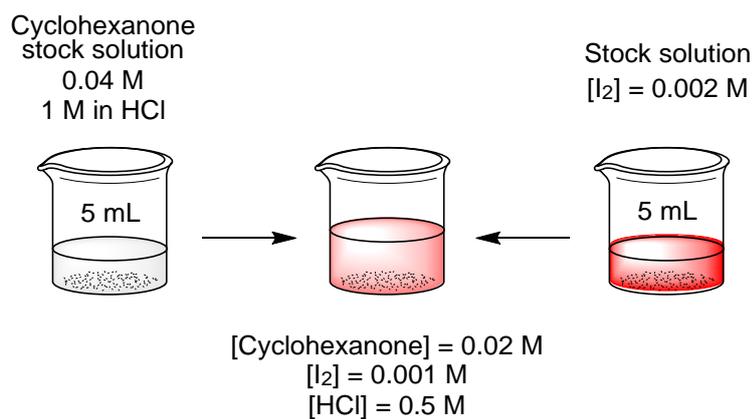


Figure 2. Students measure out exactly 5.0 mL of a cyclohexanone solution with the help of a graduated or volumetric pipette and add it to a beaker. They then add 5.0 mL of the iodine solution into a second beaker. Shortly before starting the kinetics run the two solutions are combined by pouring the contents of one beaker to the other and swirling or stirring the mixture briefly to ensure that the two solutions are mixed.

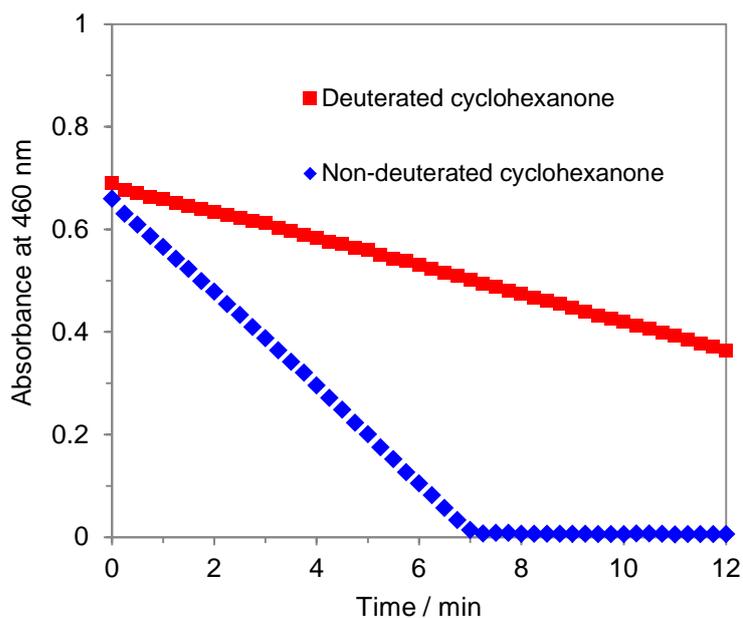


Figure 3. Student data showing the change in absorbance at 460 nm as a function of time for the acid-catalyzed reaction of iodine with cyclohexanone and cyclohexanone- d_4 . The reaction takes about 3 – 4 times longer when deuterated cyclohexanone is used. The PKIE can be easily determined from the ratio of the two slopes and equals 3.4 in the example above.

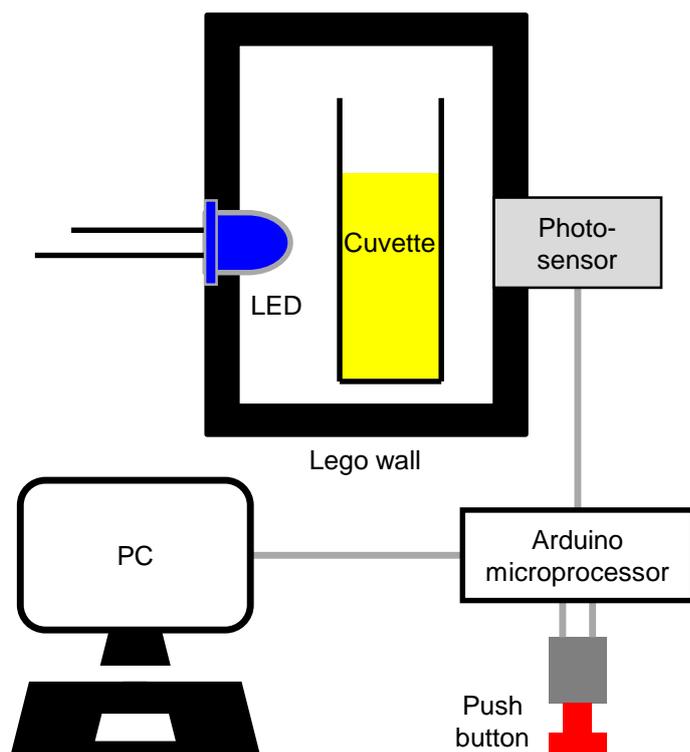


Figure 4. Schematic set-up of the colorimeter used in this work. A blue LED (emission range 455–465 nm, powered by a battery) is arranged so that its light shines straight onto an Adafruit TSL2561 digital light sensor which measures light intensities in lux. The sample cuvette is placed in between. A small wall of Lego™ bricks surrounds the cuvette and holds the LED and digital light sensor in place. An Arduino Uno microprocessor checks the light intensity made by the light sensor every second and calculates the absorbance as the logarithm of the light as the logarithm of the ratio of observed light intensity and a reference value, which is transferred to a computer. The Arduino also checks regularly whether a push button has been pressed and, if it has, the light intensity at that point is saved as the reference value and the absorbance is zeroed. This baseline correction is typically done with an empty cuvette, or no cuvette at all, placed in the light path.

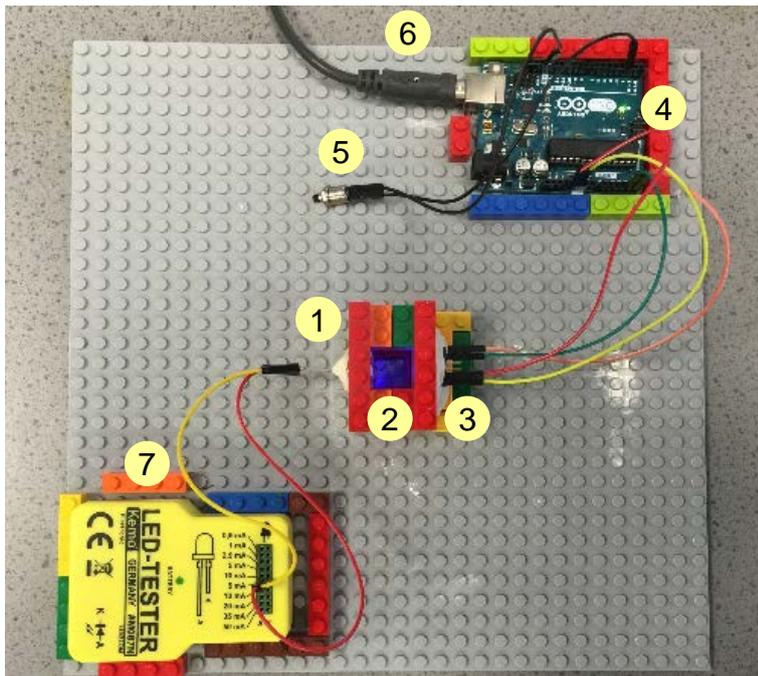


Figure 5. Photo of the colorimeter used in this work where 1 = blue LED, 2 = UV cuvette surrounded by a wall made of Lego™ bricks, 3 = photosensor, 4 = Arduino, 5 = push button, 6 = USB cable (to computer), 7 = battery.

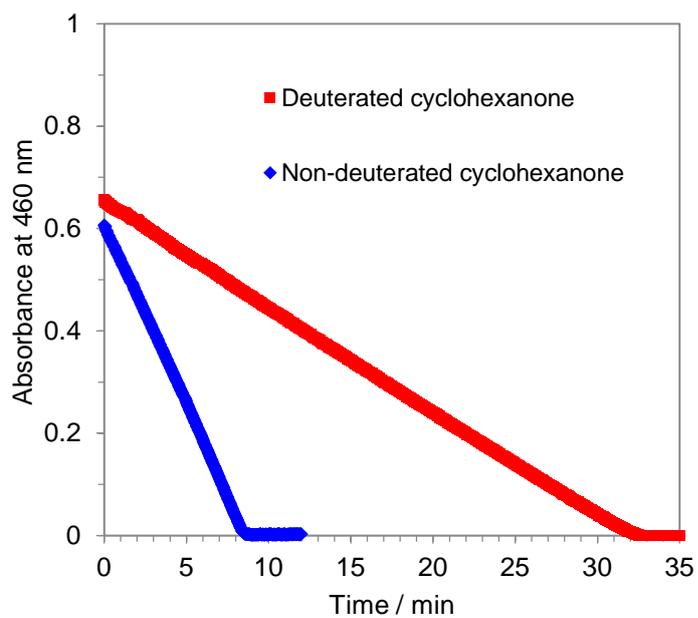


Figure 6. Change in absorbance at 460 nm as a function of time for the acid-catalyzed reaction of iodine with cyclohexanone and cyclohexanone- d_4 . The data were recorded with a homemade colorimeter. The PKIE equals the ratio of the two slopes and has a value of 3.4.

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