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Demonstration

# Hydrolysis of 2-Chloro-2-methylpropane—Demonstration Using the Quenching of Fluorescence from Fluorescein

Masatada Matsuoka* and Takashiro Akitsu

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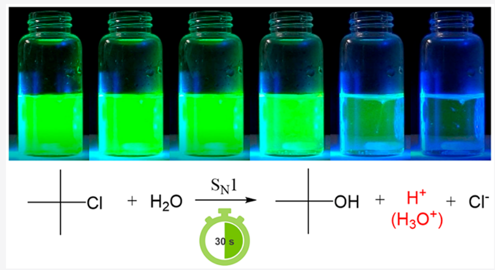
**ABSTRACT:** 2-Chloro-2-methylpropane slowly hydrolyzes in water, releasing hydrochloric acid. Therefore, the addition of 2-chloro-2-methylpropane to an aqueous solution of sodium hydroxide containing an acid–base indicator causes a color change when the solution becomes acidic. Here we report the use of fluorescein, a fluorescent dye, as an indicator for the completion of the above-mentioned reaction. Under blacklight radiation, completion of the reaction is marked by the disappearance of fluorescence. Compared with the other clock reactions, this fluorescent reaction is more advantageous because it requires inexpensive reagents and involves milder conditions, and it is expected to be utilized in chemistry outreach events. Furthermore, the demonstration can be easily understood by high school students since the reaction mechanism can be explained well by changing the pH, and it is suitable for practice in the classroom.




**KEYWORDS:** *General Public, Elementary/Middle School Science, High School/Introductory Chemistry, Demonstrations, Hands-On Learning/Manipulatives, pH, Dyes/Pigments, Rate Law*



## INTRODUCTION

An experiment in which a color change is observed after a certain period of time following the mixing of solutions is called a clock reaction. Representative examples include the reaction of potassium iodate and sodium sulfite[1] and the blue bottle experiment,[2] which are often used in demonstrations because of their visual impact. The clock reaction can be utilized as an advanced topic for understanding chemical kinetics since the color change occurs with time variation as the solution concentration is changed.

The hydrolysis of 2-chloro-2-methylpropane ($(CH_3)_3CCl$) using a color-changing acid–base indicator is a well-known reaction with a visual effect similar to that of the clock reaction.[3] When this reaction is performed in a dilute aqueous sodium hydroxide solution, the indicator changes from basic to acidic form because hydrochloric acid is generated as the reaction proceeds. Once sodium hydroxide is exhausted, the additional hydrochloric acid can lower the pH of the solution and thereby protonate the indicator. In addition, acid–base indicators are used to identify the reaction end point when the reactivities of primary, secondary, and tertiary alkyl halides during hydrolysis are compared.[4]

We have found that in the hydrolysis of 2-chloro-2-methylpropane, if fluorescein is added instead of an acid–base indicator, fluorescence is emitted under irradiation with a blacklight and disappears tens of seconds later. To the best of our knowledge, this is the first example of a fluorescent reaction utilizing the hydrolysis of 2-chloro-2-methylpropane. The purpose of this experiment is to stimulate curiosity in science by utilizing fluorescence. Furthermore, high school students can easily correlate a decrease in pH with the change in fluorescence intensity based on the reaction mechanism. An example of demonstrating the above-mentioned phenomenon is described below.

## BACKGROUND

Hydrolysis of tertiary alkyl halides proceeds in aqueous solutions via the unimolecular nucleophilic substitution ($S_N1$) reaction mechanism (Figure 1). Since hydrogen chloride is generated as the reaction progresses, when an acetone solution of 2-chloro-2-methylpropane is added to a dilute aqueous sodium hydroxide solution containing an appropriate acid–base indicator, the color changes as the indicator changes from basic form to acidic form.

Both the substitution ($S_N1$, major) and elimination (E1, minor) pathways occur in competition with each other. Thus, hydrolysis of 2-chloro-2-methylpropane yields a mixture of 2-methyl-2-propanol ($S_N1$; Figure 1, eq 3) and 2-methylpropene (E1) at a rate independent of the water concentration. When the solution is allowed to stand for a while after the reaction, small quantities of 2-methylpropene gas can be emitted.

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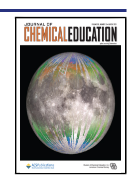






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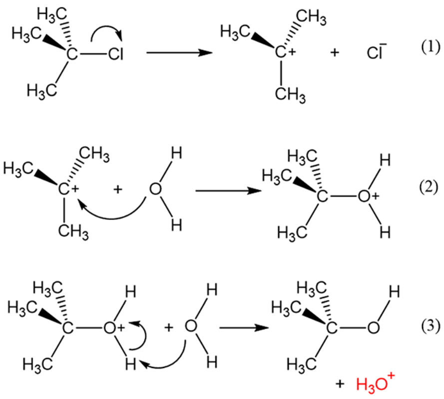


**Figure 1.** Hydrolysis reaction mechanism.

The structure of fluorescein changes depending on pH, and under basic conditions, the maximum absorption is near 491 nm, where green fluorescence with a maximum emission wavelength of 513 nm is emitted. The fluorescence intensity of fluorescein reaches its maximum at pH 9 and higher, and the fluorescence rapidly decreases at pH 8 and lower.[5] Therefore, under irradiation with ultraviolet (UV) light, the fluorescence disappears when the solution becomes acidic (Figure 2). Dynamic changes in the fluorescence can be viewed in the video in the Supporting Information.

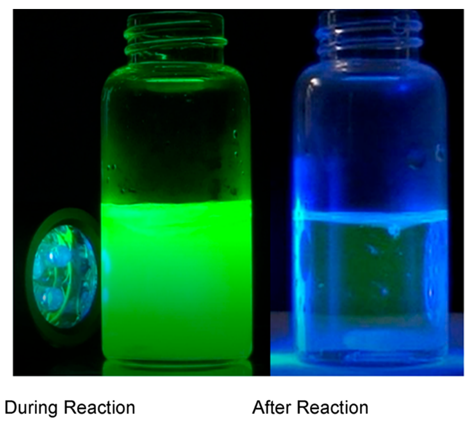


**Figure 2.** Clock reaction with fluorescein.

## ■ MATERIALS AND DEMONSTRATION

The following materials are required for this demonstration:

- 2-chloro-2-methylpropane (CAS no. 507-20-0)
- fluorescein sodium salt (uranine) (CAS no. 518-47-8)
- 0.10 mol/L sodium hydroxide solution (standard solution)
- acetone
- ethanol (96%)
- 30 mL glass vials (30 mm diameter)
- two 10 mL pipettes
- 250 μL microsyringe
- laboratory water bath
- magnetic stirrer and stir bar (1 cm)
- two thermometers (rated for use at 50 °C)
- portable blacklight (365−375 nm)
- safety goggles

The solutions used in the experiment are prepared in advance by the instructor.

### Preparation of Solutions

Fluorescein sodium salt (56 mg, 0.15 mmol) is weighed and placed in a sample bottle. Then 30 mL of water containing 10% ethanol by volume and 1.0 mL of 0.10 mol/L sodium hydroxide solution are added to the sample bottle and stirred to homogenize. The resulting solution (∼4.8 mM fluorescein at pH ∼11.5) is placed in a 30 mL glass vial (30 mm diameter), wrapped with aluminum foil, and stored in the refrigerator.

2-Chloro-2-methylpropane (700 μL, 0.60 g, 6.4 mmol) is added to a 100 mL volumetric flask, and acetone is added up to the marked line to bring the volume to 100 mL. The resulting 64 mM reagent solution is placed in a 100 mL polypropylene narrow-mouth reagent bottle.

Sodium hydroxide solution (5.0 mL of a 0.10 mol/L standard solution) is added to a 100 mL volumetric flask, and water is added to the marked line to bring the volume to 100 mL. The resulting 5.0 mM aqueous sodium hydroxide solution (pH ∼11.5) is placed in a 100 mL polypropylene narrow-mouth reagent bottle.

Ideally, the solutions should be prepared the day before the demonstration but will remain active for approximately 2 weeks if stored.

### Performing the Experiment

Safety goggles should be worn during the experiment. The acetone solution of 2-chloro-2-methylpropane and the aqueous sodium hydroxide solution should be preheated to 35 °C in a laboratory water bath.

The stir bar is added to a 30 mL glass vial (30 mm diameter). A 10 mL volume of aqueous sodium hydroxide solution is added and stirred using the magnetic stirrer. Then 100 μL of fluorescein solution is added using a microsyringe. When the portable blacklight is turned on next to the glass vial, the solution will show green fluorescence. Then the room is darkened, and 5 mL of 2-chloro-2-methylpropane in acetone is added. The fluorescence will suddenly disappear after approximately 30 s. (If there is no magnetic stirrer, a similar result can be obtained by mixing the solution, shaking it by hand, and leaving it to stand.)

## ■ HAZARDS

Students and instructors should wear lab coats, safety goggles, and suitable gloves during preparation and the demonstration. Sodium hydroxide, 2-chloro-2-methylpropane, and fluorescein sodium salt are considered irritants upon inhalation, ingestion, skin contact, or eye contact, but they are present only in low concentrations in the apparatus during the demonstration. Ethanol and acetone are flammable, even in solutions with water. The demonstration should be kept away from open flames or ignition sources. In the event of skin contact, it is advised to wash the area thoroughly with soap and water. Recent (material) safety data sheets ((M)SDSs) should be reviewed. After the demonstration, the solution should be disposed in an organic waste container.

The portable blacklight should not be viewed with the naked eye during the experiment. According to the American Conference of Governmental Industrial Hygienists, naked eye exposure to UV-A (315 to 400 nm) light should be limited to an irradiation dose of 1.0 J·cm$^{-2}$ for periods lasting less than 1000 s.[6] The maximum intensity of most commercially


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J. Chem. Educ. 2021, 98, 941−945

available portable blacklights is $3.0 \times 10^{-3}$ J·cm$^{-2}$ at the beam center, and it can be safely observed by wearing standard safety goggles.

## ■ RESULTS AND DISCUSSION

In this demonstration, the fluorescence disappears after approximately 30 s. Both the phenol and carboxylic acid functional groups of fluorescein are almost completely deprotonated in aqueous solutions above pH 9.0 (Figure 3).

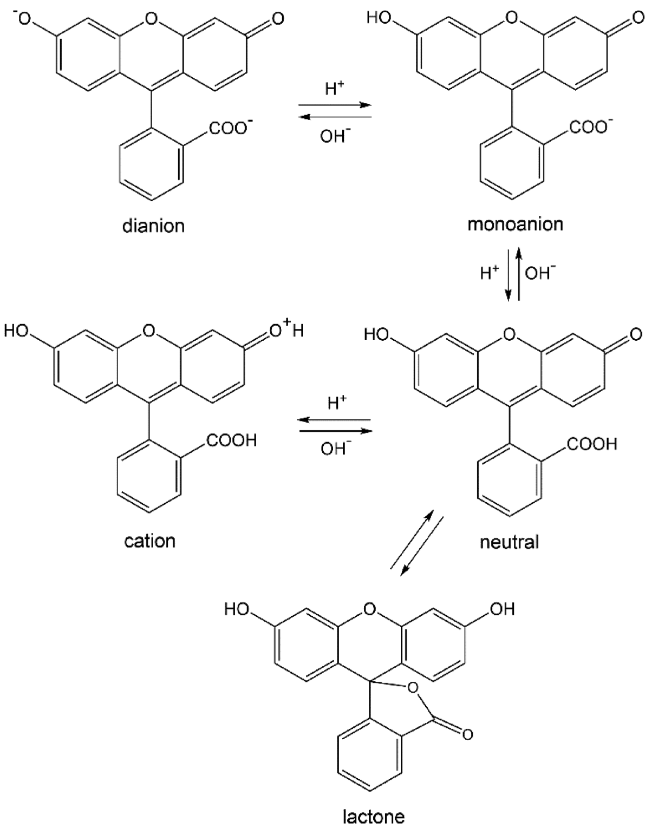


**Figure 3.** Structural changes of fluorescein in aqueous solutions.

Acidification of the fluorescein dianion first protonates the phenol (p$K_a \approx 6.4$) to yield fluorescein monoanion and then protonates the carboxylic acid (p$K_a < 5.0$) to produce the neutral species of fluorescein. Further acidification generates a fluorescein cation (p$K_a \approx 2.1$). Only the monoanion and dianion of fluorescein are fluorescent. A further equilibrium involves the formation of a colorless, nonfluorescent lactone. The lactone is formed in aqueous solution under pH 5.0 and may be the dominant form of neutral fluorescein in solvents such as acetone.[7]

We began by investigating the effect of pH on the fluorescence intensity of fluorescein. A 5 mL aliquot of hydrochloric acid or aqueous sodium hydroxide solution at a given pH was mixed with 10 mL of acetone, and then 10 $\mu$L of fluorescein solution was added with a microsyringe. With a spectrofluorometer (JASCO FP-6200), the excitation wavelength was set to 491 nm, and the fluorescence intensity at the maximum emission wavelength of 513 nm was measured. Figure 4 shows the relative fluorescence intensity based on the fluorescence intensity at pH 11. Under the experimental conditions, it was confirmed that the fluorescence rapidly decreased starting at pH 9 and disappeared at pH 4.

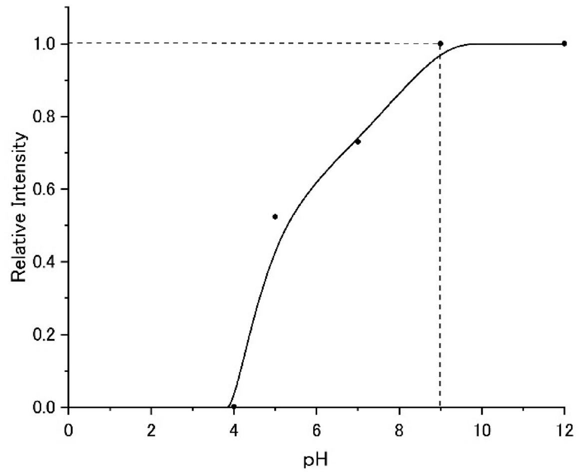


**Figure 4.** Change in fluorescence intensity of fluorescein solution as the pH is varied.

We also investigated the time variation of the fluorescence intensity using a photometer.[8] As shown in Figure 5, a wireless

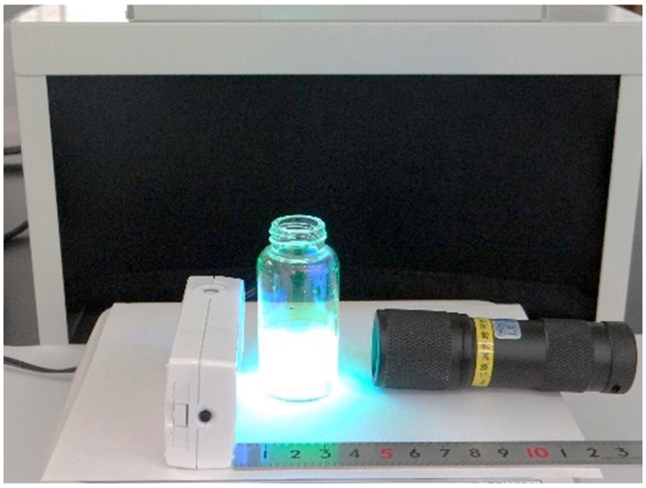

**Figure 5.** Measurement of fluorescence intensity.

optical sensor (PASCO PS-3213) and a portable blacklight were placed 5 cm apart, and a glass vial containing the chemical reaction was placed between them. The optical sensor measures the total luminous flux (measured in lux) of both constantly transmitted UV light and the variable fluorescence of fluorescein. Similar experimental results can be obtained even if different types of portable blacklights are used because the fluorescence is emitted after the excited molecule loses energy and becomes metastable (Kasha's rule).

Typical experimental results are shown in Figure 6. Immediately after the acetone solution of 2-chloro-2-methylpropane was added, strong fluorescence was emitted, and the luminous flux remained unchanged from 3 s to approximately 25 s. As the solution turned acidic, the fluorescence disappeared at 28 ± 3 s. Subsequently, the luminous flux of the transmitted UV light was observed.

In addition, the luminous flux of a blank solution prepared by adding only acetone to an aqueous sodium hydroxide solution containing fluorescein was also measured. Comparison of the two solutions shows that the fluorescence rapidly


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J. Chem. Educ. 2021, 98, 941−945

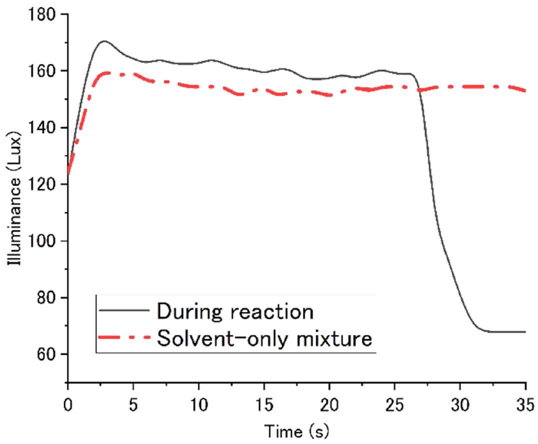


**Figure 6.** Change in fluorescence intensity during reaction.

disappears in the 2-chloro-2-methylpropane solution as the hydrolysis reaction proceeds and the solution becomes acidic.

We also used a photometer to investigate the fluorescence duration when the temperature and concentration of the sodium hydroxide solution were changed. The results are shown in Table 1. The sodium hydroxide solution was prepared by diluting the standard solution, and the duration of fluorescence was taken as the average of five measurements.

**Table 1. Time Required for the Disappearance of Fluorescence**

| Aqueous NaOH Solution Concentration (mM) | Time (s) | | |
|---|---|---|---|
| | $T$ = 25 °C | $T$ = 30 °C | $T$ = 35 °C |
| 2.50 | 29 | 18 | 13 |
| 5.00 | 62 | 38 | 29 |
| 7.50 | 95 | 57 | 41 |

The hydrolysis of 2-chloro-2-methylpropane is a first-order reaction that follows the Arrhenius equation. When fluorescein was used, the activation energy of the hydrolysis was calculated to be 58.1 kJ/mol using the data for an aqueous sodium hydroxide concentration of 5.00 mM.

In addition, a slight temperature rise (approximately 2 °C) was observed as a result of mixing. This heat generation is considered to have prevented a temperature drop of the solution during observation. In the water/acetone system, it has been reported[9] that the mixing enthalpy is maximized when the mole fraction of acetone is approximately 0.25, which is the reaction condition used here. This reaction has been attempted under many conditions,[2] and in all cases reported in the literature an acetone mole fraction of 0.20 to 0.30 has been used, presumably because of this effect.

Finally, for comparison, Table 2 lists the results of experiments performed according to the same procedure using phenolphthalein solution (5.0 mM, containing 10% ethanol by volume) instead of fluorescein solution.

The results were almost the same for both phenolphthalein and fluorescein. When phenolphthalein is used, one observes a color change from red to colorless. This is easy to observe and is therefore a widely used approach. On the other hand, fluorescein solution can be used for impactful reactions such as the clock reaction, which is performed in a dark room. When a portable blacklight is shone from a close distance, it is possible to accurately measure the time required for the disappearance of fluorescence. Although a 50−100 cm tube blacklight is not ideal for making accurate measurements of the end point, it can be used for safe chemical demonstrations.

**Table 2. Time Required for Color Change Using Phenolphthalein**

| Aqueous NaOH Solution Concentration (mM) | Time (s) | | |
|---|---|---|---|
| | $T$ = 25 °C | $T$ = 30 °C | $T$ = 35 °C |
| 2.50 | 22 | 13 | 8 |
| 5.00 | 50 | 28 | 21 |
| 7.50 | 86 | 47 | 31 |

## ■ CONCLUSIONS

We have developed a fluorescent reaction using the hydrolysis of 2-chloro-2-methylpropane. Since the solution becomes acidic as the hydrolysis progresses, the fluorescence disappears abruptly over time if fluorescein, which exhibits fluorescence under basic conditions, coexists in the solution. First, the relationship between the pH of the solution and the fluorescence intensity was clarified using a spectrofluorometer. The concentration and temperature were subsequently changed to identify suitable conditions for enabling observation by students. The experimental results showed that the time until the disappearance of fluorescence increased when the concentration of the NaOH aqueous solution was increased, and the reaction proceeded faster when the solution temperature was increased. Taking these results into consideration, we set the reaction temperature of the demonstration as slightly higher than room temperature to ensure that the changes will be complete in approximately 30 s.

Since it is possible to observe the instantaneous color change in a short period of time, this is an effective experimental teaching demonstration for stimulating the interest of middle and high school students. Middle school students, in particular, enjoy observing this fluorescence. Additional student interest can be generated by showing other objects that exhibit fluorescence when irradiated with a blacklight, such as clothing washed in detergent with UV protection, spots printed with fluorescent ink on banknotes, and seashells.

It is possible to perform a demonstration in approximately 20 min while introducing fluorescent substances that exist around us to stimulate students' curiosity. Glow sticks using the luminol reaction and bis(2,4,6-trichlorophenyl) oxalate are well-known examples of chemical luminescence, and the fluorescent reaction introduced here is expected to have the same curiosity-inducing effect. Figure 7 illustrates the performance of a chemical demonstration, including this fluorescent reaction for a chemistry outreach event, which was hosted by high school students belonging to the chemistry club. This event was held for the local junior high school and elementary school students. The chemistry club students prepared the script and decoration materials for the demonstration after school hours. The experiment was guided by the instructor in advance. The participating children provided favorable feedback such as "The explanations were easy to understand," "Amazing!/Wonderous," "They (student instructors) were kind," "I want to know how the reaction works," and "It was like magic!"

It is also worth noting that in chemistry education, experiments and observations in the laboratory can be used


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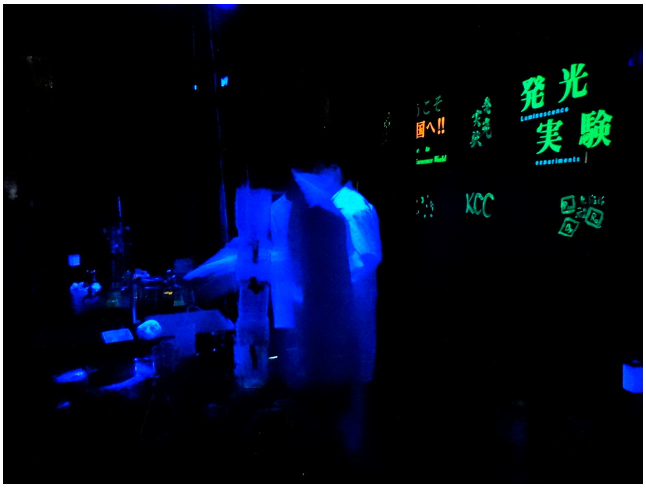


**Figure 7.** High school students perform the chemical demonstration for a chemistry outreach event.

to analogize and improve the ability to explain concepts, but the influence of COVID-19 has made it difficult to perform laboratory activities as before. Such demonstrations in a dark room can be provided as remote lessons, which will allow multiple students to safely observe it at the same time.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.0c01255.

Video showing the demonstration in Figure 2 (MP4)

## ■ AUTHOR INFORMATION

### Corresponding Author

**Masatada Matsuoka** − *Department of Chemistry, Faculty of Science, Fukuoka University, Fukuoka 814-0180, Japan;* orcid.org/0000-0002-9555-5062; Email: mtokmstd@fukuoka-u.ac.jp

### Author

**Takashiro Akitsu** − *Department of Chemistry, Faculty of Science, Tokyo University of Science, Shinjuku-ku, Tokyo 162-8601, Japan*

Complete contact information is available at:
https://pubs.acs.org/10.1021/acs.jchemed.0c01255

### Notes

The authors declare no competing financial interest.

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