

# Chemiluminescence Demonstration Illustrating Principles of Ester Hydrolysis Reactions

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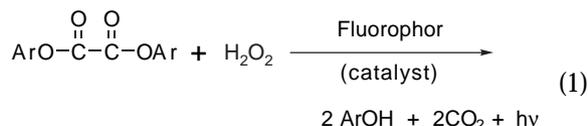
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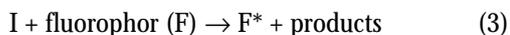
## Background

Peroxyoxalate chemiluminescence (PO-CL) forms the basis of Cyalume "light sticks" used for emergency and underwater lighting, fishing lures, and various novelty items such as glow necklaces. Since 1980, the PO-CL reaction has become widely used as an analytical tool for trace analysis (1, 2) and is currently the most sensitive and versatile chemiluminescence detection method for liquid chromatography. The overall reaction may be represented by



where Ar is an electronegative aryl group such as 2,4-dinitrophenyl or 2,4,6-trichlorophenyl.

Whereas most chemiluminescence reactions involve emission from a reaction intermediate derived from one of the reagents, the peroxyoxalate reaction transfers energy to a variety of fluorescent molecules, which in turn emit light during relaxation from the first singlet excited state. The general reaction scheme can be characterized by



The first substitution of H<sub>2</sub>O<sub>2</sub> for a phenol (ArOH) in the oxalate ester is rate limiting, and as a result the chemiluminescence duration is indicative of the rate of reaction 2. The nucleophilic substitution of H<sub>2</sub>O<sub>2</sub> is analogous to the hydrolysis of this ester by water, and thus mechanistic aspects of the kinetics of ester hydrolysis may be demonstrated visually using the peroxyoxalate reaction.

The reaction of an oxalate ester with hydrogen peroxide produces at least one, but possibly two or more, highly energetic intermediates capable of generating the excited singlet state of fluorescent molecules. Radiative decay of the singlet-excited fluorescent molecule produces the observed light emission, and a variety of fluorophors may be used to produce a range of colors. Fluorophors used to demonstrate PO-CL include rubrene (orange-yellow emission), 9,10-bis(phenylethynyl)anthracene (green emission), and 9,10-diphenylanthracene (blue emission).

The PO-CL reaction vividly demonstrates chemical reaction concepts that can be catered to a wide range of audiences. Mohan and Turro used the PO-CL reaction to demonstrate the effect of catalysis and temperature change on the rates of chemical reactions (3). They also published a synthesis for bis(2,4,6-trichlorophenyl) oxalate (TCPO) and bis(2,4-dinitrophenyl) oxalate (DNPO) that could be modified for an organic chemistry lab experiment. Both esters have since become commercially available (Aldrich, Milwaukee, WI). Chemiluminescence demonstrations and experiments using Cyalume light sticks, including a detailed description of the relevant chemical reactions, have also been described (4-6). In this experiment, we use the peroxyoxalate chemiluminescence reaction to demonstrate how the p*K*<sub>a</sub> of the leaving group and nature of the catalyst affect the rate of ester hydrolysis reactions.

## Experimental Details

This demonstration is designed for an audience of approximately 100 people, but the volumes of reagents may be scaled according to the size of the audience and the directions modified to suit the purpose of the demonstration. Although many colors can be produced using PO-CL, the orange-yellow emission from rubrene is the most easily observed for larger audiences or in rooms that are not completely dark. This demonstration is divided into two experiments: (i) the effect of leaving group on the kinetics of the uncatalyzed reaction of DNPO and TCPO with H<sub>2</sub>O<sub>2</sub> and (ii) comparison of the effects of sodium salicylate (a general-base catalyst) with imidazole (a nucleophilic catalyst) on the reaction of TCPO with H<sub>2</sub>O<sub>2</sub>.

## Materials

Organic solvents: ethyl acetate and acetonitrile

Hydrogen peroxide, 30% in water

Bis(2,4-dinitrophenyl) oxalate (DNPO)

Bis(2,4,6-trichlorophenyl) oxalate (TCPO)

Imidazole and sodium salicylate

Fluorophors such as 9,10-bis(phenylethynyl)anthracene, 9,10-diphenylanthracene, and rubrene

4 reagent bottles and 3 small test tubes that can be immersed inside the reagent bottles

**CAUTION:** The organic solvents are flammable, and care should be taken in their use and disposal. In particular, they should be stored in well-ventilated cabinets or hoods separate

from acids and oxidizing agents and isolated from sparks and flames. Organic solvents containing hydrogen peroxide should not be evaporated to dryness, as explosive organic peroxides may form. The fluorophors suggested in this study are suspected carcinogens. The 30% hydrogen peroxide used in this experiment is caustic, and gloves must be worn when preparing the 1% solution.

**Oxalate/fluorophor stock solution.** Fill four reagent bottles with 90 mL of an 80% ethyl acetate, 20% acetonitrile solution. Add 150 mg of DNPO to one bottle and 150 mg of TCPO to each of the other three bottles. Add 10 mg of rubrene to the DNPO solution and to one of the bottles containing TCPO. To the other two TCPO bottles add a similar amount of rubrene or a different fluorophor such as bis(phenylethynyl) anthracene. The TCPO solution is shelf stable for several weeks, but the DNPO stock solution must be freshly prepared. The solid oxalates decompose when exposed to moisture for long periods of time. Rubrene and other fluorophors are photooxidized in room light, and solutions must be stored in the dark.

**Hydrogen peroxide stock solution.** Prepare a nominally 1% by weight solution of  $\text{H}_2\text{O}_2$  in acetonitrile by adding 1.5 mL of 30%  $\text{H}_2\text{O}_2$  to a 50-mL volumetric flask and diluting to volume with acetonitrile. This solution is stable for several months.

#### Procedure

The PO-CL reaction is initiated by adding 10 mL of the hydrogen peroxide solution to the oxalate ester-fluorophor solution. Gently adding the hydrogen peroxide so that solution mixing is minimized allows the reagent bottle to be capped and swirled before the solution is completely brightened. DNPO reacts rapidly with hydrogen peroxide to produce bright chemiluminescence, whereas observable chemiluminescence using TCPO requires a catalyst.

To demonstrate the effect of the leaving group on the reaction rate, add 10 mL of the hydrogen peroxide solution to the DNPO-fluorophor solution and to one of the TCPO-fluorophor solutions. The lack of observable light from the bottle containing TCPO provides a visual comparison of how the phenolate leaving groups (2,4-dinitrophenolate vs 2,4,6-trichlorophenolate) affect the reaction rate. Bright, localized chemiluminescence is achieved for the TCPO reaction by adding a test tube containing 0.20 g of sodium salicylate to the reagent bottle and allowing the upright test tube to fill with solution. The addition of sodium salicylate to the TCPO reaction vividly demonstrates reaction catalysis.

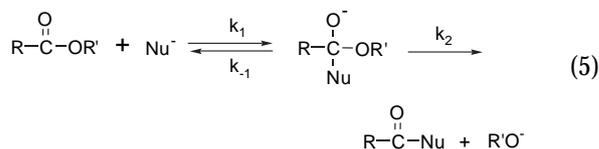
The two remaining bottles of TCPO-fluorophor can be used to compare nucleophilic and general-base catalysis. Add 10 mL of 1%  $\text{H}_2\text{O}_2$  to each of the TCPO-fluorophor solutions (a different fluorophor may help present the change of topic). Into one reagent bottle place a test tube containing 0.20 g of sodium salicylate, and into the other bottle place a test tube with a similar number of moles (0.087 g) of imidazole. Allow the test tubes to fill and then swirl the reagent bottles to completely mix the catalyst and produce homogeneous brightening of the solution. The chemiluminescence of the imidazole-catalyzed reaction fades rapidly relative to the salicylate-catalyzed reaction, visually demonstrating the much greater effect of nucleophilic catalysis on the rate of this reaction. Figure 1 shows plots of chemiluminescence intensity vs time, measured using a photomultiplier tube as a detector, for the reaction

of TCPO with  $\text{H}_2\text{O}_2$  catalyzed by imidazole and sodium salicylate. The same initial concentrations as in the demonstration are used. The light intensity decays much more rapidly using imidazole as the catalyst. The area under the emission profile is proportional to the total number of photons emitted. Note that even though imidazole causes the reaction to occur faster and to be slightly brighter initially, the quantum yield of the reaction is less in the case of imidazole catalysis.

## Discussion

### Effect of the Leaving Group on the Reaction Rate

The PO-CL reaction is analogous to ester hydrolysis in which  $\text{H}_2\text{O}_2$  instead of  $\text{H}_2\text{O}$  serves as the substituting nucleophile. Nucleophilic substitution at a carbonyl carbon generally proceeds through a tetrahedral intermediate, as shown below.



The substituent and  $\text{p}K_a$  of the leaving group affect the relative rates of the three reaction steps, as described by  $k_1$ ,  $k_{-1}$ , and  $k_2$ . An electron-withdrawing substituent in the leaving group favors addition of the nucleophile, increasing the value of  $k_1$  and of the equilibrium constant,  $K = k_1/k_{-1}$ . The rate of decomposition of the tetrahedral intermediate to products is strongly affected by the relative  $\text{p}K_a$ 's of the nucleophile and leaving group (7). The decomposition of the addition intermediate to form products ( $k_2$ ) is favored for leaving groups that are weak bases (i.e., conjugate bases of strong acids).

TCPO and DNPO contain electron-withdrawing substituents in the aryl group that promote addition of hydrogen peroxide. The inductive effect of 2,4-dinitrophenol (DNP) is greater than that of 2,4,6-trichlorophenol (TCP), thereby

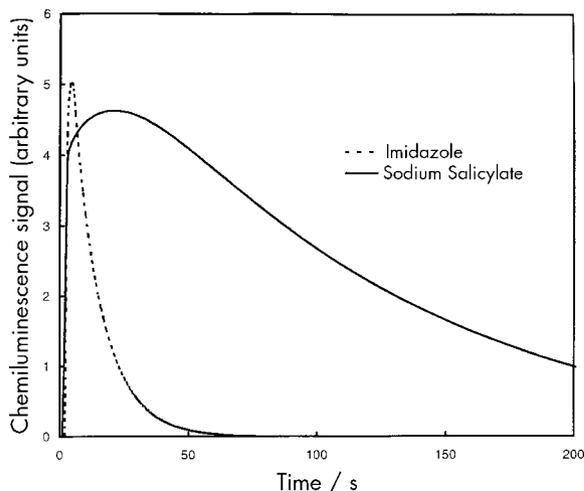


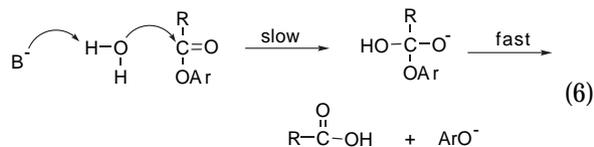
Figure 1. Chemiluminescence intensity vs time profiles comparing the effect of sodium salicylate (A) and imidazole (B) on intensity and kinetics of the reaction of  $\text{H}_2\text{O}_2$  and TCPO in the presence of 9,10-diphenylanthracene (DPA). Conditions: (A) 3.3 mM TCPO, 29 mM  $\text{H}_2\text{O}_2$ , 0.19 mM DPA and 13 mM sodium salicylate; (B) 3.3 mM TCPO, 29 mM  $\text{H}_2\text{O}_2$ , 0.19 mM DPA, and 13 mM imidazole. Chemiluminescence signals are in arbitrary units.

enhancing  $k_1$  and  $K$  for DNP relative to TCP. Also, for DNPO the elimination reaction is expected to be faster because of the acidity of DNP relative to TCP. DNP is much more acidic than TCP in water ( $pK_a$ 's of 4.08 and 7.42, respectively) (8), dimethyl furan ( $pK_a$ 's of 6.4 and 12.4) (9), and dimethyl sulfoxide ( $pK_a$ 's of 5.1 and 10.2) (9). Unfortunately, the  $pK_a$ 's in acetonitrile appear not to be known, but based on the above comparisons, DNP is predicted to be much more acidic than TCP. Thus, both formation of the addition intermediate and decomposition of the intermediate to form chemiluminescent products are enhanced for DNPO relative to TCPO.

The difference in kinetics observed for the reactions of TCPO and DNPO with hydrogen peroxide is illustrated qualitatively in the reaction coordinate diagram shown in Figure 2. The addition product is lower in energy and is formed with a smaller activation barrier for the DNPO reaction. Also, the decomposition of this intermediate to form products has a smaller barrier. Thus, the uncatalyzed nucleophilic substitution reaction of  $H_2O_2$  with DNPO and TCPO provides a basis for discussing leaving group effects on the analogous ester hydrolysis reactions.

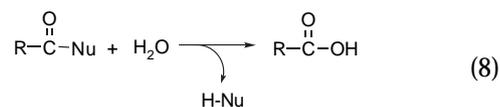
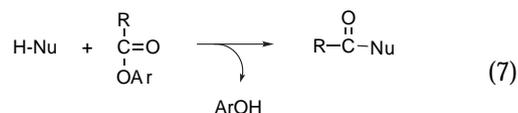
#### Nucleophilic and General-Base Catalysis

Ester hydrolysis can be promoted by different types of catalysis. Catalysts provide a reaction path having a lower activation energy and thereby increase the rate of one or more steps in a reaction. The type of catalysis can affect the mechanism and intermediates formed in a reaction. In general-base-catalyzed hydrolysis, a participating base promotes addition of water through partial proton transfer. The hydroxide ion is a better nucleophile, and  $k_1$  is favored.



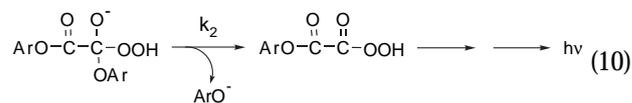
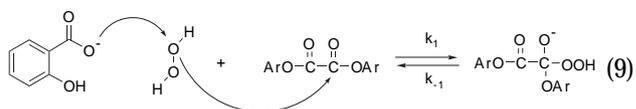
In nucleophilic catalysis, a nucleophile (H–Nu) such as an amine substitutes for phenol in the ester, forming an acyl

intermediate that is more rapidly hydrolyzed than the starting compound.



The imidazole-catalyzed hydrolysis of esters bearing good leaving groups has been shown to proceed by a nucleophilic-catalysis pathway with a second-order dependence on imidazole (10–12). In this demonstration, sodium salicylate is used as a general-base catalyst and imidazole as a nucleophilic catalyst.

In the general-base-catalyzed PO-CL reaction, sodium salicylate promotes the formation of the hydroperoxy anion ( $\text{HOO}^-$ ), a stronger nucleophile than  $\text{H}_2\text{O}_2$ . Expulsion of  $\text{HOO}^-$  from the tetrahedral intermediate is less favorable than expulsion of the phenoxide, thereby increasing the rate of product formation ( $k_2 > k_{-1}$ ).



Further reactions of the peroxy acid,  $\text{ArO}(\text{CO})_2\text{OOH}$ , lead to the observed chemiluminescence. The exact mechanism is still not completely understood but probably involves the formation of one or more cyclic intermediates that form charge-transfer complexes with the fluorophor (1, 13, 14).

Imidazole undergoes a rapid nucleophilic substitution reaction with TCPO to replace both aromatic phenols and form 1,1'-oxalyldiimidazole (ODI) (15–17). ODI is much more reactive with  $\text{H}_2\text{O}_2$  than is TCPO. As a result, the chemiluminescence occurs faster in the presence of imidazole.

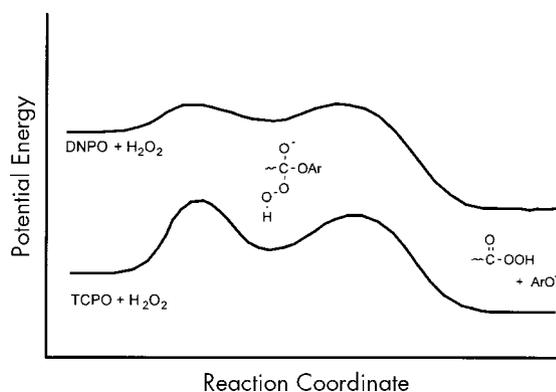
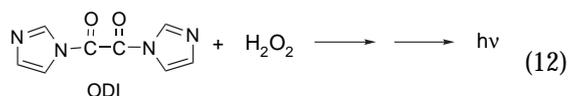
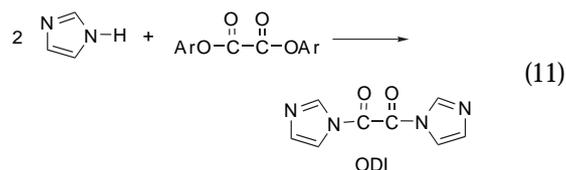


Figure 2. Reaction coordinate diagram illustrating the qualitative differences between reactions of TCPO and DNPO with  $\text{H}_2\text{O}_2$ . The two diagrams are offset vertically by an arbitrary amount. The DNPO reaction is expected to have a lower activation barrier for formation of the tetrahedral intermediate, a lower energy of the intermediate relative to reactants, and a lower barrier to formation of products. Both reactions are exothermic.

Addition of imidazole to TCPO is promoted by proton transfer to another molecule of imidazole, with the result that a second-order dependence on the imidazole concentration is observed. As further evidence for nucleophilic catalysis, sterically hindered bases of similar aqueous  $pK_a$  as imidazole, 2,4-dimethylpyridine and 2,4,6-trimethylpyridine, fail to

catalyze the reaction of TCPO and H<sub>2</sub>O<sub>2</sub> to a significant extent (17). Further details of the PO-CL mechanism have been described (1, 13, 14). The difference in reaction pathways characterized by general-base and nucleophilic catalysis is easily and vividly demonstrated with the peroxyoxalate chemiluminescence reaction.

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