



## Bromination of Fluorescein – A Facile Model Experiment for Electrosynthesis in Chemistry Classes

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### Abstract

*Since electrochemical processes inherently benefit from the increasing share of renewable energy sources for power generation in the grid, they can play a valuable role in future processes in chemical industry helping to make production more sustainable and efficient. However, due to often complex setups and the lack of striking experiments, examples for electrosynthesis are rarely discussed in science classes.*

*The experiment presented herein describes the electrosynthetic bromination of fluorescein to Eosin Y (tetrabromo fluorescein) via reactive bromine generated in-situ [1]. The developed experiment makes use of bromide ions which are oxidized at the anode to form a reactive intermediate and is therefore avoiding the direct handling of hazardous bromine by school students. The reaction of fluorescein with bromine is well-established since it can be monitored easily by changes in both adsorbed and fluorescent colour and is commonly used as a detection reaction for bromine. Within several minutes at low voltages of around 3 V, a significant change in colour from yellow to red is observable and at the same time the intense yellow green fluorescence under UV light vanishes at the given slightly basic pH condition. The product can be precipitated by lowering the pH and subsequently collected by filtration. In comparison with fluorescein, the tetrabromo derivative shows a greatly increased solubility in water which can serve as a suitable example for the concept of structure-properties-relations. Furthermore, a semi-quantitative analysis of the Fluorescein/Eosin Y ratio can be performed by thin-film chromatography where both components are easily distinguishable by their respective fluorescence colour under UV irradiation. This experiment can be expanded to a set of experiments by synthesizing the fluorescein in the lab course via reaction of resorcinol with phthalic anhydride as well as using the Eosin Y product as a photoredox organocatalyst [2, 3].*

Keywords: Electrosynthesis, fluorescence, model experiment

### 1. Introduction

In this contribution, we present an electrosynthetic bromination experiment involving two distinctly colored dyes. In these experiments, we convert fluorescein into eosin Y using electrochemically generated bromine. This *in-situ* formed reagent reacts immediately with the fluorescein, ensuring a low concentration of free bromine. Since the electrical energy production is shifting more and more to renewable sources [4], a process like this using electrical power to drive chemical reactions offers the possibility to be more sustainable than conventional synthesis strategies. These aspects, which can be discussed on the example of our model experiment, fit well to concepts from the green chemistry approach: Apart from the use of renewable energies, the in-situ generation of low amounts of toxic and reactive species reduces hazard risk, leading to a safer process for accident prevention as demanded in the green chemistry principles [5]. The following described experiments consist of a quick to perform synthesis of eosin Y, thin layer chromatography for semi-quantitative analysis, a protocol for synthesis and recovery of the solid product and a detection of intermediately formed bromine.

#### 2.1.1 Electrosynthesis of Eosin Y

In this experiment, eosin Y is produced by electrosynthesis from fluorescein.

**Equipment:** voltage source, cables, stir bar, magnetic stirrer, iron electrode, graphite electrode, crocodile clips, voltmeter, beakers, spatula, scale, UV lamp (354 nm)

**Chemicals:** water, sodium hydrogen carbonate, potassium bromide, fluorescein (sodium salt)



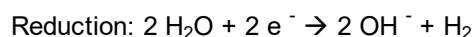
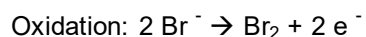
**Procedure:** 20 g sodium hydrogen carbonate, 10 g potassium bromide and 40 mg fluorescein were dissolved in 200 mL water under stirring and gentle heating. The solution is then divided equally among four beakers. After cooling to room temperature three of these solutions are electrolyzed for different times at 3 V DC, with the graphite electrode as the anode and the iron electrode as the cathode. The samples are electrolyzed for 30 seconds, 1 minute and 2 minutes, respectively. The fourth sample is used as a reference.

**Observations:** The reference solution is strongly yellow colored and emits an intense green light under UV irradiation. It can be observed that with longer electrolysis times, the color becomes more red (figure 1). Analogous to this color change, the fluorescence intensity decreases with increasing reaction time.



Fig. 1: Fluorescein solution, electrolyzed solutions after 30 seconds, 1 minute and 2 minutes, respectively.

**Interpretations:** At the cathode, water is reduced to hydroxide ions and hydrogen gas. At the anode, bromide ions are oxidized to elemental bromine. The evolution of bromine under similar conditions can be tested by addition of bromine to alkenes in chapter 2.3.



However, this bromine does not accumulate in solution, but reacts *in-situ* with fluorescein in the solution to yield tetrabromofluorescein (eosin Y). The increasingly red color indicates the progress of this bromination.

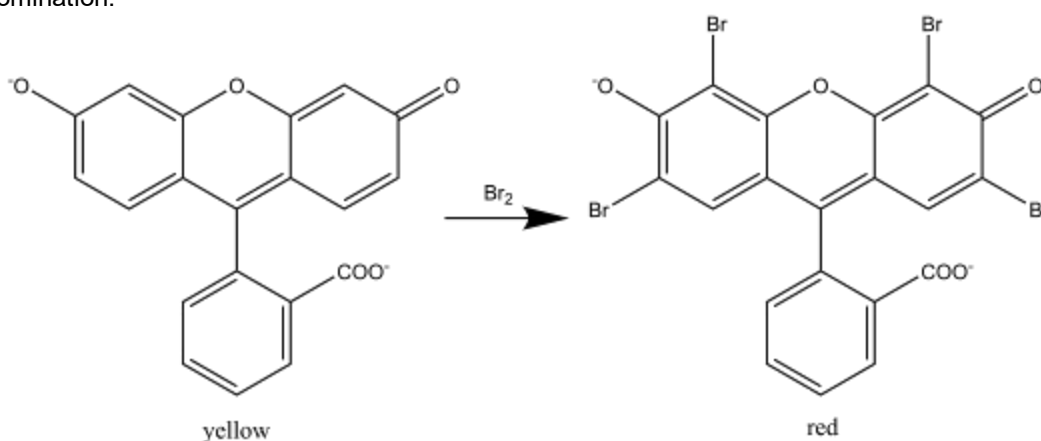


Fig. 2: Bromination of Fluorescein.



### 2.1.2 Thin layer chromatography (TLC)

To show the effect of different reaction times semi-quantitatively, a TLC is performed to analyze the products from the previous experiment.

**Equipment:** thin layer chromatography (TLC) plate (silica gel), TLC chamber, glass capillaries, pipettes, beaker, graduated cylinder, UV lamp (354 nm)

**Chemicals:** solutions from experiment 2.1.1, butanone, concentrated ammonia solution (25 wt%), ethanol

**Procedure:** The mobile phase consists of 24 mL butanone, 2 mL concentrated ammonia solution and 10 mL ethanol. The TLC chamber is filled to a height of approx. 0.5 cm with the mobile phase and kept closed to enrich the atmosphere with the mobile phase.

A small drop of each solution is placed on the plate 1 cm from the bottom using a capillary. The plate is then placed in the chamber and removed when the mobile phase approaches the end of the plate. After drying, the samples are examined under UV light.

**Observations:** Figure 3 shows the results of the TLC both under daylight as well as UV light, with the sample on the left representing the reference solution with additional eosin Y added for comparison. While sample 1 shows three spots, samples 2 and 3 each five spots. In sample 4, only one spot is visible.

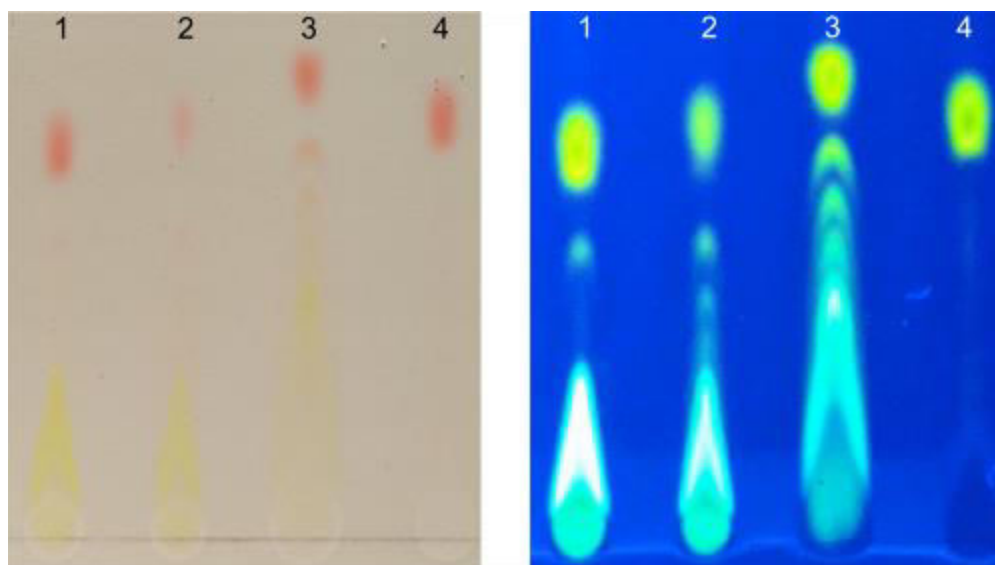


Figure 3: left: reference solution with added eosin Y, electrolyzed solutions after 30 seconds, 1 minute and 2 minutes, respectively; right: same TLC plate under UV light.

**Interpretations:** In each case, the green fluorescent spot at the lower end can be attributed to fluorescein. The upper spot (whose orange color under UV cannot be properly displayed by the camera) represents the fully brominated species eosin Y. The intermediate spots can be assigned to the three incompletely brominated intermediates. In sample 4, all of the fluorescein has been converted to eosin Y. The additional spot in sample 1 is due to impurity of commercial eosin Y.

### 2.2.1 Synthesis and product isolation

While the experiment 2.1.1 illustrates the progress of the reaction, the concentrations are too low to yield a solid product. Therefore, the experiment described herein uses a higher concentration of fluorescein to make precipitation of the product possible. However, due to the high fluorescein concentration, the color gradient during bromination is not as visible as in experiment 2.1.1.

**Equipment:** voltage source, cables, stir bar, hotplate stirrer, iron electrode, graphite electrode, crocodile clips, voltmeter, beakers, spatula, scale, büchner flask, paper filter, büchner funnel, vacuum pump, vacuum tube

**Chemicals:** water, sodium hydrogen carbonate, potassium bromide, fluorescein (sodium salt), hydrochloric acid (semi-concentrated)

**Procedure:** 5 g sodium hydrogen carbonate, 2.5 g potassium bromide and 0.4 g fluorescein were dissolved in 50 mL water under stirring and gentle heating. After cooling to room temperature the



solution is then electrolyzed under stirring at 3 V DC for at least 5 minutes with the graphite electrode as the anode and the iron electrode as the cathode.

The product is precipitated by acidifying the solution with semi-concentrated hydrochloric acid and collected using a Büchner funnel. After washing the red solid with cold water it can be dried by gentle heating on a hot plate.

**Observations:** The orange solution (due to high fluorescein concentration) turns increasingly red in the course of the electrolysis. During addition of hydrochloric acid, gas evolution leads to the formation of foam. After neutralisation a red-orange solid precipitates, which turns more yellow-orange after drying.

**Interpretations:** As in experiment 2.1.1, the fluorescein is brominated to orange-red eosin Y. Acidifying the solution protonates the carboxylate groups of the residual reactants and products and thus form carboxylic acid groups. This lowers the solubility in water by reducing the polarity of the molecules and causes them to precipitate.

### 2.2.2 Thin layer chromatography of the isolated product

The isolated product can also be analyzed by TLC.

**Equipment:** thin layer chromatography (TLC) plate (silica gel), TLC chamber, glass capillaries, pipettes, beaker, measuring cylinder, UV lamp (365 nm)

**Chemicals:** precipitated solid from 2.2.1, butanone, concentrated ammonia solution, ethanol, sodium hydrogen carbonate, water, fluorescein, eosin Y

**Procedure:** The same mobile phase is used as in experiment 2.1.2. A few crystals of the precipitated solid are dissolved in a small amount of an aqueous sodium hydrogen carbonate solution and then applied to the plate as in 2.1.2. Fluorescein and, if available, purchased eosin Y can also be applied as a comparison.

**Observations:** Figure 4 shows the results of the TLC under UV light. As in experiment 2.1.2 the different spots can be observed. In comparison to 2.1.2 the separation of these spots is more clearly visible.

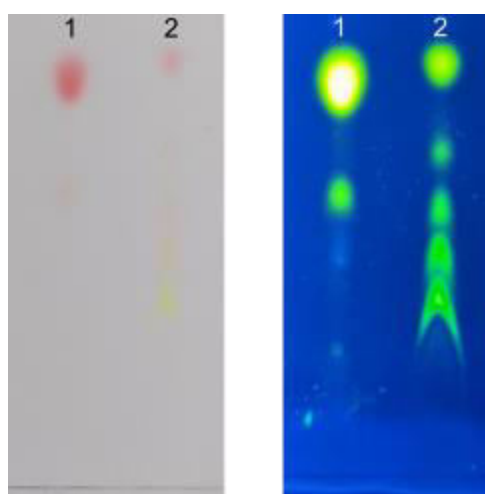


Figure 4: left: solution with eosin Y and solution of the product; right: same TLC plate under UV-light.

**Interpretations:** The sample must be treated with basic solution to convert the carboxylic acid group back to a carboxylate group and make it more soluble. In comparison to 2.1.2 the formation of eosin Y can be confirmed. However, due to the higher amount of reactant, despite longer reaction time full bromination cannot be achieved.

### 2.3 Verification of bromine as an *in-situ* reactant

The synthesis instruction is performed without fluorescein to prove the generation of elemental bromine under the given electrochemical conditions.

**Equipment:** voltage source, cables, stir bar, magnetic stirrer, iron electrode, graphite electrode, crocodile clips, voltmeter, beakers, spatula, scale

**Chemicals:** water, sodium hydrogen carbonate, potassium bromide, alkene (e.g. cyclohexene)





**Procedure:** 5 g sodium hydrogen carbonate and 2.5 g potassium bromide and were dissolved in 50 mL water under stirring and gentle heating. The electrogeneration of bromine is carried out according to the procedure described in 2.1.1 for 2 minutes. Several milliliters of this solution are thoroughly mixed with a few drops of alkene (e.g. cyclohexene).

**Observations:** The solution turns yellowish during electrolysis. After adding the alkene and shaking briefly, the solution turns colorless (figure 5).



Figure 4: left: solution after electrolysis; right: colorless solution after the addition of the alkene.

**Interpretation:** In the absence of fluorescein, the yellow color of the bromine is clearly visible, since it can accumulate in the electrolyte. The decolorization of this solution by reaction with an unsaturated hydrocarbon proves the presence of elemental bromine.

### 3. Didactical considerations

The series of experiments is valuable to discuss the benefits of electrosynthetic methods in a school environment. From a practical point of view, the experiments are facile and inexpensive to perform, so that they can be easily established in chemistry classes. The bromination experiment can be performed in 5 minutes and shows that even organic chemical reactions can be driven by electrical energy. The students can monitor the progress of the reaction by observing the pronounced change in color without any further analysis techniques. However, this experiment can be extended in order to cover analytical aspects and mechanistical considerations: Via TLC the students can verify the reaction progress semi-quantitatively and confirm that the reaction is completed after several minutes. Furthermore, the intermediate bromination products (mono-, di-, tribromofluorescein) can be identified as well. Independently, the hypothesis of bromine acting as the *in-situ* formed bromination species can be confirmed by the students (for example as part of an exploratory approach) using a detection method they are already familiar with.

The experiments enable the training of numerous competences: They cover typical laboratory skills such as the isolation and purification of products. Using the example of TLC, theoretical aspects of chromatography and underlying structure-property relationships can be addressed here. Structure-property relationships also includes altering the solubility through protonation used for precipitation. As an interesting side note, the TLC analysis reveals that commercial eosin Y contains the same impurity as observed in our experiment.

### 4. References

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