

# Catalysis Revisited

## An Overview of Classical School Experiments and New Approaches for a Better Conceptual Understanding

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**Abstract** The topics of kinetics and catalysis represent important contents in the chemical education of students and form fixed components of current school curricula and syllabi. Students need to acquire specific content knowledge and an adequate conceptual understanding of kinetics and catalysis in order to understand and evaluate chemical processes from nature and technology, especially in the current contexts of green and sustainable chemistry. To promote such an adequate conceptual understanding, this article presents five school experiments. The experiments allow high school students to acquire the concepts of kinetics and catalysis in a technically correct manner using kinetic measurements. By analyzing the recorded kinetic data using suitable spreadsheet programs, students leave the qualitative observation level and use mathematical models that allow them to draw conclusions about the underlying molecular processes. The use of spreadsheet programs underlines the interdisciplinary nature of this approach. The presented selection of experiments will provide students with an overview of all types of catalyses and their specific properties as well as common, even-numbered reaction orders. The newly acquired knowledge through the experiments enables the students to discuss economic and ecological problem situations and reflect on, and evaluate, courses of action in chemistry. The knowledge also enables students to develop adequate decision-making strategies and to communicate them properly, e.g., in the context of green and sustainable chemistry.

**Keywords:** *Catalysis, kinetics, reaction order, homogeneous, heterogeneous, enzyme catalysis, photometry, BYOD, content knowledge, conceptual understanding, classical school experiments, new approaches, hands-on experiments, easy to perform*

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### 1. Introduction

Kinetics and catalysis are fundamental topics in the educational plans for middle school chemistry classes [1-3], where the students are given a phenomenological approach to catalysis. This approach is expected to enable students to assess the role and use of catalysts in chemical reactions and to evaluate these in ecological contexts from nature and technology. In middle school chemistry, the concept of catalysis is introduced by considering the change in activation energy that allows reactions to take place under milder conditions when catalysts are used. A classically drawn picture is the minimization of the potential energy caused by the use of catalysts in comparison with non-catalyzed reactions. In this context, the foundations are usually also laid for the reaction path to be changed by the use of a catalyst. Following the catalyzed reaction path, the catalyst takes part in the

reaction but is not consumed. This characteristic of the catalyst is usually demonstrated by using heterogeneous-catalyzed reactions. An outstanding experiment in this context is, for example, the catalytic decomposition of hydrogen peroxide on the surface of platinum group metals or manganese dioxide [4], while some middle school curricula on topics of "Fuels - oil and natural gas" suggest clarifying the chemical processes of cleaning the exhaust gases from combustion engines. The topic of exhaust gas purification by the car catalyst was itself included in the PISA study as a released item in 2006. The results on this item in the PISA study were researched [5] and led to some interesting experimental approaches for higher school chemistry classes on the underlying processes [6].

In high school chemistry, these phenomenological approaches of middle school chemistry are expanded by the students' growing technical knowledge. The definitions from middle school are now confirmed by

kinetic measurements to verify the proceeding molecular processes. In the context of chemical equilibrium, students learn about chemical industrial-scale productions and the wide-ranging use of catalysts in the processes, making them both more economical and ecological. Even the catalysts' influence on the activation energy and the reaction kinetics forms part of the topic. In high school chemistry, different types of catalysis are also discussed and a distinction is made between homogeneous, heterogeneous and enzyme catalysis [7-9]. The reaction order is mostly unrepresented however, and is only available as optional content.

To better understand the molecular processes involved in chemical reactions, chemists run kinetic measurements that verify or falsify their assumptions about the molecular processes taking place.

This article gives an overview of the kinetic measurements on well-known and novel experiments in school chemistry. The recorded measurement data are analyzed using a spreadsheet program (here, Excel). Using such programs, the students work on mathematical models and, thus, depart from the classical observation level. In the mathematical modeling cycle [10], the measured values are first recorded and then evaluated using mathematical methods. Here, the result of the evaluation is an approximation of the reaction kinetics. To interpret the results, the students use their knowledge of chemistry and, thus draw conclusions about the molecular processes. In summary, the observations made during the reaction are examined and analyzed at the molecular level by interpreting the results of the kinetic measurements, which are performed volumetrically and optically.

The aim of this article is to promote in students an adequate conceptual knowledge of the kinetics and catalysis of chemical reactions by performing the experiments presented herein. In the experiments, the students learn about the three types of catalysis, homogeneous, heterogeneous and enzyme catalysis, and their underlying even-numbered reaction orders. Furthermore, the following statements on kinetics and catalysis, as found in a large number of textbooks, are supported experimentally and through the use of kinetic measurements.

- 1) The catalyst accelerates the chemical reaction by lowering the activation energy.
- 2) Catalysis provides an alternative lower energy reaction pathway.
- 3) Energy is not a catalyst.
- 4) The rate constant of a chemical reaction is temperature dependent.
- 5) The rate of second order chemical reactions depends on the concentration of two reactants.
- 6) The catalyst is not consumed in the course of the reaction.

The understanding of concepts gained through the experiments enables the students to evaluate the use of catalysts in chemical reactions in the laboratory, everyday life and nature. The knowledge also enables them to discuss green and sustainable chemistry in the contexts of process optimization and economic and ecological process management.

## 2. Homogeneous catalysis

**Experiment No. 1: Energy does not catalyze a reaction – Zero order reactions:** The decomposition of water in the Hofmann voltameter demonstrates the electrolysis of water into its components: hydrogen and oxygen. During the electrolysis, electrical energy is converted into chemical energy. A certain amount of energy must first be applied for the electrolysis to take place. This energy is referred to as the activation energy. The fact that pure energy - in this case in the form of electrical current - does not catalyze the electrolysis, but that a catalyst in the form of sulfate ions does, is shown by the following experiments on water decomposition in the Hofmann voltameter.

### Experimental:

**Materials:** Hofmann voltameter, power supply, electric wire, platinum electrodes, burette funnel, plastic tub

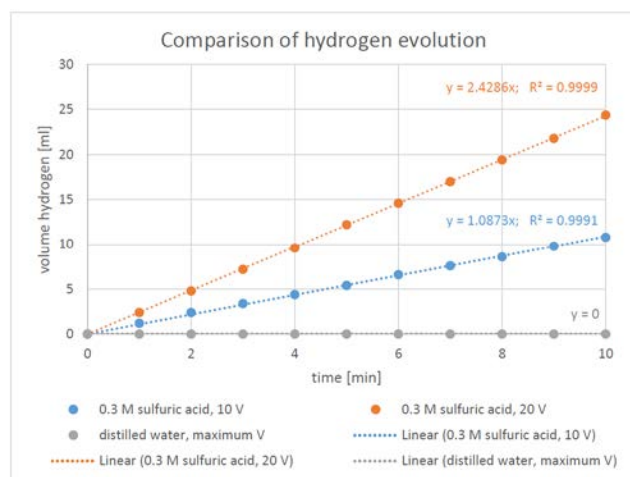
**Chemicals:** 0.3 M sulfuric acid (GHS05)

**Procedure:** The experiment is split into three parts. In the first part of the experiment, the platinum electrodes are plugged into the Hofmann voltameter which, thereupon, is filled with distilled water, connected to the power supply by the electrical wires and finally connected to the mains. The water is electrolyzed with a direct current of 0-20 V and 12 A. To prevent erroneous readings, it is important to electrolyze the water and the aqueous sulfuric acid solution for about 5 minutes with the taps of the electrodes open before starting the measurements. After this period of time, the gas volumes generated at the electrodes are read off for 10 minutes, every 60 seconds, starting with  $t_0 = 0$  s. In the second part of the experiment, the distilled water is replaced by 0.3 M sulfuric acid and electrolyzed at 10 V DC. In the third part, the electrolysis of 0.3 M sulfuric acid is carried out at 20 V DC. In accordance with parts one and two of the experiment, the gas volumes are determined at the electrodes and noted.

**Results:** Faraday's First Law of Electrolysis illustrates the interdependence between the amount of substance,  $n$ , (and the charge of its ion  $z$ ) deposited during electrolysis at one of the electrodes and the electrical charge,  $Q$ , passing through the electrolyte. The constant of proportionality is the Faraday's constant,  $F$ . The first law is described by the following equation:

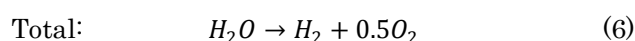
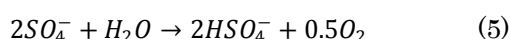
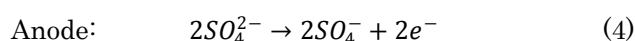
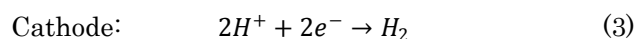
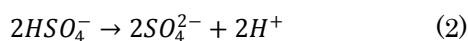
$$Q = n \cdot z \cdot F \quad (1)$$

Figure 1 displays the volume of developing hydrogen at the cathode of the Hofmann voltameter recorded during the electrolysis as a function of time. The graphic includes the data of the electrolysis of distilled water at maximum V DC (20 V, first part of the experiment, gray) and the data of the developing hydrogen gas during the electrolysis of the diluted sulfuric acid at 10 V DC and 20 V DC (part two, blue, and part three, orange, of the experiment). The complete data, including the evolution of oxygen gas at the anode, are shown in the supporting information. The developing reaction products at the anode and cathode can be tested by common test reactions.



**Figure 1.** Comparison of the hydrogen production in the Hofmann voltammeter.

As shown in Figure 1, no measurable amount of hydrogen gas is generated by the electrolysis of distilled water at 20 V DC. Although the electrolysis takes place, the amount of produced gases at the electrodes is still too small to detect. Looking at the values from the electrolysis of the 0.3 M  $H_2SO_4$ , compared to the electrolysis of distilled water, an evolution of hydrogen gas can be observed at the cathode. Even though the voltage in the case of the diluted sulfuric acid is equal to or less than the voltage used for electrolyzing the distilled water, the reaction does take place. The sulfate ions act as a catalyst in the electrolysis of water and lower the activation energy. The catalytic properties of the sulfate ions can be summarized by the following equations [11].



Assuming that the resistance,  $R$ , of the 0.3 M  $H_2SO_4$  stays constant during the reaction, twice the voltage,  $U$ , causes a doubling in the amperage. This principle can be visualized in an equation combining the Faraday's First Law and Ohm's law.

$$n = \frac{U \cdot t}{R \cdot z \cdot F}; R = \frac{U}{I} = \text{const.} \quad (7)$$

Equation (7) also shows that twice the amount of gases,  $n$ , is produced at the electrodes during the electrolysis by doubling the voltage  $V$ . This relationship can be qualitatively verified by dividing the slopes of the linear approximations of the 0.3 M  $H_2SO_4$  at 10 V and 20 V DC. The ratio of the slopes is  $1:2.23 \approx 1:2$ . The deviation of the calculated ratio with the expected value is caused by thermal effects in the electrolyte. Conducting at a higher current heats up the electrolyte and, thus its conductivity around the electrodes increases.

**Experiment No. 2: Reaction of sodium thiosulfate with hydrochloric acid – First order reaction and the Arrhenius equation:** The reaction of sodium thiosulfate with hydrochloric acid in aqueous solution is another

classical experiment in school chemistry. In the reaction colloidal sulfur is produced, thus making the solution become turbid. By measuring the turbidity as a function of time, the rate of the reaction can be determined. The measurement of the turbidity as a function of time at different temperatures also allows the verification of the Arrhenius equation and, therewith, the calculation of the activation energy.

### Experimental:

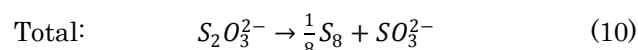
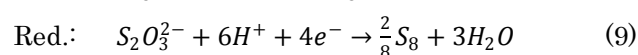
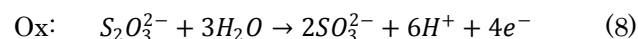
**Materials:** 100 ml Erlenmeyer flask (8x), 50 ml volumetric flask (2x), 10 ml volumetric flask (1x), hotplate, thermometer, white sheet of paper, water resistant marker.

**Chemicals:** 0.2 M sodium thiosulfate solution, 2 M hydrochloric acid (GHS05)

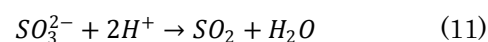
**Procedure:** In the first part of the experiment, a dilution series of sodium thiosulfate solution is prepared. Therefore, 10 ml, 20 ml, 30 ml, 40 ml and 50 ml of 0.2 M  $Na_2S_2O_3$  solution are transferred into separate 100 ml Erlenmeyer flasks. Each Erlenmeyer flask is then filled up to a volume of 50 ml with distilled water; the concentrations of the dilution series are now 0.04 M, 0.08 M, 0.12 M, 0.16 M and 0.2 M. At room temperature, successively, each solution is placed on a white sheet of paper with a drawn cross on it. The reactions and, therewith, the time measurement starts by adding 5 ml of 2 M  $HCl$  to each one of the solutions and stops by complete turbidity of the reaction mixture, indicated by the disappearing cross on the sheet of paper. For each concentration, the time until complete turbidity is noted.

In the second part of the experiment, three 20 ml volumes of the 0.2 M  $Na_2S_2O_3$  solution are transferred to three separate 100 ml Erlenmeyer flasks. The flasks are filled up to a volume of 50 ml with distilled water. In a fridge, one of the flasks is chilled down to approximately 5 °C (278 K), another flask remains at room temperature (20 °C, 293 K) and the third is heated up to approximately 40 °C (313 K). Subsequently and successively, each of the solutions at different temperatures is placed on a white sheet of paper with a drawn cross on it. The reactions and the time measurement start by adding 5 ml of 2 M  $HCl$  to each of the solutions and finishes when complete turbidity of the reaction mixture occurs. For each concentration, the time until complete turbidity is achieved is noted as well as the corresponding temperature of the reaction mixture.

**Results:** The reaction to form elemental sulfur is acid-catalyzed, as shown by the redox reactions (8) - (10) [12]:



The sulfite anion ( $SO_3^{2-}$ ) of the acid-catalyzed disproportionation reaction reacts further with protons in the reaction mixture to form  $SO_2$  and  $H_2O$  [13].



Due to possible  $SO_2$  (GHS05, GHS06) evolution, it is proposed as being necessary to work in the fume hood.

Table 1 shows the measured values of the first part of the experiment, which explain the observations from Figure 2 about the development of turbidity as a function of time. The transparent reaction mixture becomes turbid with a yellowish tint, indicating the formation of elemental sulfur. The measured times until complete turbidity is reached are plotted as a function of  $c(\text{Na}_2\text{S}_2\text{O}_3)$  (Figure 3, left). The values can be approximated by an exponential fit using the solving function in MS excel (see supporting information). The exponential curve fits exactly to the measured values, thus, the first order rate law with respect to the concentration of  $\text{Na}_2\text{S}_2\text{O}_3$  can be verified.

Table 1. Measured values of the first part of the experiment.

$c(\text{Na}_2\text{S}_2\text{O}_3)$ [M]:	0.20	0.16	0.12	0.08	0.04
t [s]:	21.98	24.72	31.86	50.52	99.25
$t^{-1}$ [s <sup>-1</sup> ]:	0.047	0.040	0.031	0.020	0.010

Figure 3 (right) illustrates the linear interdependency between the inverse time and the concentration of sodium thiosulfate. This interdependency leads to a first order reaction in  $c(\text{Na}_2\text{S}_2\text{O}_3)$  (see supporting information). Equation (12) is the logarithmized form of a first order rate function in the concentration of sodium thiosulfate.

$$\ln(c_t(\text{S}_2\text{O}_3^{-2})) = -k' \cdot t + \ln(c(\text{S}_2\text{O}_3^{-2})) \quad (12)$$

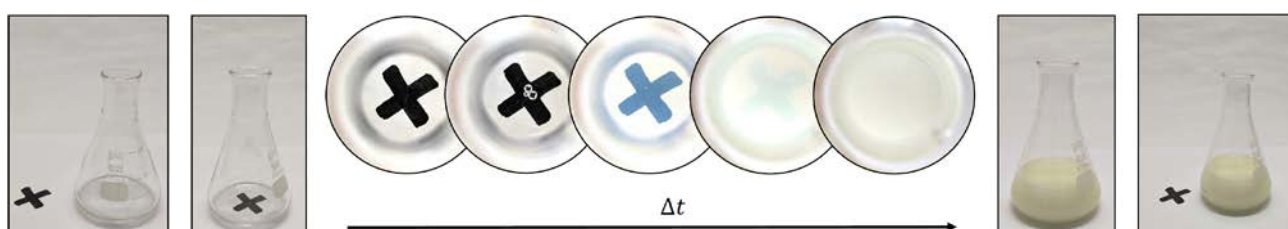


Figure 2. Developing turbidity as a function of time.

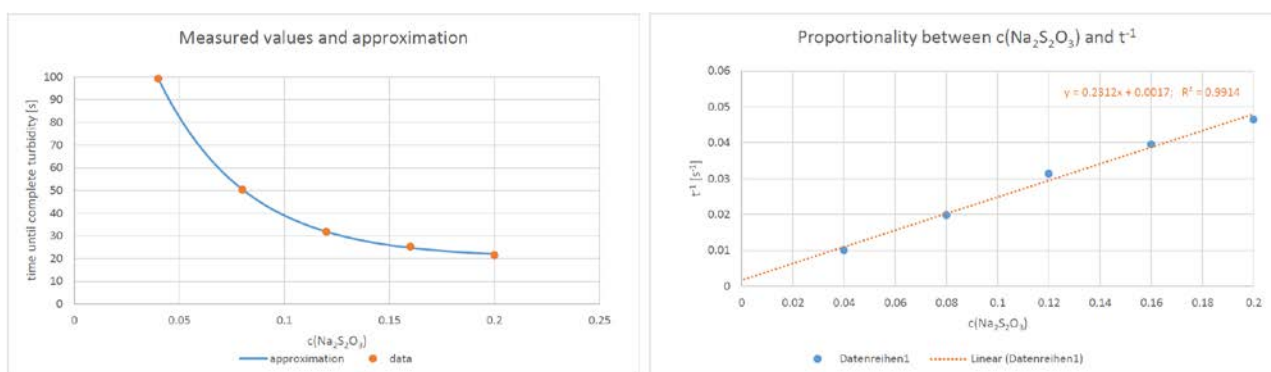


Figure 3. Graphical representation of the values from Table 1 as functions of  $c(\text{Na}_2\text{S}_2\text{O}_3)$  (left), and proportionality between  $c(\text{Na}_2\text{S}_2\text{O}_3)$  and  $t^{-1}$  (right).

Using the assumption described in the supporting information and the fact that  $c_0(\text{S}_2\text{O}_3^{-2}) = 0.2 \text{ M}$ , with respect to equation (12), for each temperature  $k'$  can be calculated by:

$$k' = \frac{\ln(0.2M)}{t} \quad (13)$$

Table 2. Temperature dependent measured and calculated values.

T [K]	t [s]	$1/t$ [s <sup>-1</sup> ]	$k'(T)$ [s <sup>-1</sup> ]
278	144.58	0.0069	0.011
293	50.35	0.0200	0.032
313	18.89	0.0530	0.085

The rate of the reaction increases with increasing temperature. This principle corresponds to the Arrhenius equation. Its logarithmized form is a linear graph. After converting the Arrhenius equation into its logarithmized form, and with respect to the values for the rate constants,  $k'$ , we can also calculate the activation energy using equation (14).

$$k' = A \cdot e^{-\frac{E_A}{R \cdot T}} \Leftrightarrow \ln\left(\frac{k'}{A}\right) = -\frac{E_A}{R} \cdot \frac{1}{T} \quad (14)$$

After plotting the calculated values  $\ln(k'(T))$  as a function of the inverse temperature  $\frac{1}{T}$  (Figure 4) the slope of the linear graph corresponds to the activation energy divided by the universal gas constant. The activation energy can be calculated by multiplying the slope of Figure 4 with  $R = 8.3145 \frac{\text{J}}{\text{K} \cdot \text{mol}}$ .

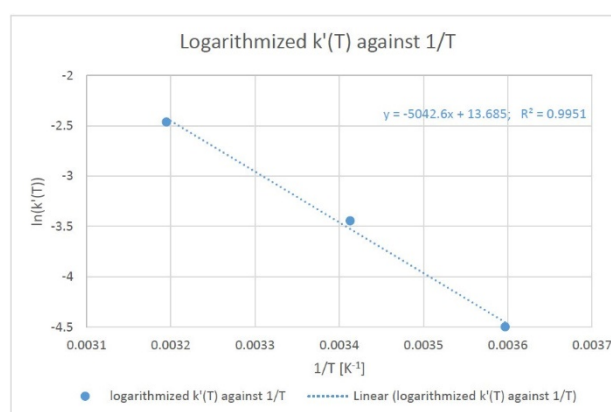


Figure 4.  $\ln(k'(T))$  as a function of  $1/T$ .

The calculated activation energy for the reaction is:

$$E_A = 5042.6 \text{ K} \cdot 8.3145 \frac{\text{J}}{\text{mol} \cdot \text{K}} = 41.9 \text{ kJ/mol}$$

**Experiment No. 3: The interdependence between two reactants – A pseudo-second order reaction:** The iodination of acetone is a very famous school experiment. Due to the striking observable discoloration of the reaction mixture, it is often used to introduce the topic of chemical reaction kinetics. By using a BYOD photometer [14] it is possible to monitor the discoloration of the reaction mixture. Thus, kinetic data can be recorded and afterwards evaluated by a spreadsheet program like MS Excel. In this experiment, the discoloration of the reaction mixture is influenced by the variation of both reactants, hydrochloric acid and acetone. The results provide information about the interdependence of both the reactants and, therewith, about the fundamental characteristics of second order reactions.

#### Experimental:

**Material:** 4 ml PE cuvette (3x), 3 ml disposable pipette (6x), stopwatch, BYOD photometer

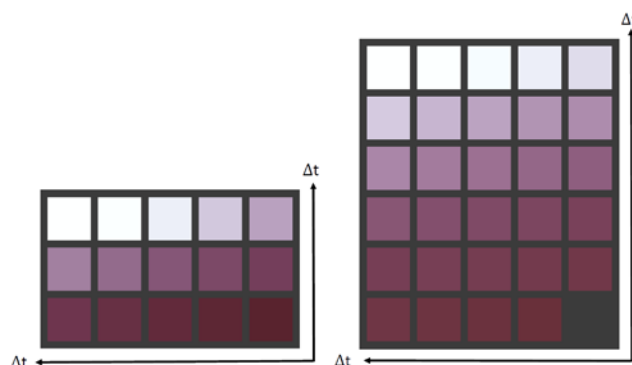
**Chemicals:** 2 M and 4 M aqueous acetone solutions (GHS02, GHS07), 0.01 M iodine-potassium iodide solution, 0.5 M and 1 M hydrochloric acid (GHS05), 0.1 % w/v starch solution

**Procedure:** With disposable pipettes, transfer 1 ml of starch solution and 1 ml of iodine-potassium iodide solution to each of the three cuvettes. Insert the cuvettes successively into the box of the BYOD photometer. Before adding the hydrochloric acid and acetone to the reaction mixtures, site the smartphone in front of the photometer box for taking the measurements. First add the hydrochloric acid to the reaction mixture and then the acetone using the following different ratios:

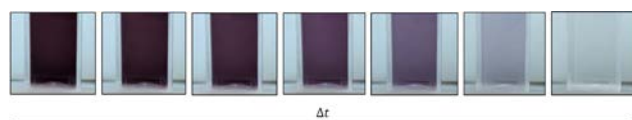
- cuvette 1: 1 ml hydrochloric acid (1 M) and 1 ml acetone solution (4 M)
- cuvette 2: 1 ml hydrochloric acid (0.5 M) and 1 ml acetone solution (4 M)
- cuvette 3: 1 ml hydrochloric acid (1 M) and 1 ml acetone solution (2 M)

The acetone solution must be added vigorously to ensure the adequate mixing of the reaction mixture. After the addition of the acetone solution, start measuring the discoloration in intervals of 10 seconds for one minute. Note the recorded RGB values as a function of time for each of the three measurements.

**Results:** Figure 5 represents the discoloration as a function of time for cuvettes 1 and 3, as recorded by Color Grab (Android). Figure 6 shows the discoloration of the reaction mixture as a function of time recorded by the camera.



**Figure 5.** Reaction course of the iodination of acetone, as recorded by Color Grab.



**Figure 6.** Time course of the iodination of acetone, as recorded by the BYOD photometer.

The kinetic data are evaluated as described by Rodriguez and Lühken [15]. Thus, the extinctions can be calculated by equation (15) using the measured RGB values.

$$E = \log \left( \frac{255}{\frac{1}{3}(R+G+B)} \right) \quad (15)$$

By plotting the extinctions,  $E$ , calculated for each measured RGB value, divided by the extinction  $E_0$  of the reaction mixture at  $t = 0 \text{ s}$  as a function of time, the kinetic data can be evaluated and compared.

The kinetic data for all three cuvettes (Figure 7) show  $\left(\frac{E}{E_0}\right)$  as a function of  $t[\text{s}]$ . The amounts of hydrochloric acid and acetone are varied, doubling the concentration of one of the reactants in each of cuvette 2 and 3 compared with cuvette 1. Cuvette 1 is the reference; relative to the amount of acetone, the amount of hydrochloric acid in cuvette 1 is twice the amount in cuvette 2, therefore, relative to the amount of hydrochloric acid, the amount of acetone in cuvette 1 is twice the amount of that in cuvette 3. By comparing the slopes of the linear approximations, it can be seen that doubling in one of the reactants, relative to the other, results in twice the slope of the linear curve. Thus, the rate of the reaction doubles.

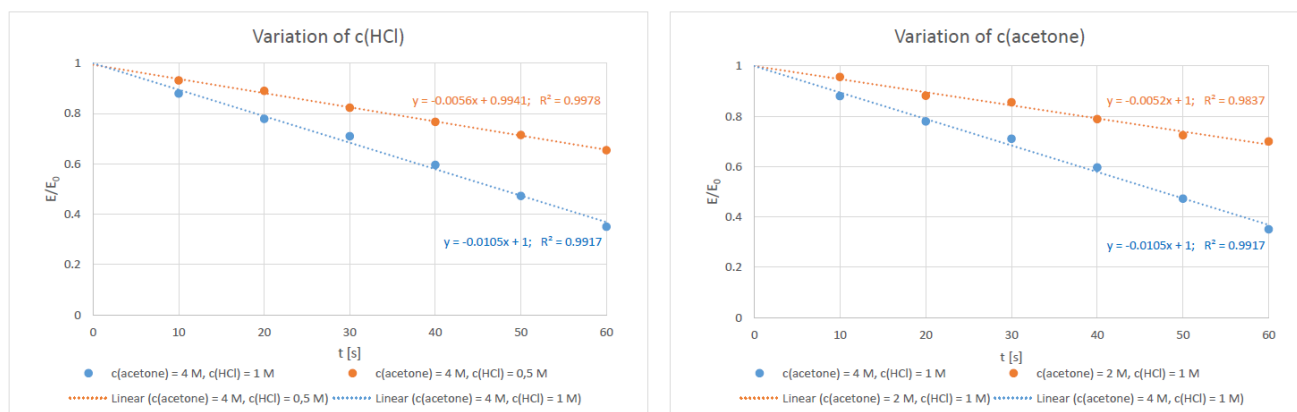
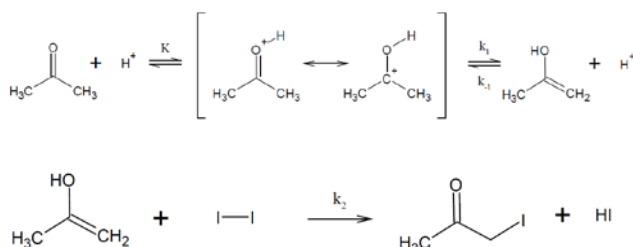


Figure 7. Variation of the HCl concentration (left) and variation of the acetone concentration (right).

These observations can be verified by employing mathematical investigations on the reaction mechanism. The reaction mechanism is shown below:



The mathematical investigations (supporting information) prove the second order kinetics, as shown in equation (16). The rate of the reaction depends on the concentrations of acetone and hydrochloric acid, and is of zero order with respect to iodine. The iodine is consumed very quickly while, relative to its concentration, the concentrations of acetone and hydrochloric acid remain nearly at constant. Thus, as we double the concentration of either reactants, the rate of the reaction and, therefore, the slope of the linear rate function doubles equally (equations (16) - (18)). The conclusions made by the mathematical investigations are verified by the kinetic measurements.

$$\frac{d[\text{I}_2]}{dt} = v = K' \cdot [\text{Ac}] \cdot [\text{H}^+] \quad (16)$$

$$K' \cdot (2 \cdot [\text{Ac}]) \cdot [\text{H}^+] = 2 \cdot v \quad (17)$$

$$K' \cdot [\text{Ac}] \cdot (2 \cdot [\text{H}^+]) = 2 \cdot v \quad (18)$$

### 3. Heterogeneous catalysis

**Experiment No. 4: Catalytic discoloration of Azorubin – A pseudo-zero order reaction:** Platinum group metals, along with Raney nickel, have been used since the foundation of the heterogeneous catalytic hydrogenation of organic substances. Not only can C=C double bonds, but also unsaturated hetero-atomic bonds like the azo bridge, consisting of an N=N double bond, be hydrogenated in the presence of the above-mentioned, finely divided platinum group metals. The azo bridge is hydrogenolized and two primary amines are formed. Since the azo bridge is a chromophore group in azo compounds, the dyes lose their coloration. The kinetics of the reaction can be analyzed by photometric measurements of the

absorbance of the sample as the reaction proceeds. The kinetic measurements are performed using the BYOD photometer.

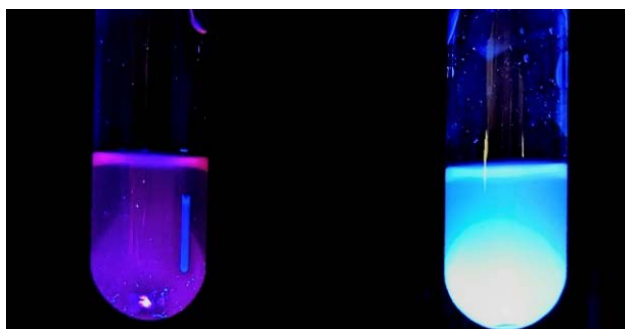
#### Experimental:

**Materials:** mortar, pestle, spatula, 50 ml graduated cylinder, 100 ml beaker, 100 ml volumetric flask, test tubes, stopper, 3 ml disposable pipettes (2x), BYOD photometer

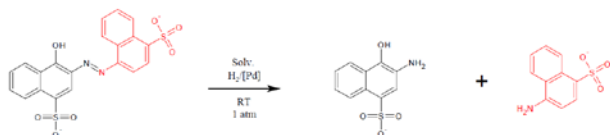
**Chemicals:** catalyst beads (Hedinger, 0.15 % Pd – 0.15 % Pt), Azorubin (E122) (GHS07), Hydrogen (GHS02, GHS04)

**Procedure:** Pestle some catalyst beads (Hedinger [16], 0.15 % Pd - 0.15 % Pt) to a fine powder. Suspend 0.1 g of the powder in a 100 ml beaker with 50 ml distilled water. To prepare the Azorubin solution, transfer 10 mg of azorubin to a 100 ml volumetric flask and make up to volume with distilled water. To run the hydrogenation, 1 ml of the catalyst suspension and 4 ml of azorubine solution are added to the test tube and mixed. The test tube is now placed in the photometer and the absorbance of the sample is determined by measuring the RGB values. The measured absorbance corresponds to the initial value of the measurement ( $t_0 = 0$  s). Accordingly, the gas chamber of the test tube is flooded with hydrogen and quickly sealed with a stopper. The kinetic measurement starts by beginning to vigorously shake the test tube ( $t_0$ ) for approximately 5 s and immediately putting it into the photometer. From  $t_1 = 10$  s onwards, record the RGB values of the sample at intervals of 10 s for 4 min. Set the reaction mixture aside and let it react until complete discoloration is reached. View the discolored reaction mixture under UV light.

**Results:** In Figure 8, the colorless and fluorescent reaction product (right) is compared to the red colored and non-fluorescent educt (Azorubin, left). The change in these physical properties clearly shows that a chemical reaction has taken place.

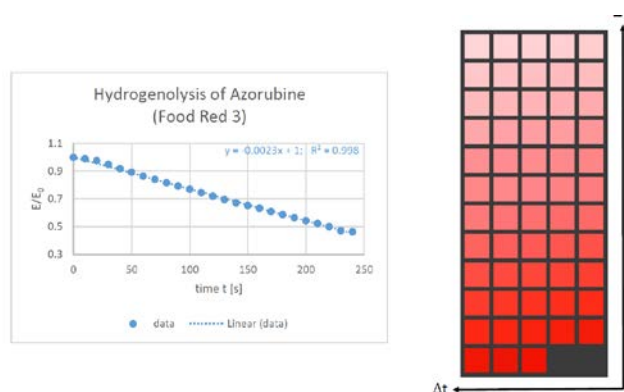


**Figure 8.** Non-fluorescent educt (left) before and fluorescent product (right) after hydrogenation.



**Figure 9.** Reaction equation of the hydrogenolysis of Azorubin into the products 3-Amino-4-hydroxy-1-naphthalenesulfonic acid (GHS07) [17] (left) and 4-Amino-1-naphthalenesulfonic acid (GHS05) [18] (right).

The kinetic data are illustrated in Figure 10. The extinctions,  $E$ , are calculated by equation (15) from the recorded RGB values. The linear approximation matches the measured values. Thus, the reaction is of zero order with respect to the concentration of Azorubin. This reaction order is characteristic for the initial rate of heterogeneous catalyzed hydrogenations since the rate of the reaction initially depends only on the diffusion processes in solution around the catalyst [19]. At maximum catalyst loading, the concentration of the dye in solution has no influence on the reaction rate [20]. For this reason, even the hydrogen concentration can be assumed to be constant. The rate of the reaction is independent of both the Azorubin and hydrogen concentrations and is, therefore, of zero order.



**Figure 10.** Graphical discoloration of Azorubin as a function of time (left), recorded by Color Grab (right).

The catalyst can be filtered off or centrifuged and washed with distilled water. It is, thus, reusable for several times and demonstrates a very important characteristic of heterogeneous catalysis: The catalyst is not consumed during the reaction and can be easily regenerated for further use.

## 4. Enzyme catalysis

### Experiment No. 5: Enzyme kinetics – Determination of the bromelain concentration of an unknown sample:

The Michaelis-Menten equation can be used to approximate the kinetics of an enzyme reaction. With this equation the substance-specific Michaelis-Menten constant and the maximum turnover rate can be determined. By mathematical transformations based on simple assumptions, the Michaelis-Menten equation can be adapted in order to draw conclusions about enzyme concentrations by using photometric measurements. In this experiment, the enzyme bromelain [21] reacts with azocasein to form a colored, water-soluble product [22]. The concentration of the product is determined photometrically and allows conclusions to be drawn about the enzyme concentration of two bromelain sources, pineapple and bromelain pills [23]. For a better overview, the experiment is divided into two experimental parts.

### Experiment No. 5.1: Enzyme kinetics – Calibration

#### Experimental:

**Materials:** micro centrifuge tubes (2 ml), piston pipettes, pipette heads, heating plate, water bath, centrifuge, glass centrifuge tubes (10 ml), 100 mL volumetric flask, 4 mL cuvettes, photometer

**Chemicals:** bromelain (GHS07 GHS08), phosphate buffer solution ( $c = 0.1$  M,  $\text{pH} = 7$ , GHS07), azocasein solution (1 % w/v in ethanol 4 % v/v and phosphate buffer, GHS02 GHS07), trichloroacetic acid (5 % w/v, GHS05 GHS09), pineapple (ripe), bromelain (pills)

**Procedure:** To prepare a  $2.5 \frac{\text{g}}{\text{L}}$  bromelain stock solution, 250 mg bromelain are transferred to a 100 mL volumetric flask and the volume made up with phosphate buffer solution. In micro centrifuge tubes, a dilution series of the bromelain solution with concentrations from  $0.25 \frac{\text{g}}{\text{L}}$  to  $2.5 \frac{\text{g}}{\text{L}}$  in steps of  $0.25 \frac{\text{g}}{\text{L}}$  is prepared by combining the stock solution with the phosphate buffer solution in different ratios. A volume of 1 mL of each solution is needed. 0.5 ml of azocasein solution is added to each tube and mixed with the bromelain solutions. All tubes are then placed in a  $45^\circ\text{C}$  water bath. After 40 minutes, the content of each micro centrifuge tube is immediately added to a separate, larger glass centrifuge tube containing 3 ml of trichloroacetic acid and mixed to precipitate out the remaining azocasein. The mixture is centrifuged at 6000 rpm for 10 minutes; 4 ml of the centrifugate is then transferred to a cuvette. The cuvettes are successively put into the BYOD photometer. The RGB values are measured for each sample, using distilled water as a blank. The extinctions are plotted as a function of bromelain concentration.

## Experiment No. 5.2: Enzyme kinetics – Determination of bromelain in pineapple and pills

### Experimental:

- Materials:** micro centrifuge tubes (2 ml), piston pipettes, pipette heads, heating plate, water bath, centrifuge, glass centrifuge tubes (10 ml), 100 mL volumetric flask, 4 mL cuvettes, photometer, mortar and pestle, pineapple, bromelain pill (e.g., Bromelain-POS®), blender, Büchner funnel
- Chemicals:** phosphate buffer solution (c = 0.1 M, pH = 7, GHS07), azocasein solution (1 % w/v in ethanol 4 % v/v and phosphate buffer, GHS02 GHS07), trichloroacetic acid (5 % w/v, GHS05 GHS09), ethanol (GHS02 GHS07)

**Procedure:** Pestle the bromelain pill, transfer the powder into a beaker and suspend it in 50 ml of buffer solution. Filter the bromelain suspension into a volumetric 100 ml flask, wash the residue with buffer solution and make the flask up to the volume of 100 ml.

Crush ¼ of a ripe pineapple (skin and leaves included) finely with a bit of water using a blender and note the mass of the quarter pineapple for further quantitative evaluation. The pineapple puree is filtered through a Büchner funnel. Note the volume of the filtrate and the volume of water added before. 10 mL of the filtrate are centrifuged at 6,000 rpm for 10 minutes. Mix the centrifugate with ethanol until the mixture reaches 70 % (w/w) ethanol/water. The mixture is stored for 30 minutes in the freezer at – 20 °C (253 K). Take it out of the freezer, mix it well and transfer 10 mL of the resulting cold suspension to a micro centrifuge tube and centrifuge at 6,000 rpm for 10 minutes. The liquid on top is discarded and the solid is suspended in 10 mL phosphate buffer solution.

To both 1 ml volumes of the prepared solutions (pill and pineapple), 0,5 mL azocasein solution is added. Add the tubes to a 45 °C water bath for 40 minutes. After this period of time, the contents of each micro centrifuge tube are immediately added to larger glass centrifuge tubes, each containing 3 ml of trichloroacetic acid and mixed to precipitate out the remaining azocasein. The mixture is centrifuged at 6,000 rpm for 10 minutes; 4 ml of centrifugate is then transferred to a cuvette. The cuvettes are successively put into the BYOD photometer. The RGB values are measured for each sample. From the calculated extinctions we can finally calculate the bromelain concentration in the samples.

**Results:** The extinction,  $E$  is calculated by equation (15) for each of the recorded RGB values. The extinctions,  $E$ , are plotted as a function of the initial bromelain concentration of the probe. To approximate a curve to the values of  $E$ , the Michaelis-Menten equation can be rewritten with respect to the absorbance and initial concentration of the enzyme,  $c_0(E)$ .

$$E = \frac{v'_{[max]} \cdot c_0(E)}{K_M + c_0(E)} \quad (19)$$

**Verification:** Equation (19) is the result based on two fundamental considerations. The first consideration is that we examine the average speed of the reaction. We determine how much of the azocasein is consumed by the enzyme over a period of 40 minutes. With respect to the temperature and the concentrations of the reactants, in this period of time the average speed of the reaction corresponds to the initial speed of the enzyme reaction,  $v_0$ . The average speed of the reaction and, thus, also the initial speed are proportional to the concentration of the product and, therefore, proportional to the measured extinction. We conclude:

$$v_0 = \theta \cdot E; \quad (20)$$

$\theta$ : Factor of proportionality,  $E$ : extinction

$$E = \frac{1}{\theta} \cdot v_0 \quad (21)$$

From the steady state approximation, it can be deduced that the substrate concentration in this quasi-steady state is proportional to the initial concentration of the enzyme.

$$c(s) = \phi \cdot c_0(E); \quad (22)$$

$\phi$ : Factor of proportionality

Equations (21) and (22) inserted into the Michaelis-Menten equation give equation (19). By approximating the measured values by equation (19),  $v'_{[max]}$  and  $K_M$  can be calculated. Using equation (19) with the calculated values for  $v'_{[max]}$  and  $K_M$ , an unknown enzyme concentration can be determined by measuring its extinction. By approximating hyperbola (19) to the measured values, the coefficients of determination in the approximations are  $R^2_{smartphone} = 0.9879$  and  $R^2_{photometer} = 0.9646$ .

Table 3 contains the calculated and measured values of the extinctions of all the described samples as well as the calculated concentrations. The values as well as the approximations are illustrated in Figure 11. The concentrations of bromelain in 100 ml of both extract solutions are displayed in Table 3. The total volume of the bromelain extract from ¼ pineapple was about 280 ml. Therefore the quantity of bromelain in ¼ pineapple is about 36.7 mg ( $m = 2.8 \cdot 13.1 \frac{mg}{100ml} = 36.68 mg$ ), the amount of bromelain in a whole pineapple is about 146.75 mg, calculated from smartphone photometer (SP) data.

**Table 3. Calculated and measured values of the extinctions of all the described samples.**

Sample	c(Enzyme) [mg/100 ml]	$E_{smartphone}$	$E_{photometer}$
Calibration	0	0.0006	0.000
	25	0.0186	0.172
	50	0.0306	0.287
	75	0.0411	0.390
	100	0.0480	0.448
	125	0.0537	0.515
	150	0.0576	0.539
	175	0.0628	0.572
	200	0.0602	0.556
	225	0.0621	0.561
250	0.0654	0.591	
pineapple extract	13.1 / 16.8 (SP/PH)	0.0115	0.139
Bromelain pill	67.2 / 68.3 (SP/PH)	0.0386	0.368



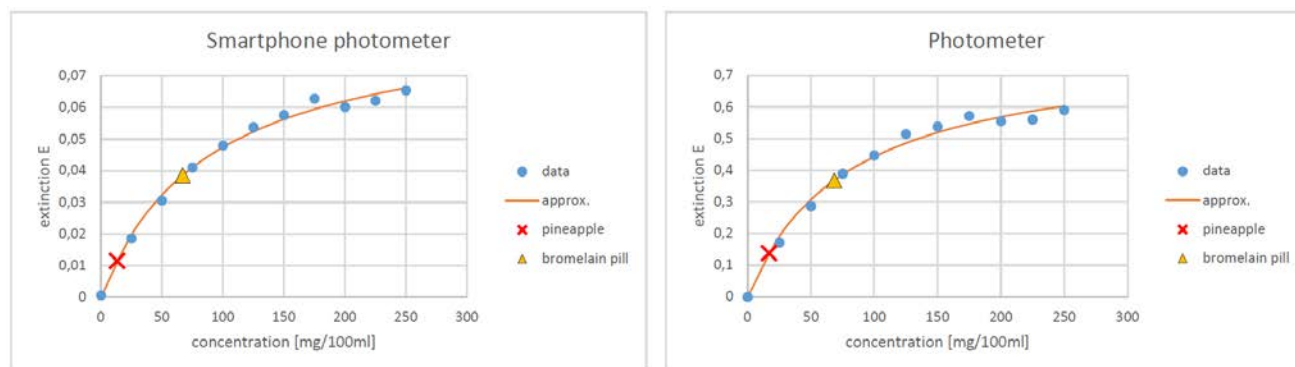


Figure 11. Graphical illustration of the measured values by smartphone (SP) (left) and photometer (right) (PH), including the approximations.

## 5. Discussion

By performing and evaluating the experiments presented in this article, the students learn about the three types of catalysis, homogeneous, heterogeneous and enzyme, and the properties of the different reaction orders. The students learn about selected experiments that illustrate the aforementioned statements about kinetics and catalysis.

The first experiment shows that energy is not a catalyst, but the use of sulfate ions catalyzes the electrolysis of water. The catalyzed reaction pathway is of lower activation energy which permits the electrolysis of water under mild conditions.

The reaction of sodium thiosulfate with hydrochloric acid clearly demonstrates the properties of first order kinetics by variation of the amount of sodium thiosulfate solutions in the reaction mixtures. In this simple, hand-held experiment, the temperature dependence of the reaction rate can, likewise, be verified by the Arrhenius equation. The students learn that by varying the reaction temperature, a reaction can be accelerated to achieve a better product yield.

In experiment 3, the students can observe that the reaction rate of some reactions depends on more than one reactant. By varying the concentration of both reactants, they gain a simplified insight into the more complicated second order kinetics. The mathematical investigations confirm the assumption derived from the observations that the reaction rate doubles when the concentration of one of the reactants doubles.

Through the heterogeneous catalytic hydrogenolysis of azo dyes, the students gain experimental insights into the kinetics of heterogeneous catalysis. The experiment also demonstrates the reusability of the catalyst as it is not "consumed" but can be regenerated and reused. The catalyst used is not a waste product at the end of the synthesis; it continuously serves as a resource in the production process. This principle is of fundamental importance in a large number of industrial processes in order to make production processes more economical and ecological and, thus, greener and more sustainable.

In the fifth experiment, the catalytic action of the bromelain enzyme is used here to determine its concentration in natural substances or everyday products. The extraction of bromelain from pineapple can be

discussed here from an economic and ecological point of view since most of the bromelain is found in the inedible parts of the pineapple fruit. In terms of the principles of green and sustainable chemistry, the possible synthesis of bromelain can be discussed in contrast to the bromelain extraction from fruits.

Thus, the experiments presented in this article can be used to promote an adequate and multifaceted understanding of the kinetics and catalysis of chemical reactions that will enable students to evaluate and communicate chemical processes properly, including in the context of a green and sustainable chemistry.

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