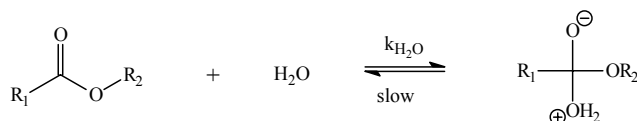


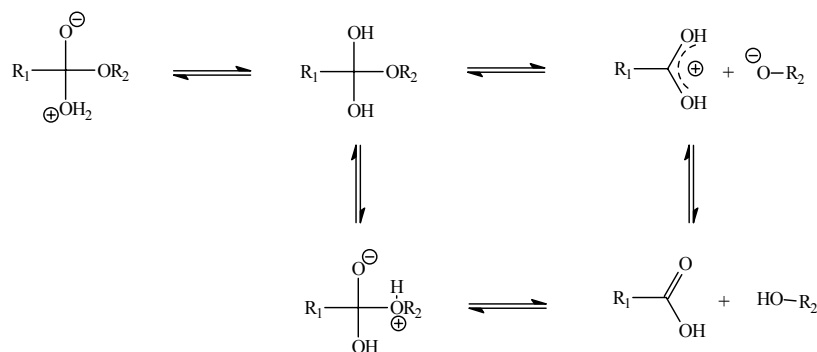
## Hydrolysis of a Carboxylic Acid Ester: Neutral and Base Enhanced Reaction of p-Nitrophenyl Acetate

### Background:

The investigation of anthropogenic organic chemicals in the environment, includes both biotic and abiotic chemical transformations that take place under environmental conditions. The major abiotic environmental processes by which pollutants are transformed are hydrolysis, photolysis, oxidation and reduction. A primary pathway for the transformation of organic esters in aquatic environments is hydrolysis. Hydrolysis reactions are normally sensitive to a variety of catalytic influences that include specific acid and base catalysis, general acid and base catalysis, nucleophilic catalysis, metal oxide surface catalysis and metal ion catalysis (1,2). Hydrolysis (reaction with water) is usually the most important reaction for molecules susceptible to nucleophilic attack. In freshwater, hydroxide ion and water are the dominant nucleophiles with OH<sup>-</sup> being about 10,000 times more reactive than H<sub>2</sub>O in substitution at carbon (1,2). The hydrolysis of a carboxylic acid ester may proceed by a number of different mechanisms, depending on the substrate structure, the pH and the presence of catalyzing species (3-6). Under neutral conditions, the reaction generally proceeds via *addition* to the carbonyl carbon to produce a tetrahedral intermediate. The slow step is attack by water.



Once formed, this intermediate may proceed through a number of proton transfer steps and elimination of an alkoxide ion or alcohol leaving group.



The kinetics of hydrolysis at constant pH (natural systems are usually well buffered) can be described by *pseudo first order* kinetics;

$$\text{Rate (overall)} = - \frac{d[\text{ester}]}{dt} = k_h [\text{ester}]$$

Where  $k_h$  is the overall *pseudo first order* hydrolysis rate constant (s<sup>-1</sup>). The hydrolysis of a carboxylic acid ester can proceed via three distinct *bimolecular* mechanisms; acid catalyzed, neutral and base enhanced.

$$\text{Acid catalyzed rate} = k_A [\text{H}^+] [\text{ester}]$$

$$\text{Neutral rate} = k_{\text{H}_2\text{O}} [\text{H}_2\text{O}] [\text{ester}]$$

$$\text{Base enhanced rate} = k_B [\text{OH}^-] [\text{ester}]$$

where  $k_A$ ,  $k_{\text{H}_2\text{O}}$  and  $k_B$  are second order rate constants (M<sup>-1</sup> s<sup>-1</sup>).

The overall hydrolysis rate will be the sum of these individual rates. Thus;  

$$\text{Rate (overall)} = \{k_A [\text{H}^+] + k_{\text{H}_2\text{O}} [\text{H}_2\text{O}] + k_B [\text{OH}^-]\} [\text{ester}]$$

And the *pseudo first order* rate constant is given by;

$$k_h = k_A [\text{H}^+] + k_{\text{H}_2\text{O}} [\text{H}_2\text{O}] + k_B [\text{OH}^-]$$

Since in aqueous solution, the concentration of  $\text{H}_2\text{O}$  remains virtually constant at  $\sim 55.5$  M, the neutral contribution is generally expressed as a *pseudo first order* constant,  $k_N = k_{\text{H}_2\text{O}} [\text{H}_2\text{O}]$ .

$$k_h = k_A[\text{H}^+] + k_N + k_B[\text{OH}^-]$$

Ester hydrolysis has been shown to be accelerated by both acid and base so the rate is pH dependent as shown below. At high pH, the dependence of  $\log k$  vs pH increases with a slope of +1 (specific base 'catalysis'). In general, reaction with  $\text{OH}^-$  is important even at pH values below pH 7. Specific acid catalysis is relevant only at relatively low pH's and only for compounds showing rather slow hydrolysis kinetics under neutral conditions (2).

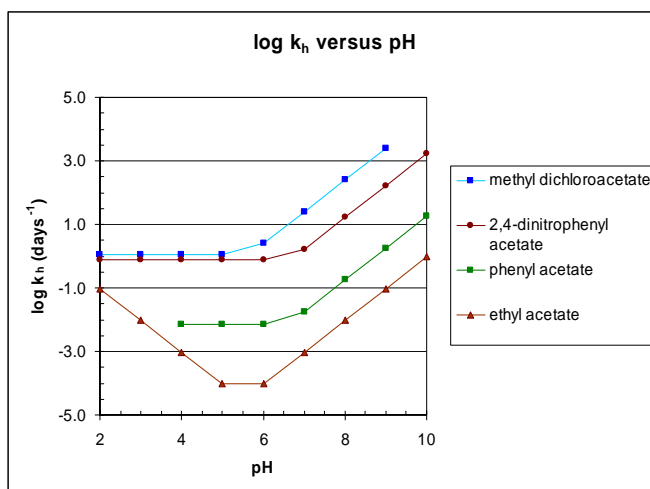


Figure 1: Dependence of observed hydrolysis rate constants ( $k_h$ ) on pH for several carboxylic acid esters.

At any given pH, the overall rate of ester hydrolysis is generally dominated by one or two of these terms. For most esters, the rate of hydrolysis under environmental pHs ( $\sim 5 - 9$ ), is dominated by neutral and base accelerated components only. Thus;

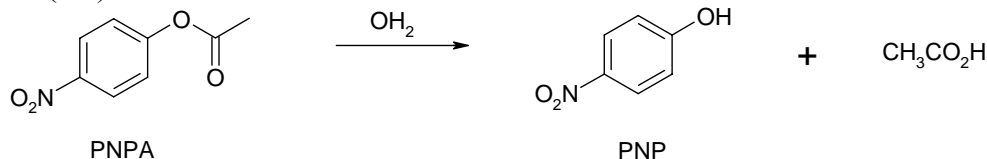
$$k_h \approx k_N + k_B[\text{OH}^-]$$

And it can be shown that the pH where both the neutral and base enhanced reactions contribute equally is given by;

$$\text{pH} (I_{\text{NB}}) = \log \left( \frac{k_N}{k_B K_w} \right)$$

where  $K_w$  is the autoionization constant for water.

In this experiment, we will study the hydrolysis of para-nitrophenyl acetate (PNPA) to para-nitrophenol (PNP) using measured changes in the uv/vis absorption of reaction solutions (7,8).



To evaluate  $k_B$ , we will carry out the reaction at high pH, where  $k_B[\text{OH}^-] \gg k_N$  and hence  $k_h \approx k_B[\text{OH}^-]$ . The slow step in the mechanism for base hydrolysis is attack by the hydroxide ion on the ester and thus the reaction is bimolecular overall.

$$\text{Rate} = k_B[\text{OH}^-][\text{PNPA}]$$

If the reaction is carried out at conditions where the  $[\text{OH}^-] \gg [\text{PNPA}]$ , the reaction will be *pseudo first order* and the rate expression is simply;

$$\text{Rate} = k_h [\text{PNPA}] \quad (\text{where } k_h = k_B[\text{OH}^-])$$

Using the integrated form of the first order rate equation;

$$\ln ([A]_t/[A]_0) = -k t$$

a plot of  $\ln [\text{PNPA}]$  versus time will provide a slope equal to  $-k_h$ .

We then carry out a similar experiment at or near neutral pH, where both the  $\text{H}_2\text{O}$  and  $\text{OH}^-$  mechanisms may contribute, the slope of  $\ln [\text{PNPA}]$  versus time will give the *pseudo first order* rate constant,  $k_h$  under conditions where;

$$k_h = k_N + k_B[\text{OH}^-]$$

since we now know  $k_B$  and  $[\text{OH}^-]$ , we can calculate  $k_N$ .

Rate constants and half-lives for hydrolysis of some carboxylic acid esters at 25°C (2)

$R_1$	$R_2$	$k_A (\text{M}^{-1} \cdot \text{s}^{-1})$	$k_N (\text{s}^{-1})$	$k_B (\text{M}^{-1} \cdot \text{s}^{-1})$	$t_{1/2} (\text{pH } 7)$
Methyl	Ethyl	$1.1 \times 10^{-4}$	$1.5 \times 10^{-10}$	0.11	2 yr
Methyl	<i>tert</i> -butyl	$1.3 \times 10^{-4}$		$1.5 \times 10^{-3}$	140 yr
Methyl	Phenyl	$7.8 \times 10^{-5}$	$6.6 \times 10^{-8}$	1.4	38 d
Methyl	2,4-dinitrophenyl		$1.1 \times 10^{-5}$	94	10 h
chloromethyl	Methyl	$8.5 \times 10^{-5}$	$2.1 \times 10^{-7}$	140	14 h
dichloromethyl	Methyl	$2.3 \times 10^{-4}$	$1.5 \times 10^{-5}$	$2.8 \times 10^3$	40 min
dichloromethyl	Phenyl		$1.8 \times 10^{-3}$	$1.3 \times 10^4$	4 min

## EXPERIMENTAL

### Objectives:

- observe uv/vis absorption spectral changes during the hydrolysis of PNPA
- investigate the pH dependence of PNPA hydrolysis
- evaluate  $k_N$  and  $k_B$  for PNPA at room temperature

### Procedure:

The following solutions are provided:

- 3 M KCl
- phosphate buffers at pH 7, 8 and 9
- 0.0100 M NaOH (pH 12)

You will need to prepare 100 mL of each of the following solutions:

- $1.00 \times 10^{-4}$  M PNPA containing 3.0% (v/v) methanol
- $1.00 \times 10^{-4}$  M PNP containing 3.0% (v/v) methanol
- an aqueous blank containing 3.0% (v/v) methanol
- 0.00100 M NaOH (pH 11)

The PNPA is not very soluble in water and a stock solution should be prepared in 30. mL of methanol and then made up to 100.0 mL with distilled water. Then make the appropriate second dilution with water. Since the PNPA contains methanol, the PNP should be prepared in the same manner. All spectra and kinetic runs will be carried out at a constant ionic strength using a 1:1:1 mix of 3M KCl/buffer or NaOH solution/ $10^{-4}$  M substrate.

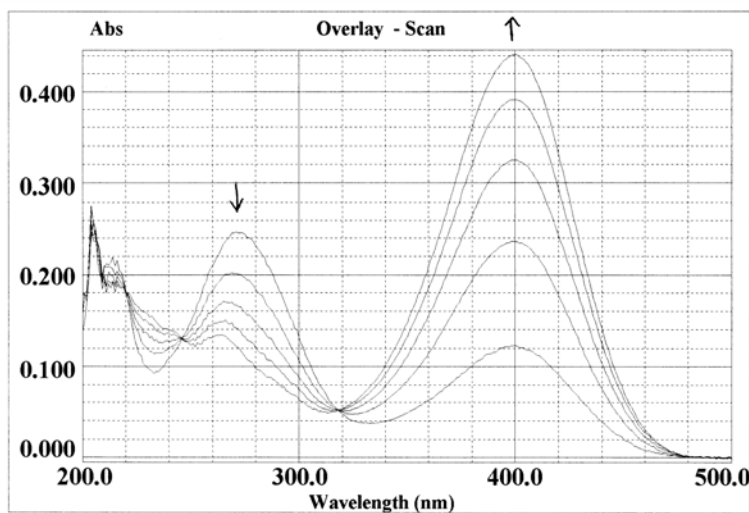


Figure 2: Overlay spectra showing the disappearance of PNPA with concomitant increase of PNP at pH 9,  $\Delta t = 60$  mins,  $[\text{PNPA}]_0 = 1.0 \times 10^{-4}$  M

### Single Scan Absorption Spectra:

Prepare a PNPA solution by mixing 1.0 mL each of 3 M KCl, pH 7 buffer and  $1.0 \times 10^{-4}$  M PNPA. Zero the spectrophotometer using a blank solution containing 1.0 mL of 3% aqueous methanol, 1.0 mL of pH 7 buffer and 1.0 mL of 3 M KCl. Obtain absorption spectra for PNPA between 200 and 500 nm to determine  $\lambda_{\text{max}}$  at pH 7. In a similar manner, obtain absorption spectra for PNP at pH 7, 8 and 9 buffer. Record the absorption of PNP at 400 nm and calculate the molar extinction coefficient ( $\epsilon_{400}$ ) at pH 7, 8 and 9 using Beer's Law;

$$A = \epsilon l c$$

where  $\epsilon$  is the molar extinction coefficient,  $l$  is the optical path length (1 cm) and  $c$  is the molar concentration of PNP in the cuvette.

### Multiple Scan Overlay Absorption Spectra:

In overlay scan mode, obtain a series of spectra between 200 and 500 nm to follow the hydrolysis reaction of PNPA. Mix 1.0 mL of each of 3 M KCl, 0.00100 M NaOH and  $1.0 \times 10^{-4}$  M PNPA directly in the UV cuvette sample cells. Record and overlay spectra at 60 sec intervals over a 15 min period using an appropriate blank solution.

### Time Drive (measuring absorption at a fixed wavelength over time):

Follow the hydrolysis of a solution containing 1.0 mL of  $1.0 \times 10^{-4}$  M PNPA, 1.0 mL of 0.010 M NaOH (pH 12\*) and 1.0 mL of 3 M KCl in a 1 cm optical glass cuvette recording the absorption at 400 nm every 10 sec over a 5 min period. Use an appropriate blank solution to zero the instrument. At this pH the reaction is over in about 4 mins, so prepare the PNPA solution last, mix quickly mix and place in spectrophotometer and immediately initiate recording absorption changes.

If time permits, repeat the timedrive using the pH 11 solution using an appropriate blank and the table below as a guide.

At the end of the laboratory period, set up an overnight timedrive to follow the hydrolysis at one of pH 7, 8 or 9 using the table below as a guide.

<b>pH</b>	<b>Time interval</b>	<b>Time period</b>
12*	10 sec	300 sec
11**	30 sec	20 min
9	10 min	12 hr
8	30 min	24 hr
7	60 min	48 hr

\* note the concentration of  $\text{OH}^-$  in the reaction solution is actually  $3.3 \times 10^{-3}$  M

\*\* note the concentration of  $\text{OH}^-$  in the reaction solution is actually  $3.3 \times 10^{-4}$  M

### Data Analysis:

In order to analyze the kinetics of the hydrolysis reaction and use the integrated form of the first order rate equation to calculate  $k_h$ , we will need to know the concentration of the ester as the reaction progresses (i.e.,  $[\text{PNPA}]_t$ ). Since it is easier to monitor the concentration of the yellow coloured product PNP, we will use the fact that the hydrolysis is a 1:1 reaction and express the concentration of the ester as its initial concentration minus the concentration of product formed;

$$[\text{PNPA}]_t = [\text{PNPA}]_o - [\text{PNP}]_t$$

#### When Absorption at the end of the reaction is known:

At high pH (11 and 12) the reaction will be complete after 5 – 20 mins. In this case, the absorption of PNP at completion ( $A_{\infty}^{400}$ ) is proportional to  $[\text{PNP}]_{\infty}$ , which will be equal to the  $[\text{PNPA}]_o$ . Hence, it can be shown that;

$$\frac{[\text{PNPA}]_t}{[\text{PNPA}]_o} = \frac{A_{\infty}^{400} - A_t^{400}}{A_{\infty}^{400}}$$

And a plot of  $\ln \{(A_{\infty}^{400} - A_t^{400})/A_{\infty}^{400}\}$  versus time will yield a straight line with slope =  $-k_h$ . The observed *pseudo first order* rate constant for hydrolysis can then be evaluated from a least squares analysis of the slope.

#### When Absorption at the end of the reaction is not known:

At neutral pH's the reaction is much slower and may take several days to go to completion. If the absorption of PNP at completion ( $A_{\infty}^{400}$ ) can be measured, the approach given above can again be used. However, if the reaction is stopped before completion, the rate constant can be obtained using the experimentally determined molar extinction co-efficient ( $\epsilon_{400}$ ) to convert the absorbance of PNP to the molar concentration of PNP with Beer's Law;

$$[\text{PNP}] = A / \epsilon$$

So for a 1.0 cm cell,

$$\frac{[\text{PNPA}]_t}{[\text{PNPA}]_o} = \frac{[\text{PNPA}]_o - \frac{A_t^{400}}{\epsilon^{400}}}{[\text{PNPA}]_o}$$

And a plot of  $\ln \{([\text{PNPA}]_o - A/\epsilon)/[\text{PNPA}]_o\}$  versus time will yield a straight line with slope =  $-k_h$ . The observed *pseudo first order* rate constant for hydrolysis can then be evaluated from a least squares analysis of the slope.

### Data Summary:

- Plot  $\log k_h$  versus pH to illustrate the pH dependence of the hydrolysis of PNPA
- Calculate the rate constants for the base enhanced ( $k_B$ ) and the neutral ( $k_N$ ) reaction using time units of seconds and compare your values with the literature.
- Calculate the percentage the neutral mechanism contributes at the pH 12 and the percentage of the base enhanced mechanism contributes at pH 7.

Be careful about the reactant concentrations.

**Questions:**

1. Which is the dominant mechanism of hydrolysis of PNPA at pH 7?
2. What is the hydrolysis half-life of PNPA at pH 7?
3. At what pH will the rates of base enhanced hydrolysis and neutral hydrolysis of PNPA be equal?
4. Provide stepwise mechanisms for *specific* acid catalyzed hydrolysis, neutral hydrolysis and base enhanced hydrolysis of PNPA. What is meant by the terms *general* acid and *general* base catalysis?
5. Would the hydrolysis of 2,4-dinitrophenyl acetate be faster or slower than PNPA? Give reasons.

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