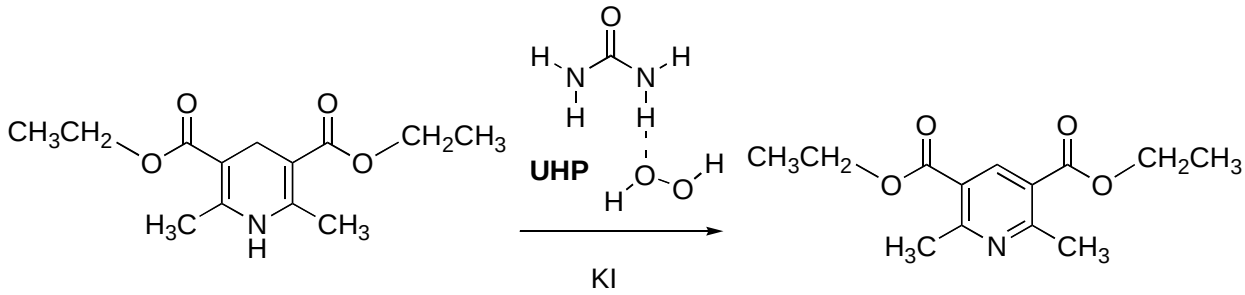
PRACTICAL PROBLEMS

PROBLEM 1 (Practical)

Reaction of Hantzsch Ester with Urea Hydrogen Peroxide

In this experiment, you are required to synthesize a pyridinedicarboxylate derivative from 1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylic acid diethyl ester (1,4-DHP or Hantzsch ester) by oxidation with urea hydrogen peroxide (UHP), an environmentally-friendly oxidant.



1,4-DHP

Chemicals

- Hydrochloric acid, c = 0.5 mol dm^{-3}, 50 cm^3
- 1,4-dihydro-2,6-dimethylpyridine-3,5-dicarboxylic acid diethyl ester, $C_{13}H_{19}NO_4$; (1,4-DHP), powder, 1 g
- 1,4-DHP for TLC, 3 mg
- Ethanol, C_2H_5OH, 10 cm^3
- Ethyl acetate, $CH_3COOC_2H_5$, 25 cm^3
- Heptane, C_7H_{16}, 20 cm^3
- Potassium iodide, KI, 150 mg
- Sodium metabisulfite, $Na_2S_2O_5$, 1 g
- Sodium hydrogen carbonate, saturated aqueous solution, 25 cm^3
- Urea hydrogen peroxide, $CH_4N_2O{\bullet}H_2O_2$, (UHP), 1 g

Procedures

(1) Place a 22 mm magnetic stirring bar in a 100 cm^3 test tube. Fix the test tube on a magnetic stirrer using a clamp. Add 1,4-DHP (1 g) (labelled as 1,4-DHP powder), and

potassium iodide (150 mg) to the test tube, followed by ethanol (5 cm^3), with a 5 cm^3 graduated pipette.

(2) Add 1 g UHP (wear gloves) and stir the mixture. (Caution: this reaction is exothermic.)

(3) For thin layer chromatography (TLC) analysis, prepare a mixture of ethyl acetate and heptane (1 : 2 in volume) using a measuring glass and place an appropriate amount of the mixture in a TLC developing chamber. Add 1 cm^3 of ethyl acetate to the vial (labelled as 1,4-DHP TLC) to dissolve 1,4-DHP (3 mg).

(4) Check your TLC plates before using. If they are damaged, they can be replaced without penalty. Draw a start line on the lower portion of a TLC plate with a pencil (see Fig. 1.1).

(5) During the reaction, the reaction mixture becomes clear (usually within 20 min). When the reaction mixture becomes clear (the precipitates may form when it cools, but precipitates will not affect the TLC analysis), take a small portion of the mixture using a glass capillary and load it to make two spots in the centre and right positions on the TLC plate. Load an appropriate amount of the 1,4-DHP solution prepared in procedure (3) in the centre and left positions, so that there are three spots on the plate, with the centre spot containing both the reaction mixture and 1,4-DHP (see Fig. 1.1). Develop the TLC plate in the TLC chamber (see Figs. 1.1 and 1.2). Mark the solvent front with the pencil. Visualize the spots using a UV lamp (254 nm) and draw a line around the UV-active spots on the TLC clearly with the pencil. Assess the completion of the reaction based on the TLC results. Repeat the TLC analysis after ten minutes, if you find significant amounts of 1,4-DHP in the reaction mixture. [Note that you will perform TLC analysis again in procedure (8).] Place the last TLC plate in a zipper storage bag marked “A.”

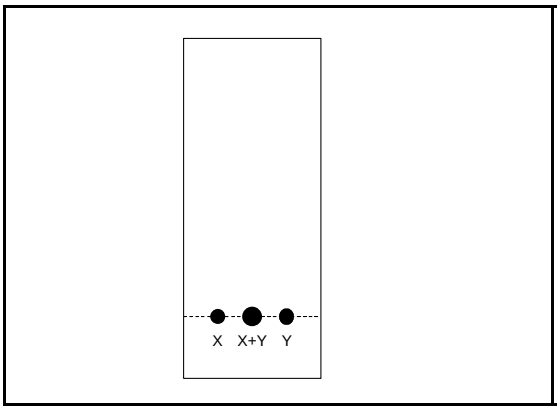


Fig. 1.1 Spots on the TLC plate before development;
X: 1,4-DHP, Y: Reaction mixture.

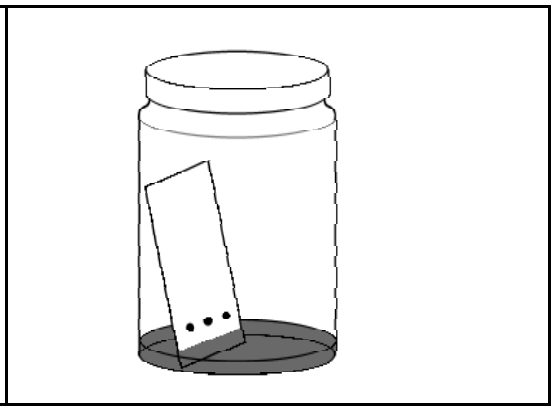


Fig. 1.2 TLC plate placed in the TLC developing chamber.

(6) Set up the suction filtration equipment (see Fig. 1.3). Connect the suction flask to the diaphragm vacuum pump. Place a Büchner funnel fitted with a rubber adapter onto the suction flask. Place a glass microfiber filter sheet on the funnel.

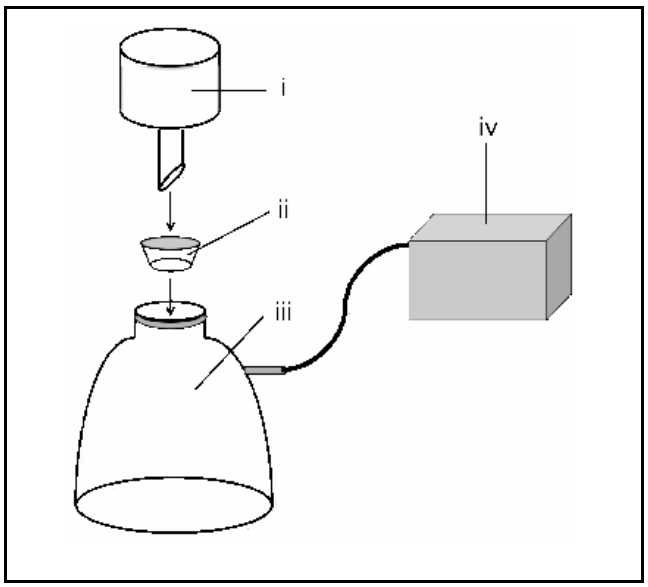


Fig. 1.3 Suction filtration equipment:
i - Büchner funnel; ii - rubber adopter;
iii - suction flask; iv - diaphragm vacuum pump.

(7) Add water (5 cm^3) to the reaction mixture using a 10 cm^3 plastic graduated cylinder. Add sodium metabisulfite (1 g), transfer the contents of the tube (including the stirring bar) into a 200 cm^3 conical beaker and wash the test tube with water (30 cm^3). Place the 200 cm^3 conical beaker on the magnetic stirrer and stir the solution. Add saturated sodium hydrogen carbonate solution in small portions using a 2 cm^3 graduated pipette until the pH of the aqueous phase becomes just over 7 (check the pH with pH test paper). Filter the precipitate formed through the Büchner funnel with suction using the diaphragm vacuum pump, and wash the precipitate with a small portion of water. Suck air through the precipitates for a minute to dry the product.

(8) Transfer the filtrate from the suction flask to a 300 cm^3 conical beaker. Transfer the filtrate (2 cm^3) to a 10 cm^3 test tube using a 2 cm^3 graduated pipette. Place a 10 mm magnetic stirring bar in the test tube and fix it securely with the clamp. Add 1 cm^3 of ethyl acetate to the test tube using a 2 cm^3 graduated pipette and stir the solution vigorously for 30 seconds on the magnetic stirrer. Stop stirring and wait for the solution to separate into two layers. Analyze the upper layer by TLC to see if there is any product remaining in the filtrates. Spot the filtrates on the plate in the same way as procedure (5). Mark the solvent front and the spot(s), if any. Place the TLC plate in a zipper storage bag marked “B.” If you detect the product on the TLC plate, add more saturated sodium hydrogen carbonate solution.

(9) At this stage, if you find a precipitate formed, filter and wash it. If you find no precipitate, skip this filtration process.

(10) Suck air through the precipitate for 10 minutes to dry the product. Place your product and the glass microfiber filter sheet in the crystallization dish. Cover the dish with the lid marked with your code. Avoid placing the stirring bar in the dish. Place the crystallization dish with the lid in a zipper storage bag marked “C.”

1.1 Copy (sketch) the TLC plate in bag “A” on your answer sheet.

1.2 Determine and record the R_f values (to the 2nd decimal place) of the spots on the TLC plate in bag “A.”

1.3 Draw the structural formula of the organic cation before adding sodium hydrogen carbonate.

1.4 What is (are) the final product(s) derived from UHP? Give the chemical formula(e) of the product(s).

1.5 Submit the following:

i) TLC plate in bag “A”.

ii) TLC plate in bag “B”.

iii) Your product and filter paper in the crystallization dish placed in bag “C”.

SOLUTION

1.1 Indication of the solvent front line and the base line is expected.

Some points will be subtracted if:

i) there are less than three spots loaded on the base line,

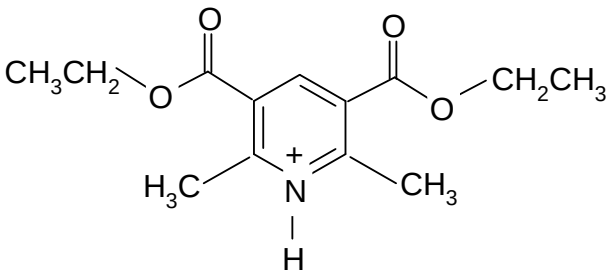
ii) the spots are not separated on the TLC after development,

iii) the solvent front line and/or the base line is missing.

1.2

Spot	R_f value
1,4-DHP	0.32 – 0.42
Product	0.61 – 0.71

1.3



1.4 H_2O and CH_4N_2O

1.5 The following rules for evaluation of the practical results in this problem were suggested by the organizer and approved by the International Jury:

<u>Evaluation of the TLC plates</u>.

Points will be subtracted:

- if the outline to be drawn with a pencil around the UV-active spots, is unclear or missing,
- if the solvent front line and/or base line is missing.

<u>Evaluation of the product and filter paper in the crystallization dish (in bag “C”)</u>.

i) A technician should weigh the product and calculate the percent yield after drying at 60 ℃.

ii) In most cases the sample is pure and dissolved in $CDCl_3$ completely. The special calculation based on the percent yields and an approved graph is

applied only if no 1,4-DHP or by-products are observed in the ^{1}H NMR spectrum and the product is completely soluble in $CDCl_3$.

iii) If there are peaks of 1,4-DHP (ca δ 2.19 ppm) and the corresponding pyridine product (ca δ 2.85 ppm) in the ^{1}H NMR spectrum and the percent yield is 100 % or less, the actual percent yield is calculated by an equation proposed by the organizer and approved by the International Jury.

iv) Some points subtraction is suggested:

- if insoluble material remains after the addition of $CHCl_3$ for ^{1}H NMR measurements,
- if by-products are detected evidently in the ^{1}H NMR spectrum.

PROBLEM 2 (Practical)

Determination of Fe(II) and Fe(III) by visual colorimetry

In this experiment, you are required to determine Fe(II) and Fe(III) in a given sample solution which simulates a dissolved magnetite ore by visual colorimetric analysis involving a colour reaction between Fe(II) and 2,2'-bipyridine (bpy) to form an intensely red complex, $Fe(bpy)_3^{2+}$.

The amount of $Fe(bpy)_3^{2+}$ complex can be quantified by visual colorimetric measurement using Nessler tubes. This is a quite simple technique that was employed before photoelectric instruments were generally available, but an accuracy of less than ± 5% can be achieved. In this technique, a pair of Nessler tubes is used; one is filled with a reference solution, and the other is filled with a solution to be tested. The depths of colours of the two solutions are balanced by adjusting the heights of liquid columns of the solutions.

When the colours look the same, the concentration can be calculated from that of the reference solution with a known concentration and the height of the column of each solution based on the Lambert-Beer law:

$$A = \varepsilon c\, l$$

where A is the absorbance, c is the concentration, l is the pass length and ε is the molar absorption coefficient. First, you will learn to employ this technique by conducting measurements **A** and **B**, and then you will determine the concentrations of Fe(II) and Fe(III) with measurements **C** and **D**.

Chemicals

- Sample solution
- Standard $Fe(bpy)_3^{2+}$ solution 1 containing 2.0 mg of iron in 1 dm^3 of the solution.
- Standard $Fe(bpy)_3^{2+}$ solution 2 containing 3.0 mg of iron in 1 dm^3 of the solution.
- Acetate buffer solution, pH 4.6, 1 : 1 mixture of acetic acid and sodium acetate.
- Disodium hydrogen phosphate solution, c = 0.1 mol dm^{-3}.
- 2,2'-bipyridine aqueous solution, w = 0.2 %.
- Sodium thioglycolate.

Procedures

(1) Add 5 cm^3 of acetate buffer solution, 5 cm^3 of disodium hydrogen phosphate solution (to mask Fe(III)), 5 cm^3 of 2,2'-bipyridine solution and 10.00 cm^3 of sample solution into a 50 cm^3 volumetric flask using appropriate pipettes for each and dilute the resulting solution with water to the 50 cm^3 mark. Then stopper the flask and mix the solution well. Allow it to stand for at least 20 min to fully develop colour. This solution is named "sample 1."

(2) Add 5 cm^3 of acetate buffer solution, 5 cm^3 of 2,2'-bipyridine solution and 5.00 cm^3 of sample solution into a 50 cm^3 volumetric flask. Then add 20 mg of sodium thio-glycolate powder (in excess) to reduce Fe(III) to Fe(II). Dilute the solution with water to the 50 cm^3 mark, stopper the flask and mix the solution well. Allow it to stand for at least 20 min. This solution is named "sample 2."

(3) Perform visual colorimetric measurements A – D based on the "Instructions for visual colorimetric measurement" shown below.

Instructions for visual colorimetric measurement

Set a pair of Nessler tubes on a Nessler tube rack placed on an LED light box (do not remove it from the bag at any time) and turn on the light (see Fig. 2.1). Pour the provided "standard $Fe(bpy)_3^{2+}$ solution 1" into one tube to an appropriate height (70 – 90 mm is recommended) from the bottom (etched marks on the tube indicate fixed heights from the bottom in mm) and use this as a reference for measurements **A** – **D**. Pour the solution to be measured into the other tube, and then compare its depth of colour with that of the reference solution by looking downward through the solutions toward the LED light box.

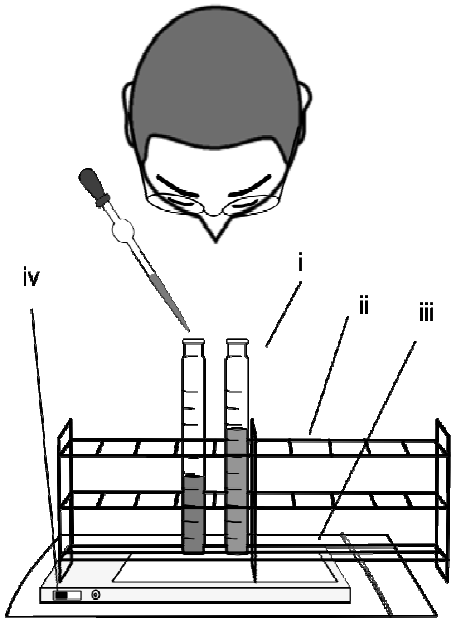


Fig. 2.1
Visual colorimetric measurement:
i: Nessler tube;
Ii: Nessler tube rack; iii: LED light box in a zipper storage bag;
Iv: power switch.

Adjust the height of the liquid column of the test solution by adding or removing the solution with a graduated pipette until the depth of colour in the two tubes is identical. Estimate your

reading to at least 1 mm.

Note that the depths of colour in a certain range may be recognized as identical to human eyes. The appropriate value for the height of the test solution, *h*, should be determined by taking the range into the consideration. For example, if you adjust the height of the liquid column of the test solution only by increasing (or decreasing) the volume, you could reach a lower (or higher) value than the true one. A possible way to estimate the true value is to take an average between the values of lower and higher limits.

Measurement A:

Perform a measurement using "standard $Fe(bpy)_3^{2+}$ solution 1" as both the reference and the test solutions. In this measurement, pour the reference solution into a Nessler tube to achieve an appropriate height, and then pour the test solution into the other Nessler tube until the colours of the two solutions match each other. (When the colours match, the heights should ideally be the same.) Then add more test solution until you recognize that the colours have become different from each other. Report both the lower and higher limits of the height of the liquid column of test solution with the same depth of colour as the reference solution.

2.1 Report your results for measurement A using the table provided on the answer sheet.

Measurement B:

Perform a measurement of "standard $Fe(bpy)_3^{2+}$ solution 2" as a test solution using "standard $Fe(bpy)_3^{2+}$ solution 1" as a reference.

2.2 Report your results for measurement B using the table provided on the answer sheet.

Measurement C:

2.3 Perform the measurement of sample 1.

Report your results for measurement C using the table provided on the answer sheet.

Measurement D:

2.4 Perform the measurement of sample 2.

Report your results for measurement D using the table provided on the answer sheet.

2.5 Express the concentration, *c*, of the test solution, using the concentration of the reference solution, *c'*, and the heights of each liquid column, *h* and *h'*.

2.6 Calculate the concentrations of Fe(II) and Fe(III) in the original sample solution in mg dm^{-3}.

———————————

SOLUTION

2.1– 2.4

The competitors were requested to summarize their results in four tables for measurements A, B, C, and D, respectively. It was necessary to record the height, *h*', (in mm) of the standard solution and the height, *h*, of the test solution.

A distribution graph was proposed and applied for evaluations of the competitors' results.

2.5 $c = \dfrac{c'\,h'}{h}$

2.6 For Fe^{2+}:

$$c(Fe^{2+}) = \frac{2.0\ \text{mg dm}^{-3} \times h'_C \times 50\ \text{cm}^3}{h_C \times 10\ \text{cm}^3}$$

$c(Fe^{2+})$: concentration, (mg dm^{-3}), of Fe^{2+} in the sample solution,

h_C: experimental height, (mm), of the liquid column of the test solution in the measurements C,

h'_C: experimental height, (mm), of the liquid column of the standard solution in the measurements C.

For Fe^{3+}:

$$c(Fe^{3+}) = \frac{2.0\ \text{mg dm}^{-3} \times h'_D \times 50\ \text{cm}^3}{h_D \times 5\ \text{cm}^3} - c(Fe^{3+})$$

$c(Fe^{3+})$: concentration, (mg dm^{-3}), of Fe^{3+} in the sample solution,

h_D: experimental height, (mm), of the liquid column of the test solution in the measurements D,

h'_D: experimental height, (mm), of the liquid column of the standard solution in the measurements D.

Concentrations of Fe^{2+} and Fe^{3+} used for particular original sample solutions:

	$c(Fe^{2+})$ / mg dm^{-3}	$c(Fe^{3+})$ / mg dm^{-3}
Sample 1	8.16	18.0
Sample 2	8.60	19.0
Sample 3	9.08	17.7

PROBLEM 3 (Practical)

Polymers in Analysis

Polymers can be used in various analyses. In this task, you are first required to analyze a polysaccharide using a polymer-polymer interaction, which will then be utilized to identify polymers in the second part.

I. Analysis of Polysaccharide by Colloid Titration

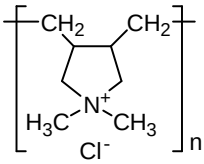
You are provided with a solution of a polysaccharide containing sulfonate ($-SO_3^-$) and carboxylate ($-COO^-$) groups. You are asked to determine the concentrations of these two groups by colloid titration under the basic and acidic conditions based on the differences in the protonation behaviour of these acid groups. A back-titration technique is utilized.

When these acid groups are ionized, the polysaccharide becomes a polyanion. Upon addition of polycation, poly(diallyldimethylammonium) (provided as its chloride salt, PDAC), it forms a polyion complex. PDAC solution is standardized using the standard solution of potassium poly(vinyl sulphate) (PVSK). At the endpoint of colloid titration, the number of anionic groups becomes equal to that of cationic groups.

Chemicals

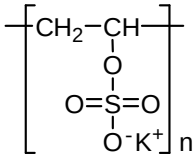
- Polysaccharide solution, 50 cm^3
- Poly(diallyldimethylammonium chloride), (**PDAC**), aqueous solution

  Formula:



- Potassium poly(vinyl sulfate) aqueous solution, c = 0.0025 mol dm^{-3}; (monomer unit concentration)

  Formula:



- sodium hydroxide, aqueous solution, c = 0.5 mol dm^{-3}
- toluidine blue, $C_{15}H_{16}N_3SCl$, (TB), aqueous solution, c = 1 g dm^{-3}
- Solutions X-1 – X- 5 for Part II**.**

Procedure

(1) Take precisely 20 cm^3 of the PDAC solution using a volumetric pipette into a 100 cm^3 conical beaker. Add 2 drops of toluidine blue (TB) into the conical beaker. Titrate the resulting blue solution with the standard solution of PVSK (c = 0.0025 mol dm^{-3} - monomer unit concentration). At the endpoint the colour turns purple. Note that the solution becomes gradually turbid as the endpoint approaches. The endpoint is determined when the colour remains purple for 15 – 20 seconds. Repeat if necessary.

3.1 Report the PVSK solution volume (**z** cm^3) consumed in the standardization of PDAC. Record your reading to 0.05 cm^3.

_____

(2) Take precisely 5 cm^3 of the polysaccharide solution and 20 cm^3 of the PDAC solution using volumetric pipettes into a conical beaker. Add 0.4 cm^3 of NaOH solution (c = 0.5 mol dm^{-3}) and 2 drops of TB to the solution. Titrate the resulting blue solution with the PVSK standard solution in a similar manner. Repeat if necessary.

(The appearance of coagulation may be different, depending on the pH of the solution.)

3.2 Report the PVSK solution volume, (**x** cm^3) consumed in the titration under basic conditions. Record your reading to 0.05 cm^3.

3.3 Mark the acid group(s) ionized under the basic conditions on the answer sheet.

_____

(3) Repeat procedure (2) above with the addition of 0.5 cm^3 of HCl solution (c = 0.5 mol dm^{-3}) instead of NaOH solution (c = 0.5 mol dm^{-3}).

3.4 Report the PVSK solution volume (**y** cm^3) consumed in the titration under acidic conditions. Record your reading to 0.05 cm^3.

3.5 Mark the acid group(s) fully ionized under acidic conditions on the answer sheet.

3.6 Calculate the concentrations of the $-SO_3^-$ (or $-SO_3H$) groups and the $-COO^-$ (or –COOH) groups (in mol dm^{-3}) in the given polysaccharide solution.

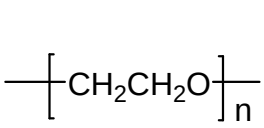
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II. Identification of compounds

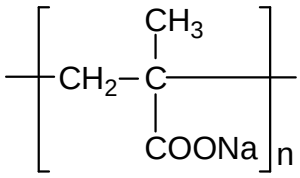
You are provided with five solutions **(1 – 5)** and each solution contains one of the compounds below (all of which are used). The concentration is 0.05 mol dm^{-3} (for polymers, monomer unit concentration). Your task is to identify all the compounds by carrying out the following procedures.

$HOCH_2CH_2OCH_2CH_2OCH_2CH_2OH$

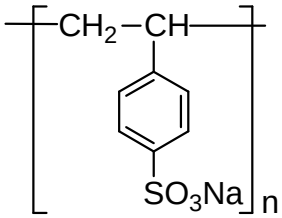
(TEG)



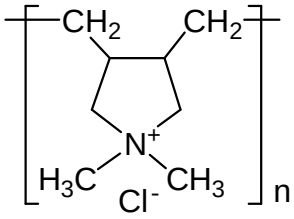
(PEO)



(PMANa)



(PSSNa)



(PDAC)

Abbreviations:

TEG = triethylene glycol;

PEO = poly(ethylene oxide);

PMANa = poly(sodium methacrylate);

PSSNa = poly(sodium 4-styrenesulfonate);

PDAC = poly(diallyldimethylammonium chloride) MW. stands for molecular weight.

Helpful comments

i) Aggregates observed in part I could be observed when mixing two polymer solutions in an appropriate combination in which an interaction takes place between the two polymers. They can be utilized to identify polymer samples.

ii) The volume of a solution measuring 5 mm in height from the bottom of the vial is approximately 1 cm^3. Remember that you have only 10 cm^3 of each solution.

Procedures

(4) Mix similar volumes of two solutions together in a vial.

(5) If necessary, you can acidify the resulting mixture. Ten drops of hydrochloric acid (c = 0.5 mol dm^{-3}) from a plastic Pasteur pipette are sufficient for this purpose.

3.7 Identify the compound in each solution based on the experimental results. Mark one of the five boxes to indicate your identification of the samples.

Sample					
1	❑ TEG	❑ PEO	❑ PMANa	❑ PSSNa	❑ PDAC
2	❑ TEG	❑ PEO	❑ PMANa	❑ PSSNa	❑ PDAC
3	❑ TEG	❑ PEO	❑ PMANa	❑ PSSNa	❑ PDAC
4	❑ TEG	❑ PEO	❑ PMANa	❑ PSSNa	❑ PDAC
5	❑ TEG	❑ PEO	❑ PMANa	❑ PSSNa	❑ PDAC

SOLUTION

I. Analysis of Polysaccharide by Colloid Titration

3.1 $V = 20.06\ cm^3$; z = 20.06

3.2 Sample A: $V = 13.14\ cm^3$; x = 13.14
Sample B: $V = 12.07\ cm^3$ x = 12.07
Sample C: $V = 10.91\ cm^3$ x = 10.91

3.3 Under basic conditions: The acid groups are $–SO_3H$ and –COOH.

3.4 Sample A: $V = 15.26\ cm^3$; y = 15.26
Sample B: $V = 14.61\ cm^3$ y = 14.61
Sample C: $V = 13.59\ cm^3$ y = 13.59

3.5 Under acidic conditions: The acid group is $–SO_3H$.

3.6 The concentrations (in mol dm^{-3}) of:
$–SO_3^-$ (or $–SO_3H$) groups = 0.0005 (z – y)
$–COO^-$ (or –COOH) groups = 0.0005 (y – x)

II. Identification of compounds

PMANa and PSSNa are polyanions and they interact with a polycation (PDAC) to form a precipitate. Under acidic conditions the carboxylate ($–COO^-$) groups in PMANa undergo protonation and PMANa changes to protonated poly(metacrylic acid) (PMA). The resulting carboxy groups (–COOH) interact with the ether oxygen atoms in PEO through hydrogen bonding to form a precipitate. Since the protonated PMA is no longer a polyanion, the precipitate (the complex between PMANa and PDAC) disappears after the addition of HCl.

On the other hand, PSSNa does not exist as a protonated form even under acidic condition.

The expected results of mutual reactions are summarized in the table below. The signs in upper rows correspond to the reactions before addition of HCl while those in lower rows to the reactions after addition of HCl.

	TEG	PEO	PMANa	PSSNa	PDAC
TEG					
PEO	– –				
PMANa	– –	– +			
PSSNa	– –	– –	– –		
PDAC	– –	– –	+ –	+ +	

Note: (+) precipitation is formed; (**–**) no precipitation occurs (or the precipitation disappears)