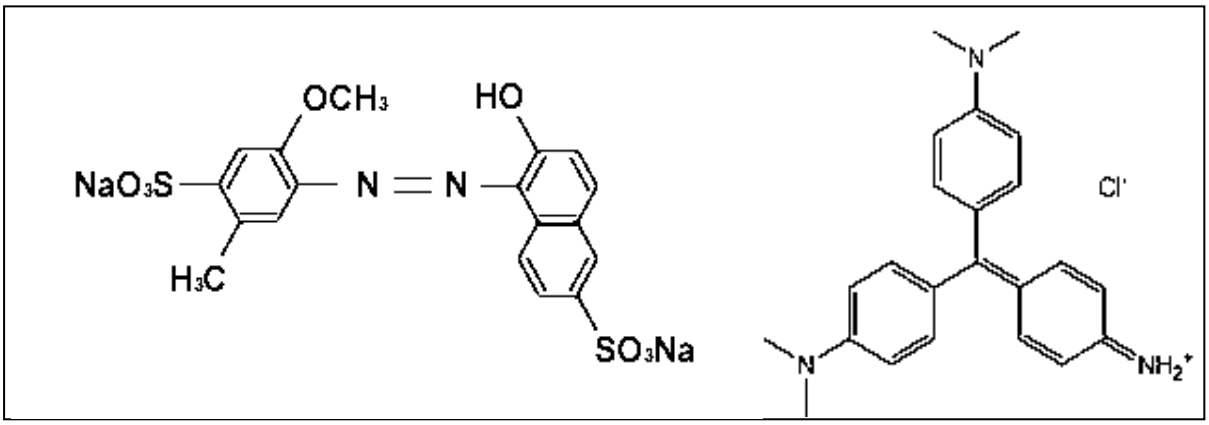
PRACTICAL PROBLEMS

PROBLEM 1 (Practical)

Reverse-phase Chromatography: Spectrophotometric Analysis

Chromatographic separation followed by spectrophotometric analysis is one of the most widely practiced analytical techniques in chemical laboratories around the world. For example, organic compounds in a complex mixture are often analyzed by reverse-phase liquid chromatography with spectrophotometric detection. In reverse-phase chromatography, hydrophobic interactions between the stationary phase material (usually octadecyl group) and the non-polar moiety of the analyte is utilized. The chromatogram can be simplified and the compound of interest selectively determined by proper choice of the detector wavelength. In this part of the Practical Test, spectrophotometric analysis of dyes, with and without separation, will be performed.



Food Red No. 40 Methyl Violet 2B

1.1 Spectrophotometric Analysis of R and B in a Mixed Solution

a) Measure absorbance of both Solutions R (3.02×10^{-5} M) and B (1.25×10^{-5} M) (Fig. A & B). Fill in the Table in the Answer Sheet with your measurements. Draw absorption spectra for the red dye in red ink and for the blue dye in blue ink (Fig. 1.1).

b) Repeat absorbance measurements for Solution MD. Solution MD is a mixture of Solution R and B in a certain ratio. Add the spectrum in black ink to Fig. 1.1.

c) Based on the Beer-Lambert law, determine the molar concentration of both dyes in Solution MD using the data in the Table. Do not determine the fraction of one dye by subtracting the fraction of another dye from 1.

1.2 Chromatographic Separation Followed by Spectrophotometric Analysis

a) Elute the cartridge with about 10 cm^3 of Solution E using 10 cm^3 syringe (Fig. C).

b) Load 1.00 cm^3 of solution MD onto the cartridge (Fig. D).

c) Using 1 cm^3 syringe, elute with Solution E (Fig. E). Collect the solution eluting through the outlet in a 10 cm^3 volumetric flask. Repeat until the red compound is completely eluted and collected.

d) Fill the flask to the 10 cm^3 mark with Solution E and mix. Call this Solution F.

e) Obtain the absorption spectrum of solution F as in Experiment 1.1. Dilution takes place during elution. Therefore, multiply the measured absorbance by 10 when drawing the spectrum for Solution F. Draw spectrum with broken line in Fig. 1.1 in red ink.

f) Dilute Solution R as necessary and construct a calibration curve, at a wavelength of your choice, for analysis of the red dye (R) in Solution F. Draw a calibration curve in the answer sheet (X-axis, concentration; Y-axis, absorbance, Fig. 1.2). Indicate the wavelength used. The calibration curve must have three points in addition to the origin. Mark the position of Solution F on the calibration curve.

g) Report the concentration of R in the original Solution MD.

h) Compare this concentration with the value you obtained in Experiment 1-1 and report the recovery (amount eluted/amount loaded) associated with chromatography.

PROBLEM 2 (Practical)

Reverse-phase Chromatography:

Acid-Base Titration of Acetic Acid and Salicylic Acid

Acetic acid (AA) and salicylic acid (SA) are slightly different in polarity and thus can be separated on a reverse-phase cartridge using distilled water as eluent. AA is eluted first. The total amount of AA and SA in a mixed solution will be determined by titration. Then, AA and SA will be separately determined following chromatographic separation.

2-1. Determination of the Total Amount of AA and SA in a Mixed Acid (MA) Solution

a) Titrate 10 cm^3 of distilled water with the NaOH (< 5 mM) solution provided. Report blank acidity in 1 cm^3 of distilled water in terms of the volume of the NaOH solution. Take this blank acidity into account for all solutions in subsequent data analyses. Show corrections in the calculation part in the answer sheet.

b) Standardize NaOH solution with 2.00 cm^3 of the standard KHP (potassium hydrogen phthalate) solution (1.00×10^{-2} M) provided. Repeat and report the concentration of the NaOH solution. Show how you accounted for the blank acidity.

c) Withdraw 1.00 cm^3 of Solution MA and determine the total acidity. Repeat and report the total number of moles of AA and SA combined in 1.00 cm^3 of Solution MA.

2-2. Reverse-phase Separation and Titration

a) Elute a new C-18 cartridge with about 10 cm^3 of distilled water using 10 cm^3 syringe.

b) Load 1.00 cm^3 of Solution MA onto the cartridge. Collect the liquid eluting at the outlet in tube 1 (Fraction 1).

c) Elute with 1 cm^3 of distilled water. Collect the eluent in a test tube (Fraction 2). Repeat until Fraction 20 is collected. You will have 20 test tubes with about 1 cm^3 liquid in each tube.

d) Titrate acidity in each test tube. Report volume of the NaOH solution consumed and the amount of acid(s) in each test tube. Make a graph in the answer sheet (Fig. 2-2) showing the amount of acid(s) in each test tube.

e) Blank acidity and the background (due to leaching out of residual materials from the column) must be subtracted. In determining the amount of eluted AA, disregard tubes containing only trace amounts of acids. Tube 2 and 3 contain most AA. Calculate the total amount of AA eluted by adding the amount of AA in tubes. Similarly calculate the total amount of SA eluted. Indicate, in Fig. 2-2, which fractions you used to get the amount of each acid.

f) Calculate the mole percent of AA in solution MA.

PROBLEM 3 (Practical)

Qualitative Analysis of Organic Compounds

In this experiment your task is to identify seven solid unknowns from the list of compounds on page 7 that are common drugs in everyday life and valuable agents in organic chemistry. To achieve this, perform chemical tests on unknowns according to the following procedures and analyze your results.

Procedure

Helpful Comments

a) The weight of a spatula tip-full of a solid is about 15~20 mg.
b) Wipe spatula cleanly with Kimwipe between uses.
c) After adding any reagent described below to a solution of an unknown sample, mix the contents thoroughly and observe the resulting mixture carefully.
d) To get full marks, you should perform all the tests and fill out the table.

Test 1: Solubility test

To a test tube, add a spatula tip-full (15~20 mg) of an unknown sample and 1 cm^3 of CH_3CN. Shake the test tube and report the solubility. Repeat the test with 1M HCl, water, and 1M NaOH.

Test 2: 2,4-DNPH test

Place about 15~20 mg of an unknown sample in a test tube and dissolve with 2 cm^3 of 95 % EtOH. (For the water soluble unknowns, dissolve about 15~20 mg of an unknown in 1 cm^3 of water.) Add five drops of the 2,4-dinitrophenylhydrazine solution in concentrated sulphuric acid and 95% ethanol (labelled as 2,4-DNPH).

Test 3: CAN test

Mix 3 cm^3 of the cerium(IV) ammonium nitrate solution in dilute HNO_3 (labelled as CAN) with 3 cm^3 of CH_3CN in a test tube. In another test tube add about 15~20 mg of an unknown sample in 1 cm^3 of the mixed solution. (For the water soluble unknown samples, dissolve about 15~20 mg of an unknown sample in 1 cm^3 of water first, and then add 1 cm^3 of CAN.) If there is a colour change in the solution, the solution may contain alcohol, phenol or aldehyde.

Test 4: Bayer test

In a test tube, dissolve about 15~20 mg of an unknown sample in 2 cm^3 of CH_3CN. (For the water soluble unknown samples, dissolve about 15~20 mg of an unknown in 1 cm^3 of water.) To the solution, slowly add five drops of the 0.5 % $KMnO_4$ solution, drop by drop while shaking.

Test 5: *pH* test

In a test tube, dissolve about 15~20 mg of an unknown sample in 2 cm^3 of 95 % EtOH. (For the water soluble unknown samples, dissolve about 15~20 mg of an unknown sample in 1 cm^3 of water). Measure the pH of the solution with pH paper.

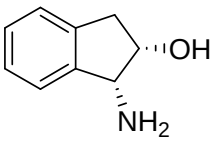
Test 6: Iron(III) chloride test

Take the solution from Test 5 and add five drops of a 2.5 % $FeCl_3$ solution.

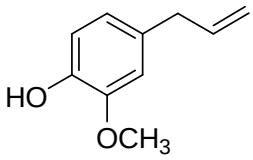
Results

1. Record your test results in the answer sheet. Write *O* if soluble and *X* if insoluble for the solubility tests. Write (+) for the positive reactions and (–) for the negative reactions for tests 2 ~ 4 and 6. Write *a*, *b* and *n* for acidic, basic or neutral, respectively, for pH test 5.
2. Based on your test results, identify the most plausible structures for the unknown compounds from the provided list of compounds. Write the compound initial in appropriate box.

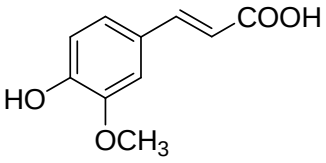
Possible Unknown Compounds (next page):


OH
NH2

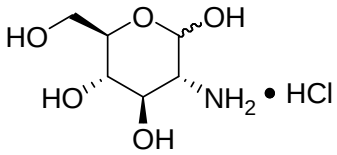
(A)


HO
OCH3

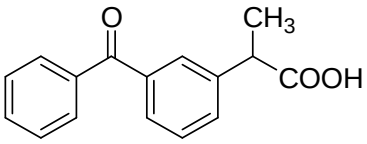
(E)


COOH
HO
OCH3

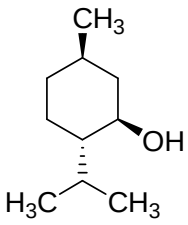
(F)


HO
O
OH
HO
NH2 • HCl
OH

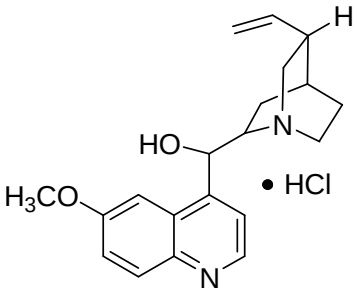
(G)


O
CH3
COOH

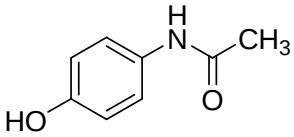
(K)


CH3
OH
H3C
CH3

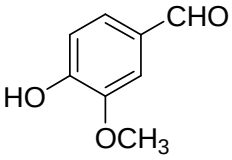
(M)


H
N
HO
• HCl
H3CO
N

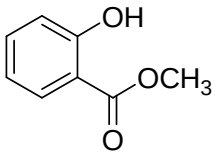
(Q)


H
N
CH3
O
HO

(T)


CHO
HO
OCH3

(V)


OH
OCH3
O

(W)