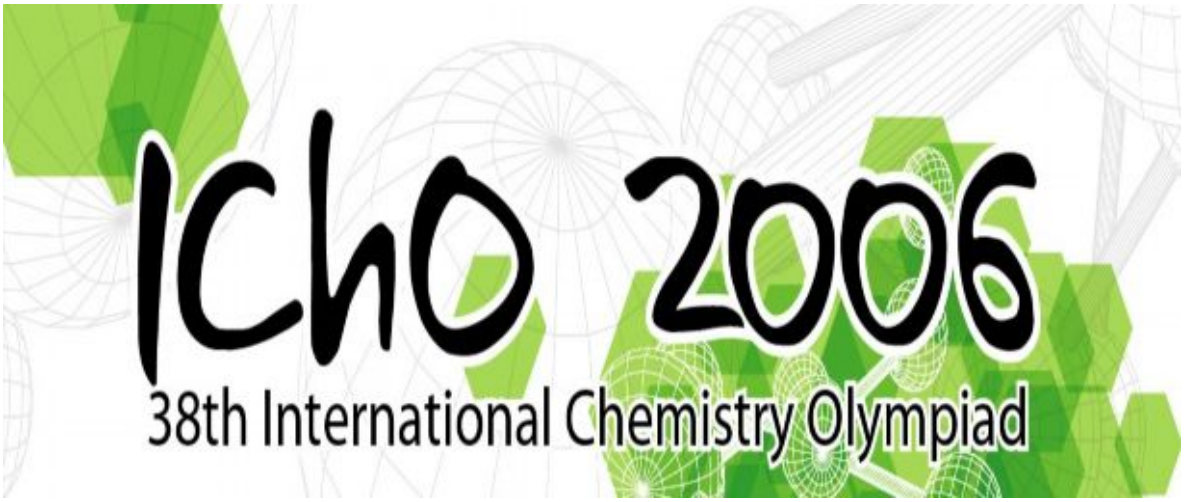
Chemistry for Life,

Chemistry for better Life



Practical Test

2006. 7. 5

Gyeongsan, Korea

General Directions

- **You have 5 hours to finish the test. Manage your time wisely. You might spend about one hour for Test 1 (10 points), two hours for Test 2 (15 points), and two hours foTest 3 (15 points).**
- **Write your name and code number on each page of the Answer Sheet.**
- **There are 7 pages of Test and 7 pages of Answer Sheet.**
- **Write answers and calculations within the designated box.**
- **Use only the pen, ruler, and calculator provided.**
- **An English-language version is available.**
- **Figures to supplement User's Instructions for the spectrophotometer, C-18 cartridge, and pipet filler are provided in a separate sheet.**
- **Additional samples or supplies will be provided with 1 pt penalty for each item. (except distilled water)**
- **You may go to the restroom with permission.**
- **After finishing the test, place all sheets (Test and Answer Sheets) in the envelope and seal.**
- **Remain seated until instructed to leave the room.**
- **You may take the pencil case, pen, ruler, calculator, and C-18 cartridges home.**

Safety and Disposal

- **Wear safety goggles and lab coat.**
- **No hazardous chemicals are used. All acid, alkali, and dye solutions are dilute. However, it is better to minimize contact with skin. Wipe off with wet Kimwipe in case of contact.**
- **Do not sniff reagents.**
- **Dispose used chemicals in the plastic bottle labeled “DISPOSABLE”. Discard used test tubes and broken glasses in the “Waste Basket”.**

Apparatus, Chemicals and Supplies

Test-1,2 (white basket)

spectrophotometer		1
cuvet (1 cm path-length)		1
C18 cartridge		4
10 mL syringe		1
1 mL syringe		1
pasteur pipet		3
1 mL pipet		1
5 mL pipet		1
pipet filler		1
10 mL volumetric flask		2
buret		1
test tube		20
test tube rack		1
50 mL Erlenmeyer flask		1
100 mL beaker		2
silicone bulb		2
three-color pen, ruler		1
sqeeze bottle		3
labeled as	Solution E	33% ethanol in water
	NaOH solution	less than 5 mM
	water	distilled water
100 mL bottle		6
labeled as	Solution R	red dye in Solution E
	Solution B	blue dye in Solution E
	Solution MD	mixed dye of B and R
	Solution MA	mixed acids; acetic acid & salcylic acid in water
	KHP	potassium hydrogen phthalate solution
	phenolphthalein	0.05% solution

Test-3 (black basket)

test tube		95
test tube rack		1
spatula		2
1.5 mL graduated pipet (polyethylene)		15
tweezers		1
pen (for writing on a test tube)		1
pH test paper		1
100 mL bottle		3
labeled as	95% EtOH	95% ethanol
	CH_3CN	acetonitrile
	water	distilled water
30 mL dropping bottle		6
labeled as	1M HCl	1M HCl solution
	1M NaOH	1M NaOH solution
	2,4-DNPH	3% 2,4-dinitrophenylhydrazine solution
	CAN	20% ceric ammonium nitrate solution
	0.5% $KMnO_4$	0.5% $KMnO_4$ solution
	2.5% $FeCl_3$	2.5% $FeCl_3$ solution
10 mL vial		7
labeled as	Set□ U-1	
	Set□ U-2	
	Set□ U-3	
	Set□ U-4	
	Set□ U-5	
	Set□ U-6	
	Set□ U-7	

The spectrophotometer has three compartments, the light source, the detector, and the cuvet holder. You will find the cover of the cuvet holder open. Leave it open. A cuvet is placed with the label facing the light source (Fig. A). Use this orientation throughout the experiment. The spectrophotometer has been stabilized and is ready for use. Follow the procedure below to take absorbance readings.

a) Fill the cuvet about 3/4-full with Solution E and insert into the cuvet holder. Do not close the cover of the cuvet holder.

b) Using the mouse of the computer, move the cursor to REFERENCE and click three times. Then click MEASURE three times and you will get absorbance readings close to zero at ten wavelengths between 470 and 650 nm at 20 nm intervals (Fig. B).

c) Fill the cuvet with sample solution and click MEASURE three times. You will get absorbance readings for your sample at the same wavelengths. Record absorbance values in the Table in the Answer Sheet.

How to Use the C18 Cartridge

a) The cartridge has an inlet and an outlet (Fig. C). The inlet has a larger diameter.

b) To wash or elute, first withdraw the liquid with a proper syringe and connect the syringe to the inlet of the cartridge. Then push the liquid slowly with a plunger into the cartridge. (Fig. C & E)

c) To load the sample, attach the 10 mL syringe to the inlet of the cartridge. Using a 1 mL pipet, transfer 1.00 mL aliquot of a sample solution to the syringe (Fig. D). Load the sample onto the cartridge using the plunger. Make sure that no amount of sample remains on the syringe. Try to avoid air entering into the cartridge after sample loading.

d) The cartridge can be reused after washing with Solution E.

e) Separate the syringe from the cartridge when removing the plunger from the syringe.

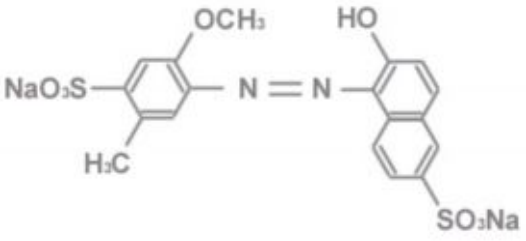
How to Use the Pipet Filler

Move the dial downward to fill the pipet and upward to release the liquid (see Fig. F).

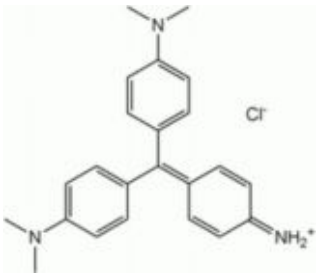
Practical Test 1

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 nonpolar 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 mL of Solution E using 10 mL syringe (Fig. C).

b) Load 1.00 mL of solution MD onto the cartridge (Fig. D).

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

d) Fill the flask to the 10 mL 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.

Practical Test 2

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 mL of distilled water with the NaOH (< 5 mM) solution provided. Report blank acidity in 1 mL 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 mL of the standard KHP (potassium hydrogen phthalate) solution (1.00 x 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 mL of Solution MA and determine the total acidity. Repeat and report the total number of moles of AA and SA combined in 1.00 mL of Solution MA.

2-2. Reverse-phase Separation and Titration

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

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

c) Elute with 1 mL 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 mL 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.

Practical Test 3

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.

- Unknowns Labeled
Set□ U-1, Set□ U-2, Set□ U-3, Set□ U-4, Set□ U-5, Set□ U-6, Set□ U-7

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 mL 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 mL of 95% EtOH (For the water soluble unknowns, dissolve about 15~20 mg of

an unknown in 1 mL of water). Add five drops of the 2,4-dinitrophenylhydrazine solution in concentrated sulfuric acid and 95% ethanol (labeled as 2,4-DNPH).

Test 3: CAN test

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

Test 4: Baeyer test

In a test tube, dissolve about 15~20 mg of an unknown sample in 2 mL of CH_3CN (For the water soluble unknown samples, dissolve about 15~20 mg of an unknown in 1 mL 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 mL of 95% EtOH (For the water soluble unknown samples, dissolve about 15~20 mg of an unknown sample in 1 mL of water). Measure the pH of the solution with pH paper.

Test 6: Iron(III) chloride test

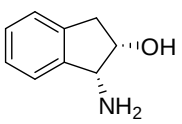
Take the solution from Test 5 and add five drops of a 2.5% $FeCl_3$ solution.

Results

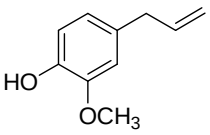
1. Record your test results in the answer sheet. Write *O* if soluble and *X* if insoluble for the solubility tests. Write (+) for the positive reactions and (–) for the negative reactions for tests 2 ~ 4 and 6. Write *a*, *b* and *n* for acidic, basic or neutral, respectively, for pH test 5.

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



(A)



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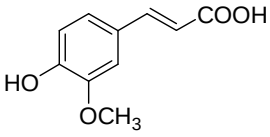

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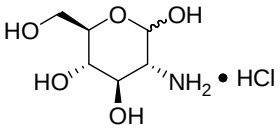
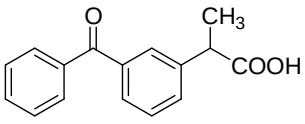


(G)



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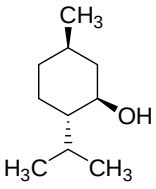
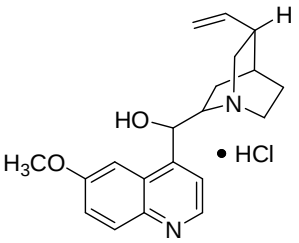


(M)



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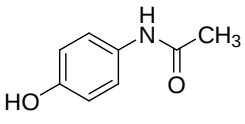

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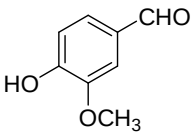

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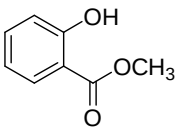

(W)