LABORATORY MANUAL

INSTRUMENTAL ANALYSIS FOR WATER POLLUTION CONTROL

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REGIONAL OFFICE FOR EUROPE

APRIL, 1969

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ATOMIC ABSORPTION ANALYSIS OF COPPER, CADMIUM, LEAD AND ZINC

I. Objectives

The purpose of this experiment is to demonstrate the use of atomic absorption spectrophotometry (AAS) in the analysis of an aqueous solution containing a mixture of copper, cadmium, lead and zinc. Direct analysis in water will be performed. In addition these metals will be chelated and extracted with an organic solvent, in which the analyses will be repeated, in order to demonstrate the increase in detectability obtained, both due to the increase in concentration and the improvement in sensitivity. A river water sample will be analyzed and interferences determined.

II. Apparatus

Atomic absorption spectrophotometer
Burner gaskets for organic solvents
Hollow cathode tubes: copper, cadmium, lead, zinc
Acetylene tank (containing approximately 8500 liters at S.T.P.; minimum pressure of 75 psi)
Acetylene regulator valve (maximum gauge pressure of 15 psi)
Clean air supply and regulator, 75 psi
pH meter and electrodes
Volumetric Flasks: 1000 ml, 8
250 ml, 2
100 ml, 10
Burets: 5 ml (graduated to 0.01 ml), 2
10 ml (graduated to 0.02 ml), 2
25 ml (graduated to 0.1 ml), 2
Pipets: 5 ml, 2
10 ml, 2
25 ml, 2

Beakers: 125 ml, 50
400 ml, 5
1500 ml, 2
Separatory funnels: 500 ml, 2
Filter paper (20 cm)
Filter funnel (10 cm)
Buret clamps, 2
Ring stand
Bunsen burner

III. Solutions and Reagents

100 mg/l cadmium ($CdCl_2$ in 4.5 N HCl), one liter
100 mg/l copper ($Cu(NO_3)_2$ in dilute HNO_3), one liter
500 mg/l lead ($Pb(NO_3)_2$ in 1% HNO_3), one liter
100 mg/l zinc ($ZnCl_2$ in dilute HCl), one liter
Methanol, reagent grade, one liter
Methyl isobutyl ketone (MIBK), reagent grade, 20 liters
Distilled water
Concentrated HCl, one liter
0.1 M HCl, 5 liters
Buffer, pH 4, 100 ml
Sodium diethyldithiocarbamate (DEDC), reagent grade, 100 g.
(prepare one liter of 5% aqueous solution)
Glacial acetic acid, 50 ml
Ammonium acetate, reagent grade, 250 g.
(prepare 250 ml of ammonium acetate-acetic acid buffer by dissolving 62.5 ammonium acetate in 200 ml distilled water; add 17.5 ml gracial acetic acid; dilute to 250 ml with distilled water; remove metal contaminants by adding 20 ml of 5% DEDC solution and extracting with two 50 ml portions of MIBK.)
River Water, 5 liters

IV. Procedure

A. Standard curves

1. Prepare the aqueous calibrating solutions for each metal by pipetting the appropriate volumes of the acidified aqueous standards into 100 ml volumetric flasks and diluting with 0.1M HCl to 100 ml so as to obtain the following solutions:

   Cu: 0, 1, 5, 10, 15 and 20 mg/l
   Pb: 0, 5, 10, 20, 30, and 40 mg/l
   Cd and Zn: 0, 0.5, 1, 2, 3 and 5 mg/l
   (separate solutions for Cd and Zn)

2. Prepare the MIBK calibrating solutions for each metal by first placing 5 ml of methanol in a 100 ml volumetric flask, then adding by buret the appropriate volume of the acidified aqueous standard, and diluting to 100 ml with MIBK to obtain the following solutions:

   Cu, Cd and Zn: 0, 0.2, 1.0, 2.0, 3.0, and 4.0 mg/l
   (separate solutions for Cu, Cd and Zn)

   Pb: 0, 1, 2, 4, 6, and 8 mg/l

3. Using the appropriate and hollow cathode tube and instrument settings according to the manual instructions, measure the absorbance for each of the aqueous calibrating solutions. Always measure the most dilute solution first. After a given metal has been analyzed, aspirate distilled water for one minute through the burner. Run each sequence of metal standards twice.

4. Repeat step 3 with the MIBK solutions.

5. For each metal in water and MIBK prepare a calibration curve, plotting absorbance versus metal concentration.

B. Extraction procedure

1. Prepare an aqueous mixture of all 4 metals by pipetting the appropriate volume of its acidified aqueous standard into a 1 liter volumetric flask and diluting to 1 liter with 0.1M HCl so as to obtain the following concentrations:
   Cu, Cd and Zn: 0.25 mg/l each
   Pb: 1 mg/l

2. Transfer exactly 250 ml of this aqueous mixture to a 400 ml beader and adjust the pH to 2.5 with the ammonium acetate-acetic acid butter solution. Transfer this solution in toto to a 500 ml separatory funnel. Add 20 ml of the 5% DEDC solution and shake for one minute. Add 20 ml of MIBK and shake for one minute. Separate the MIBK solution; then repeat with a second 20 ml portion of MIBK. Place the two 20 ml solutions of MIBK in a 50 ml volumetric flask and dilute to 50 ml with methanol.

3. Repeat step 2 two additional times.

4. Analyze the methanol-MIBK solutions containing the metal-DEDC extracts for each metal using the atomic absorption spectrophotometer. Before each metal analysis measure the MIBK calibrating solutions prepared in Part A. Calculate each metal concentration in the MIBK extract.

5. Analyze the aqueous mixture prepared in step 1 by the atomic absorption spectrophotometer. Before each metal analysis measure the aqueous calibrating solutions prepared in Part A. Calculate each metal concentration in the aqueous mixture.

C. Analysis of river water sample

1. Acidify a 1000 ml sample of river water by adding 10 ml of concentrated (11.6M) HCl. Boil for 5 minutes. Cool and filter.

2. Proceed as in Part B.

D. Test for interferences

1. Acidify a 1000 ml sample of river water by adding 10 ml of concentrated (11.6M) HCl. Boil for 5 minutes. Cool and filter.

2. Add the appropriate volumes of acidified aqueous standards to the filtrate so as to add 0.1 mg/l each of Cu, Cd, Pb, and Zn.

3. Proceed as in Part B.

V. Data Treatment

A. Using the MIBK calibration curves calculate the percent recovered in the MIBK extractions for each metal in Part IV., B.4.

B. Calculate the increment added for each metal in Part IV., D. as determined by the atomic absorption measurement. Compare to the known added concentration.

VI. Questions

A. What is the overall increase in sensitivity for each metal using the MIBK extraction procedure?

B. Are there any significant interferences present in the river water sample?

VII. References

1. H.L. Kahn, J. Chem. Ed., 43(1), A7 (1966).
2. Perkin-Elmer Corp., "Analytical Methods for Atomic Absorption Spectrophotometry", Revised Edition, Norwalk, Conn., 1968.
3. J.A. Platte, "Trace Inorganics in Water," R.F. Gould, ed., Chap. 14, Advances in Chem. Series No. 73, American Chemical Society, Washing5on, D.C., 1968.
4. J.W. Robinson, "Atomic Absorption Spectroscopy," Marcel Dekker, Inc., New York, 1966.

UV AND VISIBLE ABSORPTION SPECTROPHOTOMETRIC ANALYSIS OF NICKEL AND COPPER

I. Objectives

The purpose of this experiment is to demonstrate the simultaneous determination of nickel and copper in industrial water by the use of UV and visible absorption spectrophotometry. The principal absorption peak of the nickel diethyldithiocarbamate complex is in the UV, while that of the copper is in the visible region. However, since each complex does absorb somewhat at the principal peak of the other, it is necessary to perform measurements at each peak and make corrections in order to determine the concentration of each metal. The metal complexes are extracted from water with carbon tetrachloride, in which the absorption is measured.

II. Theory

Nickel and copper are readily complexed by diethyldithiocarbamate (DEDC) at a pH of 8.5 to 9 and can be quantitatively extracted by carbon tetrachloride. The principal absorption peaks of these complexes in carbon tetrachloride are at widely separated wave lengths, but each does absorb to some extent at the other's principal peak. Values of the molar extinction coefficients, ϵ, at these two wave lengths are for copper: $\epsilon_{436} = 12{,}850$, $\epsilon_{328} = 2{,}230$; for nickel: $\epsilon_{436} = 1{,}720$, $\epsilon_{328} = 35{,}210$ (2). The subscripts refer to the wave lengths in mμ. For a mixture of the two metal complexes in carbon tetrachloride the equations of the total absorbance, A, at each of these wave lengths, assuming a 1 cm. cell, are:

$$A_{328} = C_{Ni} \times \epsilon^{Ni}_{328} + C_{Cu} \times \epsilon^{Cu}_{328} \quad (1)$$

$$A_{436} = C_{Cu} \times \epsilon^{Cu}_{436} + C_{Ni} \times \epsilon^{Ni}_{436} \quad (2)$$

In order to utilize these equations in absorption measurements, it is more convenient to solve them simultaneously and put them in the form:

$$C_{Ni} = K^{Ni}_{328} \times A_{328} - K^{Ni}_{436} \times A_{436} \quad (3)$$

$$C_{Cu} = K^{Cu}_{436} \times A_{436} - K^{Cu}_{328} \times A_{328} \quad (4)$$

These K values are functions of the four molar absorptivities given above, and may be determined from absorbance measurements at 328 and 436 mμ for two different mixtures of nickel and copper of known concentrations by solving Equation 3 and 4 simultaneously. Having determined the four K values, the sample containing the unknown concentrations of copper and nickel is measured at the two wave lengths, and the nickel and copper concentrations then calculated from Equations 3 and 4.

Cobalt and bismuth also form DEDC complexes which are extracted by carbon tetrachloride in which they have major absorption peaks both around 367 mμ and 328 mμ. They thus can interfere with the nickel and copper analyses, but these interferences can be subtracted if their concentrations are known. Cyanide at all concentrations can also interfere by complexing the copper and nickel in competition with the DEDC.

Other metals which either precipitate in alkaline solution or with the DEDC can also interfere. The former include iron, titanium, uranium and aluminum, which may be kept in solution by the addition of sufficient citrate.

Similarly the addition of pyrophosphate prevents interference by manganese. If there are excessive amounts of cadmium, mercury, tin and zinc present, they may form precipitates with the DEDC which pass into the organic layer, but these can be removed by centrifugation.

For nickel alone the optimum range of the method is 0.007 to 0.05 mg/l; for copper alone, 0.025 to 0.18 mg/l.

III. Apparatus

Spectrophotometer for measuring at 328 and 436 mμ

Hydrogen or deuterium lamp for spectrophotometer

1 cm matched silica cells, (2)

Ring stand

Bunsen burner

Analytical balance and weights

Mortar and pestle

Filter funnel, 10 cm

Filter paper, 20 cm

Separatory funnel, 125 ml, (2)

Graduated cylinder, 100 ml

Dropper syringe, (4)

Watch glass for 250 ml beaker, (2)

Beakers:

150 ml, (2)

250 ml, (2)

400 ml, (2)

10 ml (4)

25 ml (4)

bottles, glass stoppered

1 liter (4)

100 ml (4)

250 ml (4)

IV. Solutions and Reagents

0.2M $CaCl_2$ stock solution (1 liter)

0.02M $CaCl_2$ stock solution (1 liter)

0.2M $MgCl_2$ stock solution (250 ml)

0.02M $CuCl_2$ stock solution (250 ml)

pH 5 buffer (100 ml)

(Prepare by mixing 23.9 ml 0.1M NaOH and 50 ml 0.1M potassium acid phthalate and dilute to 100 ml)

pH 8.4 buffer (100 ml)

(Prepare by mixing 8.5 ml 0.1M NaOH and 50 ml 0.1M H_3BO_3 solution and dilute to 100 ml)

0.4M $NaClO_4$ solution (2 liters)

Tap water (1 liter)

V. Procedure

A. Activity coefficient corrections

1. Prepare several 100 ml Ca^{++} solutions in the range of 0.1 to 0.001 M by diluting the $CaCl_2$ stock solutions.

Hydrochloric acid (1/1), (250 ml)

Phenol red indicator solution, (250 ml)

(Prepare by grinding 0.05 g phenol red in a mortar with 1.4 ml of 0.1N sodium hydroxide solution; dilute to 250 ml with water)

Sodium diethyldithiocarbamate (DEDC) solution, (250 ml)

(Prepare by dissolving 0.1 g of NaDEDC in 250 ml water and filter; extract twice with 20 ml portions of carbon tetrachloride; filter through wet, water washed filter paper)

Carbon tetrachloride, analytical grade, (1 liter)

Tetrasodium pyrophosphate solution, (100 ml)

(Prepare by dissolving 4 g of $Na_4P_2O_7 \cdot 10\ H_2O$ in water and dilute to 100 ml)

River water, (5 liters)

V. Procedure

A. Calibration

1. Prepare 250 ml of each of the following 4 calibrating solutions by pipetting the appropriate volumes of the copper and nickel standard solutions into a 250 ml volumetric flask and diluting with distilled water:

   Calibrating Solution A: 0.02 mg/l Ni; 0.025 mg/l Cu

   Calibrating Solution B: 0.02 mg/l Ni; 0.050 mg/l Cu

   Calibrating Solution C: 0.03 mg/l Ni; 0.025 mg/l Cu

   Calibrating Solution D: 0.03 mg/l Ni; 0.050 mg/l Cu

2. Extract each of these solutions and measure their absorbances at

the nickel peak in the vicinity of 328 mμ and the copper peak in the vicinity of 436 mμ, following the procedures in Part B. Determine the exact wavelengths of these two peaks using Calibrating Solution B, and perform all subsequent measurements at these wavelengths.

3. Using the absorbance measurements at the two wave lengths for Calibrating Solutions A and B, determine the 4 K values in Equations 3 and 4 in Part II. Repeat with Calibrating Solutions C and D, averaging the two values obtained for each K.

B. Extraction and measurement

1. Pipet 100 ml of the sample into a 125 ml separatory funnel. Add 2 drops of phenol red indicator, 4 ml of tetrasodium pyrophosphate solution, and 1 ml of citric acid solution; swirl solution between additions. Add ammonium hydroxide dropwise until the solution is violet (requires 12 to 15 drops). Add 5 ml of the DEDC solution, mix and add exactly 5 ml of carbon tetrachloride. Shake for 5 minutes, then allow 10 minutes for separation. Transfer the carbon tetrachloride extract to a 10 ml glass stoppered flask.

2. Repeat step 1 with 100 ml of distilled water, the final extract to be used as a blank.

3. Measure the absorbances of the extract from step 1, using that from step 2 as the reference solution, the two wave lengths in the vicinity of 328 and 436 mμ to be selected by scanning as

indicated in Part A.2.

4. For the unknown solutions calculate the copper and nickel concentrations, using Equations 3 and 4 from Part II, with the K values from Part A.3.

C. Analysis of river water sample

1. Acidify a 1000 ml sample of river water by adding 10 ml of concentrated (11.6M) HCl. Boil for 5 minutes. Cool and filter.

2. Proceed as in Part B.

D. Test for interferences

1. Acidify a 1000 ml sample of river water by adding 10 ml of concentrated (11.6M) HCl. Boil for 5 minutes. Cool and filter.

2. Add the appropriate volume of the standards to the filtrate so as to add 0.02 mg/l each of nickel and copper.

3. Proceed as in Part B.

VI. Data Treatment

Calculate the increment added for the copper and nickel in Part V.D, by subtracting the results of the analysis from Part V.C. Compare to the known added concentrations.

VII. References

1. American Society for Testing and Materials, "Standard Method of Test for Nickel in Industrial Water," A.S.T.M. No. D 1886-65.

2. J. M. Chilton, Annal. Chem., 25, 1274 (1953).

3. C. N. Reilley and D. T. Sawyer, "Experiments for Instrumental Methods," McGraw-Hill Book Co., Inc., 1961.

ANALYSIS WITH DIVALENT CATION SELECTIVE ELECTRODE

I. Objectives

The purpose of this experiment is to demonstrate the use of a divalent cation selective electrode, both for the measurement of calcium ion concentration and total hardness. The effects of pH and ionic strength will be measured, as well as interfering cations. The relationship between ion activity and concentration will be considered; also a comparison will be made of a direct potentiometric measurement and an incremental technique for determining calcium concentration.

II. Theory

The divalent cation selective electrode[a] is equally sensitive to calcium and magnesium ion activity, but is also sensitive to other divalent ions to varying extents, as well as to hydrogen and other univalent cations. In using this electrode in conjunction with a reference electrode and measuring the total cell potential with a high impedance volt meter, such as pH meter, the cell potential may be expressed as (1):

$$E_{CELL} = \text{const.} + (RT/2F) \times \ln \{(Ca^{++}) + (Mg^{++}) + \textstyle\sum_i K_i (i)^{2/Z_i}\} \quad \text{(D-2)}$$

where E_{CELL} is in volts, RT/2F at 25°C is 0.0295 volts, and ( ) refers to the activity of an ion; Z_i is the charge of the i ion and K_i is its selectivity constant. Some reported values of K are for Zn^{++}, 3.5; for Cu^{++}, 3.1; for

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a. Model 92-32, Orion Research, Inc., U.S.A.

for Ba^{++}, 0.94; and for Sr^{++}, 0.54 (2). The sensitivity to Ca^{++}, Mg^{++} and Cu^{++} will be measured in this experiment. The K value for H^{+} is very high, and, therefore, the electrode should not be used at low pH. It should be used at a minimum pH of 5, depending on the divalent ion activity, but preferably over 7. The effect of pH will also be measured in this experiment.

Because this electrode is equally sensitive to Ca^{++} and Mg^{++} and these usually constitute the major source of hardness ions in natural waters, it may frequently be used, therefore, to measure the hardness. However, such a measurement is expressed in concentration units, rather than activity; therefore, it is necessary to convert the activity reading obtained from the potential measurement to concentration, either by using an activity coefficient, f_i, which is the ratio of the activity to molar concentration according to

$$(i) = [i] \times f_i \qquad \text{(D-3)}$$

where [i] refers to concentration of the i species; or perform the calibration of the electrode at the same ionic strength as that of the test solution, so that f_i is the same during calibration and measurement. In that case the E_{CELL} is a measure of concentration, even though it is sensing activity. The ionic strength may be held constant by adding an electrolyte like 0.1M and 0.05M $NaClO_4$, and this will be done in this experiment. One difficulty here is that the electrode also senses Na^{+} concentration in the test and calibrating solutions are the same and the divalent ion concentration is not much less than 1/100 that of the Na^{+}, this should present no difficulties.

Finally, an incremental method of analysis will be utilized and compared to the direct potentiometric method. In this technique the Nernst slope, b,

is measured for a particular ion, with b = RT/2F in Equation D-2. Having done that a known incremental concentration of the test ion, in this experiment Ca^{++}, is added to the test solution, the potential being measured before and after the increment. Assuming a constant value of b and the same f_i in both measurement, it may be shown that

$$\Delta\ m/m = 10^{\Delta E/b} - 1 \qquad \text{(D-4)}$$

where Δm is the known incremental concentration, m is the unknown concentration of test ion in the original solution, and ΔE is the measured change in potential (1).

III. Apparatus

Divalent cation activity electrode, Orion Model 92-32 Calomel or silver-silver chloride reference electrode pH meter or specific ion meter

graph paper, semi log:

2 cycle

3 cycle

volumetric flasks:

100 ml (50)

200 ml (5)

1000 ml (5)

beakers:

250 ml (25)

pipets:

2 ml (2)

1000 ml

1500 ml

Volumetric flasks, glass stoppered:

100 ml, (2)

250 ml, (4)

1000 ml, (2)

Bottles, glass stoppered:

10 ml, (10)

250 ml, (10)

1000 ml, (4)

Pipets, delivery:

1 ml, (2)

5 ml, (2)

10 ml, (2)

25 ml, (2)

50 ml

Pipets, graduated:

5 ml

10 ml

IV. Solutions and Reagents

1000 mg/l copper ($Cu(NO_3)_2$ in dilute HNO_3), (one liter)

1000 mg/l nickel ($Ni(NO_3)_2$ in dilute HNO_3), (one liter)

Ammonium hydroxide solution (1/1), (250 ml)

Nitric acid solution (350 g per liter), (100 ml)

2. Using the divalent cation electrode, reference electrode, and meter, measure the potential for each of these solutions. Repeat twice.

3. Using 3 cycle semi-log paper, plot E versus Ca^{++} concentration (the latter on the semi-log scale) for each sequence of measurements.

4. For each solution calculate the Ca^{++} activity coefficient using the Davies equation

$$- \log f = 2 \times \left(\frac{\sqrt{I}}{1 + \sqrt{I}} - 0.2\ I\right)$$

where $I = 1/2 \sum_i M_i \times Z_i^2$

5. Plot E versus Ca^{++} activity for this series of solutions.

B. Effect of pH

1. Prepare a series of Ca^{++} solutions at pH 8.4 and an ionic strength of 0.1M by pipetting into a 100 ml volumetric flask 2 ml of pH 8.4 buffer, 25 ml of 0.4M $NaClO_4$ and the appropriate volume of $CaCl_2$ stock solution; dilute to 100 ml with distilled water. The final Ca^{++} concentrations are to be:

   0.02, 0.01, 0.005, 0.0025, and 0.00125M.

2. Repeat step 1 with pH 5.0 buffer.

3. Using the divalent cation electrode, reference electrode and meter, measure the cell potential for all the prepared solutions.

4. Using 2 cycle semi-log paper plot E versus Ca^{++} concentration (the latter on the semi-log scale) for each pH.

C. Effect of ionic strength

1. Prepare a series of Ca^{++} solutions at an ionic strength of 0.04M and pH 8.4 by pipetting into a 100 ml volumetric flask 2 ml of pH 8.4 buffer, 10 ml of 0.4M $NaClO_4$ and the appropriate volume of $CaCl_2$ stock solution; dilute to 100 ml with distilled water. The final Ca^{++} concentrations are to be:

   0.005, 0.0025, 0.00125 and 0.0005M

2. Measure these solutions with the divalent cation electrode.

3. Plot the results on semi-log paper and compare the results with those from Part B.

D. Incremental technique

1. Prepare 100 ml of a 0.00125M $CaCl_2$ solution in distilled H_2O.

2. Pipet 25 ml of this solution into a 100 ml volumetric flask, adding 25 ml of 0.4M $NaClO_4$, 2 ml of pH 8.4 buffer, and diluting to 100 ml with distilled water.

3. Repeat step 2, but adding an increment of $CaCl_2$ stock solution equivalent to 0.0005M Ca^{++} after the final dilution.

4. Measure the potentials of the solutions prepared in steps 2 and 3. Use Equation D-4 to calculate the Ca^{++} concentration of the solution prepared in step 1.

E. Hardness measurements

1. Prepare 100 ml of a mixture of 0.002 Ca^{++} and 0.002 Mg^{++} from the stock solutions.

2. Pipet a 25 ml aliquot of this mixture into a 100 ml volumetric flask, adding 2 ml of pH 4 buffer and 25 ml of 0.4M $NaClO_4$; dilute to 100 ml with distilled water.
3. Prepare 100 ml of a mixture of 0.002M Ca^{++}, 0.002M Mg^{++} and 0.002M Cu^{++} from the stock solutions.
4. Repeat step 2 witn the mixture from step 3.
5. Measure the potentials of the solutions prepared in steps 2 and 4, calculating the total divalent ion concentrations from the calibration curve prepared in Part B.1.

F. Measurement of tap water

1. Dilute a 25 ml aliquot of tap water as in Part B.1.
2. Measure the potential of this solution and calculate the divalent ion concentration by comparison with the calibration solution measured and plotted in Part B.4.
3. Dilute a 25 ml aliquot of tap water as in Part D.2.
4. Dilute a 25 ml aliquot of tap water as in Part D.3.
5. Measure the potentials of these solutions and calculate the divalent ion concentration as in Part D.4.
6. Compare the results obtained in steps 2 and 5.

VI. Questions

A. Are the effects of ionic strength and pH important enough in divalent ion measurements that these variables should be controlled when measuring environmental samples?

B. Is the incremental technique as accurate as that of direct potential

measurement? In what way might it be more useful?

C. Under what circumstances is the divalent ion electrode not a good sensor for total divalent ion concentration?

VII. References

1. J. B. Andelman, J. Water Poll. Control Fed., 40, 1844 (1968).

2. Orion Research, Inc. Manual for Divalent Cation Activity Electrode, Model 92-32.

POLAROGRAPHY

I. Objectives

The purpose of this experiment is to demonstrate the analytical feasibility of polarographic technique for metal analysis in natural and waste waters. The experiment also points out some of the important aspects of polarography such as the verification of the Ilkovic equation, effect of oxygen and surface active agents and methods employed for obtaining quantitative results.

II. The Ilkovic Equation

The equation commonly used to describe the diffusion current relationships using the dropping mercury electrode is the equation,

$$i_d = \left\{k\ n\ D^{1/2}\ m^{2/3}\ t^{1/6}\right\} C \qquad (1)$$

where

i_d = average diffusion current, amp,
t = droptime, sec,
m = flow of mercury, mg/sec,
D = diffusion coefficient of ion under investigation cm^2/sec,
C = concentration of ion under investigation, M/l,
n = number of electron equivalents per mole of reactant,
k = constant equal to 0.607.

Temperature effect is not included in this equation and the temperature should be maintained constant during the calibration and the analysis of samples. The constant "k" should be equal to 0.607 at temperatures between 15^oC and 40^oC, when other quantities are expressed in the units indicated above.

A fundamental constant, specific for any given substance, can be derived from equation 1.

$$I = k\ n\ D^{1/2} = \frac{i_d}{C\ m^{2/3}\ t^{1/6}} \qquad (2)$$

where I is referred to as the "diffusion current constant". The constant I is independent of the characteristics of the electrode.

In the case of a metal ion reversibly reduced to the metallic state and for using an amalgam, the electrode potential can be given by the following relationship,

$$E_{M^{n+}/M} = E^{o}_{M^{n+}/M} + \frac{RT}{nF} \ln \frac{[M^{n+}]\,(Hg)}{[M]_{Hg}} \qquad (3)$$

where

$E^{o}_{M^{n+}/M}$ = standard reduction potential, volts,

R = gas constant

T = absolute temperature

F = the Faraday

Under these conditions it is possible to relate the electrode potential (applied voltage), half-wave potential ($E_{1/2}$) and the current by the following relationship

$$E_{de} = E_{1/2} + \frac{0.059}{n} \log \left[\frac{(i_d - i)}{i} \right] \qquad (4)$$

where

E_{de} = potential of the dropping mercury electrode, volts

$E_{1/2}$ = half-wave potential,

i = current at E_{de} (corrected for residual current),

i_d = diffusion current,

$0.059 = \frac{RT}{F}$ at 25°C.

By plotting $\log \left[\frac{(i_d - i)}{i} \right]$ versus E_{de}, equation 4 can be represented by a straight line the slope of which is equal to $\frac{0.059}{n}$ and an ordinate intercept equal to $E_{1/2}$. This is illustrated by Figures 1 and 2. In Figure 1 the current-potential curve for a reversible redox system is plotted showing numerical values for both i_d and $(i_d - i)$. These values were used in equation 4 to plot the straight line in Figure 2 from which the values of $E_{1/2}$ and n

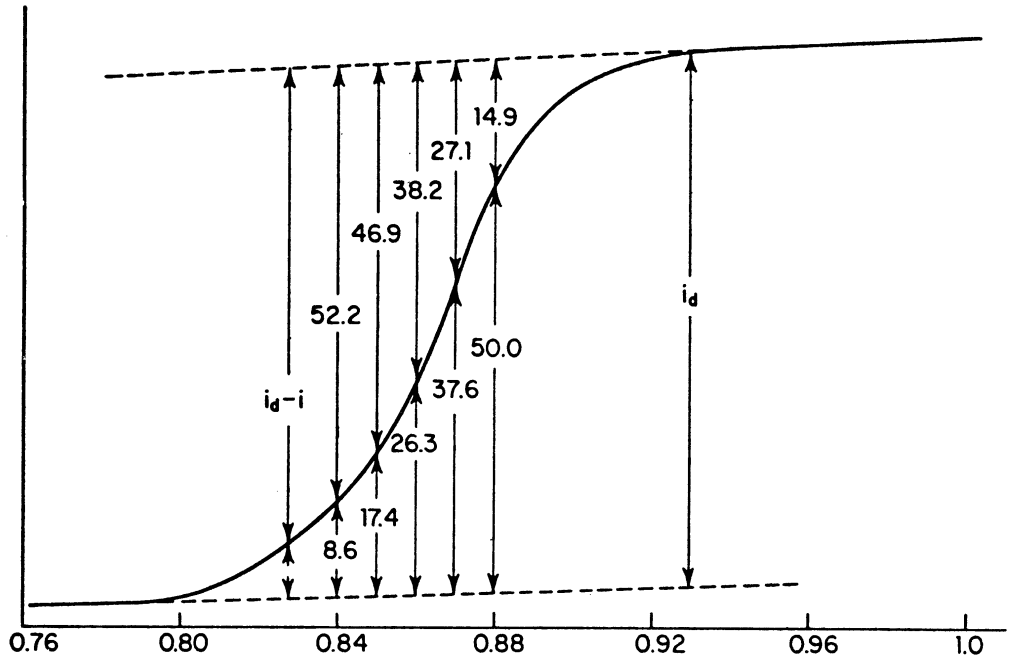

Fig. 1. Method for obtaining data for the determination of "n" and $E_{1/2}$

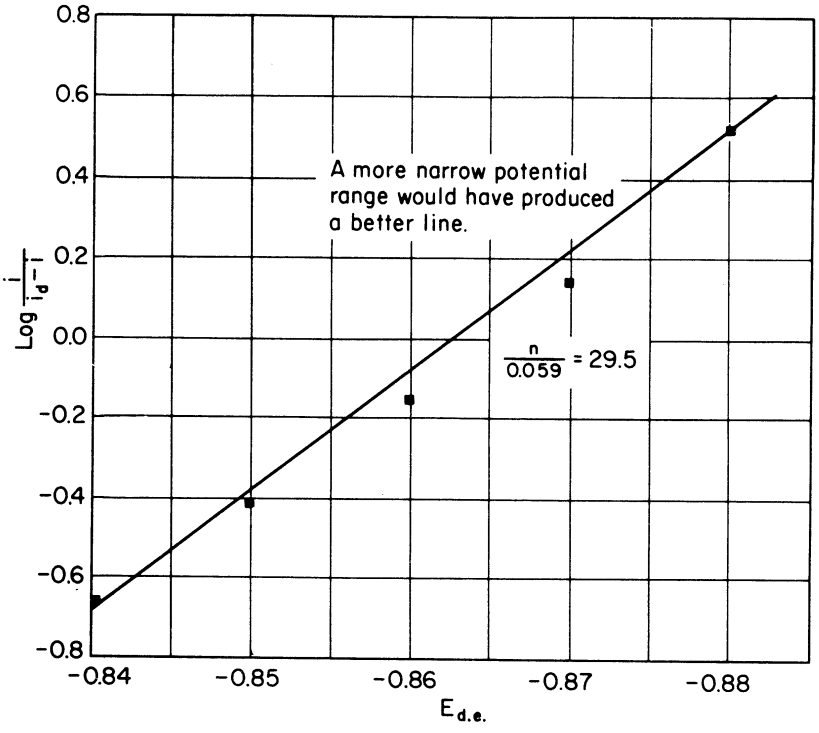

Fig. 2. A plot of log $\left[\frac{i}{i_d-i}\right]$ vs. $E_{d.e.}$ to obtain "n" and $E_{1/2}$.

were easily calculated and found to be equal to 1.8 and -0.62V, vs. S.C.E., respectively.

Apparatus

Recording polarograph
Dropping mercury electrode system
Polarographic cell with a reference electrode
Nitrogen system for deaerating the test solution
Volumetric flasks (3), 100 ml
Pipets, one each, 1 ml, 5 ml, 10 ml, 15 ml

Solutions

HNO_3, 6M
KCl, 0.001M
Triton x-100, 0.2% (surface active agent)
HCl supporting electrolyte, 0.2M (17 ml concentrated HCl/liter)
$CdCl_2$, 0.02M standard solution

Procedure

1. Prepare three dilutions, 0.001M, 0.002M and 0.003M of the $CdCl_2$ solution. Exactly pipet 5 ml, 10 ml and 15 ml portions of 0.02M $CdCl_2$ standard solution into three separate 100 ml volumetric flasks and dilute exactly to the mark with 0.2 M HCl supporting electrolyte.

2. Deaerate each solution and determine its polarogram between -0.1 and -1.2 volts versus saturated calomel electrode.

3. Determine the halfwave potential $E_{1/2}$ which is the point of inflection in the polarogram for each solution.

4. Determine the diffusion current from the polarograms of the 0.01M, 0.002M and 0.003M $CdCl_2$ solutions (as illustrated in Figure 3). Plot the value of the diffusion current against the concentration of $CdCl_2$ solutions. A straight line shows adherence to the Ilkovic equation (Figure 4).

5. Plot the values of the half-wave potentials, $E_{1/2}$, against the diffusion current for the three $CdCl_2$ solutions. Draw a straight line through these three values and extrapolate to zero diffusion current. The ordinate intercept should be equal to the true half-wave potential.

6. Plot $\log \left[\frac{(i_d - i)}{i} \right]$ against potential for the polarogram of the 0.001M $CdCl_2$ and claculate the values of n and $E_{1/2}$.

III. Analysis for Cu(II), Cd(II), Ni(II), and Zn(II)

There are several methods for instrument calibration to obtain quantitative measurements.

1. Absolute Method. This is based on using the value of the diffusion current constants "I" given in the literature. By determining both "m" and "t" for the dropping mercury electrode system the concentration of the electroactive ion in the test solution can be determined from equation 2.

2. Method of Standard Samples. This technique is based on determining the diffusion current for a number of standard samples and plotting a diffusion current-concentration calibration curve.

3. Method of Standard Addition. When only a few samples are to be analyzed, the method of standard addition appears to be the most convenient. Two polarograms are required; one for the unknown and one for the unknown containing an added known amount of the same material. Figure 4 indicates how the experimental data can be utilized. The volume of the solution changes upon the addition of the standard and this produces a corresponding change in the concentration. The result is that a correction for this dilution must be made as follows:

$$C_{unk} = \frac{i_d \, v \, C_{std}}{[\Delta i(V+v) + i_d v]} \qquad (5)$$

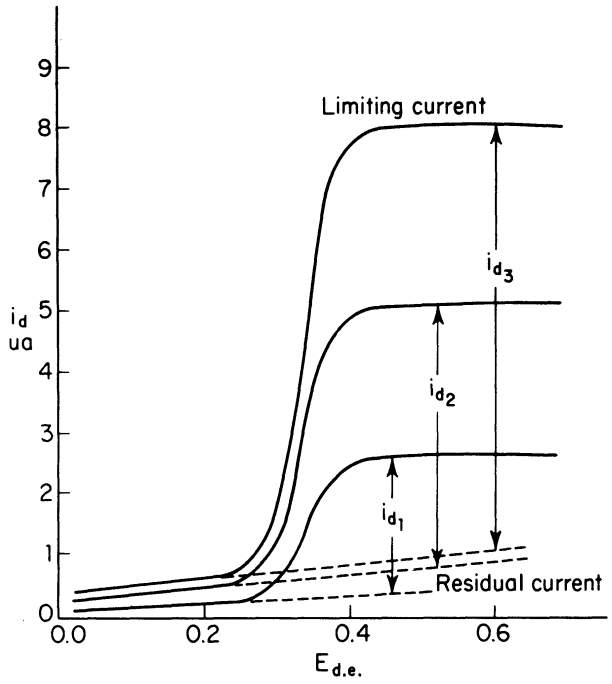

Fig. 3. Polarographic curves at three different concentrations.

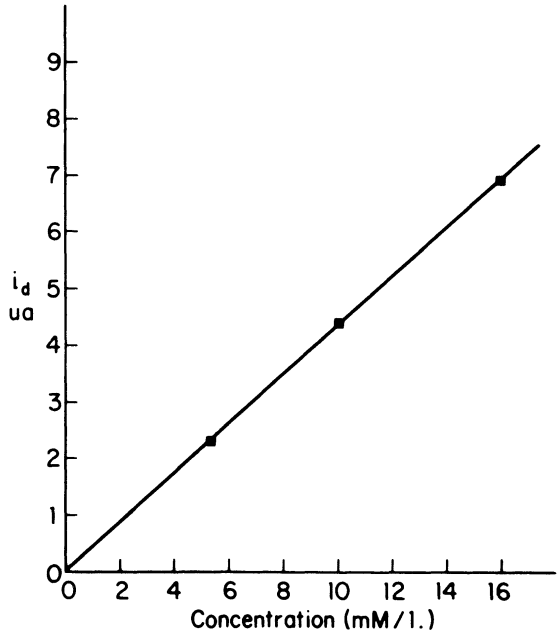

Fig. 4. Calibration curve obtained from the data in Figure 3.

where i_d = the diffusion current of the unknown alone,

Δi = the increase in the diffusion current due to the added standard,

V = original volume of the solution,

v = volume of the standard added,

C_{unk} = concentration of the unknown,

C_{std} = concentration of the standard.

4. Method of Internal Standard. This method is probably the least used method of any presented thus far. However, since the principle involved is rather interesting it is included. Two substances present in a solution in equal concentrations will each have a diffusion current. The ratio of these diffusion currents will be independent of the capillary used, providing the usual parameters of temperature and supporting electrolyte are kept constant. Once this ratio has been established, a known amount of one of the substances can then be placed in a solution of an unknown of the other substance, thus acting as an internal standard, and the unknown concentration can be evaluated. This is expressed by equation 6.

$$\frac{i_{d(1)}}{i_{d(2)}} = \frac{I_1 C_1}{I_2 C_2} \qquad (6)$$

where $i_{d(1)}$ and $i_{d(2)}$ are the diffusion currents of the internal standard and the unknown, respectively. (I_1/I_2) is the ratio of the diffusion current constants ($I=607\ n\ D^{1/2}$) and C_1 and C_2 are the concentrations of the standard and the unknown, respectively. The I values can be calculated from the relationship, $I = 607\ n\ D^{1/2}$ if the diffusion coefficients are known. An easier and more accurate way of evaluating I is to prepare solutions of known strength and calculate the ratio based on equation 6.

Apparatus

Recording polarograph
Dropping mercury electrode system
Polarographic cell
Nitrogen for deaerating test solution

Solutions

KCl, 0.001M
Triton X-100 0.2% (surface active agent)
Tap water equilibrated to room temperature
NH_4Cl, anhydrous salt
Ammonia, concentration solution
Standard solutions of Cu(II), Cd(II), Ni(II) and Zn(II), 0.001 M each,
A sample of wastewater which contains Cu(II), Cd(II), Ni(II) and Zn(II).

Procedure

1. Run a polarogram on a sample of 0.001M KCl from 0 to -1.9 volts versus saturated calomel chloride.

2. Add two drops of 0.2% of Triton x-100 (surface active agent) to a sample of 0.001M KCl, stir well, and determine polarogram as above.

3. Run a polarogram on a tapwater sample equilibrated to room temperature.

4. Characterize and discuss the two-step reduction waves of molecular oxygen in the above samples.

5. Withdraw samples of the Cu(II), Cd(II), Ni(II) and Zn(II) solutions, adjust to about 0.1M in ammonium chloride and 1M in ammonia and add sufficient maximum suppressor of Triton X-100. (Before determining the polarograms, each sample solution should be stripped of dissolved oxygen.)

6. Run polarograms on the copper, cadmium, nickle and zinc samples, separately, and determine the half-wave potential $E_{1/2}$ for each case.

7. Prepare a sample containing a mixture of Cu (II), Cd (II), Ni (II) and Zn (II) and adjust the medium as outlined in item 5. Run a polarogram of the sample solution for the simultaneous determination of the metal ions. Characterize the reduction wave for each metal and compare with results obtained from item 6.

8. Evaporate till dryness on exact volume of the wastewater sample, dilute with distilled water and adjust the medium as outlined in item 5. Prepare a polarogram for the sample and characterize types and quantities of metal ions present.

IV. Dissociation Constants and Complex Formation:

Polarography provides a unique technique for the study of metal complexes and the determination of their formula and stability constants. Procedures for investigating metal complexes are based on the fact that the characteristic half-wave potential, $E_{1/2}$, of a free metal ion is shifted when the metal ion undergoes complex formation. The degree of the shift in the half-wave potential is a function of the concentration of the ligand. Hence by measuring the shift in $E_{1/2}$ at various ligand concentrations, it is possible to characterize the metal complex.

It is assumed that a single complex species predominates over a wide range of concentration e.g. chelate complexes; according to the following equation

$$MX_p^{+n} + ne \rightleftharpoons M + pX$$

the shift in half-wave potentials can be estimated as follows

$$\left(E_{1/2}\right)_c - \left(E_{1/2}\right)_s = \frac{0.0591}{n} \log K + p\frac{0.0591}{n} \log C_X \qquad (7)$$

where

$\left(E_{1/2}\right)_c$ = half wave potential of the complex ion

$\left(E_{1/2}\right)_s$ = half wave potential of the simple metal ion

K = dissociation constant of the complex

p = number of ions involved in the complex

C_X = molar concentration of the complex ion

Apparatus

Recording polarograph
Dropping mercury electrode system
Polarographic cell with a reference electrode
Nitrogen system for deaerating the test solution
Volumetric flasks (5), 100 ml
Pipets, one each, 2 ml, 10 ml, 20 ml, 30 ml and 50 ml.

Solutions

$Pb(NO_3)_2$, 0.02M
KNO_3, 1.0M
Organic ligand "X" (extracted from pharmaceutical waste effluent).

Procedure

1. In each of the five volumetric flasks, pipet 2.00 ml of the lead nitrate solution. Into each of these flasks also place

Flask	Substance
1	10 ml of 1.0MKNO_3
2	10 ml of Organic Ligand Solution
3	20 ml of Organic Ligand Solution
4	40 ml of Organic Ligand Solution
5	80 ml of Organic Ligand Solution

Mix each solution thoroughly.

2. Run polarograms on deaerated samples of each of the above solutions.

3. Determine half-wave potential for each curve and test for the reversibility of the wave by plotting $[(i_d-i)/i]$ vs. E from which calculate the value of n.

4. From a plot of $E_{1/2}$ vs. log X determine p.

5. Calculate the stability constant for the complex using equation 7.

Questions

1. Discuss the effect of organic matter frequently present in waste water, on metal ions determination by the above procedures.
2. Compare the analytical feasibility of the polarographic dropping mercury electrode and membrane electrodes for the analysis for copper ions and dissolved oxygen.
3. Explain the reason for testing the polarographic waves in experiment IV for reversibility of the electrode reaction?

IV. Salinity Effect

Procedure:

1- Prepare seven samples, one liter each, place in separate beaker labelled as follows

A- Distilled Water E- 0.5M NaCl

B- 0.001 M NaCl F- 1.0M NaCl

C- 0.01 M NaCl G- 2.0M NaCl

D- 0.1M NaCl

2- Allow the seven sample solutions to equilibrate overnight with air at constant temperature (room temperature).

3- Determine the dissolved oxygen content of each sample by the membrane electrode at optimum stirring rates and constant temperatures.

4- Determine the dissolved oxygen content of each sample by the Winkler titration technique.

5- Plot dissolved oxygen measurements by the membrane electrode and by the Winkler titration as a function of salt content.

6- Calculate the salting out coefficient (K_s)

7- Construct a calibration curve to account for the salting out effect.

V. Temperature Effect

Procedure:

1- Equilibrate a large volume of tap water (5 liters) with air at about $5^{o}C$ in a large glass or plastic container.

2- By means of a magnetic stirrer (or a motor-driven stirrer) mix the solution at a constant rate.

3- Immerse the membrane electrode in the water solution and determine the dissolved oxygen content at about $5^{o}C$.

4- Simultaneously determine the dissolved oxygen content of the test solution by the Winkler method.

5- Slowly increase the temperature of the test solution up to about 30°C while simultaneously determine the dissolved oxygen content by the membrane electrode and by the Winkler test at approximately 5°C intervals. Be sure that the test solution is in equilibrium with air at all temperatures.

6- Plot the dissolved oxygen content by membrane electrode and by the Winkler test as a function of temperature.

7- Discuss the effect of temperature on each method of analysis for dissolved oxygen. Differentiate between the effect of temperature on the solubility of molecular oxygen and the effect on the measurement technique.

8- Contruct a calibration curve to account for the temperature effect.

VI. BOD Determinations

Procedure:

1- Using an assumed or estimated BOD value for raw sewage as a guide, calculate the factors for a range of dilutions to cover the desired depletions. Those dilutions which exhibit a depletion of 2 mg/l and a residual of 1 mg/l are most reliable. At least three dilutions in duplicate should be used for an unknown sample.

2- Into a larger container measure accurately the required amount of sample to give five liters of diluted waste. Fill to the mark with dilution water (Std. Meth., 12th Ed., p. 416). Carefully mix, avoiding the entrapment of air bubbles.

3- Siphon the mixture from the cylinder into fourteen 300 ml glass stoppered bottles, filling the bottles to overflowing.

4- Determine the DO concentration on two of the bottles by the appropriate Winkler modification and record as "Intial DO".

5- Incubate the twelve remaining bottles at 20°C in

complete darkness. The incubated bottles should be water-sealed by inversion in a tray or by using a special water-seal bottle.

6- For a period of 5 days of incubation, at desired intervals, determine the DO on the bottles. Preferably run duplicate samples at daily intervals. Average the DO concentration of the duplicates and report as "Final DO".

7- Insert two D.O. membrane electrodes in the remaining two BOD bottles and continuously monitor the DO content in each bottle over a period of 5 days. The two BOD bottles should be immersed in a $20^{o}C$ water bath and sufficient mixing should be maintained in each bottle by means of magnetic stirrers.

8- Calculate the 5-day BOD from data obtained by the membrane electrode and the Winkler titration.

9- Calculate the BOD rate constant "k" from data obtained by the membrane electrode and the Winkler titration.

VIII. Questions

1- Explain the descrepancy between dissolved oxygen measurements by the membrane electrode and the Winkler titration techniques in waters of varying salinity and varying temperatures.

2- If you are a fish in a stream, would you be more concerned with the activity level of molecular oxygen or the concentration level? Why?

3- What is the effect mixing on the BOD test?

ANALYSIS OF CHEMICAL OXYGEN DEMAND (COD)

I. Objective:

In the COD test dichromate is reduced to chromic ion by the reaction

$$Cr_2O_7^{-2} + 14H^+ + 6e^- \longrightarrow 2Cr^{+3} + 7H_2O$$

In the manual test the excess dichromate is determined by titration with ferrous iron. Since both species of chromium are colored, the extent of the reaction may also be measured by the decrease of the orange $Cr_2O_7^{-2}$ or the increase in the green Cr^{+3}. The absorbance maximas of $Cr_2O_7^{-2}$ and Cr^{+3} are 420 and 600 mμ respectively. Calibration curves will be prepared at both 420 and 600 mμ and the effect of chloride will be determined using the Auto Analyzer. River and waste water samples will be analyzed by both the manual and automated proceedure.

II. Apparatus:

Auto Analyzer
Manifold for automated COD (figure1)
Volumetric flasks:
- 1000 ml
- 100 ml

Burets:
- 25 ml (graduated to 0.1 ml)

Pipets:
- 1 ml
- 5 ml
- 10 ml
- 25 ml

Erlenmeyer flasks:
- 500 ml

Boiling flasks with 24/40 joint:
- 500 ml

Fredrichs Reflux condenser 24/40 joint:
Ring stand
Buret clamp
Glass beads

III. Reagents:

A. Automated Method

Digestant: 7.5 g Ag_2SO_4 and 3.07 g $K_2Cr_2O_7$ per liter of concentrated sulfuric acid.

Mercuric Sulfate: 3.75 g $HgSO_4$ /liter

Glucose Standard: Dissolve 3.754 g glucose/liter of distilled water. This solution has 4000 ppm COD.

Chloride Solution: Dissolve 16.48 g NaCl/liter water. This solution contains 10,000 ppm Cl^-.

B. Manual Method

Potassium dichromate solution (0.250N): Dissolve 12.259 g $K_2Cr_2O_7$, which has been dried for 2 hours at 103° C, in distilled water and dilute to one liter.

Sulfuric Acid Reagent: 8.4 g Ag_2SO_4 /liter conc. H_2SO_4. Requires 1 to 2 days to dissolve.

Ferrous O-phenanthroline indicator

Mercuric sulfate

Ferrous ammonium sulfate (approx. 0.250N)-Dissolve 98g $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ in distilled water. Add 20 ml conc. H_2SO_4, cool and dilute to one liter.

IV. Experimental Procedure:

A. Prepare a standard series of 0, 40, 80, 120, 160, and 200 ppm COD. Analyze samples by the AutoAnalyzer manifold shown in Figure 1 without dilution at both 420 and 600 mμ . At each wavelength analyze the several river and waste water samples.
(Some samples may have to be manually diluted to be analyzed).

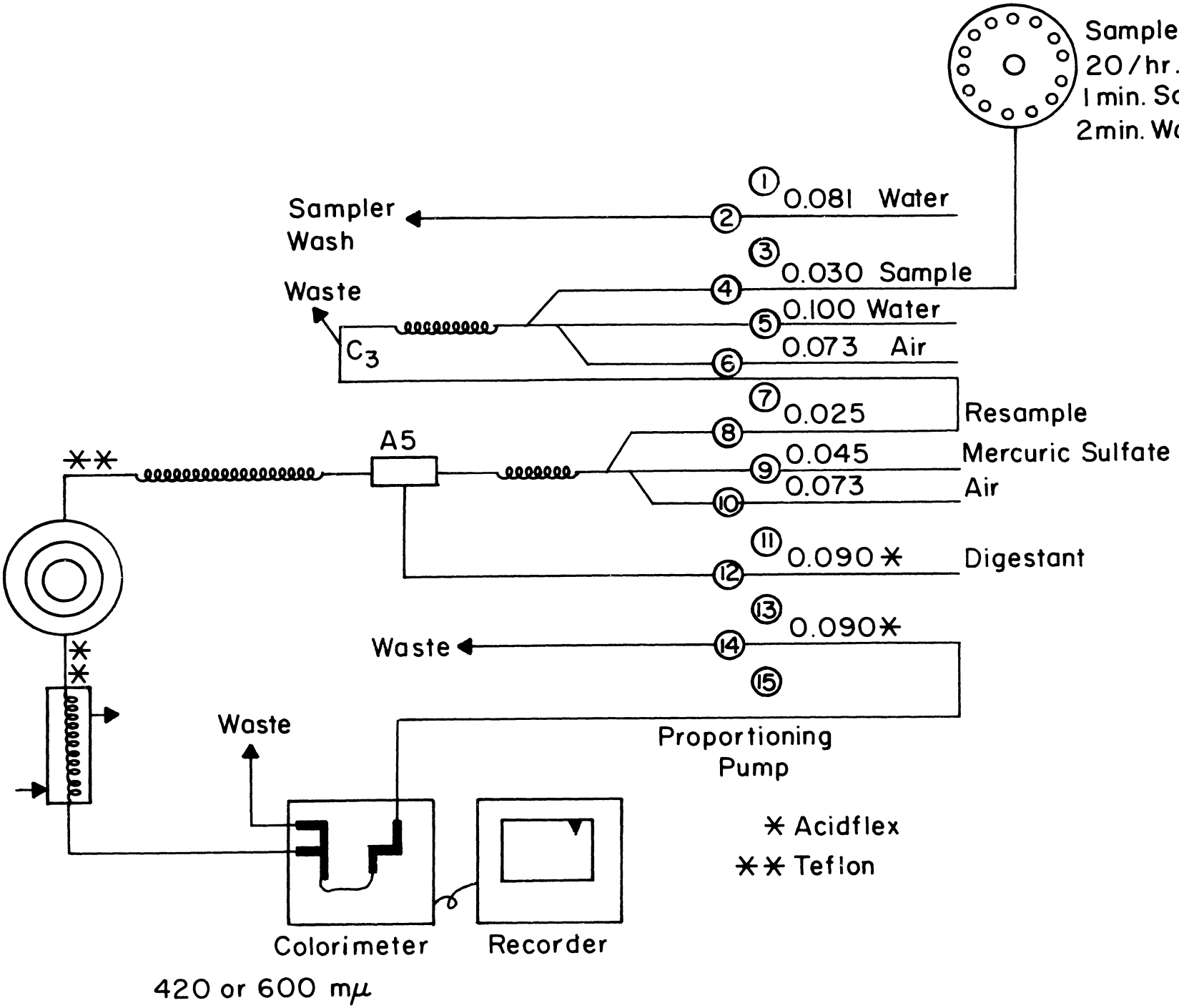

FIGURE 1. AUTO ANALYZER CHEMICAL OXYGEN DEMAND MANIFOLD.

B. Determine the interference of chloride by analyzing at 600 mμ a series of samples containing 120 ppm COD and 200, 400, 600, 800, and 1000 ppm Cl^-. For this experiment the mercuric sulfate should be replaced by water. Replacing the tube in mercuric chloride analyze solutions containing 120 ppm COD and 500, 1000, 1500, 2000, 2500, and 3000 ppm Cl^-.

C. Connect the dilution line and analyze at 600 mμ series of samples containing 0, 400, 800, 1200, 1600, and 2000 ppm COD. Analyze the river and waste water samples at 600 mμ.

D. Standardize the ferrous ammonium sulfate solutions by pipeting 25 ml of 0.25N $K_2 Cr_2 O_7$ into a 500 ml Erlenmeyer flask and adding 250 ml of distilled water and 50 ml of concentrated sulfuric acid. After the solution has cooled add 10 drops of ferrous O-phenanthroline indicator and titrate to a red end point with ferrous ammonium sulfate.

E. Analyze a 400 ppm COD standard, a blank, and each of the river and waste water samples by:

Placing 0.4g $HgSO_4$ in the reflux flask, adding 20 ml of the sample and mixing. Add 10.0 ml of the potassium dichromate. Carefully add 30 ml of the sulfuric acid reagent and mix. Add glass beads to prevent bumping during the refluxing. After refluxing the mixture for 2 hours, cool and wash down the condenser with distilled water.

Dilute the mixture to about 100 ml, add 2-3 drops of indicator and titrate with the ferrous solution. At the end-point the color will change from blue-green to red brown.

V. Calculations:

A. Normality of ferrous ammonium sulfate solution=

$$\frac{25 \times 0.25}{\text{ml ferrous solution}}$$

B. Calculations of COD

$$\text{COD mg/l} = \frac{(a - b) \times \text{normality of ferrous soln.} \times 8000}{\text{ml of sample}}$$

a = ml ferrous ammonium sulfate used for blank
b = ml ferrous ammonium sulfate used for sample

Questions:

What are the advantages and disadvantages of using each of the wavelengths in the COD determination?
How can the sensitivity of an automated colorimetric procedure be increased?
Why does the chloride interfer in the COD test? Why does mercuric ion prevent its interference?

References:

1) M.H. Adelman, "Automation in Analytical Chemistry; Technicon Symposia 1966," Vol. I Mediad, New York, N.Y. 1967, p.552

2) American Public Health Association, "Standard Methods for the Examination of Water and Wastewater," 12th ed., New York, N.Y. 1965, p. 510.

3) A.H. Molof and N.S. Zaleiko, Proc. 19th Ind. Waste Conf., 540 (1964).

TOTAL CARBON ANALYSIS

I. Objectives

This experiment illustrates the utility of a commercial total carbon analyzer for the rapid determination of small concentrations or organic carbon in water regardless of the organic structures present and in the presence of inorganic carbon forms.

II. Apparatus

Total Carbon Aanlyzer, with regulated oxygen supply

Assorted beakers and erlenmeyer flasks

Magnetic stirrer with stirring bar

Regulated nitrogen supply for sparging

Pipets (3), 5, 10 and 20 ml volumetric

Volumetric flasks (10), 100 ml

Microliter syringes (2), 25 and 50 µl capacities; Hamilton 702N and 705N with standard 2" needles (non-tapered point) with Chaney adapters.

Kimwipe laboratory tissue

III. Reagents

Concentrated HCl

Na_2CO_3 reagent grade; 1000 mg/l as C

Potassium acid phthalate, reagent grade; 1000 mg/l as C

Glucose; 1000 mg/l as C

Acetic acid, glacial; 1000 mg/l as C

IV. Theory

Pertinent chemical theory and the interpretation of total carbon data were presented in Chapter X. A block flow diagram for the total carbon analyzer is shown in figure 1, and the function of most components is self explanatory. The detector system is a non-dispersive infrared analyzer sensitized to CO_2 (figure 2). Two IR sources direct emitted radiation through sample and reference tubes directly onto both sides of a divided cell charged with equal concentrations of CO_2 and separated by a pressure sensitive diaphragm. Both beams are simultaneously chopped at 10 cycles/sec. When there is no CO_2 in the sample (or when both beams are blocked by the chopper) there is an equal intensity of radiation striking both sides of the divided cell and the pressure increase due to absorption of radiation by the CO_2 is equal resulting in no movement of the diaphragm. If CO_2 appears in the sample stream a pressure imbalance will be created across the diaphragm due to CO_2 absorption in the sample tube and the diaphragm will distend toward the sample side. As the beams are once again blocked, however, the diaphragm will return to a balanced condition. Thus the motion of the diaphragm is the source of electrical signal as it constitutes one plate of a two plate variable capacitor. The other plate is a stationary metal button near the center of the diaphragm. The variable capacitance produced modulates a radio frequency signal from an oscillator which is then demodulated, amplified and used to drive a recorder.

The unit is non-dispersive in the sense that it contains no monochromator and the IR sources are broad band emitters. Actually the pressure sensitive

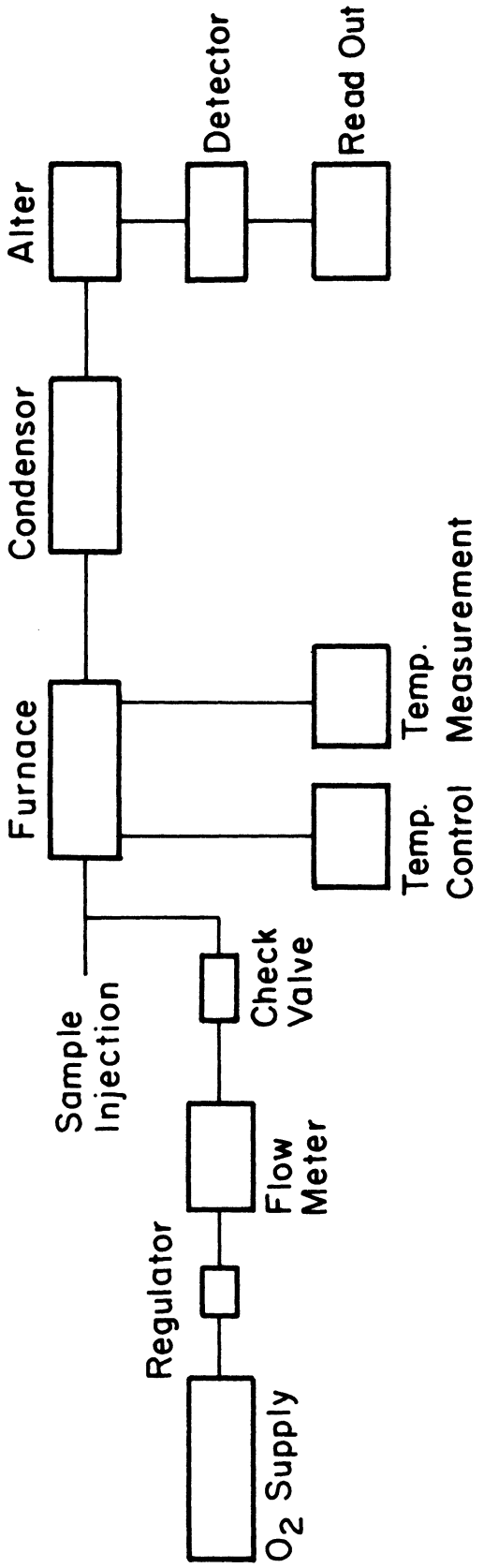

Figure 1. Block Diagram of Total Carbon Analyzer

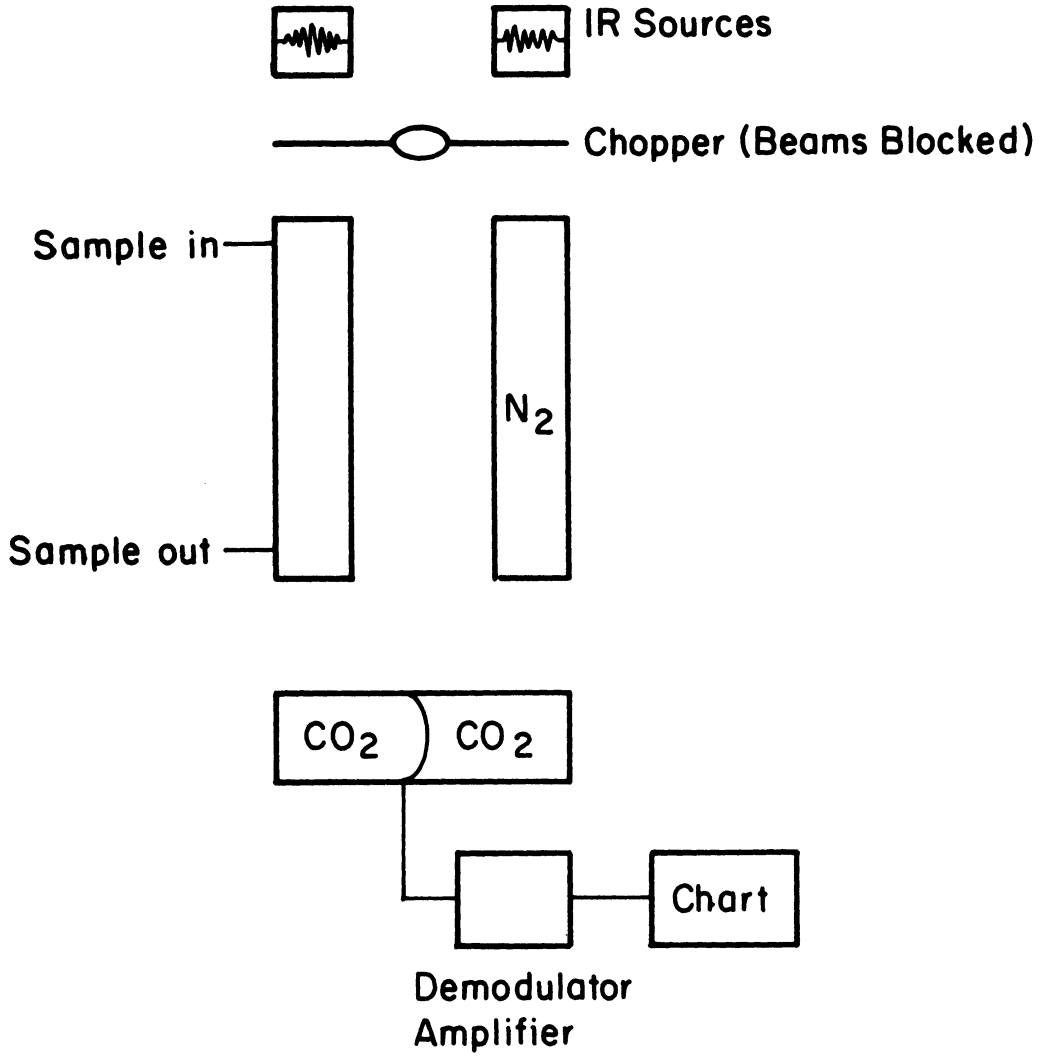

Figure 2. Non-Dispersive Infrared Analyzer

detector cell acts as it's own monochromator being affected only by the presence or absence of wavelengths absorbed by CO_2. Thus the only interference possible in the sample stream would be caused by gases which have overlapping IR absorption bands with CO_2 (near 3.0 μ). Since H_2O vapor absorption partially overlaps this region it is removed by condensation prior to admission to the IR detector.

V. Procedure

The analyzer unit should be warmed up at least 2.0 hours prior to use with the oxygen flow adjusted to 50 ml/min and furnace temperature adjusted to 950°C. Prepare dilutions of the potassium acid phthalate standard to yield 25, 50, 100, 150 and 200 mg C/l operating standards.

a. Practice the injection procedure by injecting replicate 20 μl samples of the 100 ppm standard with the gain control adjusted to yield mid-scale recorder response, until uniform response is obtained. The use of the Chaney adaptor will allow reproducible volumes to 0.1 - 1.0%. Carefully observe what injection technique gives uniform response (i.e., speed of injection, syringe position, etc.) Peak reproducibility of 1% or less should be obtained for mid-scale deflection.

   Similarly inject each operating standard in triplicate and prepare a standard curve by plotting peak height versus carbon concentration. Be certain to correct all samples for a distilled water blank.

b. Prepare 100 mg C/l operating standards from the stock solutions of

sodium carbonate, glucose, and acetic acid. Inject 20 µl samples of each in triplicate and compare the results to the standard curve obtained with potassium acid phthalate without readjusting the gain setting used for the standard curve.

c. Mix equal volumes of the 100 mg/l sodium carbonate and the 100 mg/l glucose operating standards. Inject 20 µl samples and determine carbon concentration from the standard curve. Add 3 drops of concentrated HCl to 100 ml of the mixture and sparge with nitrogen for 3-5 minutes at 200 ml/min. Inject 20 µl samples of the spurged solution and determine carbon concentrating from the standard curve.

d. Repeat (c) above for an equal volume mixture of the 100 mg/l operating standards of glucose and acetic acid.

VI. Questions

1. Does the acidification and sparging procedure appear to remove inorganic carbon?
2. What affect does the acidification and sparging procedure have on the concentration of a volatile acid such as acetic acid?
3. Speculate on the possible effect of acidification and sparging on aqueous solutions containing small concentrations of ethanol, acetone, phenol and benzene.
4. Estimate the lowest concentration of dissolved carbon detectable with your procedure.

VII. References

1. C. E. VanHall, J. Safranko and V. A. Stenger. Anal. Chem. 35, 315 (1963).

2. C. E. VanHall, D. Barth and V. A. Stenger. Anal. Chem. 37, 769 (1965).

3. R. B. Schaffer, et al. Jour. Water Poll. Control Fed., 37, 1545 (1965).

VOLATILE ACID ANALYSIS

I. Objectives

In this experiment we shall determine the concentrations of various short chain (volatile) acids which are found in anaerobic biological treatment units. Both the concentration of total volatile acids and the relative amounts of the various individual acids are of importance in maintaining anaerobic digestor control.

It has been found that when the total volatile acid concentration in such units exceeds about 2000 mg/l, digester failure may occur. By maintaining a daily analyses of total volatile acids in the unit, the onset of potential problems can be discovered by noting an increase in total volatile acids before the concentration approaches 2000 mg/l. Necessary corrective measures can then be undertaken to reduce the volatile acid level well before problems arise.

The relative concentrations of individual volatile acids are also important from a digester stability standpoint. It has been noted (1) that when the ratio of propionic acid to all other acids increases, the anaerobic unit may be approaching an unstable condition. Often the total volatile acid increase may be so small as to seem insignificant, however, if the percent of propionate is also increasing, corrective measures should probably be undertaken.

II. Apparatus

Gas chromatograph - hydrogen flame detector
10 ul Hamilton syringe
Column - carbowax 20 M (9%) - phosphoric acid (1%) on 60-80 mesh chromosorb
W. 12 ft long - 1/8" O.D. stainless steel.

III. Solutions

Volatile acid mixtures of known concentration

Acetic, Propionic, Butyric, Valeric, Caprionic in Water

3 standards approximately	100 mg/l each
	400 mg/l each
	700 mg/l each

IV. Theory

The general principles of gas chromatography have been presented in the theory section.

V. Procedure

The column, injector port, and detector should be turned to the desired settings and allowed to heat at least three hours before use.

Temperature Settings:	
Column	130°C
Injection port	200°C
Detector	200°C
Carrier Gas Flow Rate:	20 ml/min
Hydrogen Flow Rate:	20 ml/min
Air Flow Rate:	300 ml/min
Sample Size:	5 ul
Carrier Gas	Helium

About ten minutes before use, turn on carrier gas then hydrogen and air to the detectors.

Inject the lowest concentration of the volatile acid mixture. Adjust the range and attenuation switches if necessary so that the maximum peak height just stays on the chart. Follow each volatile acid injection with three 10 ul injections of distilled water to remove "ghost" peaks. Inject each of the known concentrations mixtures three times.

Determine the peak area and height for each volatile acid peak. Plot both peak area area versus concentration of volatile acid and peak height versus concentration for each of the five volatile acids.

Measure the distance on the chart from the point of injection to the center of each volatile acid peak.

The identification of each volatile acid in an unknown sample can be determined by locating the peak from the point of injection. The concentration of any volatile acid can be determined by first measuring the peak area then using the peak area versus concentration plot to determine the concentration.

Three samples of unknown volatile acid concentrations have been provided. These unknowns represent samples which have been taken from an anaerobic digester on three consecutive days. Determine the type and concentration of volatile acids in each of the samples. Plot the results in the following manner: (1) individual volatile acids concentration versus days; (2) percent individual volatile acids versus days; (3) total volatile acids versus days.

VI. Questions

1. Is the digester becoming more stable, remaining constant, or becoming unstable?
2. What are the indicators which tell how the digester is changing and how have these indicators changed?
3. Determine the concentrations of volatile acids in the unknowns using peak heaight rather than peak area. Are the concentrations higher, lower, or about the same?
4. What would you expect to happen to peak location if (a) the carrier gas flow was increased, (b) the column temperature was decreased, (c) the sample volume was decreased?
5. If a fatty acid of 8 carbon chain length was injected, what would you expect its location to be in relation to the other peaks?

VII. References

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THIN LAYER CHROMATOGRAPHY

I. Plate Preparation

A. Laboratory Preparation

1. Glass plates can be purchased commercially or simply cut in the lab in appropriate sizes, 2x8", 8x10", etc.
2. Solid phases are available in various stock quantities. Silica Gel G (Warner-Chilcott Laboratories) can be obtained with or without gypsum binder and/or a phosphor.
3, Application to the plate. A slurry containing 5 gm silica gel to 10 ml water can be prepared in a mortal and poured onto the glass plates that have been lined on all edges with strips of masking tape. Spreading of the layer is accomplished by rolling in one direction with a glass rod (0.5 cm diameter). After drying at room temperature for 15 minutes the plates can be oven-dried at 100^{o}C for 30 minutes, cooled and stored in a dessicator until use.

   Uniformity of thickness on any one plate can be obtained remarkably well with a little practice; however thickness will vary from plate to plate.

   Spray attachments are available for applying slurries to glass plates; we have had little success with this procedure.

   Maximum uniformity in thickness of the static phase can be obtained with the use of any of several mechanical applicators that are commercially available.

B. Precoated Sheets

Sheets precoated with a variety of chromatographic supports are available and are extremely convenient. However, our experience with precoated silica gel sheets

indicated they have longer development times and are not as amenable to the application of relatively large volumes of sample.

II. Application of Sample

A. Choice of Solvent

Most of the phenols and phenolic acids are soluble in volatile organic solvents such as ethyl ether or 95 percent ethanol. Generally, the more volatile the solvent the smaller the spot size after application will be. A gentle stream of dry air directed onto the plate greatly facilitates the application of sample.

B. Applicator

1. Lambda micropipettes are relatively inexpensive and good for quantitative spotting.
2. Automatic streakers are available and, although expensive, provide uniform applications for preparative work.
3. Pyrex tubing drawn to a capillary tip provides the fastest, most inexpensive means of applying samples. Not sufficient for quantitative spotting, however.

C. Spot Location

Proximity of spots on the same plate is a function of sample concentration but a minimum horizontal separation of 2.5 cm was found to be adequate in our study. All spots were placed 1-2 cm above the solvent reservoir to prevent draining druing development.

III. Development

A. Developing Chamber

Almost any ordinary vessel can be used; one gallon jars, drinking glasses, or commercially available containers of different sizes covered with plastic caps, glass

plates or saran wrap. Solvent can be placed in the vessel which is then capped so that an approach may be made to air saturation with solvent vapor. Plates containing the sample plus known standard compounds can then be rapidly can then be rapidly inserted for development. R_f values will vary from run to run using this procedure although $R_{standard}$ values are reasonably reproducible.

If reproducible R_f values are desired, constant temperature and air saturation with the solvent system must be provided. A simple means of accomplishing this is to cap the chamber with a two-hole stopper fitted with a stop cork and a separately funnel with the tip bent to touch the inside of the chamber. A prepared plate with the bottom 3.0 cm free of Silica Gel can be placed in the chamber which contains solvent standing to a depth of 1.5 cm. After air saturation is obtained solvent can be run in from the separatory funnel to begin development.

B. Solvent Systems

1.	Ethyl ether	- - -	
2.	Benzene/ethanol	98/2; 95/5; 90/10	vol/vol
3.	Benzene/methanol/ acetic acid	95/8/4	vol/vol
4.	Benzene/dioxand/ acetic acid	90/25/4	vol/vol
5.	Benzene/acetic/ water	125/72/3	vol/vol
6.	Isopropanol/ammonium/ hydroxide/water	200/10/20	vol/vol

The first two solvent systems were particularly useful for separating aldehydes and ketones in the pH 6.5 extract; the remainder were generally useful systems for phenols and phenolic acids.

IV. Detection

A. Drying

Plates can usually be air dried in 5-10 minutes except where acid-base indicators are to be sprayed and the solvent contains an acidic or basic component. In the latter cases oven drying may be needed.

B. Fluorescence

Dried plates should be observed before spraying for any visible fluorescence under a UV hand lamp. Many of the phenolic acids fluoresce brilliantly and their location can be marked with small identations in the silica gel surface. In some cases exposure to NH_3 fumes greatly enhances fluorescence.

C. Chromogenic Reagents

Spraying must be done carefully to prevent draining of the silica gel. Sometimes oven heating enhances color formation and in other cases the fading or changing of a color with time may be indicative of certain compounds. The chromogenic reagents employed in our study and the structural implications inferred from them are listed in Table 1, while the color reactions of some typical phenols and phenolic acids with these reagents are shown in Table 2.

V. Effectiveness of Separation

Typical R_f values of the phenolic degradation products identified during our study are shown in Table 3. In actual practice, little reliance was placed on absolute R_f value as known pure compounds were in all cases co-chromatographed on the same plate using several solvent and spray systems. Nevertheless, the values in Table 3 indicate the extent of separation obtained.

VI. Data Recording

It is important to establish a system for recording the large aniybt if data accumulated during a thin layer chromatographic investigation. Several techniques were found to be useful during the course of this study.

A. Photocopy

The dried and finished plates can be photocopies with certain copy machines. This procedure can be tedious, however, and of limited value, depending on the size of the plate and the brand of copy machine.

B. Color Photography

This procedure is excellent, although expensive, for recording relative R_f values on the same plate as well as the color development.

C. Tracing

Using thin sheets of typewriter copy paper of vellum, the dried finished plates can easily be traced. The color of each spot and other pertinent experimental details can conveniently be inscribed on the copy sheet before filing in the data notebook.

TABLE 1

USEFUL CHROMATOGRAPHIC SPRAYS FOR THE IDENTIFICATION OF PHENOLIC COMPOUNDS

	Spray		Structural Implication
1.	Ferric chloride-ferric cyanide spray; 3% $FeCl_3$ in water followed by 3% $K_3Fe(CN)_5$		Phenols in general, particularly those of catechol-pyrogallol type.
2.	2,4-dinitrophenylhydrazine 0.1% in 2N HCl		Aldehydes and ketones
3.	Diazotized p-nitraniline		
	a. p-nitraniline, 0.3% in 8% HCl	50 ml	Phenols in general, particularly mono-hydroxy phenolic compounds
	b. $NaNO_2$, 5% in water	3 ml	
	c, Na_2CO_3, 10% in water	50 ml	
4.	Tetrazituzed benzidine		
	a, Benzidine solution 5 gr dissolved in 14 ml conc. HCl and diluted to 1 liter.	1 Vol.	Phenols in general, particularly those of resorcinol-phloroglucinol type
	b. Aqueous 10% sodium nitrate	1 Vol.	
5.	Vanillin Reagent		Phloroglucinol-resorcinol phenols not having carbonyl or carboxylic acid groups on the ring or conjugated with it.
	a, Vanillin, 10% in 95% ethanol	2 Vol.	
	b. Conc. HCl	1 vol.	

TABLE 2. COLOR REACTION OF THE IDENTIFIED COMPOUNDS WITH THE LOCATION REAGENTS

SPRAY / COMPOUND	$FeCl_3$-$K_3Fe(CN)_6$	2,4-DNP	p-Nitraniline	TAB	Vanillin/HCl
Vanillin	Blue	Yellow	Pinkish-Yellow	Yellow	---
Vanillic Acid	"	---	Yellow	"	---
Syringic Acid	"	---	"	"	---
3,4-DHBA	"	---	Pink	Brown	---
3,5-DHBA	"	---	Yellow	Orange	---
Resorcinol	"	---	"	Brown	Red
Catechol	"	---	Pink	"	---

Notes: --- indicates no immediate color development

-DNP = -dinitrophenylhydrazine

-DHBA = -dihydroxybenzoic Acid

TAB = Tetrazotized benzidine

TABLE 3. R_f VALUES OF THE IDENTIFIED COMPOUNDS IN DIFFERENT SOLVENT SYSTEMS. SILICA GEL G LAYERS

(R_f taken at the leading edge of color spots)

COMPOUND \ SOLVENT	1	2	3	4	5	6
Vanillin	0.89	0.59	0.68	0.70	0.67	0.45
Vanillic Acid	0.26	0.10	0.57	0.59	0.68	0.27
Syringic Acid	0.03	0.03	0.51	0.53	0.64	0.22
3,4-DHBA	0.37	0.05	0.29	0.31	0.40	0.08
3,5-DHBA	0.20	0.00	0.24	0.37	0.32	0.25
Resorcinol	0.98	0.31	0.48	0.56	0.47	0.75
Catechol	0.96	0.41	0.55	0.58	0.57	0.61

DHBA = Dihydroxybenzoic acid

ANALYSIS WITH SULFIDE ION SELECTIVE ELECTRODES

I. Objectives

The purpose of this experiment is to demonstrate the use of the sulfide ion selective electrode by direct potentiometric analysis, potentiometric titration, and an incremental technique. The effects of pH and ionic strength will be determined.

II. Theory

The sensing material of the sulfide ion selective electrode[a] is a solid silver sulfide membrane whose potential is related to the silver ion activity (Ag^+) in solution according to (3):

$$E = \text{const.} + (2.3\ RT/F)\ \log\ (Ag^+) \qquad \text{(S-1)}$$

where 2.3 RT/F has the value 0.059 volts at 25°C. In the absence of silver ions in the test solution there is a slight dissolution of the solid silver sulfide at the solution interface; this thin layer of solution becomes saturated with silver sulfide so that the electrode then becomes sensitive to sulfide ions in the bulk of the test solution because of the relationship $(AG^+)^2 = K_{sp}/(S^{--})$ which, when inserted into Equation S-1 changes the latter to

$$E = \text{const.} - (2.3\ RT/2F)\ \log\ (S^{--}) \qquad \text{(S-2)}$$

The constant in this equation is different from that of Equation S-1; also note the minus sign and the addition of the factor 1/2. The measurement of E is made in conjunction with a calomel reference electrode and a high impedance volt meter.

The electrode may be used to measure sulfide concentration, $[S^{--}]$, by utilizing the relationship between activity concentration

$$(S^{--}) = f \times [S^{--}] \qquad \text{(S-3)}$$

---

[a]Model 94-16, Orion Research, Inc., U.S.A.

where f is the sulfide ion activity coefficient. After measuring the sulfide activity its concentration may be determined by using Equation S-3, provided that the activity coefficient is calculated from a relationship that requires knowledge of ionic strength; alternately, the electrode may be calibrated with solutions of known sulfide concentration whose ionic strengths are identical to that of the test solution. Both methods will be utilized in this experiment.

Another important solution variable is the pH. Since H_2S is a dibasic weak acid, the fraction of the total sulfide concentration $[S]_T$ (which is equal to $[H_2S] + [HS^-] + [S^{--}]$), which is present as S^{--} depends on the pH. At 25°C and at an ionic strength of 0.1 M, the values of pK_1 and pK_2 (concentration ionization constants, not activity) are 6.8 and 14.4, respectively (2). This means that at a pH below 12 a negligible fraction of the $[S]_T$ is present as S^{--}, while it is necessary to have a highly basic solution for most of the $[S]_T$ to exist as S^{--}. Nevertheless, at a given pH and ionic strength a constant fraction of the $[S]_T$ exists as S^{--} and the electrode may be calibrated at these solution conditions in order to determine both S^{--} and $[S]_T$ in a test solution. The effect of pH on the electrode measurements will be determined in this experiment.

For the purpose of measuring sulfide concentration of precipitation titration may be performed in which a basic sodium sulfide solution is titrated with silver nitrate of known concentration, the end point being detected with a sulfide electrode. In the end point region the sulfide concentration is changing rapidly, so that a plot of E versus added ml of $AgNO_3$ solution will generally result in a large "greak." Such a precipitation titration will be performed in this experiment and is useful in calibrating the sulfide standard solutions.

Finally, a useful "incremental" technique will be utilized in analyzing the total sulfide concentration, $[S]_T$, this method being independent of pH or ionic strength. It depends on the fact that at any given pH and ionic strength the sulfide activity, (S^{--}), is related to $[S]_T$ by a single factor, k, such that

$$(S^{--}) = k \times [S]_T \qquad \text{(S-4)4}$$

This may be inserted into Equation S-2 to give:

$$E = \text{const.} - (2.3\ RT/2F)\ \log [S]_T \qquad (S\text{-}5)$$

with 2.3 RT/2F being now designated as b. After measuring E for the test solution, a known increment of silver nitrate is added, precipitating some Ag_2S, and thereby, reducing the $[S]_T$ by a known amount, $[S]_T$. Measuring the potential for this solution permits the calculation of $[S]_T$ in the original solution according to (1):

$$[S]_T/[S]_T = 10^{E/b} - 1 \qquad (S\text{-}6)$$

This incremental technique will also be used to measure $[S]_T$.

III. Apparatus

Sulfide ion selective electrode, Orion Model 94-16
Calomel reference electrode (double junction or sleeve type)
High impedence voltmeter (in pH meter)
Electrode holder
Graph paper, semi-log (3 cycles)
25 ml burst (2)
150 ml beaker (10)
Volumetric flask, glass stoppered:
    100 ml (25)
    250 ml (6)
1   1 liter (5)
    250 ml glass stoppered bottle (10)
    Pipets:
        5 ml (graduated) (2)
        5 ml (2)
        10 ml (2)
        25 ml (2)
        50 ml (2)

IV. Solutions and Reagents

$Na_2S \cdot 9H_2O$, reagent grade (100 g)
$AgNO_3$, reagent grade (100 g)
0.1 M Na_2S solution (1 liter)
0.1 M $AgNO_3$ solution (1 liter)
0.1 M NaOH, carbonate free (2 liters)
0.5 M NaOH, carbonate free (1 liter)
1 M $NaNO_3$ (2 liters)
0.5 M HCl (1 liter)
Solution B, I= 0.5 M, pH = 13 (liter)
(Prepare by placing 400 ml of IM $NaNO_3$, 200 ml 0.5 M NaOH solution into a 1 liter volumetric flask and diluting with distilled water.)
Solution C, I = 0.5 M, pH = 8.4 (1 liter)
(Prepare by placing 500 ml of 1 M $NaNO_3$, 17.5 ml 0.1 M NaOH, and 100 ml 0.1 M H_3BO_3 into a 1 liter volumetric flask and diluting with distilled water.)
Solution D, I = 0.1, pH = 8.4 (1 liter)
(Prepare as with solution C, but use only 100 ml of 1 M $NaNO_3$.)

V. Procedure

A. Effect of Ionic Strength

1. Pipet 5.0 ml of 0.1 M Na_2S stock solution into 250 ml volumetric flask; dilute to 250 ml with pH 13 stock solution B to give a final solution of I = 0.5 M, ph 13 and $[S]_T$ = 0.002 M.
2. Using the sulfide buffered solution prepared in step 1 prepare the following sulfide solutions by successive dilutions to 100 ml with stock solution B:
$[S]_T = 5 \times 10^{-4}$ M, 1.25×10^{-4} and 0.313×10^{-4} M

3. Repeat step 1, but diluting with 0.1 M NaOH to prepare a solution of I = 0.1 M, pH 13 and $[S]_T$ = 0.002 M.

4. Prepare solutions of $[S]_T$ as in step 2 by diluting the solution prepared in step 3 with 0.1 M NaOH.

5. NOTE: In all the previous and subsequent steps be careful to avoid prolonged exposure of the sulfide solutions to air.

6. Using the sulfide electrode, calomel reference electrode, and meter, measure the E for each of the prepared sulfide solutions.

7. At each ionic strength, using 3 phase semi-log paper plot E versus $[S]_T$ (the latter on the log scale).

B. Effect of pH

1. Pipet 50 ml of 0.1 M Na_2S stock solution into a 150 ml beaker; titrate with 0.5 M HCl to pH 8.4. Transfer the solution to a 100 ml volumetric flask and dilute with distilled water.

2. Pipet 10 ml of solution prepared in step 1 into a 250 ml volumetric flask and dilute with pH 8.4 solution C so as to obtain a solution with I = 0.5 M and $[S]_T$ = 0.002 M.

3. Using the solution prepared in step 2, dilute successively to 100 ml with pH 8.4 solution C so as to obtain the following solutions:

   $[S]_T = 5 \times 10^{-4}$ M, 1.25×10^{-4} M and 0.313×10^{-4} M.

4. Repeat steps 2 and 3 with pH 8.4 solution D so as to obtain the same series of $[S]_T$ solutions but with an ionic strength of 0.1 M.

5. Measure E for each of the prepared solutions and plot the results on semi-log paper. Compare the results to Part A.

C. Precipitation Titration

1. Pipet 10.0 ml of 0.1 M Na_2S stock solution into a 100 ml volumetric flask and dilute with distilled water.
2. Pipet 50 ml of this solution into a 150 ml beaker.
3. Measure the E for this solution.
4. Using a 25 ml buret with 0.1 M $AgNO_3$, titrate the sulfide solution, measuring E after each addition of $AgNO_3$.
5. Plot E versus ml $AgNO_3$ added.
6. Calculate the sulfide concentration from the results of the titration and compare to the known sulfide.

D. Incremental Technique

1. Prepare an "unknown" concentration of $[S]_T$ of 0.001 M by diluting 2.5 ml of the 0.1 M Na_2S stock solution to 250 ml with pH 8.4 stock solution D.
2. Pipet exactly 50.0 ml of this "unknown" sulfide solution into a 125 ml beaker and measure its E.
3. Dilute 10 ml of the 0.1 M $AgNO_3$ solution to 100 ml with distilled water.
4. Add exactly 2.0 ml of this 0.01 M $AgNO_3$ solution prepared in step 3 to the 50 ml solution of step 2; mix and remeasure E.
5. Calculate the $[S]_T$ of this "unknown" solution using Equation S-6.

VI. Questions

A. For a given $[S]_T$ are the effects of ionic strength and pH significant on the measured E? If so, are these effects systematic, and in what way?

B. Devise a method for measuring $[S]_T$ for a water sample of unknown pH and ionic strength.

C How do the precipitation and incremental techniques compare in ease of use to the direct potentiometric analysis of $[S]_T$?

VII. References

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ANALYSIS OF PHOSPHATE

I. Objectives

Most natural waters contain only small amounts of phosphate, but it is the limiting nutrient in many natural waters. Sewage, agricultural drainage, and certain industrial wastes contain high concentrates of phosphate. Phosphate may be soluble as ortho-phosphate, condensed phosphate, or organic phosphate (e.g., glucose-1-phosphate and adenosine-5-monophosphate) or it may be incorporated in the particulate material in water.

This experiment will deal with the determination of ortho-phosphate by one automated and three manual methods of analysis and the comparison of the sensitivity, accuracy and precision of these methods. Condensed and organic phosphates will not be determined in this laboratory, but they can be analyzed by the same analytical methods after suitable hydrolysis of the sample.

II. Apparatus

Autoanalyzer
Manifold for automated phosphate determination (Fig. 1)
Spectrophotometer
Volumetric flasks:
1000 ml
100 ml
Pipets:
1 ml
5 ml
10 ml
Erlenmeyer flasks:
125 ml
250 ml
Cylinders:
50 ml
100 ml
250 ml

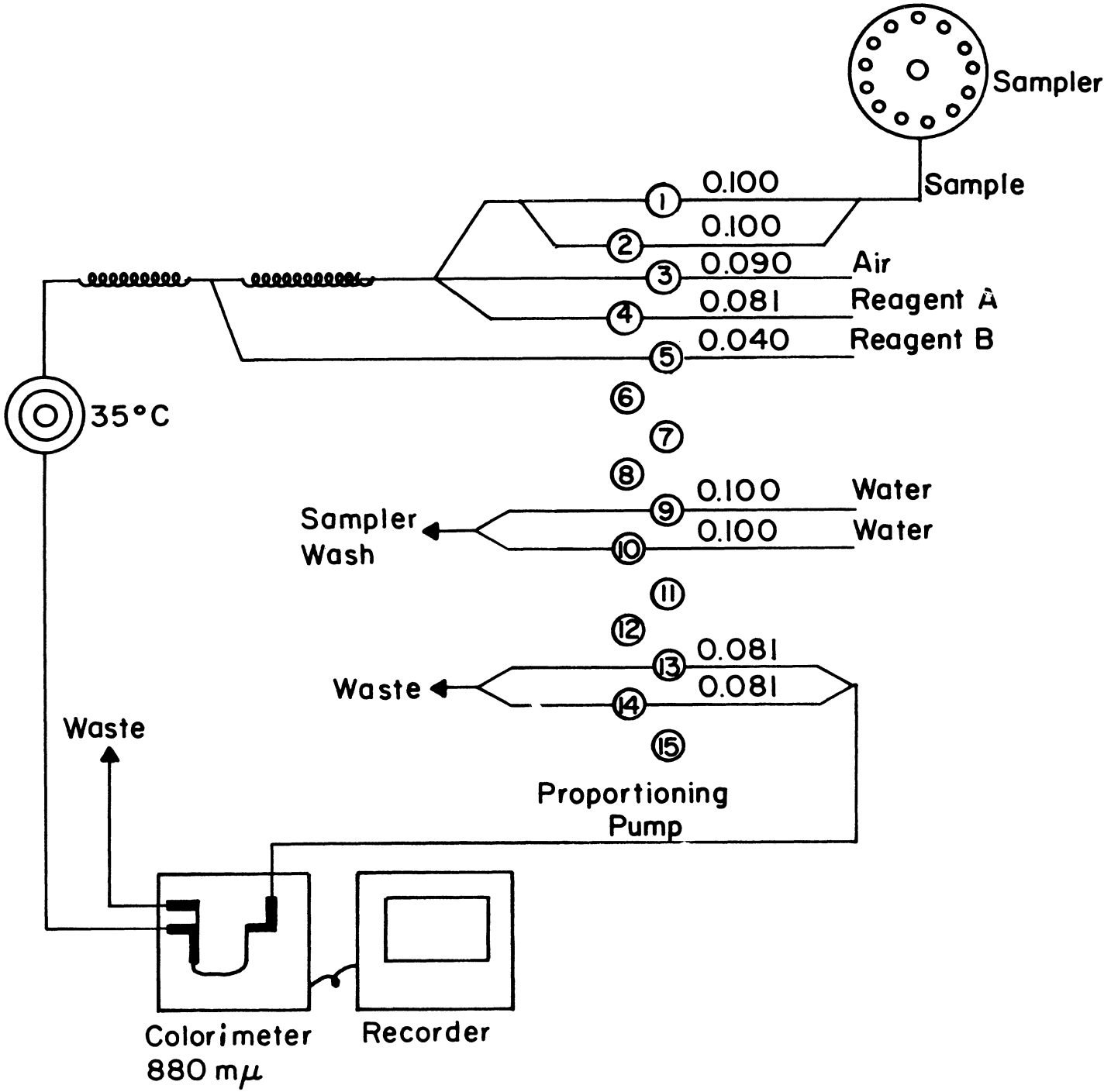

Figure 1. Auto Analyzer Phosphate Manifold

III. Reagents

A. Amino napthol sulfonic acid method

Ammonium molybdate-strong acid solution:

Dissolve 31.4 g $(NH_4)_6\ MO_7\ O_{24} \cdot 4H_2O$ in 200 ml distilled water. Add 252 ml conc. H_2SO_4 to 400 distilled water. Add 3.4 ml conc. HNO_3. Mix the acid and molybdate solutions and dilute to 1 liter.

Amino napthol sulfonic acid solution:

Dissolve 70 g $Na_2S_2O_5$ and 42 g Na_2SO_3 in about 900 ml water. Add 0.75 g finely ground 1-amino-2-napthol-4-sulfonic acid and dilute to 1 liter.

Phosphate stock standard:

Dissolve 0.7165 g KH_2PO_4 in 1 liter of water. 1.0 ml = 0.500 mg PO_4.

Phosphate working standard:

Dilute 10.0 ml of the stock standard to 100 ml with distilled water. 1.0 ml = 0.050 mg PO_4.

B. Stannous Chloride Method

Ammonium molybdate-strong acid solution:

Dissolve 25 g $(NH_4)_6\ MO_7O_{24} \cdot 4H_2O$ in 175 ml distilled water. Add 280 ml conc. H_2SO_4 to 400 ml distilled water. Add molybdate to acid solution and dilute to 1 liter.

Stannous chloride solution:

Dissolve 2.5 g $SnCl_2 \cdot 2H_2O$ in 10 ml conc. HCl and dilute to 100 ml with distilled water. Store with a few pieces of mossy tin. Discard when tin dissolves.

C. Ascorbic Acid Method

Sulfuric acid:

70 ml conc. H_2SO_4 diluted to 500 ml.

Ammonium molybdate:

Dissolve 20 g $(NH_4)_6\ MO_7O_{24}\bullet\ 4H_2O$ in 500 ml water.

Ascorbic acid:

Dissolve 1.32 g ascorbic acid in 75 ml water. This should be prepared the day it is to be used.

Potassium antimonyl tartrate (1 mg Sb/ml):

Dissolve 0.2743 potassium antimonyl tartrate in 100 ml water.

Mixed reagent:

Mix 125 ml sulfuric acid and 37.5 ml ammonium molybdate. Add 75 ml ascorbic acid and 12.5 ml of potassium antimonyl tartrate. This reagent does not keep more than 24 hours.

D. Autoanalyzer Method

Sulfuric acid, ammonium molybdate, ascorbic acid and potassium.

Antimonyl tartrate as in ascorbic acid method

Reagent 1:

125 ml sulfuric acid and 37.5 ml ammonium molydate and 12.5 ml potassium antimonyl tartrate and 175 ml water.

Reagent 2:

Ascorbic acid solution.

IV. Analytical Procedures

A. Amino Napthol Sulfonic Acid Method

To 50 ml sample add 2.0 ml molybdate-acid and mix. Add 2.0 ml sulfonic acid reagent and mix again. Measure the absorbance at 690 mμ after 5 minutes.

B. Stannous Chloride Method

To 50 ml of sample add 2 ml molybdate and 0.25 ml stannous chloride. Measure the absorbance at 690 mμ after 10 to 12 minutes.

C. Ascorbic Acid Method

Add 10 ml of the mixed reagent to 50 ml of sample. Measure the absorbance at 882 mu after 10 minutes.

D. Autoanalyzer Method

Thirty sampler per hour can be analyzed by the manifold shown in Figure 1.

V. Experimental Procedure

For each of the manual procedures and for the automated method, analyze a standard series of 0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg PO_4/liter. For a 2.0 mg PO_4/liter sample determine the rate of color development and fading by each of the manual methods (observations should not exceed 1 hour). Analyze six replicates of a river water sample by each method.

VI. Data Treatment

Determine the average concentration, standard deviation and 95 percent confidence level for the river water by each method. Determine if the results by the stannous chloride and ascorbic acid methods are statistically different.

VII. Questions

1. Which manual procedure is the most sensitive? The most reproducible?
2. What experimental conditions other than the time for color development may be important for each method?
3. Why does the reducing agent influence the sensitivity of the procedure and the wavelength of maximum absorbance?

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MEASUREMENT OF INORGANIC NITROGEN COMPOUNDS

I Objectives:

This experiment will demonstrate methods of analysis for the inorganic nitrogen species nitrate, nitrite and ammonia. These compounds are important in water since they are required nutrients and high concentrations of these substances may be indicative of pollution. Ammonia will be determined by the phenol-hypochlorite reaction catalyzed by nitroprusside. Nitrite will be determined by diazotizing the nitrite with sulphanilic acid and coupling the product with alpha-napthylamine, forming a red product. Nitrate will be determined by reducing the nitrate to nitrite on a cadmium-copper column and measuring the nitrite formed. The efficiency of nitrate reduction by the column will be determined. Nitrate will also be determined using a nitrate specific ion electrode. The concentration of the inorganic nitrogen compounds in natural water samples will be determined.

II Apparatus:

Spectrophotometer
pH meter
Nitrate electrode
Calomel reference electrode
Coarse file
Sieves-0.5 and 2 mm openings
beakers-1000 ml and 50 ml
pipets-

- 1 ml
- 2 ml
- 5 ml
- 10 ml

Erlenmeyer flasks

- 125 ml
- 250 ml

Volumetric flasks

- 100 ml
- 1000 ml

Graduated cylinders

50 ml

Burets

25 ml

Chromatography column

III. Reagents

Ammonia Method

Deionized water. The ammonia contained in distilled water is removed by passage of the water through a small column (e.g., 30 cm long by 1-2 cm inside diameter) of hydrogen form cation exchange resin just before use, and the water is stored in a tightly stoppered glass flask.

Reagent A. Dissolve 25 g of phenol in approximately 250 ml deionized water and add to this 125 mg of sodium nitroprusside, $Na_2Fe(CN)_5NO \cdot 2H_2O$ which has been dissolved in approximately 100 ml of deionized water. Dilute to 500 ml and store in a brown bottle in a refrigerator. The reagent is stable for about one month.

Reagent B. Dissolve 12.5 g NaOH in approximately 100 ml of deionized water and add 21 ml of sodium hypochlorite(chlorine bleach containing 5.25 percent active chlorine is satisfactory).

Stock Ammonia Standard. Dissolve 0.382 g NH_4Cl in 1 liter of deionized water. This solution contains 100 µg N/ml.

Working Ammonia Standard. Dilute 10 ml of stock standard to 100 ml with deionized water. This solution contains 10µg N/ml.

Nitrite Method

Sulphanilamide solution. Dissolve 5 g of sulphanilamide in a mixture of 50 ml of concentrated hydrochloric acid and about 300 ml of distilled water. Dilute to 500 ml with distilled water. Stable several months.

N-(1-naphthyl)-ethylenediamine dihydrochloride solution. Dissolve 0.5 g of the dihydrochloride in 500 ml of distilled water and store in a brown bottle. Stable one month.

Stock nitrite standard. Dissolved 0.061 g of KNO_2 in 100 ml of distilled water. This solution contains 100 µg N/ml.

Working nitrite standard. Dilute 1 ml of the stock standard to 100 ml. This solution contains 10µg N/ml.

Nitrate Method

All solutions required for nitrite method.

Cadmium filings (0.5-2mm). Prepare by melting cadmium metal and allowing it to solidify around a drill bit. Rotate the bit and metal and file off metal with a coarse hand file. Collect those filings passing a 2 mm screen but retained on an 0.5 mm screen.

Copper sulfate solution. Dissolve 10 g copper sulfate pentahydrate, $CuSO_4 \cdot 5H_2O$, in 500 ml of water.

Copper wool turnings

Hydrochloric acid - 5%

Glass wool

Concentrated ammonium chloride solution. Dissolve 175 g ammonium chloride in 500 ml distilled water.

Dilute ammonium chloride solution. Dilute 25 ml of th concentrated ammonium chloride solution to 1 liter with distilled water.

Stock nitrate standard. Dissolved to 0.722 g of KNO_3 in 1 liter of distilled water. This solution contains 100 µg N/ml.

Working nitrate standard. Dilute 10 ml of the stock standard to 100 ml. This solution contains 10 µg N/ml.

IV. Experimental Procedures

A. Ammonia Procedure:

1. Prepare ammonia standards containing 0, 100, 200, 300, 400 and 500 ppb N.
2. Add 10 ml of solution A to 50 ml of sample and mix.
3. Add 10 ml of solution B and mix.
4. Place samples in a water bath (35-40°C) for 20 minutes.
5. Allow 15 minutes for samples to reach room temperature.
6. Measure the absorbance at 625 mµ.

B. Nitrite Procedure:

1. Prepare nitrite standards containing 0, 10, 20, 30, 40, and 50 ppb N.
2. Add 1 ml of sulphanilamide solution to 50 ml of sample and mix.
3. After 2 to 8 minutes add 1 ml of naphthyl ethylenediamine solution and mix immediately.
4. Measure the absorbance at 543 mµ between 10 minutes and 2 hours.

C. Nitrate Method (colorimetric):

1. Stir about 100 g of cadmium filings with 500 ml of 2% copper sulfate until the blue color leaves the

solution and copper particles become apparent in the solution. This is enough for about 2 columns.

2. Put a small plug of very fine copper turnings in the bottom of a buret (use glass wool if the copper is not available). Fill the column with the dilute ammonium chloride solution and pour in sufficient copperized cadmium to produce a column 30 cm in length. Add the filings slowly and make sure the column is well settled. Wash the column thoroughly with dilute ammonium chloride solution and place a plug of copper wool on top of the column. Adjust the flow rate of the column so 100 ml of solution takes between 8 and 12 minutes to flow through the column. When not in use the columns should be completely covered by the dilute ammonium chloride solution
For permanent use columns with inverted siphons and reservoirs should be used. These can be constructed by attaching a 45 cm length of 10 mm inside diameter to the bottom of a 125 ml Erlenmeyer flask. Attach a length of 2mm inside diameter tubing to the 10 mm tubing. This tube should run parallel to the 10 mm tube to a point 35 cm from the bottom of the 10 mm tube. At this point it should be bent into an inverted U whose end can be constricted by heating to provide the correct flow rate.
3. When the column becomes deactivated, empty the filings from four columns into a beaker and stir with 300 ml of 5% HCl, decant and repeat. Wash with water until the pH is greater than 5 and decant to leave the metal as dry as possible. Retreat the remaining metal with copper sulfate to provide enough copperized cadmium for approximately three columns.
4. Add 2 ml of concentrated ammonium chloride solution to 100 ml of sample and mix.
5. Pour a small portion (about 5 ml) of sample onto a column which has been drained of any excess liquid

above the metal. Drain the 5 ml.

6. Add the remainder of the sample.
7. Discard the first 40 ml which passes through the column.
8. Collect 50 ml of sample and treat as in the nitrite procedure.
9. Calibrate by analyzing nitrate and nitrite standards containing 500 ppb N. Correct the absorbances of samples by that of a blank which has been passed through the column.

D. Nitrate Method (specific ion electrode):

1. Measure the potential of 10, 50, 100, 500 ppb and 1, 5 and 10 ppm nitrate-nitrogen solutions. Plot log N vs. potential.
2. Measure the potential of a 1 ppm nitrite-nitrogen solution.
3. Measure the potential of the samples.
4. Dilute the samples with equal volumes of water and measure the potential.
5. Dilute the samples with equal volumes of a 500 ppb nitrate-nitrogen solution and determine the potential.

IV. Questions

1. What are the advantages in the determination of ammonia by the phenol-hypochlorite procedure rather than by Nesslerization?
2. What percent of the nitrate is reduced to nitrite? What percent of the nitrite is reduced by the column?
3. What is the limit of sensitivity of the potentiometric nitrate method? Of the colorimetric method?
4. Is nitrite an interference in the potentiometric method? In the colorimetric method?
5. Is the correct amount of nitrate recovered in dilution and standard addition experiments?

6. Is the same nitrate concentration obtained by the colorimetric and potentiometric procedures?

VI. References

1. J.D.H. Strickland and T.R. Parsons, "A Practical Handbook of Sewater Analysis." Fisheries Research Board of Canada Bulletin 167, Ottawa, Canada, 1968, p. 71.
2. M.W. Weatherburn, Anal. Chem., 39, 971 (1967).