Microchemical Journal

devoted to the application of microtechniques in all branches of science

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Quality Control in the Pharmaceutical Industry

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Microchem. J. **19,** 115 (1974).

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Microchem. J. **19,** 130 (1974).

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Microchem. J. **19,** 163 (1974).

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Microchem. J. **19,** 168 (1974).

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Microchem. J. **19,** 179 (1974).

The Pyrolytic Identification of Organic Molecules. I. The Pyrolytic Behavior of Organic Molecules. R. BELCHER, G. INGRAM, AND J. R. MAJER, *Chemistry Department, University of Birmingham, P.O. Box 363, Birmingham, England.*

The results of a study on the pyrolysis of about 70 organic compounds of varied composition are presented and discussed. Identification of the volatile products formed was accomplished by mass spectrometry.

Microchem. J. **19,** 191 (1974).

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Polyacrylamide gel electrophoresis was used to resolve as many as three protein components from incubation mixtures containing the inhibitor, 5-fluoro-2'-deoxyuridylate, the cofactor, 5, 10-methylene tetrahydrofolate, and thymidylate synthetase. Ternary complexes of thymidylate synthetase are stable to gel filtration and are shown to undergo a relatively slow rate of breakdown on storage at 25°C.

Theoretical and Experimental Approach to Spectrofluorometric Extractive Titrations

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INTRODUCTION

Spectrophotometric extractive titrations, an analytical technique combining titration, extraction, and spectrophotometry, have been shown to be more sensitive than any of these individual methods (2). The spectrofluorometric extraction titration technique, which would employ fluorometric measurements instead of spectrophotometry, has not been previously investigated. Because of the greater sensitivity of fluorometry and the linear dependency between concentration and light intensity in fluorometry rather than the logarithmic relationship of spectrophotometry, the spectrofluorometric extraction technique should permit the determination of micro quantities of material in analyses where only small amounts or small concentrations of sample are available. A theoretical equation for the titration curve for the fluorometric method is derived and compared to experimental data. A mathematical calculation of the endpoint is also theoretically derived and tested experimentally using 8-hydroxyquinoline for the titration of gallium and aluminum.

THEORY

Extractive titrations consist of adding successively titrant to the sample and after each addition extracting the resultant solution into an immiscible phase and measuring a change in the property of the immiscible phase. For example, in a spectrophotometric extractive titration of an aqueous Ga(III) solution, 8-hydroxyquinoline (oxine) could be employed as a titrant and $CHCl_3$ as the extracting solvent. For a 100-ml sample one might add 1 ml of the titrant, extract the sample with 10 ml of $CHCl_3$, and then measure the absorbance of the $CHCl_3$ phase, return the same $CHCl_3$ phase to the sample, add another milliliter of oxine, again extract, then remeasure the absorbance, and continue in the same manner. The endpoint may be obtained graphically by plotting the absorbance vs the milliliters of oxine added. The general equation for a spectrophotometric titra-

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tion has been published (2); a similar notation will be employed for the spectrofluorometric extractive titration which will be abbreviated as SFET.

At any point during the SFET of metal ion M^{I+} in the presence of interfering metal ion N^{J+} using the chelating agent HA as titrant, the fluorescence F of the organic phase is given by:

$$F = KP_0 d(\epsilon_{MA_I} \cdot [MA_I]_{org} + \epsilon_{NA_J} \cdot [NA_J]_{org} + \epsilon_{HA}[HA]_{org}), \quad (1)$$

where $[MA_I]_{org}$, $[NA_J]_{org}$, and $[HA]_{org}$ are concentrations of the metal chelates and free HA in the organic phase, ϵ_{MA_I}, ϵ_{NA_J}, and ϵ_{HA} are molar absorptivities of the species indicated, d is the cell path length, P_0 is power of the excitation light, and K is a proportionality constant. The extraction constants are given by

$$K_M = \frac{[MA_I]_{org}[H]^I}{[M][HA]^I_{org}} \quad \text{and} \quad K_N = \frac{[NA_J]_{org}[H]^J}{[N][HA]^J_{org}}, \quad (2)$$

for the metal ions where charges have been omitted for simplicity (8).

The equilibrium concentrations of $[MA_I]_{org}$, $[NA_I]_{org}$, and $[HA]_{org}$ can be found from mass balances and the partition coefficient for the chelating agent and are

$$C_M = [M] + [MA_I]_{org} \left(\frac{V_{org}}{V}\right) \quad (3)$$

where V is the volume of the aqueous layer and V_{org} the volume of the organic layer.

$$C_{HA} = [A] + [HA] + [H \cdot HA] + I \cdot [MA_I]_{org} \left(\frac{V_{org}}{V}\right)$$
$$+ J[NA_J]_{org} \left(\frac{V_{org}}{V}\right) + [HA]_{org} \left(\frac{V_{org}}{V}\right) \quad (4)$$

$$C_N = [N] + [NA_J]_{org} \left(\frac{V_{org}}{V}\right) \quad (5)$$

$$P_R = \left(\frac{V_{org}}{V}\right) \frac{[HA]_{org}}{[HA]} \quad (6)$$

Equations (3) and (5) assume that $[MA_I]$ and $[NA_J]$ in the aqueous layer are so small that they can be neglected.

When using 8-hydroxyquinoline(oxine) as the chelating agent HA, the following ionization constants have been determined:

$$K_1 = \frac{[H][HA]}{[HA \cdot H]} \quad \text{and} \quad K_2 = \frac{[H][A]}{[HA]}, \quad (7)$$

where $K_1 = 8 \times 10^{-6}$ and $K_2 = 2 \times 10^{-10}$ (7).

The equivalent ratio of titrant to metal introduced by Flaschka (*1*) is also useful.

$$a = \frac{C_{HA}}{I \cdot C_M} \tag{8}$$

and combining Eqs. (1–8) yields Eqs. (9 and 10).

$$C_{HA} = \frac{K_2[HA]}{[H]} + [HA] + \frac{[H][HA]}{K_1} + \frac{I(V_{org}/V)C_M}{(V_{org}/V) + [(X)^I/K_M]}$$

$$+ \frac{J(V_{org}/V)C_N}{(V_{org}/V) + [(X)^J/K_N]} + P_R[HA] \tag{9}$$

and

$$F = KP_0 d \left(\frac{\epsilon_{MA_I}(C_M)}{(V_{org}/V) + [(X)^I/K_M]} + \frac{\epsilon_{NA_J}(C_N)}{(V_{org}/V) + [(X)^J/K_N]} \right.$$

$$\left. + \frac{\epsilon_{HA} P_R V[HA]}{V_{org}} \right) \tag{10}$$

where $X = \dfrac{[H]V_{org}}{P_R[HA]V}$.

Although "*a*" cannot be put in terms of a simple function of *F*, the values of *a* and *F* can be found using Eqs. (8)–(10) and substituting values in the right side of these equations.

Also by combining Eqs. (8) and (9) and finding the differentials $\partial a/\partial[HA]$ and $\partial F/\partial[HA]$ [from Eq. (10)], one can obtain the differential $\partial F/\partial a$. This is given by

$$\frac{\partial F}{\partial a} = \frac{KP_0 d \left(\dfrac{\epsilon_{MA_I} \cdot C_M \cdot I \cdot X^I}{K_M[HA][V_R + (X^I/K_M)]^2} + \dfrac{\epsilon_{NA_J} \cdot C_N \cdot J \cdot X^J}{K_N[HA](V_R + (X^J/K_N)^2} + \dfrac{\epsilon_{HA}P_R}{V_R} \right)}{\left(\dfrac{K_2}{[H]} + 1 + \dfrac{[H]}{K_1} + P_R \right)\left(\dfrac{1}{I \cdot C_M} \right) + \dfrac{I \cdot V_R \cdot X^I}{K_M[HA][V_R + (X^I/K_M)]^2} + \left(\dfrac{J \cdot C_N}{I \cdot C_M} \right)\left(\dfrac{J \cdot V_R \cdot X^J}{K_N[HA][V_R + (X^J/K_N)]^2} \right)} \tag{11}$$

where $V_R = V_{org}/V$.

Equation (11) will enable one to find the equivalence point for the SFET. This is shown by solving the Eqs. (12) and (13) for the value of *a* at equivalence point where equations

$$F_1 = \left(\frac{\partial F}{\partial a}\right)_1 a_1 + b_1 \tag{12}$$

$$\text{and} \quad F_2 = \left(\frac{\partial F}{\partial a}\right)_2 a_2 + b_2 \tag{13}$$

refer by subscript 1 to the linear region before the equivalence point and subscript 2 to the linear region beyond the equivalence point. The term b refers to the intercept of the lines on the F axis.

A program was written for a Wang model 700 for F as a function of a and also a program solving for F and a when combining Eqs. (12) and (13) (6). The second program gives the equivalence point values of F and a. Wang programs (6) were also written for the fractional species of aluminum and gallium as a function of pH; the results are reported in Figs. 1–3.
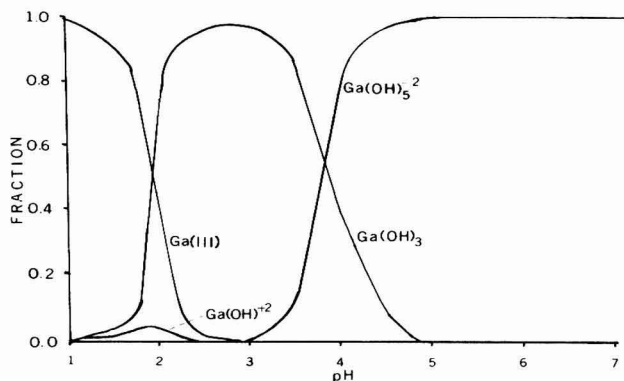
FIG. 1. Effect of pH on the fractional distribution of gallium(III) species.
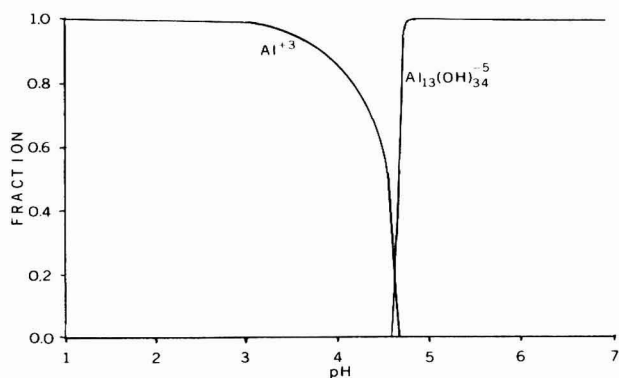
FIG. 2. Effect of pH on the fractional distribution of Al(III) species.
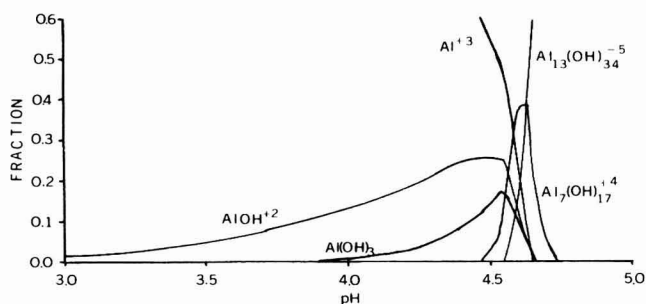
FIG. 3. Expansion of Fig. 2 in the region of pH 3–5.

MATERIALS AND METHODS

To verify the theory, experimental work was applied to the system of Ga(III) as one metal M^{i+}, Al(III) as another metal N^{j+}, and oxine as HA. The organic solvent was $CHCl_3$, and the titrant, oxine, was added to a series of 125-ml separatory funnels containing 5.0 ml of pH 4.8 acetate buffer (5 M), 3 ml of 10^{-3} M metal [Al(III) or Ga(III)], sufficient deionized water to make an aqueous volume of 100 ml after addition of oxine, and 10 ml reagent grade $CHCl_3$. The two metals were titrated separately using 10^{-2} M oxine dissolved in a 50–50 mixture of water and ethyl alcohol. The amount of oxine added varied from 0.0 to 8.0 ml and each flask was shaken well for 1 min and allowed to stand for 10 min. Fluorescence readings were taken of the $CHCl_3$ phase on a Turner Model 111 fluorometer using a Corning 7-60 as a primary filter and a Wratten 65A for the secondary filter. Gray Wratten filters when necessary were used on the secondary beam to reduce the fluorescence intensity.

The method was tried using a single separatory funnel, adding all reagents except oxine and then after adding small increments of oxine, shaking and taking fluorescence readings. The $CHCl_3$ layer used in the reading was then returned to the separatory funnel between each increment of oxine addition, further shaking performed, and another reading on the fluorometer taken. This method of adding oxine compared favorably to the method using a series of separatory funnels in that small changes in the addition of reagents such as buffer and metal did not enter significantly into the error of precision for the single funnel method as it did in the several funnel method. The multi-funnel method was used here mainly to determine experimental conditions as it was, for this purpose, the faster of the two techniques; in sample determinations the single funnel method would be faster and more precise.

RESULTS AND DISCUSSION

Results of two theoretical calculations are shown in Fig. 4. Experimental curves of similar concentrations to the theoretical are shown in Fig. 5. Agreement between experimental and theoretical SFET can be shown using the following equations:

$$\frac{F_{Al\ (exp)}}{F_{Ga\ (exp)}} = K_{exp}\ \frac{a_{Al\ (exp)}}{a_{Ga\ (exp)}}, \tag{14}$$

$$\frac{F_{Al(calc)}}{F_{Ga(calc)}} = K_{calc}\ \frac{a_{Al(calc)}}{a_{Ga(calc)}}, \tag{15}$$

where exp refers to experimentally determined values of a and F at the end point of the SFET while calc refers to theoretical calculations
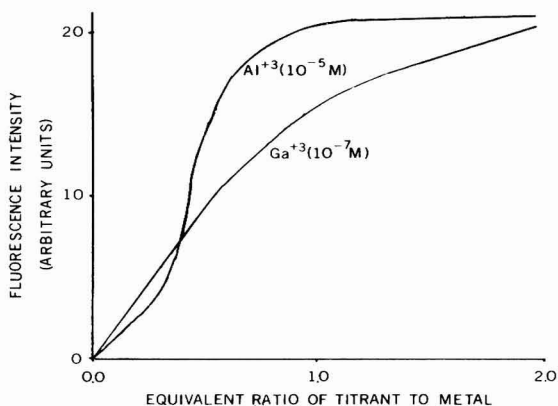
FIG. 4. Theoretical titrations of Ga(III) and Al(III) with oxine. Aluminum titrations reduced by a factor of 50 on both "F" and "a" axes.
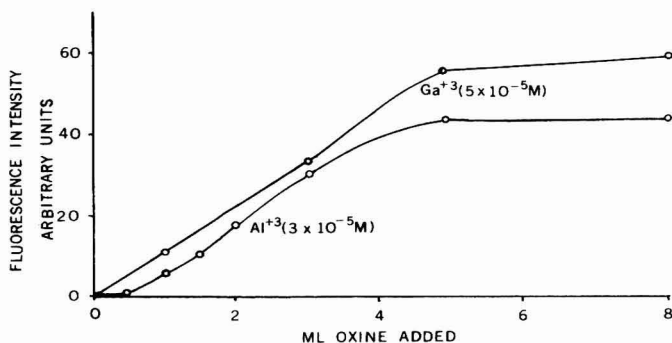
FIG. 5. Experimental titrations of Ga(III) and Al(III) with oxine.

TABLE 1
SIMILARITIES BETWEEN THEORETICAL AND EXPERIMENTAL SFET

	F_{Al}	F_{Ga}	a_{Al}	a_{Ga}	K
Exptl	1.3×10^3	1.2×10^2	4.6	3.3	7.8
Calcd	9.6×10^2	2×10^1	8.3	1.3	7.5

of a and F at the end point. Table 1 shows the similarities between the theoretical and experimental calculations using Eqs. (14 and 15). As can be seen, K is similar theoretically and experimentally and this indicates closeness of theoretical and experimental work.

SFET when tried with metals Li(I), Mg(II), Rb(I), Sc(III), and Ca(II) experimentally gave titration breaks while Be(II), Co(II), NH_4(I), and B(III) did not give titration breaks. The factor of pH was important and a slight variation in pH from the best values often gave extraneous results in these titrations. The necessity for strict pH control is apparent by examination of Figs. 1–3.

Sensitivity can be determined using a method similar to that of Mandel and Stiehler (5). The definition is

$$\gamma = |[dI/S_I dC]_{before} - [dI/S_I dC]_{after}| \tag{15}$$

where γ is the sensitivity of the titration, dI/dC is the slope of the lines and S_I is the standard deviation of the points from the regression lines. Before refers to before the end point and after to after the end point. The sensitivity was found to be 1.6×10^3 ppm^{-1} for Al–oxine titration and 1.4×10^6 ppm^{-1} for the Ga–oxine titration. A higher value means a more sensitive reaction.

Table 2 compares SFET of Al(III) and Ga(III) using oxine with SPET of Zn(II), Ag(I) and Be(III) determined by Galik (3,4) using dithizone. For the elements shown, Ga(III) is determined at a lower detection limit by SFET than any metal by spectrophotometric ex-

TABLE 2
COMPARISON OF DETECTION LIMITS OF SEVERAL IONS

Ion	Procedure	Detection limit (%)
Zn^{2+}	SPET[a]	30×10^{-6}
Ag^{1+}	SPET	17.5×10^{-6}
Bi^{3+}	SPET	5×10^{-6}
Al^{3+}	SFET	81×10^{-6}
Ga^{3+}	SFET	3.5×10^{-6}

[a] SPET stands for spectrophotometric extractive titration.

tractive titration; Al(III) is determined at a higher level. A simple comparison of the detection limits is difficult as different metals and reagents are involved.

SUMMARY

Spectrofluorometric extractive titration (SFET) is a sensitive method for determining trace amounts of certain metals. This technique combines titration, extraction and fluorometry and is more sensitive than any of these individual techniques. A theoretical treatment is derived and is compared favorably to an experimental procedure using Ga(III) and Al(III) as metals being titrated by 8-hydroxyquinoline(oxine). A sensitivity value for the experimental SFET is derived and calculated for the Ga(III) and Al(III)-oxine systems.

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The Analysis of Iron(III) Using Solvent Extraction with Phenylacetic and n-Decanoic Acids

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INTRODUCTION

Recent work (3) has shown that Fe(III) is very strongly extracted from aqueous media with high molecular weight carboxylic acids dissolved in various organic diluents. In fact, the strongest extractants for this metal out of a series of carboxylic acids studied were phenylacetic and n-decanoic acids. The organic extract phase is highly colored and hence is most suitable for use in a colorimetric method to determine small amounts of iron. In addition Fe(III) is more strongly extracted than any other metal ion investigated and so can be extracted at low pH values without interference.

Adam and Pribil (*1*) have recently reported the use of phenylacetic acid in chloroform solution for the extraction and analysis of copper.

MATERIALS AND METHOD

Reagents

Phenylacetic acid (B.D.H. Laboratory grade) was recrystallized from water (mp 76.2°C, literature value (2) 76.5°C).

n-Decanoic acid (B.D.H. Laboratory grade) was distilled under vacuum and the fraction boiling at 153°C (10 mm Hg) was collected.

The formula weights of both acids were determined by potentiometric titration in an aqueous ethanol medium (found for phenylacetic acid: 135.3, calculated: 136.1; found for n-decanoic: 172.3, calculated: 172.9).

Benzene (May and Baker "Analar" grade) was used without further purification. Chloroform (May and Baker reagent grade) was washed several times with distilled water before use.

All inorganic salts were of analytical reagent grade.

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Extraction Curves

The percentage extraction vs. pH curves for both acids were obtained for Fe(III), Ag(I), Pb(II), Cu(II), Zn(II), and Ni(II) using a 1 M solution of the appropriate acid in benzene. The initial metal ion concentration in the aqueous phase was 10^{-3} M and the aqueous phase was made 0.1 M with respect to sodium nitrate. Equilibrium pH values were measured using a Radiometer Model 28 pH meter and a combined calomel/glass electrode. Analysis of the aqueous phases for the appropriate metal ions was carried out using atomic absorption spectroscopy (Varian Techtron AA5 spectrophotometer and standard techniques). Organic phase metal ion concentrations were obtained by difference.

The Analytical Method

i. Calibration curve. Standard Fe(III) solutions were prepared using ferric ammonium sulphate dissolved in dilute sulfuric acid in the concentration range of 0.9 to 20 mg/ml. The pH value of each of the standard solutions was adjusted to 2.8 using ammonia solution. A 25-ml aliquot of each solution was shaken with 10 ml of a 1 M phenylacetic acid solution in chloroform for 1 min. After standing for 15 min to ensure complete phase separation, the chloroform solution was separated and filtered. The absorbance of each chloroform solution was measured at 340 nm using 1-cm cells against a chloroform blank solution. A calibration curve of absorbance against Fe(III) concentration in the original aqueous phase was obtained.

ii. Ferric nitrate. The iron content of a sample of ferric nitrate was determined by dissolving the salt in distilled water, adjusting the pH to 2.8, extracting with a 1 M solution of phenylacetic acid in chloroform, and measuring the absorbance at 340 nm. The iron content of the same sample was also determined by dissolving the salt in a 10^{-3} M copper nitrate solution instead of distilled water.

iii. Brass. The iron content of a sample of brass (approx 70% Cu, 30% Zn) was determined by dissolving the brass in nitric acid, adjusting the pH of the solution to 2.8, and extracting with phenylacetic acid in chloroform solution as above.

iv. Tap water. The iron content of tap water was determined by adjusting the pH to 2.8 and extracting as above. The sensitivity of the solvent extraction method can be greatly enhanced by variation of the aqueous to organic phase ratio and it was found that by using 50 ml of tap water and 10 ml of phenylacetic acid in chloroform solution an amount of iron in tap water of 0.8 μg/ml could be easily determined. A small amount of potassium permanganate was added to the acidi-

fied water before extraction to ensure that all the iron was present as Fe(III).

RESULTS AND DISCUSSION

The percentage extraction against pH curves for the extraction of Fe(III), Ag(I), Pb(II), Cu(II), Zn(II), and Ni(II) from aqueous solution by phenylacetic and *n*-decanoic acids in benzene solution are shown in Figs. 1 and 2. The $pH_{1/2}$ values have been obtained from these curves and are given in Table 1.

Phenylacetic acid is seen to be a better extractant than *n*-decanoic acid for metals which are extracted at the lower pH values; such as Fe(III), Pb(II) and Cu(II). For metals which are extracted at the higher pH values, viz, Zn(II) and Ni(II), *n*-decanoic acid is a better extractant than phenylacetic acid.

The extraction curve for Ag(I) for the phenylacetic acid case (Fig. 1) shows some anomalous behavior in that a break in the curve occurs at a pH around 2.92, whereby the distribution ratio at higher pH values is less than expected. It is possible that photodecomposition of Ag(I) is occurring to some degree at the higher pH values which would have the effect of lowering distribution ratios.
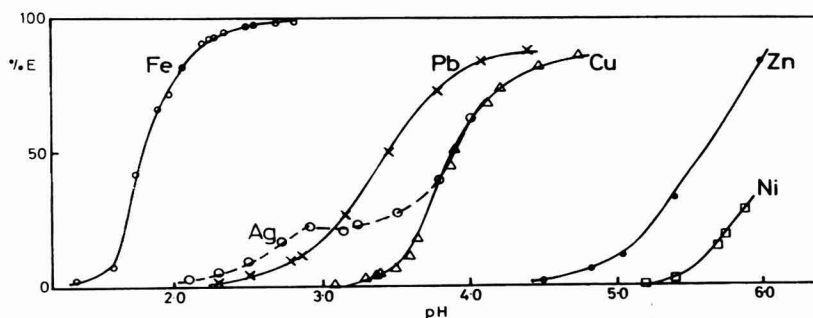
FIG. 1. Percentage of *E* against pH curves for phenylacetic acid.
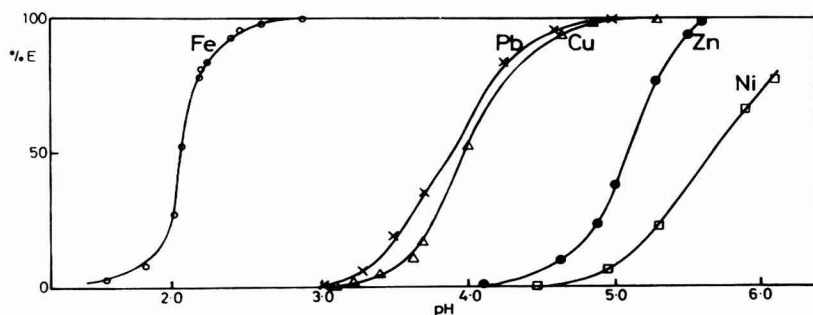
FIG. 2. Percentage of *E* against pH curves for *n*-decanoic acid.

TABLE 1
pH$_{1/2}$ VALUES FOR THE EXTRACTION OF METALS WITH
PHENYLACETIC AND *n*-DECANOIC ACIDS

| | pH$_{1/2}$ | |
Metal	Phenylacetic acid	*n*-Decanoic acid
Fe(III)	1.80	2.07
Ag(I)	3.90	—
Pb(II)	3.46	3.90
Cu(II)	3.90	3.98
Zn(II)	5.62	5.10
Ni(II)	ca. 6.2	5.68

As seen from figure 1, a minimum equilibrium pH of 2.8 is needed to achieve 100% extraction of Fe(III) using phenylacetic acid as extractant. At this pH, approximately 20% of Ag(I) and 10% of Pb(II) are also extracted, but no Cu(II) is extracted.

When using *n*-decanoic acid as extractant, a minimum equilibrium pH of 2.9 is required for 100% extraction of Fe(III). At this pH, no Pb(II) or Cu(II) is extracted, so that *n*-decanoic acid offers some advantages over phenylacetic acid in terms of selectivity by pH control.

For the present work, phenylacetic acid was chosen for the analytical technique because it is more readily available, cheaper, and easier to purify than *n*-decanoic acid. Chloroform was used as the diluent rather than benzene because of its transparency in the ultraviolet region of the spectrum. However, only a slightly better extraction efficiency is achieved using chloroform as the diluent in place of benzene.

The ultraviolet-visible spectrum of the Fe(III)-phenylacetic acid complex in chloroform shows a very broad absorption band stretching from 300 to about 400 nm. The position of the band is unaltered by a change in concentration of iron(III) in the aqueous phase in the range $(0.5 \text{ to } 2.5) \times 10^{-4}$ M, however, above a concentration of 10^{-3} M the absorption band is shifted to longer wavelengths. The wavelength of 340 nm was chosen for the analytical method because of the high absorbance at this wavelength and because of the good adherence to Beer's Law and minimal spectral interference from other metals and from phenylacetic acid itself.

The Cu(II)–phenylacetic acid complex shows significant absorbance in the region 300 to 400 nm and hence it is necessary to prevent Cu(II) being extracted simultaneously with Fe(III) by careful

control of the aqueous phase pH value. The coextraction of Cu(II) can conveniently be monitored using the strong absorption band of the copper complex at 700 nm. This is the wavelength used by Adam and Pribil (2) for the analysis of Cu(II).

Both the Ag(I) and Pb(II) phenylacetic acid complexes show some absorbance in the region that the Fe(III) complex absorbs, but the absorbance is small compared to the absorbance of the Fe(III)–phenylacetic acid complex. For example, at a wavelength of 340 nm, a solution containing approximately 0.4 μg cm^{-3} of Fe(III) would result in about the same absorbance by the organic phase as a solution containing approximately 108 μg/ml of Ag(I), and 207 μg/ml of Pb(II). Thus the spectral interference due to Ag(I) and Pb(II) will be negligible unless they are present in the sample in far greater amounts than Fe(III) (of the order of 300 times as much Ag and 1000 times as much Pb).

The calibration curve for the analytical method using a 1 M solution of phenylacetic acid in chloroform is shown in Fig. 3 as a plot of absorbance of the chloroform phase at 340 nm against the initial concentration of iron(III) in the standard aqueous solutions. Adherence to Beer's Law is obtained up to aqueous concentrations (aqueous to organic phase ratio 2.5 : 1) of about 18 μg/ml. The absorptivity of the complex in chloroform solution at 340 nm is 0.24 μg^{-1} cm^2.
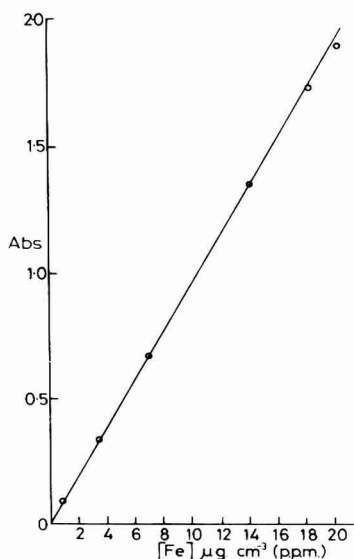
FIG. 3. Calibration curve for the analytical method.

TABLE 2
THE ANALYSIS FOR IRON OF SEVERAL TEST SAMPLES

| | Fe content | |
| | Phenylacetic acid method | Atomic absorption spectroscopy |
Sample		
Ferric nitrate	13.69%	13.72%
Ferric nitrate (in the presence of 10^{-3} M copper nitrate)	13.70%	13.74%
Brass	2.05%	2.02%
Tap water	0.81 μg/ml	0.80 μg/ml

The results obtained for the analysis of Fe(III) in four test samples (two of which contained large amounts of copper) are shown in Table 2. The results of the analysis of the samples by atomic absorption spectroscopy are also shown in the table.

The figures obtained using the extraction technique with phenylacetic acid are seen to be in good agreement with those obtained using atomic absorption spectroscopy, even in cases where there are large amounts of copper present. In these latter cases careful pH control of the aqueous solutions is critical.

SUMMARY

The extraction of Fe(III) from aqueous solutions by phenylacetic acid in chloroform solution provides a simple, rapid, and accurate method for the determination of low amounts of iron. Concentrations less than 1 μg/ml can be easily determined particularly if advantage is taken of the amplification procedure afforded by the solvent extraction technique by using a large aqueous to organic phase ratio.

A maximum concentration of iron(III) of about 18 μg/ml in the aqueous phase (aqueous to organic phase ratio of 2.5 : 1) can be determined; however, above this concentration deviations from Beer's Law occur for the absorbance of the organic phase. The method is very selective, particularly in the presence of copper, provided care is taken to adjust the aqueous pH to a value of 2.8.

n-Decanoic acid could also be used in the method in place of phenylacetic acid and would have some advantage in selectivity, particularly in cases where very large amounts of Ag(I) and Pb(II) were present.

ACKNOWLEDGMENTS

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Solvent–Polymer Interaction

I. Molecular Transport of Some Selected Organic Liquids in Polymer Membrane

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INTRODUCTION

The measurement of solvent–polymer interactions, including sorption, diffusion, and permeation, has been the subject of continuous interest for several decades. Many theoretical considerations and experimental results of the transport of solvents in various polymer systems have been reported (*4, 5, 8, 17, 19*). Probably, one of the most important findings in recent work on the diffusional transport of small molecules in polymeric solids is that at temperatures well above glass transition temperature (T_g) of polymers the kinetics of sorption and of permeation of organic vapors are invariably Fickian characteristics (*5*). However, such studies include the molecular transport of the most organic vapors, which are either solvents or swelling agents, for ordinary polymers (*5, 14, 16, 18*). Only a limited number of the published data is available for the interpretation of transport behaviors in organic liquid–polymer systems (*1, 11, 12, 15*). Therefore, a considerable additional work in this particular area is recommended. The present paper reports the measurements of the diffusional transport of some selected organic liquids in polymer membrane at various temperatures well above T_g of the polymer by sorption–desorption techniques using thermogravimetry (TG).

The use of TG as a rapid and simple means of studying drug–plastic interactions has been reported (*1*). In that study a group of organic liquid compounds were first placed in contact with a specific plastic, nylon-6/6, until equilibrium was attained. The sorbed materials were then placed into the TG instrument and the desorption was followed in a dynamic manner by increasing the temperature at a constant rate. The resultant TG-desorption curve then permitted an eval-

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uation of the equilibrium sorption concentration and the activation energy of desorption which in turn allowed a qualitative insight as to the role the structure of the compound played in the sorption process.

Hung and Autian (*12*) also reported the use of TG to the investigation of the interaction of a series of lower aliphatic alcohols (all liquids) with a specific polymer membrane, namely, polyurethane. In their work, sorption–desorption experiment was conducted at isothermal conditions. The TG-desorption experiment was performed in a static way by maintaining the temperature constant. From a single TG-desorption experiment the equilibrium sorption concentrations (saturation values) and the diffusion coefficients of alcohols at each specific temperature were determined simultaneously. In addition, the activation energy of diffusion for each alcohol in polymer matrix was evaluated from the Arrhenuis plottings. The possible mechanisms of diffusional behaviors and the influences of the molecular size and molecular weight of alcohol molecules on their diffusion rate in the polymer matrix were also demonstrated.

The present work extends the TG studies on the interactions of a wide spectrum of organic liquids, which possess general solvent properties (but no swelling capacity toward polyurethane) and have a variety of structural–functional groups, with the polyurethane membrane. These solvents are hexane, benzene, toluene, 3-methyl-1-butanol, 1-hexanol, 1-heptanol, 1-octanol, benzoic acid (satd soln), chlorobenzene, acetone, methyl acetate, and ethyl acetate. The polyurethane materials were used in this study because of their new and rapid growth both in practical and theoretical importance, and their interaction with organic liquids have not been extensively studied. The TG-desorption experiments were carried out under both isothermal (static) and nonisothermal (dynamic) conditions with micro- or semimicro scale. From these experiments, a number of thermodynamic properties and kinetic parameters such as solubility, equilibrium sorption constant, diffusion coefficient, permeability, the changes in standard enthalpy and standard entropy, activation energies of desorption and of diffusion, and so forth, in and from the polyurethane membrane are obtained. The correlation between the molecular mobilities of solvent molecules in polymer matrix and the microscopic structure and macroscopic properties of solvent molecules and of polymer segments are discussed.

EXPERIMENTAL METHODS

Reagents and Materials

Eleven organic liquid solvents: hexane, benzene, toluene, 3-methyl-1-butanol, 1-hexanol, 1-heptanol, 1-octanol, chlorobenzene, acetone,

TABLE 1
SOME PHYSICAL PROPERTIES OF ORGANIC LIQUIDS

Compound	Formula wt	Boiling point[a] (°C)	Density[a] (g/cc)	Molecular vol[b] $\times 10^{23}$ (cc/molecule)
Hexane	84.16	69.0	0.6603	21.16
Benzene	78.11	80.0	0.8790	14.75
Toluene	92.13	111.0	0.8669	17.64
3-Methyl-1-butanol (isoamyl alcohol)	88.15	130.5	0.8120	18.02
1-Hexanol	102.17	157.2	0.8186	20.72
1-Heptanol	116.20	176.0	0.8219	23.47
1-Octanol	130.23	195.0	0.8246	26.20
Benzoic acid (satd soln)	122.12	250.0	1.2659	16.02
Chlorobenzene	112.56	132.0	1.1066	16.33
Acetone	58.08	56.2	0.7920	11.77
Methyl acetate	74.08	57.3	0.9274	12.92
Ethyl acetate	88.10	77.1	0.9010	15.70

[a] Values obtained from "Handbook of Chemistry and Physics," 44th ed., Chem. Rubber Pub. Co., Cleveland, OH, 1963.

[b] Molecular volume calculated from the relationship $d = M/V$ by dividing the molecular weight by the density and the Avogadro number to yield the volume per molecule.

methyl acetate, and ethyl acetate; and one aqueous saturated solution of benzoic acid were used in this study. All of these compounds are of reagent grade or of the highest purity obtained commercially. They were used as such without further purification. Table 1 lists the compounds and summarizes a number of physical properties for each compound.

The polyurethane was obtained as a thin film from Molded Products Co, Easthampton, MA under the code number MP950. It is a thermoset solid polyurethane prepared by reacting a polyurethane prepolymer with 4,4′-methylenebis(2-chloroaniline). Its general properties are those of an elastomer.

Apparatus and Equipment

The apparatus used in sorption experiments was a glass sorption tube stoppered by a screwcap with Teflon-faced rubber liner.

The equipment used to measure desorption under dynamic condition (nonisothermal) was the Fisher Series 100 TG system manufactured by Fisher Scientific Co, Pittsburgh, PA. This system consists of Model 120P TG accessory, Model 360 linear temperature programmer, Model 260F furnace, Cahn RG electrobalance (manufac-

tured by Cahn Instrument Co, Paramount, CA), and two-channel "Servo/Riter II," 1-mV X-Y Recorder (Texas Instruments, Inc., Houston, TX). However, for desorption experiments under static (isothermal) condition "Sero-Utility" water bath (Sero-Utility Bath, Model 82, Precision Scientific Co., Chicago, IL) was replaced for Model 260F furnace. A schematic diagram of the experimental setup is shown in Fig. 1.

Procedure

The general experimental procedure setup for a TG-desorption run involved several steps. These include preparation of samples, calibration of balance and recorder, calibration of thermocouple and recorder, TG operation, and analysis of the TG curve to obtain useful information, etc.

A. Preparation of samples. Test membranes were punched from the polyurethane film with a paper punch, No. 1, 0.63 cm diameter. Cylindrical shaped membranes were cut of 0.62 cm diameter, 0.0770–0.0778 cm thickness, and 25.5–27.2 mg. In all instances, test membranes for each different solvent at each specified temperature were kept as uniform as possible and never exceeded 0.5% (thickness or weight) from sample to sample. Individual thickness and weight of the membrane were always used, however, in the treatment of the TG curves for obtaining the thermodynamic and kinetic data. Prior to use in any experiment the test membranes were soaked in 95% ethanol for 48 hr and then rinsed repeatedly with distilled water. An additional soaking in 50% ethanol for 24 hr with subsequent rinsing with
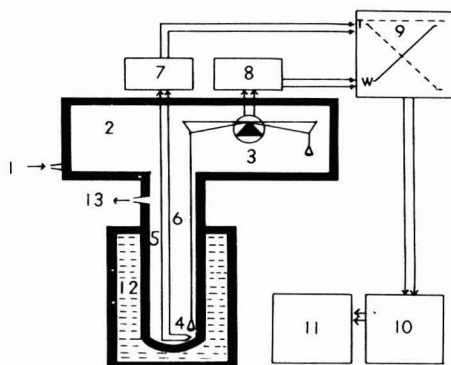
FIG. 1. Schematic diagram of TG experimental setup: (1) gas in; (2) sample atmosphere; (3) balance chamber; (4) sample; (5) sample thermocouple; (6) quartz hangdown tube; (7) reference junction; (8) balance control; (9) recorder; (T) temperature change; (W) weight change; (10) linear temperature programmer; (11) temperature control; (12) furnace or thermostat (water bath); (13) gas out.

distilled water was performed. Finally, the test membranes were rinsed with acetone and dried to constant weight. These membranes were then stored in a desiccator until ready for use.

For sorption experiment at isothermal condition, 10 pieces of approximately the same initial dry weight of membranes were placed into a glass sorption tube containing 20.0 ml of the specific liquid solvent. The tube was then stoppered and placed into a thermally controlled water bath adjusted to 30 ±0.1°C. Similar procedure and technique were employed for preparation of different sorption samples at various temperatures of 40 and 50 ±0.1°C.

Preliminary measurements indicated that the equilibrium sorption occurred within a few days to several weeks depending upon the nature of the compound and the temperature. For uniformity, however, the sorption tubes with samples were kept in the water bath for exactly 30 days. These equilibrium sorbed samples were used in all TG-desorption studies.

B. Calibration of balance, recorder, and thermocouple. Generally, before performing a TG run, the balance and the recorder were first calibrated with standard weights according to the size of the sample to be dealt with. For the size of the samples used in the present study the best recorder range is 10 mg and the "Mass Dial Range," 100 mg. After calibration, the recorder range can be expanded to 1, 2, 4, or 20 mg full scale, depending upon the sample weight, the amount of sorption, and the rate of desorption of the particular system under investigation. Furthermore, the sample measuring thermocouple (here platinum) and the recorder temperature pen were calibrated with 0°C reference junction by immersing the thermocouple into a small beaker (or Dewar flask) containing some crushed ice in equilibrium with water and then zeroing the temperature at the left side of the recorder chart. This operation sets the 100 line of the recorder chart as the reference point at 0°C.

C. Desorption studies under dynamic (nonisothermal) condition by employing TG technique. TG is a sensitive and an accurate technique which measures the changes in weight of a material as a function of temperature (dynamic or nonisothermal condition) or as a function of time at a certain specified temperature (static or isothermal condition). It is one of the most versatile methods of quantitative analysis, and is being used by many major industries for routine analysis and quality control, as well as in research. A great deal of information in a wide variety of chemical investigations can be obtained from the application of this technique (*13*) under either dynamic or static conditions. Under dynamic condition, a plot of weight change vs temperature (and/or time) is obtained. While for isothermal method, a plot of

weight change vs time is obtained at constant temperature. For desorption study under dynamic condition the TG instruments were set up as follows:

Programming rate: in increasing rate of 5°C/min.

Electrobalance sensitivity: 0.01 mg.

Recorder span: initially at 10 mg full scale; expanded scale: 1 mg full scale for the smallest amount of sorption; 2 mg full scale for a small amount of sorption; 4 mg or 10 mg full scale for a moderate amount of sorption; 20 mg full scale for a large amount of sorption.

Chart speed: 0.5 in./min for a moderate and a large amount of sorption and fast desorption processes. 8 in./hr for a small amount of sorption and slow desorption processes.

Inert gas atmosphere: flushing with nitrogen gas at a constant flow rate of 100 ml/min.

Operation temperature range: ambient temperature to 170°C.

The sampling procedure was then followed after the setup of the experimental conditions and the calibration of the instruments. A piece of sorbed polyurethane samples (sorbed samples or test samples) was then removed from the sorption tube and excess liquid on the membrane surface blotted with tissue paper. Immediately (within 70 sec) the sorbed sample was introduced into the TG instrument, the initial sample weight of sorption (equilibrium saturation values) was recorded, and a TG desorption run was started with a linear programming rate of 5°C/min. This same sampling technique was used through all of the TG desorption experiments. A stream of nitrogen gas was passed through concentrated sulfuric acid and then into the chamber containing the test sample at a constant flow rate of 100 ml/min to flush and remove any adhering gases or desorbed gaseous products during the desorption experiment. The gas (N_2) in turn was led out of the chamber into a series of gas washing bottles. The heating temperature was always kept 30 to 40°C below the melting point of polyurethane membrane so that the structure properties of polymer matrix may not be destroyed by thermal effect and the weight change of the sorbed samples as a function of temperature was merely resulting from the sorbed solvents. At least duplicate runs for desorption of each sorbed sample were made. These weight changes as a function of temperature, TG curves, were used in the determination of kinetic data.

D. Desorption studies under static (isothermal) condition by utilizing TG technique. The setup of experimental conditions and the calibration of the TG instruments for desorption study under isothermal condition are the same as those previously described as under

dynamic condition but certain modifications were made in this study. In this case the sorbed sample, the quartz hangdown tube and the sample thermocouple (to measure the temperature of the test sample) were all contained in a chamber which could be immersed into a constant temperature water bath adjusted to the same temperature of the initial 30-day sorption experiments (i.e., 30, 40, and 50 ±0.1°C). When equilibrium temperature was achieved in the chamber (10 min), the sampling process was followed. After the temperature in the chamber (containing test sample) was reequilibrated (2 min), the instrument was turned on and the weight vs time was recorded on chart paper until constant weight was obtained. It required 1.5 to 53 hr to complete a desorption experiment depending on the system and the temperature being studied. To ensure reproducibility, at least two test samples for each solvent at each sorption–desorption temperature were run. The isothermal TG curves thus obtained were used for the evaluation of the solubility and the diffusivity of the specific compound (solvent) at the corresponding temperature being studied.

RESULTS AND DISCUSSION

Solubility and Equilibrium Sorption Constant

The solubility (S) and equilibrium sorption constant (K_{sorp}) for 12 organic liquid compounds in polyurethane membrane at 30.0, 40.0, and 50.0 ±0.1°C are summarized in Table 2. All of these values are the average of at least two determinations. The reproducibility ranges from 0.5 to 2.0%. The S values were obtained from the difference between the initial readings of the recorder just before the starting of the isothermal TG runs and the weight of dry polyurethane membrane. It was based on the assumption that saturation values were maintained during the very short periods of sampling process. In general, values of S and K_{sorp} increase with increasing temperature for all solvents studied. The temperature coefficients are, with the exception of benzoic acid, ranged from 0.5 to 10.0% for each increment of 10.0°C. Benzoic acid shows a higher temperature coefficient (about 20.0% increased). Qualitatively, it is possible that the increased thermal effect might enhance the segmental motions of polymer chains and slightly increase in amorphous regions or thermal activated "holes" to accommodate more solvent molecules.

Values of K_{sorp} for solvent–polymer systems were computed from the following considerations on the equilibrium process occurring in the liquid phase at constant temperature and pressure:

$$L + P \rightleftharpoons L \rightarrow P_{(liquid \ in \ solid)}, \tag{1}$$
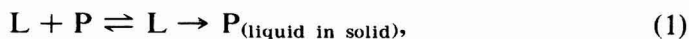
TABLE 2
SOLUBILITY AND EQUILIBRIUM SORPTION CONSTANT OF SOME SELECTED
ORGANIC LIQUIDS IN POLYMER MEMBRANES AT VARIOUS TEMPERATURES

Compound	Temperature (°C)	Solubility, S (mg solvent/ mg membrane)	Equilibrium sorption constant, K_{sorp} (mmole solvent/ g membrane)
Hexane	30	0.0825	0.980
	40	0.0867	1.03
	50	0.0934	1.11
Benzene	30	0.5585	7.15
	40	0.5593	7.16
	50	0.5608	7.18
Toluene	30	0.4874	5.29
	40	0.5030	5.46
	50	0.5058	5.49
3-Methyl-1-butanol	30	0.3561	4.04
	40	0.3722	4.22
	50	0.4093	4.64
1-Hexanol	30	0.4097	4.01
	40	0.4220	4.13
	50	0.4741	4.64
1-Heptanol	30	0.3718	3.20
	40	0.4102	3.53
	50	0.4358	3.75
1-Octanol	30	0.2984	2.29
	40	0.3309	2.54
	50	0.3663	2.81
Benzoic acid (satd soln)	30	0.2259	1.85
	40	0.2853	2.34
	50	0.3249	2.66
Chlorobenzene	30	0.9016	8.01
	40	0.9262	8.23
	50	0.9520	8.46
Acetone	30	0.3851	6.63
	40	0.4365	7.52
	50	0.4829	8.31
Methyl acetate	30	0.4378	5.91
	40	0.4586	6.19
	50	0.5030	6.79
Ethyl acetate	30	0.4115	4.67
	40	0.4190	4.75
	50	0.4275	4.85

and

$$a_{L \to P}/a_L \cdot a_P = K_{eq},   \qquad (2)$$

where L, P, and L → P denote the liquid solvent, the solid polymer membrane, and the sorbed liquid solvent in the polymer membrane, respectively; a_L, a_P, and $a_{L \to P}$ are corresponding activities in that order; K_{eq} is the thermodynamic equilibrium constant. However, for pure liquid solvent and pure solid polymer membrane, a_L and a_P are equal to unit. Therefore, Eq. (2) reduces to

$$K_{eq}(a_L \cdot a_P) = K_{sorp} = a_{L \to P}.   \qquad (3)$$

However, during the sorption–desorption process, no chemical reactions occurred in the solvent–polymer systems. The polyurethane membrane is insoluble in the solvents. It retains its original constant shape, size, weight, and structural properties. Moreover, since TG measures accurately the direct changes in mass of sorbed solvents only, Eq. (3) can be rewritten as:

$$K_{sorp} = a_{L \to P} = \frac{\text{No. of moles of solvents sorbed}}{\text{unit mass of the polymer}},   \qquad (4\text{-a})$$

or

$$K_{sorp} = \frac{\text{moles}}{\text{kg membrane}} = \frac{\text{mmoles}}{\text{g membrane}}.   \qquad (4\text{-b})$$

Thus Eq. (4-b) was used in the calculation of K_{sorp} in this study.

Effect of Molecular Weight on K_{sorp}

Figure 2 shows the relations between K_{sorp} and molecular weight (MW) of the corresponding solvents at 30.0, 40.0, and 50.0 ±0.1°C. It
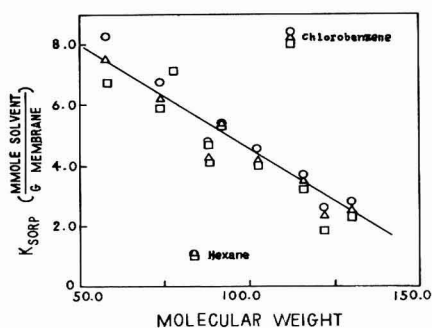
FIG. 2. Effect of molecular weight on the equilibrium sorption constants of organic liquid solvents in polyurethane membrane at various temperatures: (□) 30°C; (△) 40°C; (○) 50°C.

is evident that K_{sorp} is inversely related to MW. This is logical, since the larger molecule tends to occupy more free volume in the amorphous regions than the smaller one. However, hexane and chlorobenzene present two extreme cases. Hexane, because of its symmetrical nonpolar structural property and its characteristic hydrophobic type intermolecular forces, shows a strong resistance to be sorbed by polyurethane membrane. Thus it deviates negatively (lower solubility) from the linearity. On the contrary, the positive deviation of chlorobenzene from the linear relationship not only indicates that chlorobenzene has a strong affinity for polyurethane membrane but also implies that there are some similarities in structure and polarity between chlorobenzene molecules and polyurethane chains. [For examples, there are many polar groups such as aliphatic hydrocarbon (—CH$_2$—), ether (—O—), ester (—COO—), amide (—CONH—), urethane (—NH—COO—), aromatic hydrocarbon (—C$_6$H$_4$—), and the chlorinated aromatic derivatives

$$(-NH-COO-NH-\!\!\left\langle\!\!\bigcirc\!\!\right\rangle\!\!-CH_2-\!\!\left\langle\!\!\bigcirc\!\!\right\rangle\!\!-),$$
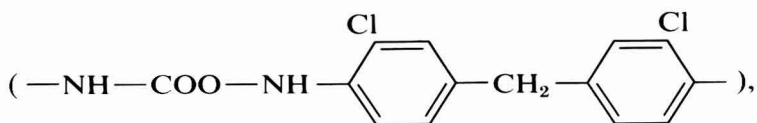
etc., in the polyurethane chains (21)]. Following the generalization, "like sorbed in like," this explanation is consistent with the experimental results.

Thermodynamic Properties of Sorption

For the present study, a basic relation between the change in standard affinity of sorption (standard chemical potential, Δu^0) and the change in standard enthalpy (heat of sorption, ΔH^0) and the change in standard entropy of sorption (ΔS^0) can be established as

$$\Delta u^0 = \Delta H^0 - T\Delta S^0, \tag{5-a}$$

or by rearrangement

$$\Delta u^0/T = H^0/T - \Delta S^0, \tag{5-b}$$

where $\Delta u^0 = -2.303\ RT \log K_{sorp}$.

By assuming ΔH^0 and ΔS^0 remaining constant over a small temperature range (30.0 to 50.0°C), a plot of $\Delta u^0/T$ versus $1/T$ producing a straight line with a slope equal to ΔH^0 and an intercept equal to $-\Delta S^0$ was obtained. These plots are presented in Fig. 3, while the numerical values of ΔH^0 and ΔS^0 are tabulated in Table 4.

As shown in Table 4, all values of ΔH^0 and ΔS^0 are positive. The positive values of ΔH^0 are interpreted as indicating that some

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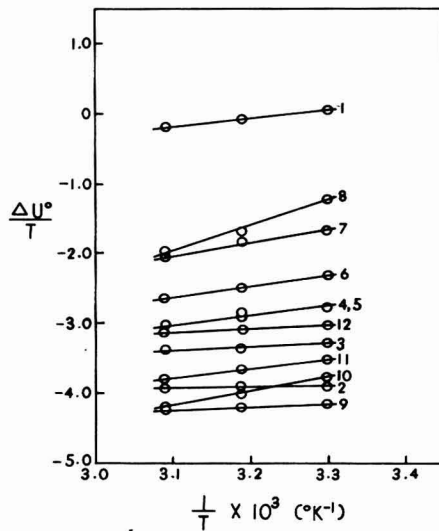
FIG. 3. Thermodynamic properties of organic liquid solvents in polyurethane membrane: (1) hexane; (2) benzene; (3) toluene; (4) 3-methyl-1-butanol; (5) l-hexanol; (6) l-heptanol; (7) l-octanol; (8) benzoic acid; (9) chlorobenzene; (10) acetone; (11) methyl acetate; (12) ethyl acetate.

amounts of thermal energies are required to induce sorption effectively by creating void volumes or holes between the segments or interchains of the polyurethane membrane. The more predominantly positive values of ΔS^0 than those of ΔH^0 highly suggest that the microstructurally correlated probability for the combination or orientation of sorbed solvent molecules with the polymer chains are high. In other words, the process of sorption of organic liquid solvents from a more closely packed homogeneous liquid phase into a more randomly oriented amorphous regions of heterogeneous solid polymer phase is a spontaneous process which increases the entropy and leads to an equilibrium sorption at the specified temperature.

Determination of Diffusion Coefficient from the Isothermal TG Desorption Curves

The mathematical model for the determination of diffusivity of solvent molecules in the polymer matrix by desorption method with TG technique has been previously derived (*12*). The final form of the equation which was used in the present determination is

$$\log [Q(t)/Q(\infty)] = \log (8/\pi^2) - [(\pi^2 D/(2.303 L^2)](t), \qquad (6)$$

where $Q(t)$ is the weight of liquid solvents per unit area of the plane sheet remaining in the solid polymer at time t, $Q(\infty)$ is the total weight
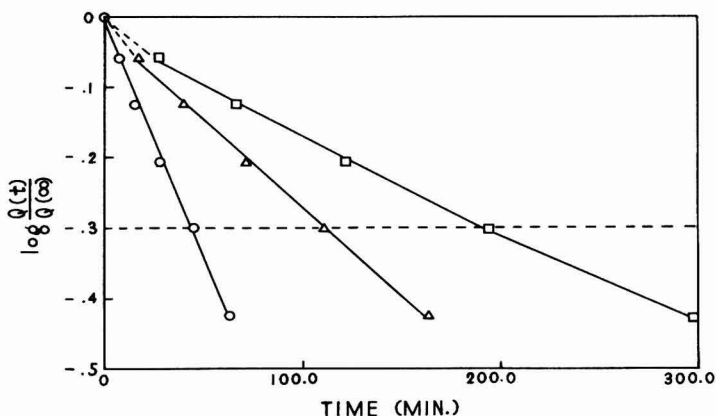
FIG. 4. Typical plots of log $Q(t)/Q(\infty)$ vs time for evaluation of diffusivity of 1-heptanol in polyurethane membrane at various temperatures: (□) 30°C; (△) 40°C; (○) 50°C.

of liquid solvents diffusing out of the plane sheet at infinite time (for a system which undergoes a complete desorption $Q(\infty)$ should equal to the solubility S), D is the diffusion coefficient or diffusivity, L is the thickness of the polymer membrane, t is time.

Equation (6) is a linear equation, thus for any desorption experiment a plot of log $[Q(t)/Q(\infty)]$ vs time t should reveal a linear relationship from which D can be calculated from the slope. In practice, desorption data were taken directly from the TG curves, recalculated as log $[Q(t)/Q(\infty)]$ and plotted vs time t. Some typical plots for evaluation of diffusivity of 1-heptanol in polyurethane membrane at three different temperatures are shown in Fig. 4. Linear relations for Eq. (6) were found to exist in the range of 0% up to approximately 50% desorption (that is, where log $[Q(t)/Q(\infty)] = -0.30$) and thus only these portions were used for calculating the slopes. The slopes were computed by the method of least-squares by means of an IBM 360 computer using Fortran IV. Furthermore, these linear plots also demonstrate that the diffusion of solvent molecules in the polyurethane membrane is Fickian characteristic and D is constant in the early stage of desorption. All other solvent–polymer systems give the same diffusional pattern of plottings as those shown in Fig. 4 and are therefore neglected.

Effect of Molecular Size on the Diffusivity

The determined D values for 12 organic liquid compounds at 30.0, 40.0, and 50.0 ±0.1°C are listed in Table 3. In the same table the calculated values of permeability (P) are also given. P values were

TABLE 3
DIFFUSIVITY AND PERMEABILITY OF SOME SELECTED ORGANIC LIQUIDS IN
AND THROUGH POLYMER MEMBRANES AT VARIOUS TEMPERATURES

Compound	Temperature (°C)	Diffusivity $D \times 10^8$ (cm²/sec)	Permeability $P \times 10^8$ (cm²/sec)
Hexane	30	5.64	0.465
	40	6.09	0.528
	50	6.75	0.631
Benzene	30	12.9	7.22
	40	18.8	10.5
	50	23.5	13.2
Toluene	30	14.3	6.97
	40	16.0	8.07
	50	19.4	9.81
3-Methyl-1-butanol	30	1.40	0.499
	40	2.97	1.11
	50	5.58	2.28
1-Hexanol	30	1.42	0.582
	40	3.07	1.30
	50	4.80	2.28
1-Heptanol	30	0.863	0.321
	40	1.61	0.660
	50	3.59	1.56
1-Octanol	30	0.497	0.148
	40	0.713	0.236
	50	1.81	0.663
Benzoic acid (satd soln)	30	0.165	0.0372
	40	0.285	0.0813
	50	0.527	0.171
Chlorobenzene	30	13.7	12.4
	40	17.7	16.4
	50	28.6	27.2
Acetone	30	14.3	5.51
	40	19.3	8.42
	50	26.5	12.8
Methyl acetate	30	11.7	5.12
	40	16.4	7.52
	50	24.3	12.2
Ethyl acetate	30	11.2	4.61
	40	17.0	7.12
	50	21.6	9.23

calculated from the simple relation

$$P = DS, \tag{7}$$

as a product of the diffusivity and the solubility. This simple relation holds for the permeation process when D obeys Fick's diffusion law and S obeys Henry's law (5, 9). It is not certain to what degree with penetrant (solvent)–polymer systems used in this study, if in fact one or both laws are actually obeyed. Thus, the P values presented in Table 3 should be considered as estimates of the permeability coefficients.

Figure 5 shows the relationship between D and the molecular volume ($\overline{V}$, or molecular size) of each of the solvents at three different temperatures studied. It is obvious that D is, in general, an inverse function of $\overline{V}$. There are two categories of compounds showing variable diffusion behaviors with respect to their molecular size. The first category contains larger molecules such as 3-methyl-1-butanol, 1-hexanol, 1-heptanol, and 1-octanol four compounds in which a polar hydroxyl (—OH) functional group is attached to one terminal of each of the molecules. This group of compounds is found to exhibit a slower diffusion rate and to show a good linear relationship between D and $\overline{V}$ ($\overline{V} = 18.02, 20.72, 23.72, 26.20 \times 10^{-23}$ cc/molecule, respectively). It seems that the interaction through hydrogen-bonding between the —OH group of these solvent molecules and the polar groups of the polyurethane chains is responsible for the slower dif-
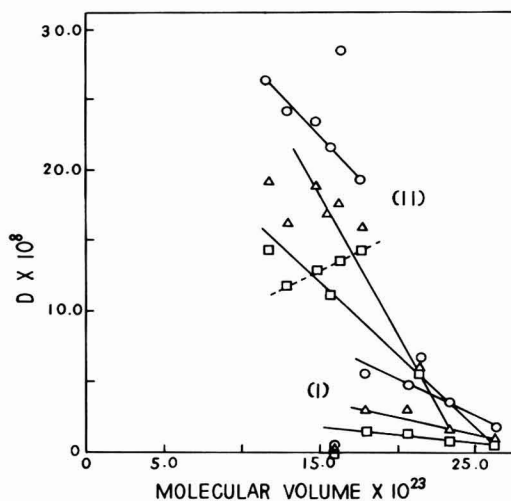
FIG. 5. Effect of molecular size on the diffusivity of organic liquid solvents in polyurethane membrane at various temperatures: (□) 30°C; (△) 40°C; (○) 50°C.
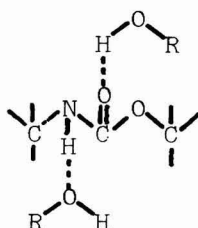
FIG. A. Possible bindings through hydrogen-bonding between alcohol molecules and polyurethane chains: R = 3-methyl-1-butyl, 1-hexyl, 1-heptyl, 1-octyl.

fusion rate of the category (Fig. A). However, it is also possible that other secondary forces such as hydrophobic bonding play a role in the interactions. These types of bondings for solvent–polymer interactions have been discussed by other investigators (5). The second category consists of smaller molecules such as benzene, toluene, chlorobenzene, acetone, methyl acetate, and ethyl acetate 6 liquid solvents. This category shows a rather scattering from linearity between D and $\bar{V}$ at lower temperatures (30.0 and 40.0°C). This is interpreted as reflecting that the variety of structural properties and functional groups in the molecules of the group cause the variable diffusional behaviors at lower temperature. However, at higher temperature (50.0°C) because of the compensation of the thermal energy to the molecular motions of individual molecules an excellent linear relation was observed. Furthermore, benzene, chlorobenzene, and toluene three compounds because of their similarity in aromatic structure reveal a good linear direct relation between D and $\bar{V}$ at 30.0°C.

In addition, two special cases should be pointed out. First, although the molecular size of hexane ($\bar{V} = 21.16 \times 10^{-23}$ cc/molecule) is about the same as that of 1-hexanol ($\bar{V} = 20.72 \times 10^{-23}$ cc/molecule), hexane molecules diffuse much faster than 1-hexanol molecules in polyurethane membrane at the same temperature (for instances, $D = 5.64, 6.09, 6.75 \times 10^{-8}$ cm²/sec for hexane; and $D = 1.42, 3.07, 4.80 \times 10^{-8}$ cm²/sec for 1-hexanol, respectively). This again, as previously described, reflects the nonpolar molecular property of hexane and the weakness of intermolecular binding forces between hexane molecules and polyurethane chains. Second, the $\bar{V}$ value of benzoic acid ($\bar{V} = 16.02 \times 10^{-23}$ cc/molecule) is almost identical to that of chlorobenzene ($\bar{V} = 16.33 \times 10^{-23}$ cc/molecule), but D values of benzoic acid ($D = 0.165, 0.285, 0.527 \times 10^{-8}$ cm²/sec) are only 1.2 to 1.8% of those of chlorobenzene ($D = 13.7, 17.7, 28.6 \times 10^{-8}$ cm²/sec) at the corresponding temperatures. Besides, if benzoic acid is classified into the —OH functional group containing compounds of the first category, it is the smallest molecule in that category but its

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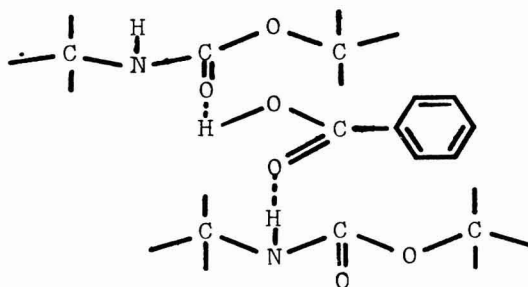
FIG. B. Possible intermolecular forces through double hydrogen-bondings between benzoic acid and polyurethane chains.

diffusion rate is also the slowest one. This phenomenon is not consistent with the generalization that D is an inverse function of $\bar{V}$ as would be expected for the first category [Fig. 5, (I)]. The possible explanation for this distinguished diffusion behavior is that benzoic acid may be bound strongly to the polar groups of polyurethane chains through the formation of double hydrogen bonds as shown in Fig. B. In other words, one molecule of benzoic acid is capable of assuming the same geometric plane as the surrounding polyurethane chains in the amorphous zone and fitting itself between two adjacent polyurethane chains.

Diffusivity as a Function of Equilibrium Sorption Constant

The variation of D with respect to K_{sorp} for the various solvent–polymer systems at three temperatures are presented in Fig. 6. It is evident that D is directly proportional to K_{sorp}. In addition, two categories of compounds containing the same kinds of molecules as those found in Fig. 5 and showing slightly different relations between D and K_{sorp} can be seen. In the first category (benzoic acid, 1-octanol, 1-heptanol, 1-hexanol, and 3-methyl-1-butanol) D values change slowly with K_{sorp}. However, larger slopes are observed for the plots of D vs K_{sorp} for the second category (hexane, ethyl acetate, methyl acetate, benzene, chlorobenzene, toluene, and acetone). Since K_{sorp} is itself an inverse function of MW (Fig. 2), Figs. 5 and 6 clearly demonstrate that both factors (MW and $\bar{V}$ in the homogeneous liquid phase) are directly correlated with each other and the effect on diffusivity in the heterogeneous solid polymer membrane are similar. The effect of MW on D may be interpreted as a direct reflection of the molecular motions of the molecules in the polymer matrix (larger molecules diffuse slowly) while the effect of $\bar{V}$ on D is generally correlated with the availability of the free volume between polymer chains for diffusion flow if Eyring's "hole theory of diffusion" is presumed (*10*).
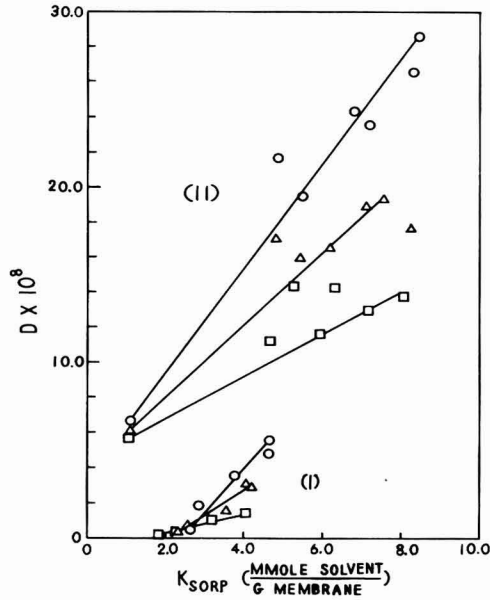
FIG. 6. Diffusivity of organic liquid solvents as a function of equilibrium sorption constants in polyurethane membrane at various temperatures: (□) 30°C; (△) 40°C; (○) 50°C.
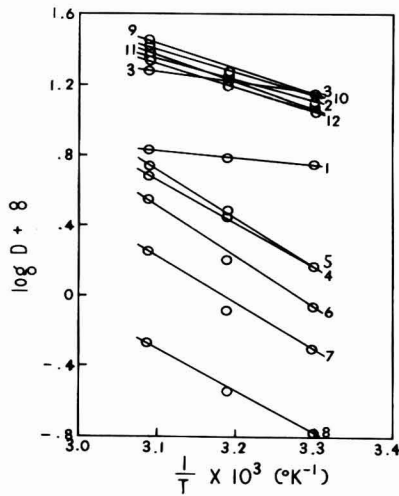
FIG. 7. Arrhenius plots for the diffusion of organic liquid solvents in polyurethane membrane: (1) hexane; (2) benzene; (3) toluene; (4) 3-methyl-1-butanol; (5) 1-hexanol; (6) 1-heptanol; (7) 1-octanol; (8) benzoic acid; (9) chlorobenzene; (10) acetone; (11) methyl acetate; (12) ethyl acetate.

Activation Energy of Diffusion

The activation energies, E_D, for solvent molecules diffusing in poly-urethane membrane were calculated from the slopes of the plots of log D vs $1/T$ following Arrhenius type equation in the form

$$\log D = \log D_0 + (E_D/2.303\ R)(1/T), \tag{8}$$

where D is diffusivity, D_0 a constant (that is, frequency factor or preexponential factor indicating the value of D at infinite tempera-ture), E_D the activation energy for diffusion, R the universal gas con-stant, and T the absolute temperature.

Figure 7 shows the good linear plots for the solvent–polymer systems studied by employing Eq. (8). The determined E_D values (Table 4) ranged from 1.69 kcal/mole (for hexane) to 13.5 kcal/mole (for 1-heptanol). Since the energy required to "open a hole" in the polymer matrix to accommodate a diffusing molecule will bear a direct relationship to the activation energy of diffusion, larger mole-cules in a related series will have larger E_D and slower diffusion rates. This is in conformity with experimental observations reported here (Figs. 5 and 6).

Moreover, it is interesting to note that most of the E_D values ob-tained here for organic solvents diffusing in the polymer membrane are comparable to the literature values of the heat of vaporization (ΔH_v, listed in Table 4) for the corresponding solvents vaporizing from the liquid phase (except hexane, benzene, and toluene). This probably suggests but not proves that the intermolecular forces (i.e., binding forces and/or bondings) play an equally important role in both phases, and the diffusion mechanism and the vaporization process are similar in these particular solvent–polymer systems studied.

Kinetics of Desorption Process at Nonisothermal Condition

In general, TG technique has been used in kinetic studies for the following solid state reaction (thermal decomposition) at noniso-thermal condition (7)

$$A(s) \rightarrow B(s) + C(g). \tag{9}$$

Similarly, the kinetics of desorption from solid polymer membrane at nonisothermal condition is expressed as

$$L \rightarrow P \longrightarrow P(s) + L(g), \tag{10}$$
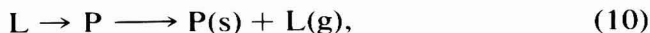
where P(s) is the solid polyurethane membrane after desorption, L(g)

TABLE 4
KINETIC PARAMETERS AND THERMODYNAMIC PROPERTIES OF SOME SELECTED ORGANIC LIQUIDS IN POLYMERIC MEMBRANE

Compound	Activation energy of diffusion E_D (kcal/mole)	Nonisothermal activation energy of desorption E_{desp} (kcal/mole)	Heat of vaporization at pure liquid state[a] ΔH_r (kcal/mole)	Standard enthalpy change of sorption ΔH^0_{sorp} (kcal/mole)	Standard entropy change of sorption ΔS^0_{sorp} (cal/mole/°K)
Hexane	1.69	7.56	7.63	1.10	3.56
Benzene	5.68	6.67	8.15	0.386	4.03
Toluene	2.87	7.61	8.58	0.356	4.50
3-Methyl-1-butanol	12.7	6.84	12.5	1.30	7.04
1-Hexanol	11.6	7.10	12.7	1.69	8.27
1-Heptanol	13.5	7.38	13.9	1.74	8.02
1-Octanol	12.1	9.79	14.3	2.04	8.35
Benzoic acid (satd soln)	11.0	6.59	15.3	5.95	20.5
Chlorobenzene	6.93	7.46	8.73	0.519	5.85
Acetone	5.84	5.28	7.65	2.26	11.2
Methyl acetate	6.92	7.60	7.25	2.70	12.2
Ethyl acetate	6.25	6.34	7.79	0.619	5.04

[a] Values obtained from "Handbook of Chemistry and Physics," 51st ed., D-146, Chem. Rubber Pub. Co., Cleveland, Ohio, 1970.

the desorbed liquid solvents from the polyurethane membrane into the gas phase.

During the past few years, several methods have been developed to allow kinetic analysis of TG data for reaction (9) (*3, 6*). However, recently, a simple, sensitive graphical method proposed by Broido (*2*) can be used in treating TG data of both decomposition and desorption processes represented by Eqs. (9) and (10). Broido's method is an approximation method which assumed that for a pure solid substance undergoing pyrolysis reaction would follow a first-order reaction kinetics and the Arrhenius rate constant equation apply. The simplified form of the Broido's method is

$$\log \log (1/Y) = -(E_{desp}/2.303\ R)(1/T) + \text{constant}, \qquad (11)$$

where E_{desp} denotes the activation energy of desorption, R and T are same as defined before. The term Y is defined as

$$Y = (W_t - W_\infty)/(W_0 - W_\infty),$$

where W_t, W_0 and W_∞ are the weights of samples at time t, at initial zero time and at infinite time t_∞, respectively. A plot of $\log \log (1/Y)$ vs $1/T$ leads to straight line from which the slope $(-E_{desp}/2.303\ R)$ is obtained. Some typical plots which obey Eq. (11) are presented in
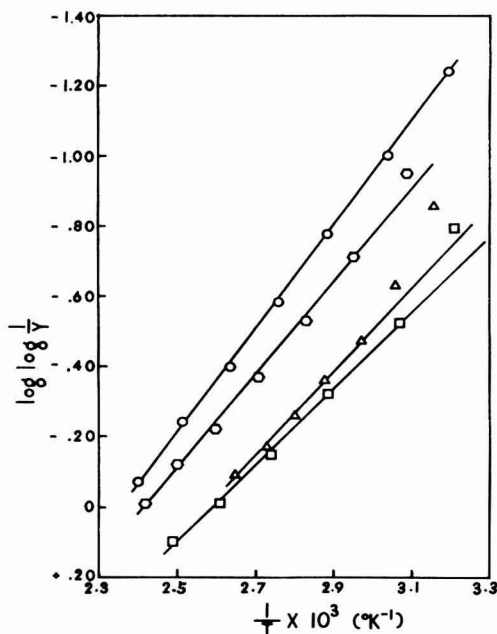
FIG. 8. Kinetics of desorption of organic liquid solvents from polyurethane membrane: (○) 3-methyl-1-butanol; (△) benzene; (□) acetone; (◯) ethyl acetate.

Fig. 8. The average values of E_{desp} from at least two TG desorption runs are shown in Table 4. The relative precision is about ±2.5%.

From Table 4, it is evident that E_{desp} values are almost constant for 12 compounds studied. It appears that the mechanisms of desorption of these solvents under dynamic condition are same and following first-order desorption kinetics as expressed by Eq. (10). Furthermore, E_{desp} values for four alcohols (the first category) and benzoic acid are approximately half of the corresponding E_D values. This indicates that for these compounds, because of the stronger intermolecular forces, more activation energies are required (for breaking hydrogen bonds) for diffusion process to occur at isothermal condition than for desorption to proceed at dynamic manner. Besides, the mechanism of molecular diffusion in the polymer matrix is far more complex than would be predicted from a simple kinetic process.

Linear Free Energy Relationships

In this study, the linear free energy relationships (LFER) for solvent–polymer interactions can be expressed as

$$\log D = m \log K_{sorp} + c, \tag{12-a}$$

and

$$\Delta G_D^* = n\Delta u^0 + d, \tag{12-b}$$

where ΔG_D^* is Eyring's free energy of activation for diffusion, m,
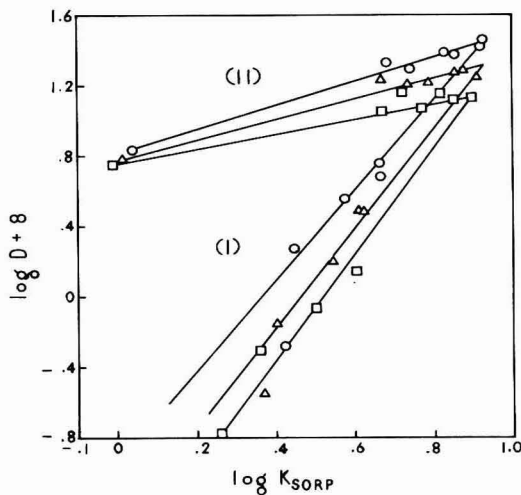
FIG. 9. Linear free energy relationship: plots of $\log D$ vs $\log K_{sorp}$ for organic liquid solvents in polyurethane membrane at various temperatures: (□) 30°C; (△) 40°C; (○) 50°C.

c, n, and d are constants characteristics of each specific system. [The definition of LFER and its application to the other systems have been described in detail by Wells (20).]

Figure 9 shows some excellent plots of log D vs log K_{sorp} for 12 solvent–polymer systems. Two groups of straight lines showing varied slopes can be seen [for examples, by Eq. (12-a) slopes, $m = 2.83$, 2.74, 1.97 for the first category; and $m = 0.46$, 0.55, 0.68 for the second category at 30.0, 40.0, and 50.0 $\pm$ 0.1°C, respectively]. The beautiful feature of these plots is that they provide a real correlation between microscopic molecular structure and macroscopic properties of solvent molecules in the polyurethane membrane. Thus one can predict D value for structurally related solvent molecules from these plots if the corresponding K_{sorp} is known and vice versa.

SUMMARY

Methods and microtechniques for determining solubility, diffusivity, thermodynamic properties, and kinetic parameters of 12 selected organic liquid solvents in polyurethane membrane by thermogravimetry (TG) are described. TG provides a simple, sensitive, rapid, and accurate microtechnique for measuring a minute change in weight (or mass) of a substance as a function of time at isothermal condition or as a function of temperature at dynamic manner. Thus from a single isothermal TG-desorption experiment, solubility and diffusivity of solvent molecules in polyurethane membrane were obtained simultaneously. Furthermore, by a dynamic TG-desorption run, kinetic parameter such as activation energy of desorption of solvent molecules from polyurethane membrane was determined. In addition, much other useful information such as equilibrium sorption constant, the changes in standard enthalpy and standard entropy of sorption, permeability, the activation energy of diffusion and so forth for solvents in polyurethane membrane are also evaluated and discussed. Finally, the correlation between the microscopic molecular structure and macroscopic properties of solvent molecules in polymer membrane is interpreted in terms of linear free energy relationships.

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Spectrophotometric Determination of Sodium Citrate in Blood

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INTRODUCTION

A relatively rapid and simple method for the analysis of sodium citrate added to blood as an anticoagulant was needed for blood shipments received here. Present methods were too involved and time consuming to be worthwhile. Tiwari and Pande (2) have shown that it was possible to quantitatively determine organic acids, such as citric acid, in an acidic medium by complexing the acids with ferric ions and analyzing by spectrophotometric methods. Pyatniski (1) has also shown that iron will form stable citrate complexes in an alkaline medium. Therefore, it was of interest to develop a quick, reliable method for the determination of citrate in blood in a manner analogous to previously published methods.

MATERIALS AND METHODS

A Beckman Acta III ultraviolet-visible recording spectrophotometer was used to determine spectra of various solutions. Measurements were made at 375 nm.

Sample solutions. A 250-mg sample of citric acid or sodium citrate was weighed to the nearest 0.1 mg and dissolved in distilled water in a 500-ml volumetric flask. Various volumes were pipetted into a 10-ml volumetric flask followed by 1 ml of ferric stock solution. A calibration curve was prepared from spectral measurements on these solutions. These solutions were run against a ferric blank containing 1 ml of ferric stock solution and 9 ml of distilled water.

Ferric ammonium sulfate solution. The method of Tiwari and Pande was followed. About 5 g of ferrous ammonium sulfate were dissolved in 200 ml of distilled water containing 3 ml concentrated sulfuric acid. The ferrous ion was oxidized to ferric by the addition of 30% hydrogen peroxide, with the excess being boiled off. The solu-

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tion was brought to a volume of 250 ml. A 10-ml aliquot of this solu-
tion was then diluted to 100 ml. This solution will be called ferric
stock solution.

An alternative method involved dissolving 2.5 g ferric ammonium
sulfate in one l of distilled water containing 3 ml of concentrated sul-
furic acid.

Normally a 10-ml aliquot of blood was taken, and 20 ml of 20%
perchloric acid were added to deproteinize the blood. The resulting
mixture was centrifuged and the supernatant liquid poured into a
small beaker. This solution was brought to pH 7.0 ± 1.0 by the addi-
tion of potassium hydroxide solution. This solution was filtered into a
50-ml volumetric flask. To one flask 1 ml of ferric stock solution was
added. Water was added to mark in both flasks and both were run
against the ferric blank solution. To obtain absorbance due to sodium
citrate in the blood, the absorbance of the blood blank (the solution
with no ferric stock solution added) was subtracted from the absor-
bance of the solution containing the 1 ml of ferric stock solution.

RESULTS AND DISCUSSION

Our results were not in agreement with those of Tiwari and Pande.
Below concentrations of 1.5 mg/ml reasonable agreement was found.
However, above this concentration absorbance changed very little
with increasing concentration, a fact which disagrees with the pre-
viously published data. Representative data is summarized in Table 1.
A number of parameters were changed in an attempt to find agree-

TABLE 1
SPECTRAL DATA FOR CITRIC ACID SOLUTIONS

Concn of citric acid[a] (mg/ml)	Amount of citric acid[b] (mg/ml)	Absorbance
0.406		0.220
	0.545	0.160
0.813		0.345
	0.875	0.260
1.219		0.440
	1.575	0.440
1.625		0.445
	1.925	0.540
2.031		0.460
	2.275	0.630
2.438		0.475

[a] Present work.
[b] Tiwari and Pande (2).

TABLE 2
SPECTRAL DATA ON CITRIC ACID AND SODIUM CITRATE STANDARD[a]

Concn of citrate (mg/ml)	Concn of citric acid (mg/ml)	Absorbance
	0.445	0.148
0.546		0.104
	0.890	0.285
1.092		0.193
	1.335	0.350
1.638		0.253
	1.78	0.391
2.184		0.299

[a] Different blank standards used.

ment between our data and the published data. This included varying the method of preparation of iron stock solution, varying the length of time between the preparation of solutions and the measurement of absorbance, and varying the pH of solutions. None resulted in the published linear relationship of absorbance vs concentration of citric acid.

It was found that there was no distinction between using citric acid (pH ~3) or sodium citrate to prepare the calibration curve. In both cases the absorbance no longer increased with the concentration past a certain concentration. Table 2 contains representative data.

Analyses for citrate in blood samples agree well with expected values (Table 3). The removal of interfering species by addition of perchloric acid is an important step, as it was observed that these species react with the ferric ion in the ferric stock solution to produce a strongly absorbing deeply yellow-brown solution with a maximum absorbance below 300 nm. Perchloric acid was used since any perchlorate anion remains after the removal of the potassium perchlorate

TABLE 3
SODIUM CITRATE CONTENT IN BLOOD

Samples	Percentage sodium citrate	
	Exptl	Calcd
1	0.106 ± 0.002	0.105
2	0.097 ± 0.001	0.097
3	0.098 ± 0.002	0.100

acts as a very weak ligand and, therefore, will not interfere with the formation of the ferric–citrate complex.

A blank of the blood solution must be run as it has a finite, but low (<0.1) absorbance at 375 nm with maxima at 287 and 198 nm. No other interferences are evident. Increments of standard citrate solution may be added for which measured absorbances agree well with calculated values.

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A Liquid Sample Container for Oxygen Flask Combustion in Organic Microanalysis

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INTRODUCTION

The oxygen flask method has been extensively used in recent years as effective method for the analysis of halogen and sulfur in organic compounds but it seems to be difficult for application to liquid samples. Samples of high boiling point (above 200°C) have been absorbed in a filter paper which was weighed and subsequently burned in the flask (2), while Schöniger (6) weighed such a liquid in a glass capillary and wrapped it in a filter paper. Various other methods have been proposed by other workers (1, 3, 7). We attempted the use of a bag-type sample container made of cellulose tape because of its easy availability, simple working, and relatively high resistance to various solvents, and examinations were made on the blank test values for each lot of commercial cellulose tapes, and on the size and the shape of the container. For these experiments, monochlorobenzene of bp above 100°C and butyl chloride of bp below 100°C were used as liquid samples for the determination of their chlorine content.

MATERIALS AND METHODS

Apparatus

Automatic titration apparatus. Metrohm potentiograph E-336 was used with silver and calomel electrodes.

Sample containers. Commercially available cellulose tape, Cellotape (Nichiban Co., Ltd., Tokyo) and Toyo Roshi filter paper No. 6 (Toyo Roshi Co., Ltd., Osaka) were used. The containers were made up in different ways as described in Results and Discussion.

Combustion flask. A 100-ml egg shaped flask with universal ground joint having a platinum gauze holder (5).

Reagents

Liquid Samples. Monochlorobenzene and butyl chloride were of special reagent grade.

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Absorption solution for combustion of samples. Standard 0.004 *N* silver nitrate solution. Other reagents were the same as described in Ref. (5).

Procedure

Liquid sample ranging 1–2 mg is absorbed in the filter paper part of the Cellotape container, the sample being transferred from a capillary tip of glass pipe. The container is closed tightly by pinching the top of the container with tweezers, and weighed. This is clamped in a platinum gauze holder with a small piece (6 × 16mm) of the filter paper (Toyo Roshi No. 6) for ignition, and is burned in a 100-ml combustion flask containing 3 ml of 1 *N* potassium hydrozide solution and 6 drops of saturated hydrazine sulfate solution. The absorbent is then transferred into a titration vessel using approximately 20 ml of wash liquid containing 50% alcohol and is submitted to potentiometric titration using 0.004 *N* silver nitrate solution by the method previously reported (5).

RESULTS

Making of Bag-type Container and Its Blank Test

Soep and Doemon (7) had already reported the use of a cellulose tape for this kind of combustion analysis. They used a strip of cellulose tape, 30 × 24 mm, lined with a piece of filter paper, 25 × 6 mm, and made it into a bag. Referring to this size, we attempted to make a bag-type container as shown in Fig. 1 using Cellotape, 18 × 30 mm, lined with a filter paper of 25 × 6 mm. This container weighed approximately 60 mg. Blank experiment was carried out by the combustion of this container without sample using a conventional 300-ml flask and it consumed 0.14–0.15 ml of 0.004 *N* silver nitrate solution. We have been using a 100-ml flask for combustion of samples for carrying out the titration with smallest volume of titrant as possible and at the same time determination with smaller sample size in the analy-
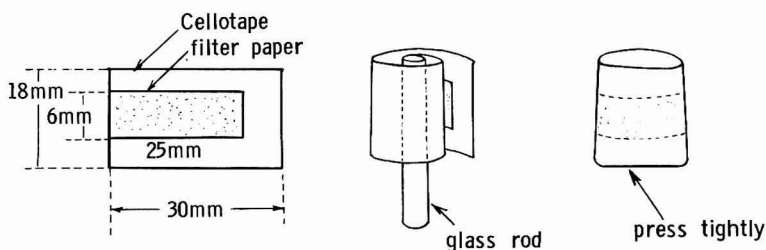
FIG. 1. Bag-type Cellotape container.

TABLE 1
BLANK VALUE OF DIFFERENT LOTS OF CELLOTAPE[a]

Lot A		Lot B		Lot C	
Container wt (mg)	0.004 N AgNO$_3$ (ml)	Container wt (mg)	0.004 N AgNO$_3$ (ml)	Container wt (mg)	0.004 N AgNO$_3$ (ml)
26.67	0.09	26.94	0.09	26.20	0.10
28.73	0.09	26.75	0.09	27.42	0.09
26.15	0.09	27.40	0.10	25.56	0.10
26.89	0.09	26.59	0.09	26.83	0.10
27.23	0.09	27.41	0.09	25.61	0.09

[a] Container: Cellotape 12 × 17 mm, filter paper 6 × 12 mm.

sis of halogens and sulfur (5). It has been known that the weight of the filter paper used for combustion of the sample in 100-ml flask should be around 30 mg, so that the Cellotape container made as above seemed to be too large. Therefore in order to comply with the use of a 100-ml flask, container of lighter weight was made from a piece of Cellotape measuring 12 × 17 mm, lined with a filter paper of 13 × 6 mm, which weighed around 27 mg and was found to be completely combustible in a 100-ml flask. The blank values were lower than the earlier one, corresponding to 0.09–0.10 ml of 0.004 N silver nitrate solution. Similar containers were also made from three lots of the Cellotape of 12 mm in width and blank values of these containers, as shown in Table 1, were in approximate agreement, indicating that there is no difference among the lots. The same result was obtained with 18-mm width Cellotape.

Analysis with Bag-Type Container

From the foregoing results, the bag-type containers were made from Cellotape of 12-mm width and were used for the analysis of liquid samples. Satisfactory determination values for monochlorobenzene (bp 131°C) were obtained in general. In the case of butyl chloride (bp 78.5°C) significantly negative results of around 1% lower than the theoretical appeared (Table 2).

Analysis with Roll-Type Container (1)

The negative analytical values using the bag-type container were considered to be due to the instantaneous vaporization of the sample from relatively thin wall of the container during the combustion especially for samples with boiling point below 100°C. Therefore a container of roll-type which had thicker wall around the sample was

TABLE 2
ANALYTICAL RESULTS WITH BAG-TYPE CONTAINER

Monochlorobenzene bp: 131–132℃, Cl% = 31.50				N-Butyl chloride bp: 78.50℃, Cl% = 38.30			
Sample (mg)	0.004 N AgNO$_3$ (ml)	Found		Sample mg	0.004 N AgNO$_3$ (ml)	Found	
		Cl (%)	Error (%)			Cl (%)	Error (%)
1.576	3.51	31.59	+0.09	1.250	3.29	37.34	−0.96
1.439	3.17	31.24	−0.26	0.892	2.34	37.21	−1.09
1.625	3.59	31.34	−0.16	1.373	3.62	37.40	−0.90
1.456	3.21	31.27	−0.23	1.445	3.84	37.70	−0.60
1.428	3.16	31.39	−0.11	1.355	3.57	37.37	−0.93

made, as shown in Fig. 2. Combustion of monochlorobenzene using this roll-type container gave approximately satisfactory values. Although the values obtained with butyl chloride were closer to theoretical values than those obtained with the bag-type container, they still showed a general tendency of low analytical values (Table 3).

Analysis with Roll-type Container (2)

The foregoing results suggested that samples with boiling point below 100°. would still be somewhat vaporized during the combustion. Therefore, in order to prevent the vaporization the Cellotape of 18-mm width was again used and a roll-type container as shown in Fig. 2 was made from a Cellotape piece of 18 × 18 mm, lined with a filter paper of 10 × 10 mm. Analysis of butyl chloride with this container gave satisfactory results, as shown in Table 4. This roll-type container, weighed about 30 mg was completed burned in the 100-ml flask, and the blank value corresponded to about 0.10 ml of 0.004 N silver nitrate solution.

DISCUSSION

Result of experiments described above showed that the bag-type container were not satisfactory because the sample was relatively ex-

FIG. 2. Roll-type Cellotape container.

TABLE 3

ANALYTICAL RESULTS WITH ROLL-TYPE CONTAINER (1: 12 mm)

	Monochlorobenzene bp: 131–132°C, Cl% = 31.50				N-Butyl chloride bp: 78.5°C, Cl% = 38.30		
Sample (mg)	0.004 N AgNO₃ (ml)	Found		Sample (mg)	0.004 N AgNO₃ (ml)	Found	
		Cl (%)	Error (%)			Cl (%)	Error (%)
1.016	2.27	31.69	+0.19	1.099	2.95	38.07	−0.23
1.072	2.38	31.49	−0.01	1.392	3.71	37.80	−0.50
0.998	2.22	31.55	+0.05	0.950	2.55	38.07	−0.23
1.017	2.26	31.52	+0.02	1.430	3.82	37.88	−0.42
1.172	2.60	31.47	−0.03	1.074	2.88	38.04	−0.26

posed and tended to be vaporized during combustion, but the roll-type container gave a thicker layer around the sample, with closer contact between the sample and container material, resulting in prevention of vaporization and increased effect of aiding combustion. Slight lengthening of the roll and wider area of the filter paper seemed to have increased the above effect and were effective in the analysis of samples with boiling point below 100°C. The bag-type container reported to date (7) is too large for future analytical method which deals smaller sizes. This roll-type container is extremely light and suitable for such micromethods.

As iterated above, there have been many kinds of container suggested for liquid samples in flask combustion analysis. The glass capillary (6) becomes fused during the combustion, sealing the sample inside and this often becomes the source of sample loss. Container made of collodion (3) and gelatin (4) are relatively affected by solvents. Kirsten's polyethylene tube (4) is being used widely but, ac-

TABLE 4

ANALYTICAL RESULTS OF N-BUTYL CHLORIDE
WITH ROLL-TYPE CONTAINER (1: 18 mm)

Sample (mg)	0.004 N AgNO₃ (ml)	Found	
		Cl (%)	Error (%)
1.060	2.86	38.27	−0.03
1.043	2.82	38.35	+0.05
1.071	2.90	38.39	+0.09
1.041	2.81	38.29	−0.01
1.066	2.87	38.20	−0.10

cording to our examination, it is difficult to obtain a uniform capillary tube, which results in difficulty of obtaining constant weight of containers and constant blank values. In addition, procedure for sealing the sample in such a tube is somewhat difficult. In contrast, containers made from cellulose tape are relatively resistant to dissolving properties of organic solvents, available material, and quite simple in handling. In addition, shown in Table 1, weight of the containers and blank values are approximately constant, and there is no scattering of values from one lot to another. In short, containers made from a transparent cellulose tape seem to be better than those made from other materials.

The present work was carried out on samples containing chlorine exclusively, but it is thought that the method can be used for liquid samples containing other halogens of sulfur as well.

SUMMARY

Containers for weighing liquid samples in the oxygen flask method were made from commercial cellulose tape (Cellotape) and examinations were made on their size and shape suitable for microanalysis. It was found that the roll-type container was better than the bag-type in minimizing vaporization of the sample, and a marked effect was obtained by making the filter paper adhered to it of larger size.

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Direct Stripping Voltammetric Determination of Lead, Cadmium and Zinc Dithizonates

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INTRODUCTION

The methods of micro and ultramicrotrace determinations play a very important role in many problems of synthesis, characterization and technology of semiconducting and magnetic materials (5). A very useful method for the element preconcentration and separation is the liquid–liquid extraction with organic solvents, as benzene and chloroform, and complexing agents, as diphenylthiocarbazone (dithizone) (2–4). In this work we have used solvent extraction and stripping voltammetry to determine lead, cadmium and zinc, components of many semiconducting and magnetic materials. We have performed a method of stripping voltammetry which necessitates neither the destruction of the metals–dithizone complex, nor the removal of the organic solvent (benzene or chloroform). Thus, we have avoided some complicated steps which usually involve losses and contamination of the sample. The analytic conditions for performing direct polarographic analysis in the organic extracted phase were achieved by adding silver nitrate, as a demasking agent, and sodium nitrate, as a supporting electrolyte, in methanol.

EXPERIMENTAL METHOD

Apparatus. The extractions were performed in pear-shaped separatory funnels with Teflon stopcocks and a capacity of 100 ml. Polarographic measurements were performed with a polarograph with hanging mercury drop electrode described in (*1*). The polarographic cell was connected to a reservoir which contained a degassed mixture of methanol and benzene (chloroform), of the same composition as the mixture in the cell. This reservoir was used to keep constant the volume of the solution in the cell in case of evaporation during analysis. All of the polarographic measurements were made at 20°C with a sweep rate of 7.2 mV/sec. The preelectrolysis potential was -1.3 V vs SCE. Degassing time was 10 min.

Reagents. A solution of the examined metallic ions was obtained

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by dissolving the appropriate salts (chlorides or nitrates) in water. The silver nitrate solution was $3 \times 10^{-4}M$ in methanol. The dithizone solution was 25 μM in benzene (chloroform). Distilled water was further treated with dithizone; methanol, benzene and chloroform were purified by repeated distillations. Following this procedure blank solutions of each solvent did not exhibit detectable traces of impurities in polarographic measurements.

Procedure. Perform solvent extraction of the examined cations as dithizonates in benzene (chloroform) by using the suitable processes for the sample to be examined. Add to the organic solution of the metal dithizonates a quantity of silver nitrate solution in methanol which is enough to react with all the dithizone present plus an excess, equal to 1.5 ~ 2 the amount of dithizone. Dilute to volume with methanol. Transfer a suitable amount of this solution into the polarographic cell. The polarographic analysis is then performed in 40 ml 0.2 N $NaNO_3$ methanol: benzene (chloroform) = 7 : 1 solution.

Results. The method described was checked by testing samples
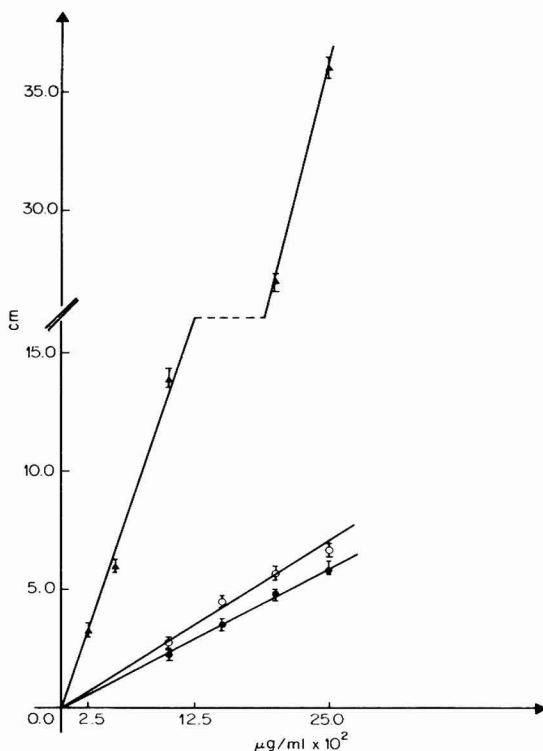
FIG. 1. Polarographic peak heights as a function of cation concentrations: (●) Pb^{2+}, (▲) Cd^{2+}, (○) Zn^{2+} in sodium nitrate methanol:chloroform 7 : 1 solution.
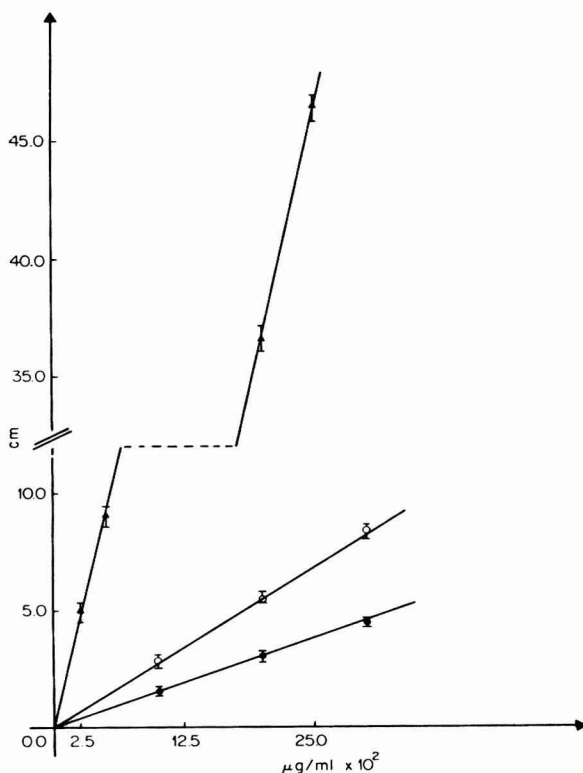
FIG. 2. Polarographic peak heights as a function of cation concentrations: (●) Pb²⁺, (▲) Cd²⁺, (○) Zn²⁺ in sodium nitrate methanol:benzene 7:1 solution.

containing known amounts of the elements to be determined. The results are summarized in Figs. 1 and 2. The electrodissolution potentials were: -0.45 V for lead; -0.60 V for cadmium and -1.00 V for zinc in both methanol-benzene and methanol–chloroform media.

DISCUSSION

After extraction cations are complexed with dithizone, in a medium of benzene (chloroform). It is therefore impossible to analyze this solution by stripping voltammetry for two reasons. First, lead, cadmium, and zinc cations are bound to dithizone and do not accumulate on the electrode. Second, it is difficult to find a base electrolyte soluble in benzene (chloroform). The first difficulty was overcome by adding to the extracted organic phase a silver nitrate solution in order to free lead, cadmium, and zinc cations from their dithizonates. In fact the silver dithizonate stability constant is much higher than those of lead, cadmium and zinc dithizonates (6). Thus, silver is able to dis-

place the cations from their complexes. Furthermore it was verified that a silver excess not superior to twice the amount of dithizone does not change the polarographic peak heights. To overcome the second difficulty, methanol was added to the extracted solution, because of its high miscibility with benzene (chloroform) and because it is a common solvent of silver nitrate and sodium nitrate. In the analytical procedure described, sodium nitrate was preferred as a supporting electrolyte because: (A) it dissolves in either benzene or chloroform methanol mixtures in a large enough quantity to be used as a base electrolyte; (B) it allows obtaining good sensitivities for the cations under examination; and (C) it does not lead to interferences in the silver solutions nor does it form insoluble precipitates. However, it was found that the percentage of benzene (chloroform) in polarographic solutions greater than 15% v/v must be avoided since they reduce sodium nitrate solubility and gradually suppress polarographic peak heights, as reported in Fig. 3. It was also observed that evaporation of polarographic solution due to strong nitrogen flux or to protracted degassing times can vary the methanol/benzene (chloroform)
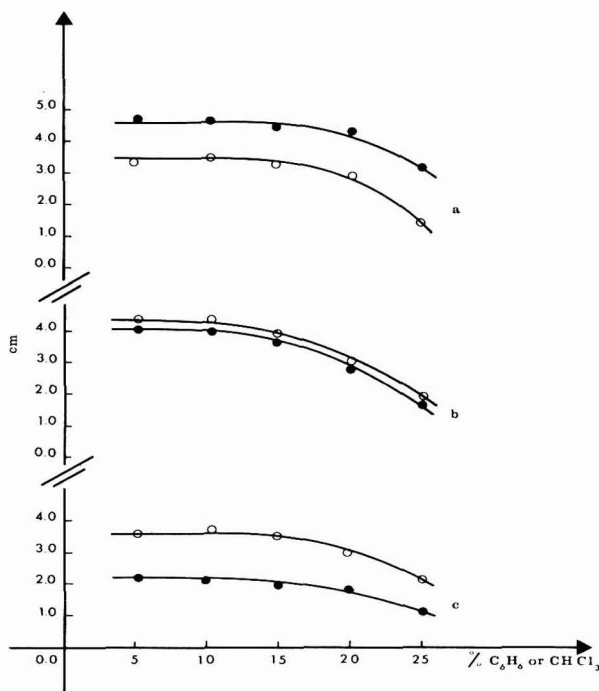
FIG. 3. Polarographic peak heights as a function of (●) benzene and (○) chloroform per cent in polarographic solution: (a) 1 μg of cadmium; (b) 5μg of zinc; (c) 5 μg of lead.

ratio. However, this variation was not considered. In fact, from Fig. 3 it is evident that in varying the concentration methanol/benzene (chloroform) around a 7 : 1 ratio, there were no notable modifications in the polarographic peak heights.

SUMMARY

A procedure is described in order to determine lead, cadmium and zinc dithizonates by stripping voltammetry. The polarographic analysis is performed in a medium of methanol and benzene (chloroform) in a ratio of 7 : 1 and in the presence of silver dithizonate. Sodium nitrate was used as a base electrolyte. By carrying out these analytic conditions it is possible to perform directly the stripping voltammetric analysis in benzene (chloroform) solution of extracted dithizonates, without further treatment. Silver nitrate in methanol is added to dithizonates in order to free lead, cadmium, and zinc from their complexes, to be determined polarographically.

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Characterization of Air-Borne Particulates[1]

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INTRODUCTION

In recent years there has been growing interest in those effluents that constitute atmospheric pollution. Research in the automotive industry has contributed to the characterization of pollutants from various sources, including emissions from automobile exhausts and from factory stacks. Because chemical analysis of emission constituents is often a precursor to their control, the analytical chemist has faced a real challenge.

Atmospheric pollutants consist of both gases and particulates. Gas analyses have been considered in detail elsewhere (*5, 6, 23*), but the analysis of emission particulates has received less attention (*10, 11*). Therefore, the purpose of this paper is to document some of our approaches that have been developed for the sampling and analysis of air-borne particulates . Emphasis is directed to the application of the methods for rapid and accurate characterization of particulate materials in the atmosphere.

EXPERIMENTAL

Sampling

Proper sampling techniques are, of course, an important aspect of particulate characterization. Particles are usually collected on filters composed of organic membranes, glass, or metal. Composition of the filter must be carefully determined and controlled because contaminants at trace levels in the filter may be excessive in terms of the element(s) being determined in small samples.

There are many ways of collecting atmospheric particulates (*19*), including paper tape samplers (*2, 24*) and cascade impactors (*19*); the selection of a sampler depends on the information required. For example, the General Motors Atmospheric Research Laboratory that

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[1] Paper presented at the International Symposium on Microchemical Techniques-1973, The Pennsylvania State University, University Park, Pennsylvania, August 19–24, 1973.
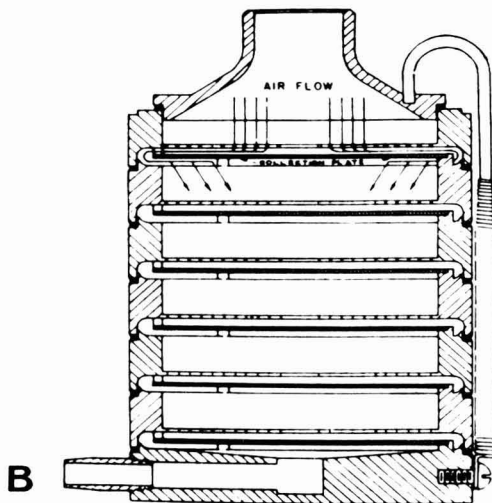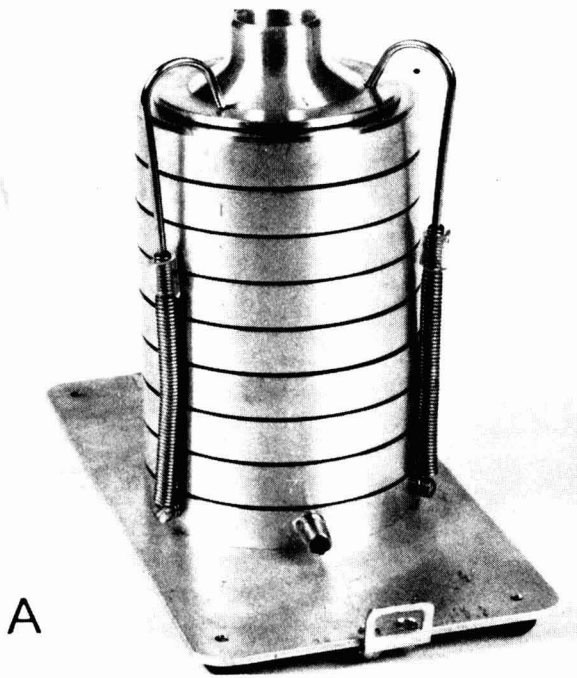
FIG. 1. Andersen air sampler. (A) External view; (B) schematic internal view (*1*).

monitors pollutants in various parts of the country uses an automatic sampler which collects particles on a fresh portion of filter paper every two hours.

Cascade impactors provide information on particle size. For example, the Andersen air sampler (1, 18) (Fig. 1) is a multi-stage, multi-jet device; each stage contains a pattern of precision holes that direct the air-borne particles toward the surface of the collection plate for that stage. The particles impact on different stages depending on their aerodynamic size and are separated into six to eight groups (depending on the number of stages). Particle size distribution can be determined from the weight of material collected on each plate after completion of the sampling. Finally, a membrane filter collects all particles that are not collected on the impaction stages.

The separation of individual particles from a mass of sample can be accomplished manually with a microscope at or below 100× magnification and a fine probe (about 15-μm tip). For particles smaller than 5 μm in diameter, a micromanipulator (4) and microscope combination may be required. Individual particles or conglomerates may be mounted on planchets or glass fibers for analysis.

Analysis

The analysis of particulate samples is usually conducted in three steps: (1) microanalysis, (2) structure identification, and (3) quantitative elemental analysis.

Microanalysis

A number of techniques are available that can provide microanalytical information about particulates. These include optical microscopy, scanning electron microscopy, and electron probe microanalysis. The first step entails examination at various magnifications with an optical microscope. Such observations can yield valuable information on the source, type, and size distribution of particles. On some occasions, direct identification is possible. For example, the particles shown in Fig. 2A were identified as cast iron dust by comparison with a representative photomicrograph (Fig. 2B) from "The Particle Atlas" (19).

Both the scanning electron microscope (SEM) with an energy dispersive spectrometer (EDS) and the electron probe microanalyzer (EPM) have proved very useful for the characterization of particles with diameters down to about 1 μm. These instruments (3, 20) provide topographical information and semiquantitative analysis for most elements in minutes. (A narrow beam of electrons is traversed over the sample surface, producing secondary electrons that give a picture
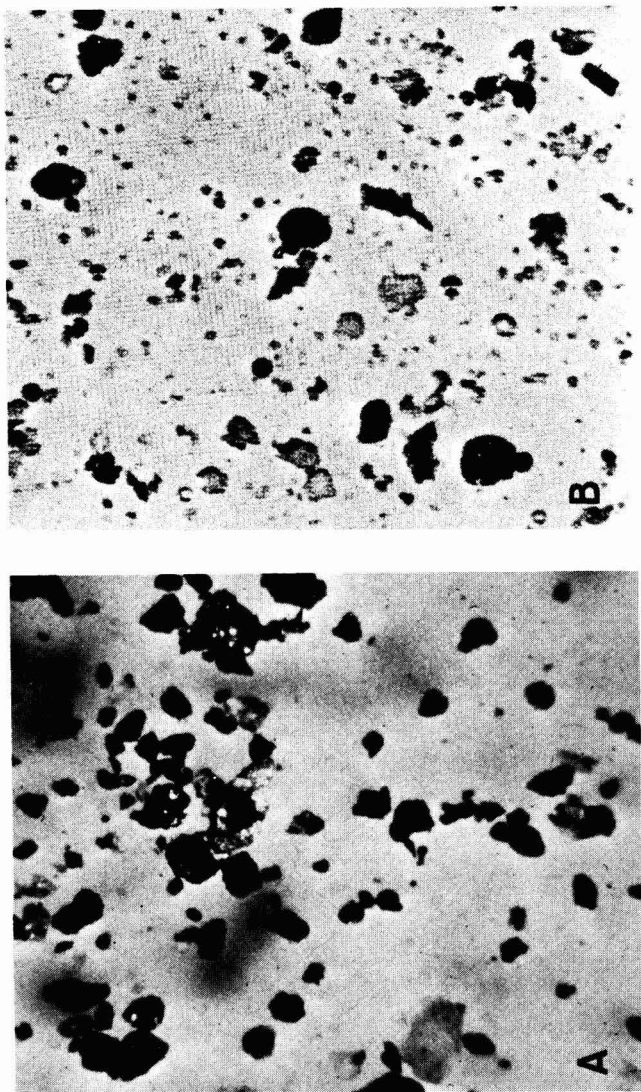
FIG. 2. Microscopic identification of particulates. (A) Factory stack sample ($\sim 160\times$); (B) cast iron dust from Reference 19 (160$\times$).

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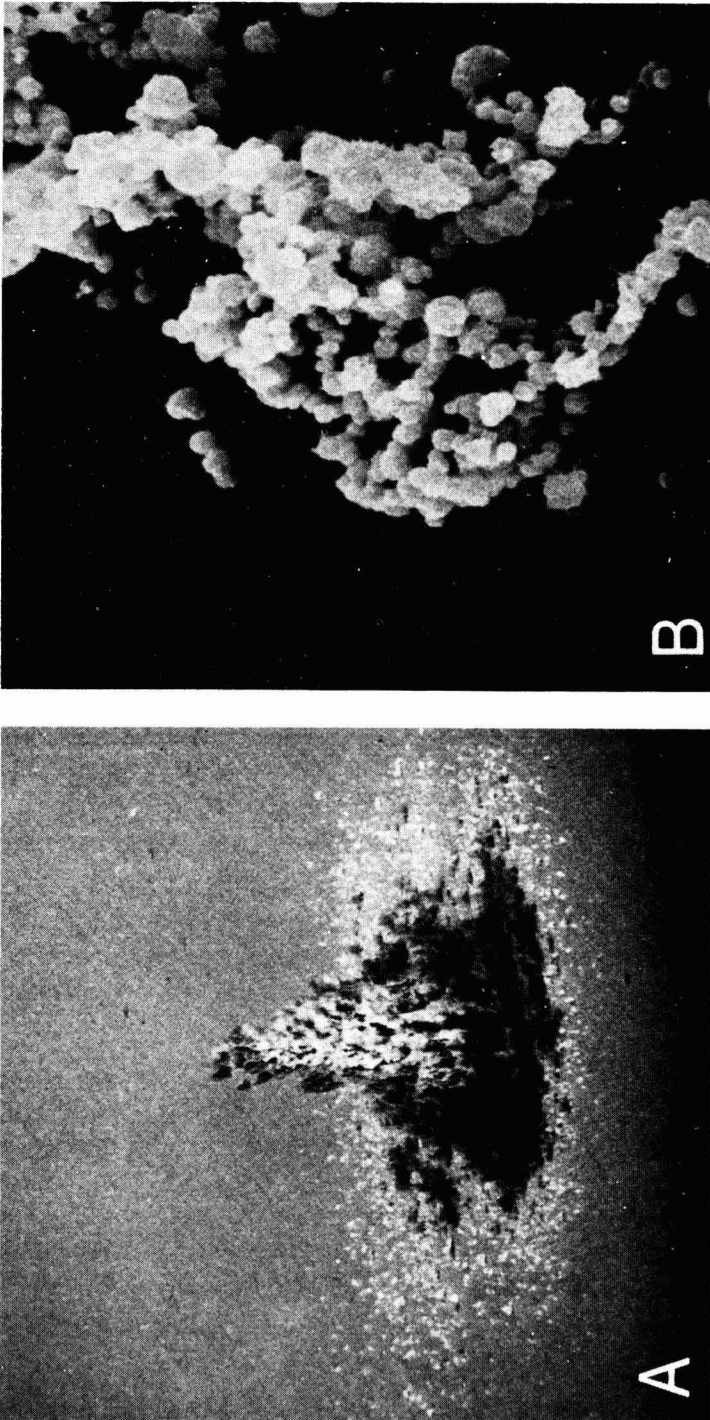
FIG. 3. Scanning electron micrographs of particles on impactor stage. (A) 60×; (B) 3000×.
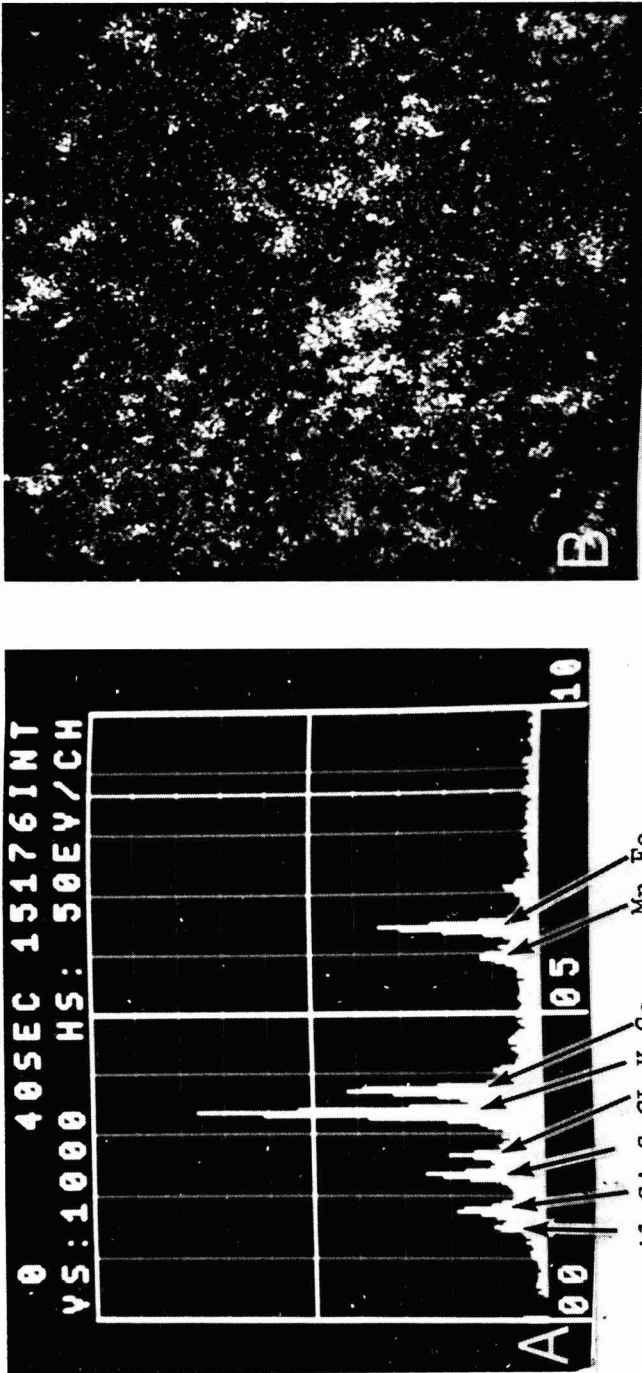
FIG. 4. X-ray analysis with energy dispersive spectrometer and electron probe microanalyzer. (A) Energy dispersive spectrum; (B) distribution of chlorine (bright areas) (500×).

of the sample and characteristic X-rays of the elements present.)
Shown in Fig. 3 are two SEM photomicrographs of particles collected
from the atmosphere; Fig. 3A (60×) shows the particles on an alumi-
num impactor plate of an Andersen sampler, and Fig. 3B (3000×)
provides greater detail of the particles of interest. A complete energy
dispersive spectrum of this particulate matter is shown in Fig. 4A; the
individual elements are designated along the abscissa, and the inten-
sity peaks along the ordinate axis are related to the concentration of
the elements present. Fig. 4B is a photograph at 500× from the EPM
which represents the distribution of chlorine (light areas) in the par-
ticulates.

The primary advantages of an SEM examination of particles are the
great depth of field and the possibility of preparing stereographic pho-
tographs for a three-dimensional view. With the EDS accessory, a
complete spectrum of elements above atomic number 11 (sodium) can
be obtained. With the EPM, individual elemental determinations
above atomic number 5 (boron) can be obtained at a limit of detection
of about 0.1% for most elements (compared with about 1% for the
EDS). Thus, these instruments complement each other in the study of
particulates.

Structure Identification

X-Ray diffraction (*16*) provides valuable information on the struc-
ture of crystalline materials which can lead to compound identifica-
tion. This identification is accomplished by comparing the diffraction
patterns with powder diffraction data (*15*). The specific techniques
used in our laboratory depend on the quantity of sample available.
When there are only a few particles, they are supported on the end of
a glass fiber about 5 μm in diameter. This fiber is fabricated with a
micro-forge technique (*26*). An example of a particle contained in
collodion and mounted on such a fiber is shown in Fig. 5. (The dark
area around the particle is the entrance port of the Debye-Scherrer
camera.) Because of the small sample size, long exposures (up to 6 hr)
to X-rays are required for a satisfactory diffraction pattern. Such ex-
posures often cause a dark background that obscures the diffraction
lines, but several different cameras have been developed to eliminate
this problem (*25*). For example, helium can be substituted for air in
the camera, or a smaller diameter camera can be used to provide
sharp detail.

If there are enough particles to cover an area about 1.5 mm in
diameter, a focusing geometry camera can be used. This technique in-
volves a Guinier camera with a parafocusing geometry and a curved
crystal monochromator (*7, 17*). With this equipment, all wavelengths
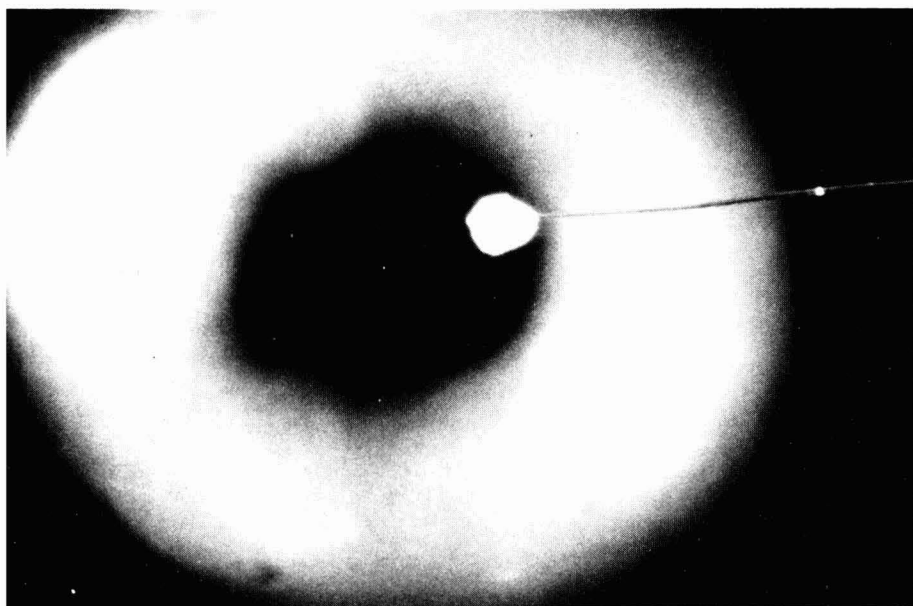
FIG. 5. Particle contained in collodion and mounted on glass fiber (70×).

other than the K_α doublet of the X-ray target tube are eliminated, and air scattering is eliminated because the sample chamber is evacuated. An exposure of only about 1 hr is needed.

Quantitative Elemental Analysis

Among the methods that have been used for the quantitative analysis of particles are X-ray fluorescence, atomic absorption spectrometry, and UV–visible–IR spectrophotometry. The choice of the method is governed by the amount and type of sample and by the elements of interest.

X-Ray fluorescence has been used in conjunction with confined-spot papers for the determination of trace and major amounts of elements in microsamples (13, 21, 22). We have previously described such methods with special emphasis on the preparation of confined-spot calibration standards that are required for the analysis of unknowns (14). In that work, sulfate was determined in particles collected from smog chamber studies of the conversion of sulfur dioxide to sulfate; also, lead and silicon were determined on paper tape spots (2-hr samples) from our Atmospheric Research Laboratory. On 100-mm spots the minimum detectable limit for sulfur is 0.15 μg; for lead and silicon it is 0.4 μg. The technique, which is simple, precise,

and rapid, should be applicable to the direct determination of other elements above atomic number 12 (magnesium).

Atomic absorption spectrometry (AAS) is another valuable technique that can provide quantitative analysis of automobile exhaust particulates that contain such metallic elements as lead, iron, calcium, and zinc. The particulate matter is removed from the filters or impaction plates by dissolution in acids. These solutions are aspirated directly into the AAS burner where neutral atoms are formed. The absorption of the radiation from a hollow-cathode tube for the element of interest is related to the concentration of the metallic element in solution. Additional elements, such as phosphorus and sulfur, can be determined by photometric methods. For example, after determination of metallic elements, phosphorus is determined by the molybdenum blue photometric method (12). The sulfur can be determined by reducing all the sulfur compounds to sulfide, which is separated by distillation and determined by the methylene blue method (8, 9).

The analytical data of Table 1 depict some of the blank problems associated with analyzing the small amounts of particulates obtained in certain studies. This problem can be observed in the data for zinc, in which a greater amount was found on the blank plate than on most of the sampling plates. It is also evident that some membrane filters (see blank) contain such high concentrations of sulfur and calcium that these elements cannot be determined reliably when present in small amounts.

In addition to the characterization of inorganic particles there is

TABLE 1

DISTRIBUTION OF ELEMENTS IN PARTICULATES FROM AUTOMOTIVE EXHAUST COLLECTED ON ANDERSEN IMPACTION PLATES

Sample	Av particle diam (μm)	Element found (μg)[a]					
		Pb	Fe	Ca	Zn	P	S
Plate 1	9.8	4	12	6	1	0.7	8
Plate 2	6.2	3	7	6	1	< 0.5	4
Plate 3	4.2	< 2	10	3	1	< 0.5	11
Plate 4	2.8	8	15	7	1	2	6
Plate 5	1.8	5	17	6	1	0.7	< 1
Plate 6	0.89	5	21	5	8	2	43
Plate 7	0.55	3	10	7	4	2	5
Plate — blank		4	8	5	4	< 0.5	4
Backup filter (membrane)		20	11	53	4	15	360
Backup filter — blank (membrane)		1	5	38	1	6	250

[a] No correction made for blanks.

often a need to identify organic matter collected in air samples. A microinfrared spectrophotometric technique has been found particularly suitable for such applications. The particles of interest are mixed with a few crystals of potassium bromide and pressed with a 5-μm die into a pellet. The infrared spectrum obtained with a beam-focusing condenser indicates which organic groups are present. Also, complex inorganic ions, such as sulfate, can be determined.

SUMMARY AND CONCLUSIONS

Reliable instrumental and chemical analysis techniques are being applied to the rapid and accurate characterization of air-borne particulates. Most of the problems associated with such analyses can be overcome with proper sampling and sample handling and with the development of suitable standards. Future improvements will undoubtedly be directed towards increased analytical sensitivity because the sample size will become smaller as the concentrations of pollutants decrease with improved emissions control. Thus, an essential feature of future programs in the analysis of airborne particulates will be the ingenuity of the analytical chemist.

ACKNOWLEDGMENTS

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Determination of Organic Carbon in Water with a Silver-Catalyzed Peroxydisulfate Wet Chemical Oxidation Method

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INTRODUCTION

Practical methods for determining organic carbon in aqueous samples usually comprise an attempt to completely oxidize the carbon, separation of the resultant CO_2, and CO_2 measurement. Two variations on this basic approach, differing in the means of oxidation, have been useful.

Wet chemical oxidation has been used as, for example, in the work of Menzel and Vaccaro (6). Wet chemical oxidation methods are capable of measuring organic carbon at levels as low as 0.05 $\mu g/ml$, primarily because large (~ 10 ml) sample volumes can be processed. The principal disadvantages of wet chemical oxidation are the long elapsed time for a determination (typically 4–12 hr) and the unknown oxidation efficiency for many oxidant-organic combinations.

Gas-phase combustion in a flowing oxygen or air stream has been suggested as an alternative method by Van Hall and co-workers (*12, 13*). The method is fast, requiring only a few minutes of elapsed time per sample. The main limitation is a maximum sample volume of about 25 μl, restricting the technique to carbon concentrations above 2 $\mu g/ml$. Although complete recovery of CO_2 has been observed for a variety of pure organic compounds (*12*), it does not appear that complete conversion of organic carbon to CO_2 has been more definitively demonstrated for this system than for many wet chemical oxidation techniques. Gas-phase combustion does offer an advantage over wet chemical methods when the samples contain volatile organic components.

A third type of system, which appears to be less commonly used, converts volatile organic compounds and CO_2 from involatile organic materials to CH_4 in a catalytic reduction process (*10*). The CH_4 is measured in a hydrogen flame ionization detector. This system is relatively complicated and, as with gas-phase combustion, is limited to

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sample volumes of about 25 μl. The detection limit is set by the system background and amounts to about 2 μg of carbon/ml.

Specific adaptations of these techniques have been reviewed by Szekielda (9) for the fields of oceanography and limnology, and by Mancy and Weber (5) for industrial wastewater analysis.

In this paper, we describe a procedure resulting from our efforts to combine the advantages of wet chemical and gas-phase oxidation techniques; namely, to increase the speed and efficiency of wet chemical oxidation while retaining the ability to use sample volumes of up to 10 ml. A room-temperature wet chemical oxidation technique, which uses peroxydisulfate catalyzed by Ag(I) as the oxidant, is described. The advantages of catalytic wet chemical oxidation are illustrated with the results of determinations on samples from an operating water treatment plant.

MATERIALS AND METHODS

A. Wet Chemical Oxidation

Many different reagents have been used for wet chemical oxidation of organic material at the microgram per milliliter level. The method described herein is a modification of the peroxydisulfate oxidation technique originally suggested by Wilson (16) and developed by Menzel and Vaccaro (6). The peroxydisulfate anion ($S_2O_8^{2-}$) is an attractive choice for the oxidant for several reasons:

(a) The reaction is apparently free of serious interferences (8).

(b) The oxidant is inexpensive, and safely and easily handled as its potassium salt.

(c) The uncatalyzed reaction rate is negligibly small at room temperature.

(d) High oxidation efficiencies have been demonstrated for a wide variety of organic compounds in milligram quantities (1, 3, 6, 8). Oxidation efficiences approaching 100% have also been noted for a variety of compounds at the microgram per milliliter level (14). Results obtained with $S_2O_8^{2-}$ oxidation compare reasonably well, for seawater samples, with those by a photolytic oxidation method (15).

(e) Certain cations, notably Ag(I), increase the rate of oxidation by $S_2O_8^{2-}$ of many organic compounds (2). Ag(I) has been used as a catalyst by previous workers (1, 3, 4, 8), in all cases at elevated temperatures. However, there has been no data on oxidation times with and without catalyst, on relative oxidation efficiency, or on effect of the catalyst on the temperature needed for the oxidation.

1. UNCATALYZED WET CHEMICAL OXIDATION

Uncatalyzed oxidation by $S_2O_8^{2-}$ at 175°C, with measurement of the CO_2 in a nondispersive infrared analyzer, was the principal referee method for this work. A commercial carbon analyzer[1] was used and is shown in simplified schematic form in Fig. 1.

Known volumes (up to 10 ml) of samples, standards, and blanks were pipetted into precleaned 10-ml borosilicate glass ampoules. 0.2 g of potassium peroxydisulfate and 0.25 ml of 6% (v/v) nitric acid solution were added. The ampoule was fitted with a purge cone and purge tube as shown in Fig. 1a. CO_2 derived from inorganic carbon was removed from the ampoules by purging for at least 5 min with purified O_2 flowing at about 70 ml/min. The ampoules were flame sealed, with continued purging of the ampoule neck region to prevent contamination by combustion products. The sealed ampoules were placed in a pressure vessel and heated to 175°C in an oven for at least 8 hr. The pressure vessel was removed from the oven and allowed to cool. The ampoules were removed from the pressure vessel, connected to the analyzing unit (Fig. 1b) and the ampoule seals broken. The CO_2 was extracted into a N_2 stream and passed through a nondispersive infrared analyzer.[2] The electrical output of the infrared analyzer was integrated with an electronic digital integrator.[3] Peak integrals for the standards, corrected for organic carbon content of the reagents and of the water used in their synthesis, were used to construct a working curve. Sample peak integrals were corrected for the reagent blank and converted to concentrations using the working curve. Detailed descriptions of apparatus and procedures are given in (7).

2. SILVER-CATALYZED WET CHEMICAL OXIDATION

The normal procedure for uncatalyzed oxidation (Sect. A.1) was modified as follows: Immediately after the purging period (Fig. 2a), the ampoule was moved to the sealing position (Fig. 2b), taking care that the purge tube and purge cone were not displaced from the ampoule neck. 1.00 ml of carbonate-free 60.0 g/liter $AgNO_3$ solution was withdrawn from the purging flask (Fig. 1a) with an automatic pipet[4] and added to the ampoule with as little disturbance as possible

[1] Oceanography International Corp., College Station, Texas, Model No. 0524A.

[2] Mine Safety Appliances Co., Pittsburgh, Pa., LIRA Infrared Analyzer Model No. 300.

[3] Autolab Div., Spectraphysics Corp., Mountainview, Calif., Model No. 6300-01.

[4] Eppendorf 1000 lambda capacity, VWR Scientific, Seattle, Wash.
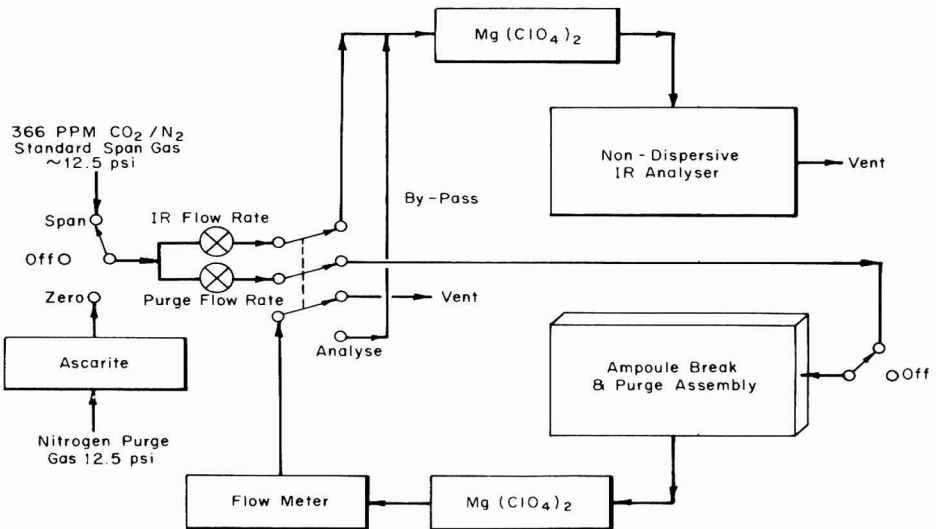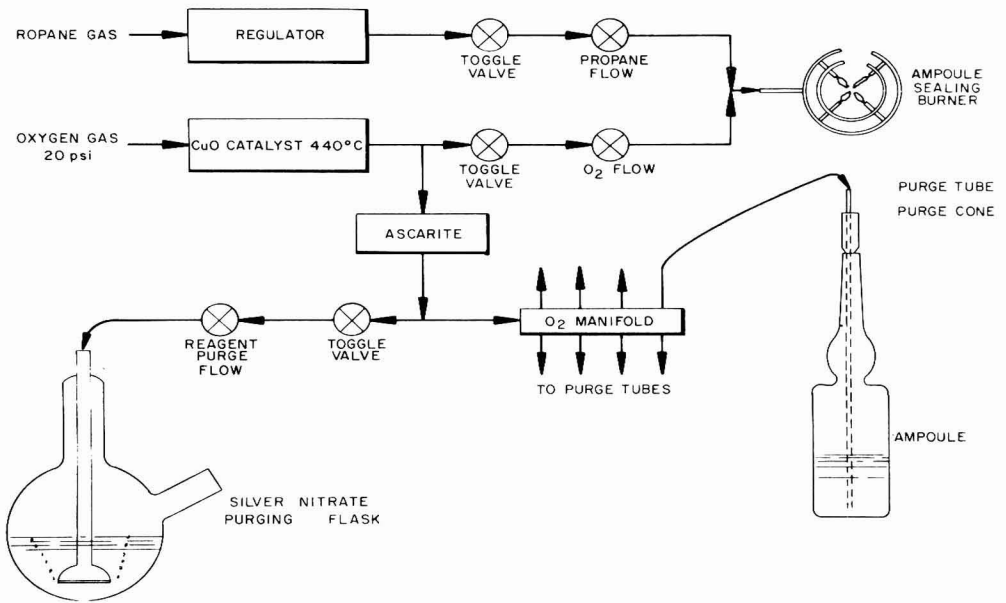
FIG. 1. Block diagram of the carbon analysis system: (a) (top) ampoule purging/sealing and reagent purging; (b) (bottom) ampoule breaking and CO_2 measurement.
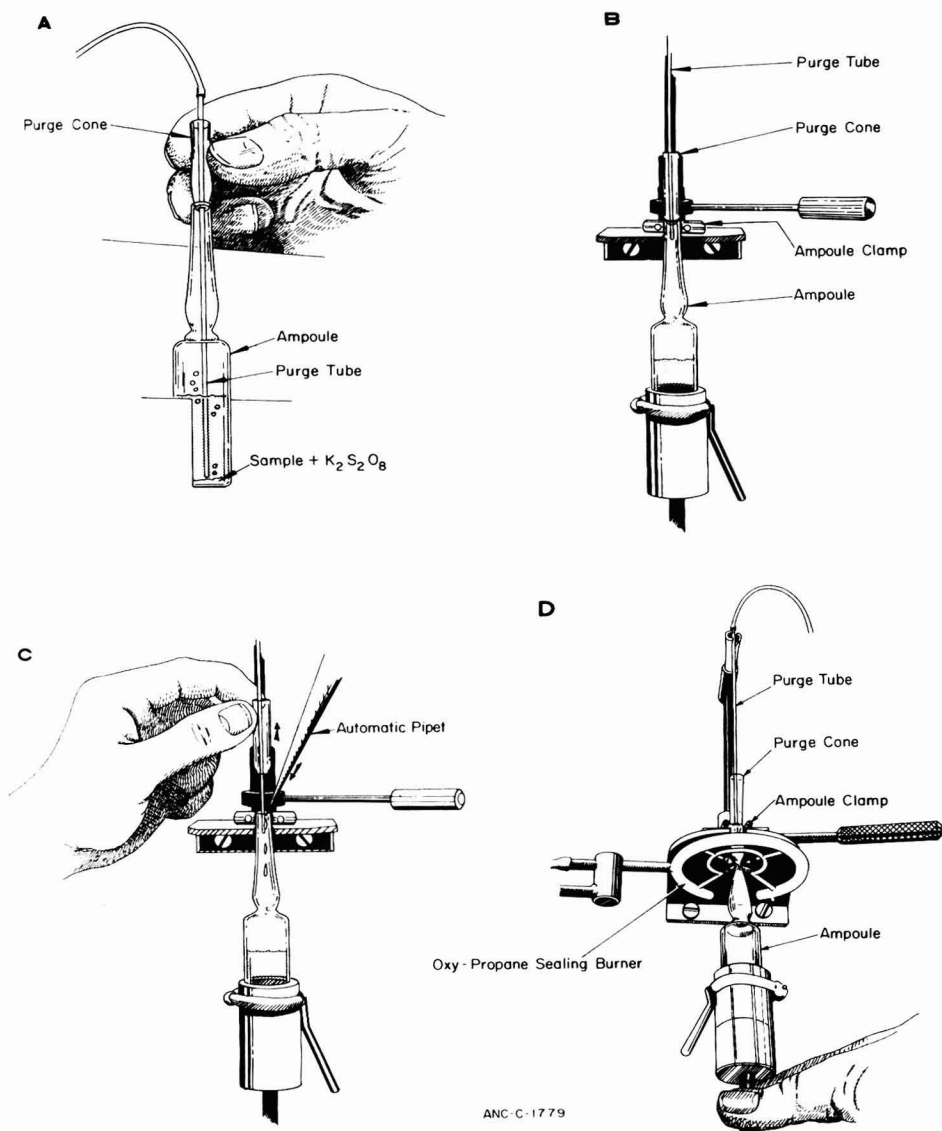
FIG. 2. Sample preparation procedure: (a) inorganic CO_3^{2-} removal, (b) ampoule positioned for sealing, (c) $AgNO_3$ addition, (d) sealing.

of the purge tube and purge cone (Fig. 2c). The sealing burner was immediately positioned and the sealing completed (Fig. 2d).

These operations must be performed quickly and smoothly because (a) entry of ambient air or combustion products into the ampoule will falsify the results and (b) conversion of organic carbon to CO_2 begins

as soon as the Ag(I) is added. It was essential that purge tubes be changed between samples to prevent carryover of $AgNO_3$ and loss of carbon during the purging period. Purge cones could be reused with no detriment.

The ampoule purging/sealing unit[5] was modified as shown in Fig. 1a to provide an additional stream of carbon-free O_2 at 100 ml/min. This stream was delivered to a 500-ml flask fitted with a sintered-glass bubbler and a side arm just large enough to admit the tip of the automatic pipet. Silver nitrate solution placed in this flask could be purged of carbonate-derived CO_2 and maintained in a carbon-free condition during the reagent dispensing step with a small continuous flow of O_2.

The heating period was omitted. Instead, the sealed ampoules were stored at room temperature for 30 min, then analyzed as in Sect. A.1.

B. Gas-Phase Combustion

Those samples with a sufficiently high carbon content were also analyzed by the method of Van Hall and co-workers (12). The apparatus was essentially similar to that previously described (13) with the exception that the syringe adapter was replaced with a septum-type injection port. This modification reduced sample blowback to more tolerable levels and allowed samples of up to 50-μl volume to be injected.

The effluent from the combustion tube was conducted to the same nondispersive infrared analyzer as was used for the wet chemical determinations.

Contrary to the previous experience (12), peak area was a better measure of CO_2 quantity than was peak height. Presumably this was due to some difference in relative time constants of the gas flow system and the infrared analyzer for the two installations. Peak areas were measured with the same integrator as in Sect. A.1.

C. Inorganic Carbon Determination

The gas-phase combustion technique of Sect. B provided a measure of total carbon. An independent determination of inorganic carbon was required to find organic carbon content. Inorganic carbon was determined by injecting a volume of sample into 6% (v/v) phosphoric acid at room temperature. The CO_2 was extracted into a nitrogen stream and measured with the infrared analyzer. The detailed procedure is given in (7). This same method was used with standard

TABLE 1
RECOVERY OF CO_2 FROM STANDARDS

Method of CO_2 production	Instrument response		Recovery	
	(μV sec/μg carbon)	σ	(%) •	σ
Inorganic	20337	672	—	
Organic, uncatalyzed[a]	20668	517	101.6	4.2
catalyzed[b]	20759	283	102.1	3.7

[a] Heated at 175°C for 8 hr.
[b] 0.032M Ag(1), room temperature for 30 min.

Na_2CO_3 solutions to generate known amounts of CO_2 for oxidation efficiency determinations.

D. Reagents

The $AgNO_3$ was CP-USP grade material[6] and was dissolved in distilled water for use. All other chemicals were analytical reagent grade and were used either directly as received or as solutions in distilled water.

The organic carbon standards were prepared from analytical reagent grade potassium hydrogen phthalate, dissolved in distilled water to give solutions containing 50.0, 10.3, and 5.6 μg of organic carbon/ml. Additional organic carbon, amounting to about 0.3 μg/ml, was contributed by the distilled water. This carbon blank was determined by the same methods used for analysis of samples and a correction was applied.

E. Sampling and Sample Storage

Stringent precautions were necessary to prevent contamination of samples with organic materials from the sample container. One-liter flint glass bottles with glass stoppers were washed with distilled water, drained, and heated in a glass annealing oven at 550°C for 8 hr. Samples were drawn directly into these bottles and analyzed as soon as possible after sampling. Standard solutions were stored in the same manner.

RESULTS AND DISCUSSION

Known amounts of organic carbon as potassium hydrogen phthalate were analyzed by both the uncatalyzed and the catalyzed wet chemical oxidation methods. The system responded linearly to

[6] Goldsmith Bros. Smelting and Refining Co., New York, N. Y.
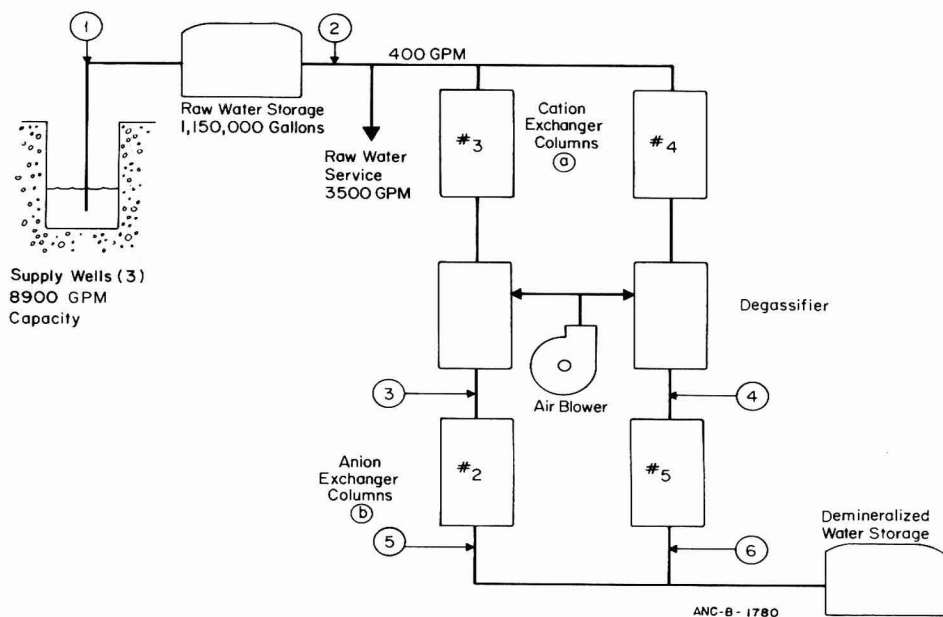
FIG. 3. Schematic diagram of water treatment plant. Circled numerals denote sampling stations. (a) Betz INX-32 gel type. (b) Betz INX-69 gel type.

amounts of carbon up to 50 μg, the largest quantity used. Linear response to up to 200 μg of carbon is expected (11). The instrument responses for the two methods are given in Table 1 as peak area per weight of carbon. Also given are the instrument response to similar amounts of carbon from Na_2CO_3 solutions and the recoveries from the organic compound relative to the Na_2CO_3. It is obvious that complete oxidation of potassium hydrogen phthalate was obtained by both methods. Similar results were obtained for solutions of succinic acid.

Some results of determinations on samples from an ion exchange water treatment plant[7] are given in Table 2. These particular results were chosen for presentation here because they provide some useful comparisons between various oxidation methods. Figure 3 is a schematic diagram of the water treatment plant, showing sampling points.

Samples taken at the well head and at the raw water storage outlet had a high organic carbon content, presumably the result of biological activity in the aquifer, since the well is located far from potential large sources of organic pollutants. Samples from these two stations gave comparable results by the uncatalyzed and catalyzed $S_2O_8^{2-}$ wet

[7] Demineralized water system, Test Reactor Facility, National Reactor Testing Station, Idaho Falls, Idaho.

TABLE 2

ANALYSIS OF SAMPLES FROM WATER TREATMENT SYSTEM

Sample station	$S_2O_8^{2-}$ (uncatalyzed. 175°C for 56 hr, except[a])		$S_2O_8^{2-}$ (Catalyzed. 0.032M Ag(I), room temp for 30 min)			High-temperature combustion, carbon ($\mu g/ml$)					
	Organic C ($\mu g/ml$)	σ ($\mu g/ml$)	Organic C ($\mu g/ml$)	σ ($\mu g/ml$)	C_{cat}/C_{uncat}	Total	σ_{total}	Inorganic	σ_{inorg}	Organic[d]	$\sigma_{organic}$
1. Well head	13.4	0.39	13.2	0.13	0.99	58.4	4.0	47.9	0.76	10.5	4.1
2. Storage tank outlet	13.6	0.34	13.5	0.21	0.99	55.8	3.1	47.4	1.3	8.4	3.4
3. Cation exchanger No. 3 outlet	0.343	0.007	0.410	0.007	1.20						
4. Cation exchanger No. 4 outlet	0.334 0.337[a]	– 0.053	0.411	0.019	1.23						
5. Anion exchanger No. 2 outlet[b]	0.258	0.035	0.294	0.112	1.14						
6. Anion exchanger No. 5 outlet[c]	0.141	0.010	0.170	–	1.21						

[a] 175°C for 8 hr.
[b] Resin loading in service for ~20 yr.
[c] Resin loading in service for ~2 yr, 4 mo.
[d] By difference.

chemical oxidation methods ($C_{catalyzed}/C_{uncatalyzed} \approx 1$). These samples contained enough organic carbon to permit use of the high-temperature combustion method. The mean organic carbon concentrations determined by this latter method were lower than those by the wet chemical oxidation methods, although the significance of the differences is doubtful on statistical grounds. The major part of the uncertainty in the organic carbon content by this last method was contributed by the total carbon determination.

Samples taken from stations downstream of the cation exchangers had a much lower organic content than those from the first two stations, possibly due to physical filtering of biological materials in the cation columns. The high-temperature combustion method had insufficient sensitivity to be used on samples taken at stations 3–6. The most striking feature of samples from these stations is the almost constant value of $C_{catalyzed}/C_{uncatalyzed}$. It is obvious that some constant fraction of the organic content is not oxidized by the uncatalyzed wet chemical procedure. It is not possible to determine from these data if the oxidation-resistant fraction was present at the first two sampling stations or was contributed from breakdown products of the cation exchanger (primarily long-chain alkyl sulfonates). Samples from station 4, oxidized without catalyst for 8 and 56 hr, showed no significant increase in oxidation efficiency with the prolonged heating period. For each particular wet chemical oxidation method, the organic carbon concentration at the two cation exchanger outlets were identical, since the operating histories of the two columns were similar. The lower organic carbon concentrations at the anion exchanger outlets reflect the general phenomenon that strongly basic anion exchangers will sorb sulfonates produced by degradation of cation exchangers (17). The lower carbon content at station 6, as compared to that of station 5, appears to be due to a decrease in the salt splitting capacity of the older anion exchanger charge (17).

Many comparative experiments such as those described above have consistently indicated oxidation efficiencies with the Ag(I)-catalyzed system as good or better than those obtained with uncatalyzed $S_2O_8^{2-}$ at elevated temperature. No interferences have been noted, but we must add that almost all our experience has been with fresh water systems. Samples with large amounts of halide have been accommodated by the simple expedient of adding $AgNO_3$ in excess of that required to completely precipitate the halide. In all of our experience to date, a 30-min oxidation time at room temperature has been sufficient to give oxidation efficiencies as good or better than those obtained with an 8-hr heated oxidation and no catalyst. The catalyzed oxidation still requires greater elapsed time and more man

hours per sample than does the high-temperature combustion method, however, the concentration of organic carbon that can be determined with reasonable precision is lower by a factor of about 50. The elapsed time for a determination is less than 90 min.

SUMMARY

The application of Ag(I) catalysis of $S_2O_8^{2-}$ to the oxidation of organic carbon to CO_2 for determination of dissolved organic carbon in aqueous samples is described. The resulting method combines to a significant degree the speed of high-temperature combustion methods and the sensitivity of wet chemical oxidation. For some samples, a higher oxidation efficiency has been observed than with an uncatalyzed wet chemical oxidation method.

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The Pyrolytic Identification of Organic Molecules

I. The Pyrolytic Behavior of Organic Molecules

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INTRODUCTION

The pyrolytic behavior of organic materials is of current interest for both the determination of their atomic constitution and their molecular structure. In oxidative decomposition procedures for the determination of the elements, the fate of the pyrolysis products is clearly defined. In those methods where an inert carrier gas is employed, such as in the determination of oxygen or nitrogen, the intermediate pyrolysis fragments are less defined. This is also relevant to pyrolysis characterization techniques currently developed for the identification of polymeric materials and some other classes of compounds. In such techniques samples are subjected to fairly low temperatures and the volatile fragments resulting from thermal decomposition are analyzed either by gas chromatography or mass spectrometry. For example, in the low-temperature decomposition of polymers, monomer and similar ordered fragments are obtained, but at high-temperatures thermal degradation is more complete, yielding simple products readily identified.

Some polymers and other classes of compounds have already been examined by thermal-degradation processes, but few attempts have been made to collect together the general principles underlying the technique of pyrolysis. There are many pyrolytic techniques, yet few investigators have carried out a systematic investigation using simple molecules in order to establish suitable conditions for general application. It seemed worthwhile to study the pyrolytic behavior of simple organic compounds under inert gas atmospheres to establish the nature of the fragments.

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The technique of pyrolysis has to be considered, especially such parameters as temperature, contact time, method of sample introduction, and weight of sample. It would seem impossible to devise a pyrolysis system suitable for all types of organic substances because of their various physical properties.

Some of the earliest pyrolysis studies were made by Berthelot (4) in 1866, and Hurd (11) has reviewed the subject up to 1929. Most of these studies were concerned with thermal syntheses, and not with possible analytical applications. Zemany (23) in 1952 first suggested the application of pyrolysis combined with instrumental analysis of the resulting products as a means of identifying organic molecules. Zemany investigated the combination of pyrolysis with mass spectrometry. This idea was extended by Davison et al. (7) in 1954, who pyrolyzed polymer samples at 650°C in an atmosphere of nitrogen. The resulting products boiling below 100°C were condensed and examined by gas chromatography. This technique has since been examined by many workers for the characterization of polymers, principally by Barlow et al (1). A review (17) of the subject was published in 1965. Pyrolysates from polymers have been analyzed by mass spectrometry by Bradt et al. (5) and by Bua and Maneressi (6). One-stage pyrolysis gas chromatography with mass spectrometry has been employed (10, 21).

Simple organic compounds such as aromatic substances were examined by Franc and Blaha (9) to a limited extent. Hydrocarbons were studied by Keulemans and Perry (16) and some aliphatic alcohols by Dhont (8) using gas chromatography. While individual compounds have been examined principally by pyrolysis gas chromatography, a more detailed study of a range of organic compounds had only been accomplished by Wolf and Rosie (22) at the time our study was carried out (14).

The process of thermal decomposition has been divided into four main categories by Beroza and Coad (3). *Thermal degradation* in the temperature range 100–300°C; *mild pyrolysis* at 300–500°C; *normal pyrolysis* at 500–800°C; *vigorous pyrolysis* at 800–1100°C. The pyrolysis products will therefore be many and varied depending on the temperature employed for a particular molecule or the size of the molecule. The more complex the molecule the greater the intricacy of the fragmentation pattern. Before analytical data from complex materials can be collected and interpreted, a knowledge of the patterns from simple molecules is essential.

Our interest in this problem began with a study of the pyrolysis process in the determination of oxygen (14) by the Shütze–Unterzaucher carbon-reduction method. Simple organic oxygenated com-

pounds were subjected to pyrolysis at 950°C in the presence of nitrogen carrier gas and the volatile pyrolysates were identified and determined semiquantitatively by mass spectrometry. The fragments collected common to all the samples were, benzene, methane, acetylene, and hydrogen. With oxygen-containing compounds either carbon monoxide, carbon dioxide, or a mixture of both products were obtained depending on the oxygen linkage in the molecule. If a nitrogroup linkage was involved then some nitric oxide and hydrogen cyanide were obtained. Some hydrogen cyanide was also produced in small quantities in all cases. Hydrogen cyanide was also produced from an amino nitrogen group associated with a functional group containing a methyl and a carbonyl group. Some carbon and nonvolatile tar-like products were also formed. Water if formed, was not easily detected unless the sample was a carbohydrate compound. An examination of our results indicated the possibility of using high-temperature pyrolysis fragmentation data as a means of identifying the structure of organic molecules.

EXPERIMENTAL METHODS

Pyrolysis Systems

Conventional vaporization pyrolysis. The apparatus and method of collecting the volatile products for mass spectrometric analysis has been described elsewhere (2). The samples were vaporized in a current of purified nitrogen gas at a flow rate of about 6 ml/min into a reaction chamber at 950 ± 10°C. Vaporization was accomplished with a Bunsen burner in the manner of a conventional.combustion procedure over a period of 10 min. A further 10 min was taken to sweep the pyrolysis fragments into the gas-sampling vessel. The sample contact time factor was of the order of 50 sec.

The weight of sample taken for each experiment was 15 ± 0.05 mg. Solids were weighed out in platinum boats and liquids in the conventional form of glass capillaries introduced into the vaporizing section of the reactor inside a short piece of silica tubing.

Direct pyrolysis. In this form of pyrolysis the sample was introduced directly into the reaction chamber by means of a silica ladle attached to an iron core for its movement into the hot zone with a magnetic pusher. The design of the reactor and sample introduction system were similar to those described by Ingram (15). The chamber had a capacity of about 85 ml and was maintained at 950 + 10°C.

Sample weights of 15 mg were taken for each experiment, weighed into the same type of containers used for the vaporization pyrolysis experiments. The pyrolysis was carried out in an atmosphere of ni-

trogen under static conditions. The volatile fragments were then swept out into the gas-sampling vessel with nitrogen over a period of 10 min at a flow rate of 12 ml/min. The contact time factor was of the order of 25 sec.

Study of the Pyrolysis Conditions

Effect of temperature. The yield of volatile products was determined by pyrolysis–mass spectrometric analysis of 15 mg samples of polyacrylonitrile over the range of 500–950°C, using the conventional vaporization pyrolysis procedure. The products obtained were those given in Table 1 and incorporated into the temperature curve (Fig. 1) in which the comparative concentrations of the products are plotted against temperature of the pyrolysis hot zone. These concentration values were calculated as the peak-height ratios of the products in respect to nitrogen (the carrier gas) obtained from the spectrograms.

Effect of flow rate. Samples of *m*-dihydroxybenzene were pyrolyzed at three different flow rates of carrier gas by the conventional vaporization procedure at 950°C. Benzene, methane, acetylene, hydrogen cyanide, hydrogen, carbon monoxide, and carbon dioxide were formed. The product–nitrogen ratios are given in Table 2 to illustrate the effect of flow-rate variation.

The concentration of each product was found to vary with flow rate indicating that contact time of the sample within the hot zone was an important factor. The higher the contact time value, the greater the decomposition of the original molecule, and the lower the concentration of the primary fragment most useful for characterization purposes. A contact time factor of 50 sec was found to be suitable for the 15 mg sample weights.

Our examination of the direct pyrolysis procedure indicated that the same volatile products were formed as in the conventional vaporization procedure. However, the amounts of some of the products

TABLE 1
COMPOSITION OF VOLATILE PRODUCTS FROM POLYACRYLONITRILE

Temp (°C)	C_6H_6	CH_2CHCN	CH_3CN	HCN	$(CH)_2$	CH_4	H_2
500	–	0.0051	0.0088	0.0026	0.004	0.0053	0.0013
600	0.0003	0.0045	0.0078	0.0033	0.0038	0.0077	0.0001
700	0.0016	0.0086	0.0114	0.0055	0.0086	0.0136	0.0023
800	0.0033	0.0015	0.0036	0.0032	0.0042	0.0174	0.003
950	0.0043	0.0004	0.0016	0.0011	0.0022	0.0161	0.0043
950	0.0045	0.0008	0.0022	0.0016	0.0021	0.0165	0.0055
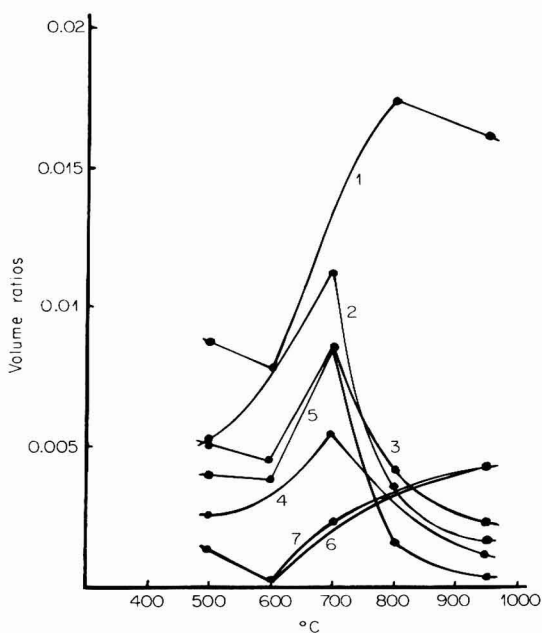
FIG. 1. Rate curves for the thermal decomposition of polyacrylonitrile. (1) Methane, (2) acetonitrile, (3) acrylonitrile monomer, (4) hydrogen cyanide, (5) acetylene, (6) benzene, (7) hydrogen.

were found to differ in the two methods of pyrolysis, presumably because of the different contact times.

Effect of carbonaceous residues. We found that the presence of deposited carbon in the reactor can influence the concentration of some of the products formed. This was particularly so in the case of

TABLE 2
EFFECT OF FLOW RATE VARIATION[a]

Product	A 12.5 ml/min	B 8.3 ml/min	C 6.3 ml/min
C_6H_6	0.0119	0.0093	0.0068
CO_2	0.0262	0.0168	0.0142
CO	0.05	0.05	0.05
HCN	0.0014	Tr	0.001
C_2H_2	0.0018	0.0018	0.0011
CH_4	0.0192	0.0155	0.0147
H_2	0.0079	0.0072	0.027

[a] Vaporization time was 5 min and sweep time was 5, 10, and 15 min, respectively, for A, B, and C.

carbon dioxide, the reaction $CO_2 + C \rightarrow 2CO$ being predominant. For quantitative work the pyrolysis tube was purged out at 950°C with oxygen after about six pyrolyses.

PYROLYSIS OF ORGANIC COMPOUNDS

Certain aspects of the results obtained from the pyrolysis of 70 organic compounds has been reported elsewhere (2). In this communication we present the mass-spectrometric-analysis results, and discuss the possibility of structural characterization with some examples from the data collected.

The product–nitrogen ratios of the volatile products from the different classes of compounds examined as calculated from their mass spectrograms are recorded in Tables 3–10. These results are of a semiquantitative nature.

In our experiments benzene was detected in significant amounts from almost all of the aromatic and aliphatic substances examined. In the case of aromatic compounds the concentration of benzene in the pyrolysates varied according to the degree of substitution. The decomposition of benzene was also studied since it was found to contribute towards the yields of hydrogen, methane, and acetylene obtained from the compounds examined.

The pyrolytic patterns produced in Tables 3–10 indicate that high-temperature pyrolysis, combined with mass spectrometric analysis could be a potential analytical tool for structural identification purposes. The different volatile products obtained are produced in varying amounts and combinations which are related to the structure of a substance, particularly to the type of functional group present in the molecule. The indication is that for a given pyrolysis condition the pattern is reproducible and hence predictable. The reproducibility is being investigated in greater detail.

TABLE 3
COMPOSITION OF GASES FROM AROMATIC C AND H COMPOUNDS

Compound	C_6H_6	HCN	$(CH)_2$	CH_4	H_2
Benzene	0.0513	0.0014	0.0012	0.0045	0.0067
	0.0502	0.0013	0.0011	0.0027	0.0058
Naphthalene	0.0016	—	0.0002	0.0059	0.025
2-Methyl- naphthalene	0.0011	0.0012	0.0004	0.0168	0.0286
2:6-Dimethyl- naphthalene	0.0007	0.0023	0.0006	0.0364	0.0335
Anthracene	0.0014	0.0003	0.0002	0.0082	0.0224

TABLE 4
COMPOSITION OF GASES FROM AROMATIC C, H, AND O COMPOUNDS

Compound	C_6H_6	CO_2	CO	HCN	$(CH)_2$	CH_4	H_2
Benzoic acid	0.028	0.0315	Tr[a]	0.0019	0.0013	0.0016	0.0079
Phenol	0.0125	Tr	0.025	0.0014	0.0011	0.0099	0.0193
o-Dihydroxy-benzene	0.0146	0.0056	0.0625	0.0012	0.002	0.02	0.0077
Cinnamic acid	0.0149	0.0214	Tr	0.0007	0.001	0.0067	0.0057
o-Toluic acid	0.0149	0.0283	Tr	0.0007	0.0008	0.0128	0.004
Thymol	0.0143	0.0104	0.01	0.0013	0.0014	0.0365	0.0104
Methyl-benzoate	0.0253	0.0262	0.025	0.001	0.0008	0.0126	0.0069
Ethyl-benzoate	0.0177	0.0226	Tr	0.0009	0.0014	0.0082	0.0061
2-Naphthol	0.0026	0.0012	Tr	—	Tr	0.0068	0.0175
1-Naphthoic acid	0.001	0.0119	0.01	0.0022	0.0006	0.0049	0.0204
1-Naphthyl-acetate	0.0035	0.0047	0.01	0.0007	Tr	0.0178	0.0131
Coumarin	0.0121	0.0042	0.0247	0.0006	0.0056	0.0076	0.0042
4-Hydroxy-coumarin	0.0056	0.0237	0.025	0.0003	0.0006	0.0053	0.0033
7-Hydroxy-coumarin	0.0092	0.0122	0.04	0.0006	0.0009	0.0066	0.0041

[a] Tr denotes a trace amount of product.

Another aspect being studied is concerned with the temperature and the method of pyrolysis. Our study of the pyrolysis of polyacrylonitrile over the range of 500–950°C (Table 1) revealed that a maximum concentration occurred with products, acrylonitrile monomer, acetonitrile, hydrogen cyanide, and acetylene at around 700°C. Methane had a maximal concentration at about 800°C, while benzene and hydrogen continued to increase with the rise in temperature. A pyrolysis temperature of 700°C produced the optimum concentration for the major volatile products which are considered to be the true distinguishing products from the polymer. Benzene in this case is derived from the free-radical fragments combining, and methane and hydrogen are considered to be the decomposition products of the compounds which had a maximal concentration at 700°C. The temperature at which the pyrolysis is carried out, is therefore, very important, since different classes of organic compounds might behave differently. The experimental evidence obtained by Wolf and Rosie (22) substantiate this opinion, since their pyrolysis results for particu-

TABLE 5

COMPOSITION OF GASES FROM AROMATIC C, H, O AND N COMPOUNDS

Compound	C_6H_6	NO	CO_2	CO	HCN	$(CH)_2$	CH_4	H_2
m-Dinitro-	0.0016	0.011	0.0256	0.025	0.0073	0.0013	0.0011	0.007
benzene	0.0018	0.0052	0.0226	0.02	0.0031	0.0012	0.0009	0.002
o-Nitro- benzoic acid	0.0062	0.0049	0.0318	0.0125	0.0071	0.001	0.0015	0.0066
3:5-Dinitro- benzoic acid	0.0007	0.0063	0.0395	0.0312	0.0085	0.0017	0.0009	0.005
o-Amino- benzoic acid	0.0102	—	0.0279	0.0062	0.0147	0.0023	0.0056	0.0198
o-Nitro- phenol	0.0029	0.004	0.0162	0.04	0.0135	0.0025	0.0038	0.0162
5-Nitroso -o-cresol	0.0029	—	0.0113	0.01	0.0038	0.0009	0.0047	0.0037
o-Nitro- analine	0.0053	0.0013	0.0058	0.0063	0.0151	0.0029	0.0043	0.0106
Benzamide	0.0344	—	0.0081	0.0416	0.0253	0.0003	0.004	0.009
Phenyl- urea	0.0095	—	0.008	0.025	0.0008	Tr[a]	0.0048	0.0031
Acetanilide	0.0134	—	Tr	0.0125	0.0121	0.0021	0.0128	0.0117
Phenacetin	0.0034	—	Tr	0.01	0.0059	0.0024	0.0102	0.0042
p-Nitro- acetanilide	0.0047	0.0008	0.0098	0.0375	0.0081	0.0014	0.0064	0.0081
8-Hydroxy quinoline	0.0079	—	Tr	0.01	0.0117	0.0017	0.0045	0.0037
-Benzildi- oxime	0.0281	—	0.0176	0.0125	0.0274	0.006	0.0046	0.0073

[a] Tr denotes trace amounts.

TABLE 6

COMPOSITION OF GASES FROM AROMATIC C, H, O, N, AND Cl COMPOUNDS

Compound	C_6H_6	CO_2	NO	CO	HCN	$(CH)_2$	CH_4	H_2
1-Chloro- 2:4-di- nitro- benzene	0.0012	0.0181	0.0151	0.0125	0.01	0.0016	0.0005	0.0026
2:6-Di- chloro- 4-nitro- phenol	0.0006	0.0095	0.0044	0.0233	0.002	0.0005	—	0.0012
p-Chloro- acetanilide	0.0077	Tr[a]	—	0.02	0.0141	0.0024	0.0132	0.0165

[a] Tr denotes a trace amount.

TABLE 7
COMPOSITION OF GASES FROM AROMATIC C, H, O, N, AND F COMPOUNDS

Compound	SiF_4	C_6H_6	CO_2	CO	HCN	$(CH)_2$	CH_4	H_2
m-Trifluoro-methyl benzoic acid	0.0148	0.0055	0.0203	0.0063	0.0004	Tr[a]	0.0006	0.0016
p-Fluoro-benzoic acid	0.0082	0.0141	0.027	0.0125	0.0006	0.0004	0.002	0.0033
Trifluoro-acetanilide	0.0155	0.0064	0.0097	0.0125	0.0003	Tr	0.0012	0.0015

[a] Tr denotes a trace amount.

TABLE 8
COMPOSITION OF GASES FROM AROMATIC C, H, O, AND P COMPOUNDS

Compounds	C_6H_6	CO_2	CO	HCN	$(CH)_2$	CH_4	H_2
Triphenyl phosphine	0.0347	—	—	0.0011	0.0009	0.0024	0.0089
Triphenyl phosphate	0.0167	Tr[a]	0.0125	0.0005	Tr	0.0024	0.0056

[a] Tr denotes a trace amount.

TABLE 9
COMPOSITION OF GASES FROM ALIPHATIC C, H, AND O COMPOUNDS

Compound	C_6H_6	CO_2	CO	HCN	$(CH)_2$	CH_4	H_2
Lauric acid	0.0073	0.0122	Tr[a]	0.0021	0.0034	0.0374	0.0062
Myristic acid	0.0078	0.0117	Tr	0.002	0.0038	0.0312	0.0082
Stearic acid	0.0064	0.0092	—	0.0015	0.0023	0.0302	0.0057
Behenic acid	0.0055	0.0083	Tr	0.0011	0.0018	0.0257	0.0039
Crotonic acid	0.0068	0.0354	0.025	0.0014	0.0027	0.023	0.0056
Sorbic acid	0.0096	0.0373	Tr	0.0014	0.0038	0.0211	0.0071
Pivalic acid	0.007	0.0285	0.025	0.0018	0.0032	0.0428	0.0086
Mannitol	0.0029	0.0105	0.0375	0.0012	0.0024	0.0108	0.0048
Sucrose[b]	0.0014	0.0199	0.025	0.0007	0.0015	0.0077	0.0021

[a] Tr denotes a trace amount.
[b] The volume ratio result for the amount of water collected was 0.0113.

TABLE 10

COMPOSITION OF GASES FROM ALIPHATIC C, H, O, N, AND Cl COMPOUNDS

Compound	C_6H_6	CO_2	CO	HCN	$(CH)_2$	CH_4	H_2
Acetamide	0.0009	0.0101	0.0125	0.0012	0.0011	0.0175	0.0032
Chloro- acetamide	—	0.0071	0.0125	0.0018	0.0007	0.0045	0.0024
Urea	—	0.0315	0.025	0.0006	Tr[a]	—	—

Methyl-
  amine
  —HCl
	—	—	—	0.0016	0.0012	0.0145	0.0042

[a] Tr denotes a trace amount.

lar groups of compounds appear to exhibit maxima over a range of 700–900°C.

Results obtained by direct pyrolysis of some aromatic and aliphatic compounds, including some compounds that had been examined using the conventional vaporization pyrolysis procedure are given in Table 11. Comparison of the product–nitrogen ratios with those in Tables 4, 5, 9, and 14 show that while the same products are formed the magnitude of the yields differed in respect to benzene, acetylene, hydrogen cyanide, and hydrogen. Methane yields also differed, but depended on the availability of a methyl group. The carbon dioxide and carbon monoxide patterns also differed. This difference in results between the two forms of pyrolysis procedure can be explained as follows:

In the conventional vaporization process, the sample in liquid form, gradually distils along the pyrolysis tube towards the hot zone aided by heat from the flame of the Bunsen burner. During this time, fragmentation of the carbon skeleton occurs as the temperature increases, and the smaller fragments are carried at low concentration into the hot zone where final decomposition into the products identified occurs. Rearrangement of the molecule, therefore, proceeds slowly, and presumably the production of methine radicals is small. For example, low yields of hydrogen cyanide were obtained when nitrogen-free compounds were pyrolyzed. Also, such products as benzene and acetylene have a greater chance of decomposing.

In the case of the direct pyrolysis, the sample is introduced immediately into the hot zone (at 950°C). Fragmentation of the carbon skeleton occurs almost at once, so that free-radical concentration must be extremely high. This is indicated by the relatively large amounts of hydrogen cyanide produced from the saturated aliphatic fatty acids and the two cyclohexane compounds. Benzene and acetylene yields are also higher because they are removed from the hot zone before decomposition can occur to any great extent.

TABLE 11
COMPOSITION OF GASES FROM AROMATIC AND ALIPHATIC
COMPOUNDS AFTER DIRECT PYROLYSIS

Compound	C_6H_6	CO_2	NO	CO	HCN	$(CH)_2$	CH_4	H_2
Benzoic acid	0.039	0.043	—	Tr[a]	0.0023	0.0018	0.0012	0.0115
o-Amino-benzoic acid	0.0112	0.0211	—	0.0125	0.0012	0.0013	0.0035	0.0047
m-Amino-benzoic acid	0.0209	0.0363	—	0.025	0.0045	0.0038	0.0045	0.0138
p-Amino-benzoic acid	0.0147	0.0282	—	0.0125	0.0019	0.0021	0.0044	0.006
Phenol	0.0073	—	—	0.0125	0.001	0.001	0.0024	0.0085
o-Nitro phenol	0.0051	0.0244	0.002	0.0416	0.0025	0.0025	0.0081	0.0119
m-Nitro-phenol	0.0071	0.0152	0.0026	0.05	0.0055	0.0029	0.0095	0.0336
p-Nitro-phenol	0.0066	0.0126	0.0014	0.05	0.0037	0.0011	0.0028	0.0245
o-Toluic acid	0.0107	0.0492	—	Tr	Tr	0.0015	0.009	0.0005
m-Toluic acid	0.0153	0.0279	—	0.0125	Tr	0.002	0.0027	0.0022
p-Toluic acid	0.0376	0.0373	—	Tr	Tr	0.003	0.0074	0.0005
Cyclo-hexanone	0.0086	Tr	—	0.0416	0.0077	0.0072	0.047	0.0983
Cyclo-hexanol	0.0102	Tr	—	0.025	0.0086	0.0081	0.0379	0.1029
Propionic acid	0.0151	0.0256	—	0.0588	0.0182	0.0108	0.0234	0.0507
n-Valeric acid	0.0217	0.0213	—	0.0416	0.0168	0.0121	0.0285	0.0476
Lauric acid	0.0158	0.015	—	0.0125	0.0089	0.0093	0.0272	0.0207
Myristic acid	0.024	0.0144	—	0.0125	Tr	0.011	0.0456	0.0425
Stearic acid	0.0136	0.0254	—	Tr	Tr	0.0084	0.0409	0.018
Behenic acid	0.0093	0.0085	—	Tr	Tr	0.0052	0.021	0.0082

[a] Tr Denotes a trace amount.

INTERPRETATION OF RESULTS

Mono-functional Benzene Compounds

The product–nitrogen ratios for a number of mono-functional ben-
zene compounds are given in Table 12, selected from the previous
tables. The results obtained from the pyrolysis of benzene indicate

TABLE 12
MONO-FUNCTIONAL BENZENE COMPOUNDS

Benzene	Refer to Table 3
Phenol	Refer to Table 4
Benzoic acid	Refer to Table 4
Methylbenzoate	Refer to Table 4
Ethylbenzoate	Refer to Table 4
Benzamide	Refer to Table 5
Phenylurea	Refer to Table 5
Acetanilide	Refer to Table 5

the stable nature of the compound and the possible reproducibility of the pyrolysis procedure. Trace amounts of hydrogen cyanide produced from the nitrogen-free compounds was presumably derived from reaction of pyrolysis carbon fragments (CH: radicals) with the nitrogen carrier gas.

Phenol and benzoic acid are distinguishable from each other by virtue of the fact that the hydroxyl functional yielded mainly carbon monoxide and the carboxylic functional group yielded predominately carbon dioxide. The amounts of methane and hydrogen also obtained from phenol were significantly greater than from benzoic acid, indicating that rupture of the benzene ring occurs in the case of phenol to eliminate carbon monoxide. Accordingly, the benzene yield is smaller because it is derived from rearrangement of hydrocarbon fragments. Ring rupture appeared to occur only to a limited extent with benzoic acid as the yield of benzene from it was much higher and the methane yield lower than from phenol. In the case of benzoic acid thermal decomposition of the molecule is seen to be straightforward with elimination of carbon dioxide and formation of benzene.

A significant difference in the pyrolysis patterns of methyl and ethyl benzoates was observed. Carbon monoxide and carbon dioxide were obtained from methyl benzoate and a high yield of carbon dioxide with only a small quantity of carbon monoxide was obtained from ethyl benzoate. The two compounds can therefore, be distinguished from each other. Methyl benzoate is reported (12) to be very stable towards heat up to a temperature of 400°C. At dull red heat (~ 600°C) it is said to decompose into benzene, diphenyl, methyl *m*- and *p*-phenyl-benzoate and trioxymethylene. Ethyl benzoate on the other hand is reported (12) to decompose at 360°C into benzoic acid and ethylene. Our results for ethyl benzoate are consistent with the literature, since a similar decomposition pattern to benzoic acid was obtained, but with a considerable amount of methane produced from high-temperature decomposition of the ethylene. A mechanism for the

thermal decomposition of these benzoates will be presented in a later communication.

· The compounds benzamide and phenylurea containing the nitrogen functional groups $-CONH_2$ and $-NHCONH_2$, respectively, are distinguishable from each other without difficulty. The former yielded a large quantity of benzene, and consequently, the methane value in relation to benzene is low. This is to be compared with a low benzene yield and a correspondingly higher yield of methane from phenylurea. Mixtures of carbon monoxide and carbon dioxide were obtained from both compounds. The most important feature was the high value for hydrogen cyanide from benzamide compared with the low value from phenylurea.

Acetanilide with its nitrogen functional group $-NHCOCH_3$, gave a different pyrolysis pattern distinguishing it from the previous nitrogen compounds. Only carbon monoxide was produced, with a trace of carbon dioxide, and the hydrogen cyanide value was greater. Methane was also produced in high yield consistent with the presence of the methyl group.

Naphthalene Compounds

The product–nitrogen ratios for naphthalene and some of its derivatives are given in Table 13. The amount of benzene produced from naphthalene and the derivatives examined was extremely small. One outstanding feature was that methane and hydrogen from the two methyl-substituted naphthalenes increased markedly with the increase in the number of methyl groups present.

The significant volatile products from 2-naphthol, 1-naphthoic acid, and 1-naphthyl acetate are derived mainly from their functional groups. The absence of large quantities of benzene and the production of tarlike residues together with carbon deposits, suggests that thermal rupture does not occur in the same way as with benzene and its derivatives. However, some differences in product ratios were found to indicate structural differences. For example, 2-naphthol yielded mainly carbon dioxide from the $-OH$ functional group,

TABLE 13
NAPHTHALENE COMPOUNDS

Naphthalene	Refer to Table 3
2-Methylnaphthalene	Refer to Table 3
2:6-Dimethylnaphthalene	Refer to Table 3
2-Naphthol	Refer to Table 4
1-Naphthoic acid	Refer to Table 4
1-Naphthylacetate	Refer to Table 4

whereas phenol yielded mainly carbon monoxide. 1-Naphthoic acid yielded a mixture of carbon monoxide and carbon dioxide compared with benzoic acid which yielded mainly carbon dioxide. There was a marked increase in the methane yield from 1-naphthyl acetate indicating the presence of the methyl group, and therefore, evidence of the presence of the acetyl group.

Isomeric Compounds

A number of isomeric substances were pyrolyzed to determine whether substitution at the ortho, meta, and para positions yielded different patterns to enable each isomer to be identified. Some results are given in Table 14. Each of the different series of isomers gave pyrolytic patterns containing possible distinguishing features.

The results obtained from the isomers of dihydroxybenzene show that resorcinol, the meta isomer, can be distinguished from the *o*- and *p*-isomers. The yield of carbon dioxide from resorcinol was much greater than from the other isomers, and consequently, the yield of carbon monoxide from the hydroxyl functional groups was less. Resorcinol being a tautomeric compound exists in the ketonic form, and therefore, its thermal decomposition proceeds possibly by a different route from that of the *o*- and *p*-isomers. These two compounds yielded the same products in the same relative proportions.

The isomers of toluic acid appear to be decarboxylated splitting off carbon dioxide and forming benzene in good yield. Considerable quantities of methane were also produced consistent with the presence of the methyl group. Unlike the pyrolytic patterns obtained when the isomers were subjected to the direct pyrolysis procedure (Table 11), the patterns obtained show very little detail to enable the isomers to be distinguished from each other.

Anthranilic acid, *o*-aminobenzoic acid, is known to be decarboxylated when heated above its melting point of 145°C yielding carbon dioxide (*13*) and aniline (*18*). It has been suggested that anthranilic acid undergoes a change through the formation of an intermediate salt (*20*):
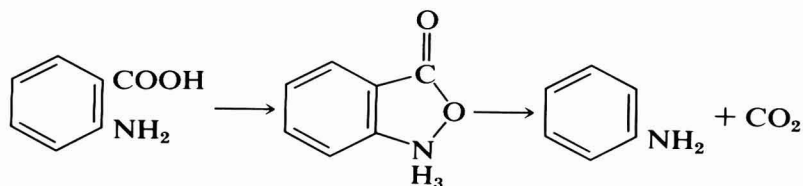

known to be complete at 205–210°C. This does not account for the formation of carbon monoxide, unless it is postulated that the intermediate can also decompose with the elimination of carbon monoxide

TABLE 14
COMPOSITION OF GASES FROM ISOMERIC COMPOUNDS

Compound	C_6H_6	CO_2	NO	CO	HCN	$(CH)_2$	CH_4	H_2
o-Dihydroxy- benzene	0.0146	0.0056	—	0.0625	0.0012	0.002	0.02	0.0077
m-Dihydroxy- benzene	0.0106	0.0208	—	0.0375	0.0009	0.0012	0.0229	0.012L
p-Dihydroxy- benzene	0.0125	0.0063	—	0.05	0.001	0.0015	0.0179	0.0076
o-Amino- benzoic acid	0.0102	0.0279	—	0.0062	0.0147	0.0023	0.0056	0.0098
m-Amino- benzoic acid	0.0057	0.015	—	0.0062	0.0086	0.0018	0.0043	0.0146
p-Amino- benzoic acid	0.0159	0.0406	—	0.025	0.0188	0.0036	0.0079	0.0198
o-Nitro- benzoic acid	0.0062	0.0318	0.0049	0.0125	0.0071	0.001	0.0015	0.0062
m-Nitro- benzoic acid	0.0071	0.0289	0.0034	0.0312	0.005	0.0008	0.0015	0.0058
p-Nitro- benzoic acid	0.005	0.0296	0.0035	0.0312	0.006	0.0011	0.0019	0.012
o-Nitro phenol	0.0029	0.0162	0.004	0.04	0.0135	0.0025	0.0038	0.0162
m-Nitro- phenol	0.0035	0.0103	0.0041	0.0625	0.009	0.0017	0.0036	0.012
p-Nitro- phenol	0.0026	0.0072	0.0018	0.0375	0.0075	0.0014	0.0025	0.0083
o-Nitro- aniline	0.0053	0.0058	0.0013	0.0063	0.0151	0.0029	0.0043	0.0106
m-Nitro- aniline	0.0068	0.0087	0.0014	0.0155	0.0106	0.0018	0.0035	0.0077
p-Nitro- aniline	0.0067	0.007	—	0.01	0.0042	0.0008	0.0023	0.004
o-Toluic acid	0.0149	0.0283	—	Tr[a]	0.0007	0.0008	0.0128	0.004
m-Toluic acid	0.0226	0.0364	—	Tr	0.0007	0.0008	0.0142	0.004
p-Toluic acid	0.021	0.0327	—	Tr	0.001	0.0008	0.0131	0.0048

[a] Tr Denotes a trace amount.

and formation of β-phenylhydroxylamine as follows:
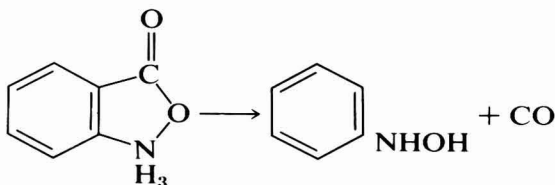
Aniline and β-phenylhydroxylamine would then fragment giving hydrogen cyanide, benzene, and the other products obtained. It is not clear how the meta and para isomers of aminobenzoic acid decompose. The o- and p-isomers are known to decompose in water at 100°C into aniline and carbon dioxide, whereas the m-isomer is stable (19). Such behavior presumably accounts for the characteristic pyrolytic patterns obtained. The other isomers, and the o-isomer pattern different from the p-isomer, the latter yielding a higher proportion of carbon dioxide and carbon monoxide. Another significant point is that the yield of hydrogen from each isomer increased through o->m->p- positions.

The isomers of nitrobenzoic acid, nitrophenol, and nitroaniline all yielded characteristic pyrolytic patterns for their possible identification. In most cases nitric oxide was produced indicating the presence of the nitro group. Hydrogen cyanide and carbon were also produced in significant amounts. Another important feature was the production of carbon dioxide in addition to carbon monoxide from the nitrophenols, and carbon monoxide in addition to carbon dioxide from the nitrobenzoic acids. A nitro group therefore, has a marked effect on the fragmentation fate of the other oxygenated functional groups in the molecule. The nitroso group appears to have a less marked effect. Thus, 5-nitroso-o-cresol (Table 4) yielded no nitric oxide and less carbon dioxide and carbon monoxide. Benzene yields from the nitro isomers were also lower because of disubstitution in the molecules. The hydrogen yield from the nitrobenzoic acid isomers increased in magnitude through the o->m->p- positions, consistent with a similar effect observed with the aminobenzoic acid isomers. In the case of the nitrophenol and nitroaniline isomers the yield of hydrogen from each series of isomer decreased in the order o-<m-<p-compounds. Methane yields were of an insignificant magnitude resulting presumably from the decomposition of benzene. The isomers containing a nitro group therefore, give pyrolysis patterns distinctly different from each other and from their corresponding parent compounds, phenol and benzoic acid. The significant distinguishing features of the ni-

trophenols is that the amount of carbon dioxide, hydrogen cyanide, and hydrogen produced decreased as the nitro group shifted its position through the o-, m-, and p- sites. The distinguishing features of the nitroanilines are differences in yields of hydrogen cyanide and hydrogen, and the concentration relationship between carbon dioxide and carbon monoxide.

Fluorinated Compounds

Interesting comparisons were obtained between trifluoroacetanilide and m-trifluoromethyl benzoic acid (Table 7) and the analogous non-fluorinated compounds acetanilide (Table 5) and m-toluic acid (Table 14). The presence of fluorine was revealed by the existence of silicon tetrafluoride in the pyrolysates. Presumably hydrofluoric acid was formed which reacted with the silica of the pyrolysis tube according to the equation:

$$4HF + SiO_2 \longrightarrow SiF_4 + 2H_2O.$$

Therefore, the thermal decomposition route may be complicated by the presence of water. Thus carbon dioxide was also formed from trifluoroacetanilide, whereas, acetanilide gave mainly carbon monoxide and a trace only of carbon dioxide. Carbon monoxide was produced from m-trifluoromethyl benzoic acid, whereas, carbon dioxide was the main oxygenated product from m-toluic acid. In addition, very little hydrogen cyanide was produced from trifluoroacetanilide indicating that the $-NHCOCF_3$ group must be fragmenting in a different way from the analogous group in acetanilide. Substitution of the hydrogens in the methyl group by fluorine had the effect of reducing the amount of methane from both of the fluorinated compounds, proving that methane is derived mainly from the methyl group and not from any other source.

p-Fluorobenzoic acid yielded silicon tetrafluoride and some carbon monoxide, and reduced amounts of benzene and carbon dioxide were obtained. These results, compared with the pattern obtained from benzoic acid (Table 4) indicate the effect of para substitution. A similar effect, can be observed by comparison of the patterns obtained from p-chloroacetanilide (Table 6) and acetanilide (Table 5). The benzene yield from the chloroderivative is much reduced, and the hydrogen yield is increased accordingly, but the yield of methane is unaffected as it is derived from the functional group. Benzene yields from trifluoromethyl benzoic acid, p-fluorobenzoic acid, and trifluoroacetanilide were halved, hydrogen yields were also reduced in significant amounts.

CONCLUSIONS

The study has shown that pyrolysis combined with mass spectrometry is a potential technique for the elucidation of the structure of organic compounds. Identification of a particular compound may be achieved through the kind of volatile products derived from a functional group contained in the molecule.

Of the two pyrolytic methods examined, we consider that direct insertion of the sample into the hot zone of the pyrolyzer will provide a more realistic pattern of the thermal degradation of a molecule.

The method of pyrolysate collection employed is not ideally suitable for quantitative measurement of the components. Gas chromatography would be more adaptable and would need less material than we have used for examination. One drawback of chromatographic analysis is that more than one column would be needed to cope with the separation of the many different kinds of components to be found in a pyrolysate.

SUMMARY

Results of a study on the pyrolysis of about 70 organic compounds of varied composition are presented and discussed. Identification of the volatile products formed was accomplished by mass spectrometry. It is shown how the pyrolytic patterns may be employed to distinguish one molecule from another. Some attention has been given to isomeric compounds and to aromatic structures containing one or more functional groups.

ACKNOWLEDGMENT

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Gel Electrophoresis as a Means of Detecting Ternary Complex Formation of Thymidylate Synthetase

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INTRODUCTION

5-Fluoro-2'deoxyuridylate (5-fluorodUMP) is the active form of the anticancer drug, 5-fluorouracil (*3, 7, 9*), and is a potent inhibitor of thymidylate synthetase (EC 2.1.1b), which catalyzes the 5,10-methylenetetrahydrofolate-dependent conversion of deoxyuridylate to thymidylate (*2, 6, 10*). Recently, both Santi and McHenry (*14*) and Langenbach, Danenberg and Heidelberger (*11*) have presented evidence which suggests that the 5-fluorodUMP-dependent inhibition of thymidylate synthetase (MW 70,000) from amethopterin-resistant *Lactobacillus casei* is the result of the formation of stable ternary complex(es) between thymidylate synthetase, 5-fluorodUMP, and 5,10-methylenetetrahydrofolate. The stoichiometry of ternary complex formation is thought to be two 5-fluorodUMP: two 5,10-methylenetetrahydrofolate:one thymidylate synthetase, a ratio which is not inconsistent with the presence of two subunits (MW 35,000) sharing an apparently identical primary structure (*12*). In this report, gel electrophoresis is shown to resolve the ternary complexes of thymidylate synthetase into two forms. Gel scanning data are used to infer the composition of these two types of complexes. As evidenced by analytical gel electrophoresis, the ternary complexes of thymidylate synthetase are quite stable entities which remain intact when isolated by gel filtration. However, storage of the isolated complexes at room temperature results in the slow breakdown of the ternary complexes, as indicated by gel electrophoretic band patterns.

EXPERIMENTAL PROCEDURE

· *Materials*

Pure thymidylate synthetase from amethopterin-resistant *Lactobacillus casei* was prepared by a modification of the method of

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Dunlap, Harding and Huennekens (5). 5-Methyltetrahydrofolate was generously provided by Drs. John Whitely and George Gapski, Scripps Clinic and Research Foundation, La Jolla, Ca. Dihydrofolate was prepared by dithionite reduction of folic acid (1). Tetrahydrofolate was prepared by the catalytic hydrogenation of folic acid in acetic acid (8). Other chemicals were obtained as follows: 5-fluoro-2'-deoxyuridine-5'monophosphate (5-fluorodUMP), Terra-Marine Bioresearch; folic acid, Calbiochem; dUMP and dTMP, Sigma Chemical Co.; amethopterin, Nutritional Biochemical Co.; Sephadex G-100 and G-25, Pharmacia Fine Chemicals.

Assay Conditions

Thymidylate synthetase was assayed spectrophotometrically essentially by the method of Wahba and Friedkin (15) as previously described (5). Specific activity is expressed as micro-moles of 7,8-dihydrofolate produced per milligram of protein per minute at 25°C. Thymidylate synthetase concentrations were determined from the absorbance at 280 nm using extinction coefficients of 1.55 mg^{-1} ml and 1.05×10^5 M^{-1} cm^{-1} (R. B. Dunlap, unpublished data).

Conditions for Ternary Complex Formation

Cofactor solutions containing 2 mM dl, L-5, 10-methylenetetrahydrofolate were prepared by dissolving 6 mg of tetrahydrofolate in 5 ml of water containing 0.25 mmoles sodium bicarbonate, 1.25 mmoles of 2-mercaptoethanol and 0.34 mmoles of formaldehyde. The concentrations of freshly prepared aqueous stock solutions of 5-fluorodUMP were determined from the absorbance at 269 nm using an extinction coefficient of 8.3×10^3 M^{-1} cm^{-1} (14). Solutions of pure thymidylate synthetase in 0.1 M potassium phosphate buffer (pH 7.0), 25 mM 2-mercaptoethanol, were inhibited by adding the appropriate volume of cofactor solution followed by the addition of 5-fluorodUMP solution required to give the desired inhibitor:enzyme molar ratios. The inhibition reaction mixtures were incubated at 25°C for times ranging from 30 min to 16 hr.

In a typical experiment, the inhibition reactions and appropriate controls were carried out in a 1.0 ml volume of 0.1 M potassium phosphate buffer (pH 7.0) containing 25mM 2-mercaptoethanol, 4.25 nmoles thymidylate synthetase (specific activity = 2.6 μmoles/mg/min), 90 nmoles 5,10-methylenetetrahydrofolate and from 0 to 9.1 nmoles 5-fluorodUMP. The amount of inhibitor in 13 samples was successively increased by a factor of 0.76 nmoles (e.g., sample 1, 0 inhibitor; sample 2, 0.76 nmoles of inhibitor; sample 3, 1.52 nmoles of inhibitor, etc.). After incubation at 25°C for 30 min, 80

μl (23 μg protein) of each sample was subjected to electrophoresis on polyacrylamide gels.

Electrophoretic Methods

Electrophoresis at pH 9.5 was performed essentially as described by Ornstein (*13*) and Davis (*4*). Gels (0.6 × 15 cm) containing 7.5% (w/v) acrylamide were run at 5–10°C at 2.5 mA/gel until the bromophenol blue marker had migrated to 1.5 cm from the bottom of the gel. Protein was visualized by staining the gels with Amido Black (*4*) followed by electrophoretic destaining. The gels were scanned at 620 nm using a Gilford Model 2410-5 linear transport equipped with a 0.2 × 2.56 mm aperture plate in conjunction with a Gilford model 240 spectrophotometer and Model 6040 recorder. The linear transport was set at a speed of 1 cm/min and the recorder chart speed was 1 in./min.

Isolation and Storage of Ternary Complexes

In a final volume of 2.6 ml of 0.1 *M* potassium phosphate buffer (pH 7.0), 152 nmoles of thymidylate synthetase (specific activity = 2.9 μmoles/mg/min), 2000 nmoles of 5,10-methylenetetrahydrofolate (obtained from the cofactor solution described above), and 338 nmoles of 5-fluorodUMP were incubated for 30 min at 25°C. The latter enzyme solution, which showed no activity after 10 min incubation time, was chromatographed at 5°C on a 1.5 by 60 cm Sephadex G-25 column, equilibrated with 0.1 *M* potassium phosphate buffer (pH 7.0). The sample was eluted from the column at a flow rate of 30 ml/hr. Two milliliter fractions were collected and analyzed for absorbance at 280 nm. Samples were taken from selected fractions across the symmetrical protein peak, which emerged from the column in the void volume. Portions of the selected samples were immediately subjected to analytical gel electrophoresis. The remainder of each sample was stored at room temperature and suitable aliquots were analyzed by gel electrophoresis at 24 hr intervals.

RESULTS AND DISCUSSION

As shown in Fig. 1, as many as three resolvable forms of thymidylate synthetase were present in the inhibition reaction mixtures. The uppermost band, I(R_f = .57), corresponds to the migration of native enzyme. Band II (R_f = 61) is located about 0.5 cm below band I; and, Band III (R_f = .66) is located about 1 cm below Band I. A more careful inspection of the gels revealed that the pattern and relative intensities of the three bands were dependent on the concentration of 5-fluorodUMP in the incubation mixture. Incremental increases in
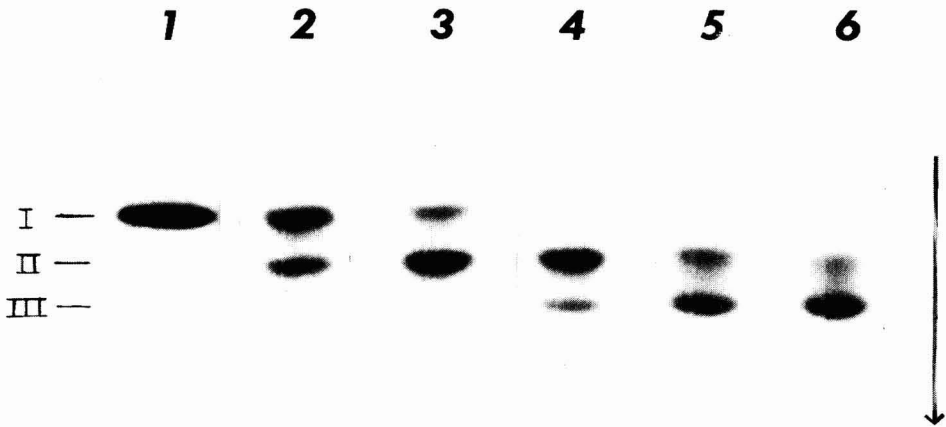
FIG. 1. Electrophoretic patterns of incubation mixtures containing various 5-fluorodUMP:enzyme molar ratios. The ratios present in the samples applied to the gels were as follows: (1) 0, native enzyme; (2) 0.36; (3) 0.72; (4) 1.04; (5) 1.43; (6) 1.97 (see text for details).

inhibitor concentration apparently resulted in first, the appearance of Band II at the expense of Band I, followed by the appearance of Band III at the expense of Band II. This initial observation was rein-forced when gel scans were used to calculate the intensities of the protein bands. Gel scans, as exemplified in Fig. 2, yielded peaks
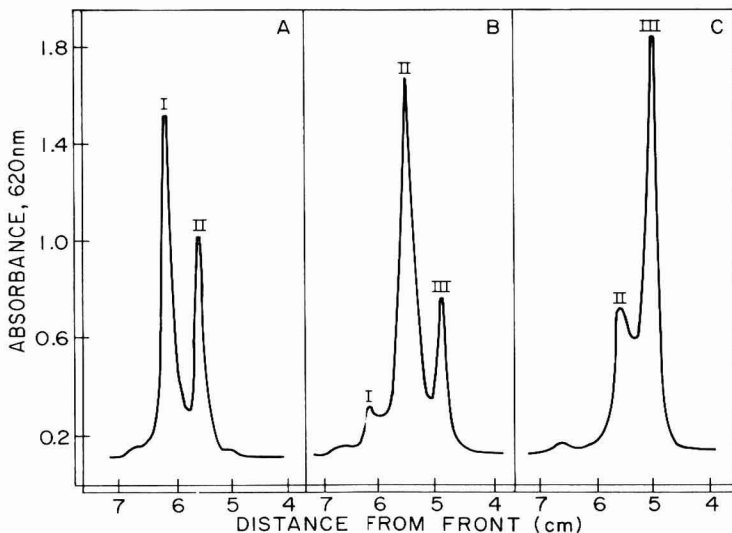
FIG. 2. Spectral scans at 620 nm of the polyacrylamide gels shown are typical of the resolution obtained using the techniques described (see "Methods") (A) gel 2 of Fig. 1; (B) gel 4; (C) gel 6.

which were resolved well enough to allow for the determination of individual peak areas, thus giving a quantitative estimate of the amount of protein present in each band. Data obtained from spectral scanning of the gels resulting from electrophoresis of the 13 inhibition reaction mixtures containing 5-fluorodUMP:enzyme ratios ranging from 0 (no inhibitor) to 2.15, are summarized in Fig. 3. The individual peak areas are expressed as percentages of the total area and are plotted against the inhibitor:enzyme ratio. It is readily apparent from Fig. 3 that at an inhibitor:enzyme ratio of 1, most of the native enzyme (Form I) has been converted to a faster migrating band (Form II); and indeed, an extrapolation of the straight portion of the line representing the disappearance of Form I leads to an intercept value of 1 on the inhibitor:enzyme axis. Thus, it can be inferred that Form II corresponds to the formation of one ternary complex per enzyme molecule. Furthermore, at an inhibitor:enzyme ratio of 1, a small amount of a third band, designated as Form III, is observed. The percentage of the protein in Form III, which migrates faster than Form II, is increased as the inhibitor:enzyme ratio is varied from 1 to 1.7, while a corresponding decrease in Form II protein is found. As shown in Fig. 3, extrapolation of the lines representing either the decrease in Form II or the increase in Form III results in intercept values corresponding very closely to an inhibitor:enzyme ratio of 2. This suggests that Form III consists of thymidylate synthetase which
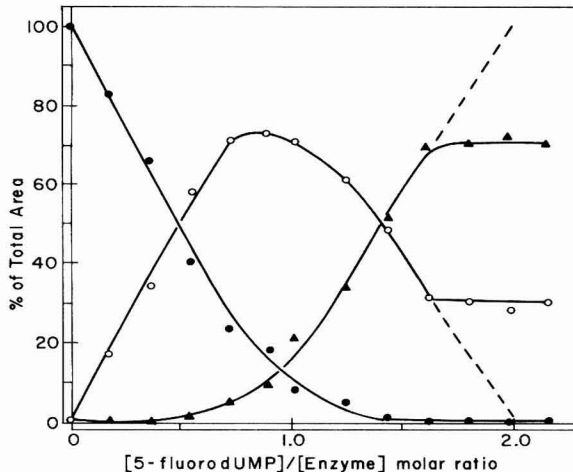

FIG. 3. The effect of varying the 5-fluorodUMP concentration on the relative quantities of the three electrophoretically resolvable forms of thymidylate synthetase present in incubation mixtures containing constant levels of enzyme and cofactor. Peak areas were calculated from gel scans and are expressed as percentages of the total area on the scan. (●—) Form I; (○—) Form II; (▲—) Form III.

has formed two ternary complexes per enzyme molecule. The fact that approximately 30% of the enzyme preparation used in these studies is apparently able to form only one ternary complex indicates that about 15% of the total binding sites are no longer viable. The faster migration of Forms II and III with respect to native enzyme (Form I) is consistent with the hypothesis that ternary complex formation involves the binding of stoichiometric quantities of 5-fluorodUMP and 5,10-methylenetetrahydrofolate, both of which are anionic in character.

No complex formation was detected on electrophoresis of enzyme solutions which had been incubated in the presence of either 5-fluorodUMP (5-fold excess) or 5,10-methylenetetrahydrofolate (20-fold excess). Incubation mixtures containing enzyme, 5-fluorodUMP (2-fold excess) and a 20-fold excess of either folic acid, dihydrofolate, tetrahydrofolate, 5-methyltetrahydrofolate or amethopterin were subjected to electrophoresis. None of these produced detectable amounts of complex except for tetrahydrofolate and 5-methyltetrahydrofolate which produced trace quantities of Form II. The possibility that the formation of Form II in these experiments is caused by trace contaminants in these folate samples is now under investigation. Incubation mixtures containing enzyme, dUMP (10-fold excess) and dihydrofolate (10-fold excess) as well as those containing enzyme, a 10-fold excess of dTMP, and a 10-fold excess of either 5,10-methylenetetrahydrofolate or dihydrofolate, all showed no stable complex formation as indicated by their electrophoretic patterns.

Inhibited thymidylate synthetase behaves identically to native enzyme when subjected to electrophoresis in the presence of sodium dodecyl sulfate (16). This indicates that the complexed enzyme is capable of dissociating into subunits and suggests that the subunits are not covalently bridged by complex formation. No gross difference in the tertiary structures of native enzyme and inhibited enzyme was indicated by the fact that both preparations behaved identically when subjected to gel filtration on Sephadex G-100.

Gel electrophoresis also provided a rapid, quantitative means of assessing the stability of the ternary complexes of thymidylate synthetase. Gel 1 in Fig. 4 illustrates the band patterns obtained from ternary complexes formed in the presence of excess 5-fluorodUMP and cofactor, while Gel 2 illustrates the electrophoretic pattern of the same sample following removal of the excess and unbound inhibitor and cofactor by gel filtration on Sephadex G-25. Scanning of Gels 1 and 2 indicates that the band patterns on both gels are highly similar; that is, the ratio of the area of Band II to the area of Band III in each gel is 0.40. In support of the notion that the ternary complexes of
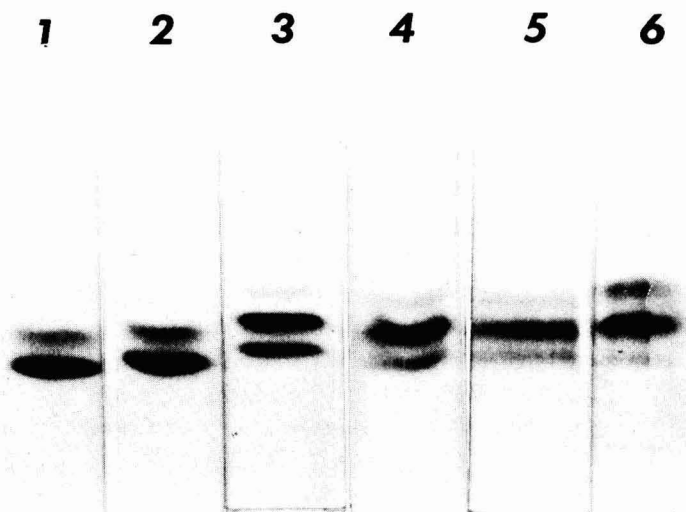
FIG. 4. Electrophoretic patterns of a sample of inhibitor–cofactor–enzyme complex isolated by gel filtration on Sephadex G-25 and stored at room temperature for various periods of time. Samples were withdrawn and subjected to electrophoresis as follows: (1) before G-25 chromatography; (2) immediately after G-25 chromatography; stored at room temperature following G-25 chromatography for: (3) 1 day, (4) 2 days, (5) 3 days, (6) 6 days.

thymidylate synthetase, isolated by either G-100 or G-25 chromatography, were homogeneous in composition, gel electrophoresis of samples selected from fractions at various positions on the elution peak gave virtually identical band patterns which exhibited a Band II area to Band III area ratio of ~0.40. These results indicate that the ternary complexes are reasonably stable and can be isolated intact by gel filtration.

In order to further investigate the stability of the ternary complexes, a sample of the ternary complexes isolated by gel filtration on Sephadex G-25 was stored at room temperature. Aliquots were removed after 1, 2, 3, and 6 day storage periods and subjected to gel electrophoresis. The band patterns of Gels 2 through 6 in Fig. 4 illustrate qualitatively that the ternary complexes undergo a slow but progressive breakdown as a function of storage time at 25°C. Data obtained from the spectral scans of these gels are summarized in Table 1. A relatively rapid appearance of Band II and a corresponding disappearance of Band III was observed after a 24 hr storage period at room temperature. As shown in Table 1, longer storage periods resulted in the slow disappearance of Band III with Band II remaining almost constant. A slow, but constant formation of Band I was observed over the entire storage period. These observa-

TABLE 1

RELATIVE PEAK AREAS OBTAINED FROM THE SPECTRAL SCANS
OF GELS 2–6 IN FIG. 4

Gel no.	Storage period[a] (days)	Relative peak areas (%)[b]		
		I	II	III
2	0	0	30	70
3	1	5	63	32
4	2	10	66	24
5	3	16	64	20
6	6	33	60	7

[a] Samples were stored at room temperature (22–27°C) in screw-capped test tubes.
[b] Peak areas are expressed as percentages of the total area on the scan.

tions strongly suggest that the inhibitor–cofactor–enzyme complex in a 1:1:1 molar ratio is more stable than the complex containing a 2:2:1 molar ratio and that the inhibitor binding sites of thymidylate synthetase are not equivalent with respect to their ability to remain complexed with this inhibitor. These preliminary observations are currently being investigated further in our laboratory.

Gel electrophoresis provides a convenient, visual method for investigating ternary complex formation of thymidylate synthetase. We are currently exploiting this technique as a means of monitoring events which alter the substrate and coenzyme binding regions of thymidylate synthetases and as a specific assay for this enzyme.

SUMMARY

Polyacrylamide gel electrophoresis was used to resolve as many as three protein components from incubation mixtures containing the inhibitor, 5-fluoro-2'-deoxyuridylate, the cofactor, 5,10-methylene tetrahydrofolate, and thymidylate synthetase. In a series of mixtures containing excess 5,10-methylenetetrahydrofolate and constant levels of thymidylate synthetase, the relative amounts of the protein components were shown to be dependent on the concentration of the inhibitor. Evidence is presented which suggests that the three protein components correspond to (1) native enzyme, (2) an inhibitor–cofactor–enzyme complex in a 1:1:1 molar ratio, and (3) an inhibitor–cofactor–enzyme complex in a 2:2:1 molar ratio, respectively. Ternary complexes of thymidylate synthetase are stable to gel filtration and are shown to undergo a relatively slow rate of breakdown on storage at 25°C.

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Book Reviews

Chemical Mutagens. Edited by ALEXANDER HOLLAENDER. Plenum, New York, 1973. xxii + 304 pp. $19.50.

This is Volume 3 of a continuing series. One of the objectives was to include more detailed discussions on techniques of some of the methods presented from a theoretical viewpoint in the first two volumes.

The Table of Contents shows the range of topics considered in this volume: History of Research on Chemical Mutagenesis; Observations on Meiotic Chromosomes of the Male Mouse as a Test of the Potential Mutagenicity of Chemicals in Mammals; Techniques for Monitoring and Assessing the Significance of Mutagenesis in Human Populations; Specific-Locus Mutational Assay Systems for Mouse Lymphoma Cells; Approaches to Monitoring Human Populations for Mutation Rates and Genetic Disease; Repair of Chemical Damage to Human DNA; *Tradescantia* Stamen Hairs: A Radiobiological Test System Applicable to Chemical Mutagenesis; Detection of Genetically Active Chemicals Using Various Yeast Systems; Total Reproductive Capacity in Female Mice: Chemical Effects and Their Analysis; Insect Chemosterilants as Mutagens; The Literature of Chemical Mutagenesis.

The first chapter on the history and the last on the literature of chemical mutagenesis should be of special interest to one contemplating entering this field. The other chapters deal in detail with the techniques that have been used.

Despite the large number of authors, this is an easy book to read.

There is an extensive author and subject index.

BILL ELPERN, *9 Surrey Way, White Plains, New York 10607*

Encyclopedia of Microscopy and Microtechniques. Edited by PETER GRAY. Van Nostrand Reinhold, New York, 1973. xi + 638 pp. $32.50.

There are few instruments so diversified as the microscope and no discipline in which it is not used in some form. Hence, a publication such as this fills a need and will find a variety of uses. Dr. Gray has done an admirable job of integrating the contributions of more than 175 collaborators, thereby earning the thanks of the entire scientific community.

The articles are in alphabetical order. Instruments and techniques are not separated. The optical microscope will be found under O; the phase microscope under P; and the electron microscope under T (transmission) and S (scanning); yet the history of them all is under H. Placenta, pineal gland, photographic materials, and photomicrography are all under P.

Come to think of it, what other system would be as logical? Moreover, there is a very comprehensive index, so there is really no confusion.

Coverage is complete and extensive. For example, there are 13 pages of fixative formulas prepared from stock solutions; and 22 pages of polychrome staining formulas citing the reagents required, the method recommended, and the results to be expected.

It is not possible to prepare a definitive review of a book of this nature. A cursory examination has revealed no errors; diagrams and pictures are very good; the type, though small, is quite readable; and the paper stock is nonreflective making perusal eas-

ier. The volume is sturdily bound to stand up under the use it will get. *Encyclopedia of Microscopy and Microtechnique* is a must for every laboratory and the comparatively reasonable price makes that possible.

DAVID B. SABINE, *484 Hawthorne Avenue, Yonkers, New York 10705*

Chromatography of Environmental Hazards. Vol. 1. By LAWRENCE FISHBEIN. Elsevier, New York, 1972. vii + 499 pp. $44.50.

Volume 1 of this three volume series deals with chromatographic and biological aspects of toxicants of environmental significance. The areas of consideration include carcinogenic, mutagenic, and teratogenic toxicants with focus on those which are pesticides, food and feed additives, drugs and industrial alkylating agents. This book presents a review of current chromatographic literature and relevant information about environmental hazards. The separation from diverse environmental sources as well as from biologic media and degradation products using TLC, GLC, and paper chromatography are described.

The book is presented as a practical text and provides a literature source for selecting chromatographic systems that can be utilized in a general sense. The chromatographic procedures are given in sufficient detail to allow the selection of an appropriate system for toxicant determination. Chapter 2 of volume 1 is a tabular summary of environmental hazards described in the following five chapters. Chapter 3 deals with alkylating agents; chapter 4 with pesticides; chapter 5 with drugs; chapter 6 with food and feed additives and contaminants; and chapter 7 with miscellaneous toxicants. The following volumes will focus on air, water, industrial pollutants, metals, pesticide residue, and drugs.

This timely survey is a comprehensive guide to chromatographic determination of toxicants of environmental significance. This work will provide an understanding of effective environmental hazard monitoring using various chromatographic methods. The book is of importance to diverse disciplines including biochemistry, biology, genetics, toxicology, molecular biology, and public health. Those interested in environmental hazard determination will welcome this well organized and written first volume. Although expensive this series is highly recommended for environmental scientists.

DAVID F. TOMKINS, *Hoffmann-La Roche Inc., Nutley, New Jersey 07110*

Quantitative Analysis by NMR Spectroscopy By F. KASLER. Academic Press, London, 1973. viii + 190 pp. £4.50.

The title is descriptive of the contents of this small (190 pages), very useful, practical book.

Part A (44 pages) consists of a concise summary of NMR theory and instrumentation. Part B (39 pages) deals with the practical aspects of type of sample, solvents, sample containers, and instrument parameters to obtain a good spectrum. Part C (71 pages) covers the quantitative determination of such properties as molecular weight, elemental analysis, isotopic composition, isomers, active hydrogen, and water; and it describes analytical procedures for alcohols, carboxylic acids, phenols, amines, olefins, and methyl group. A separate section uses examples from "pharmacy, industry, and related areas." Part D (16 pages) sketchily describes determination of polymers, wide line methods, and nuclei other than ^1H.

The author has provided an excellent, quick entry to the pertinent literature, but he

has severely handicapped the reader by providing a totally inadequate index. Failure to list Mosher's reagent for enantiomer determination is a surprising omission.

In general, the writing is clear, but the book is marred by many typographical errors.

R. M. SILVERSTEIN, *College of Environmental Science and Forestry, Syracuse Campus, Syracuse, New York 13210*

Drug Design. Edited by E. J. ARIËNS, Academic Press, New York, 1973. xv + 489 pp. $35.00.

Drug Design, Volume 11 in a continuing series of monographs on medicinal chemistry, is itself a subseries of which this is Volume IV.

Assuming we have identified a drug with respect to its chemistry and biology, there still remains the task of developing a dosage form. This is the area covered in the first five chapters of this volume. The principles which will serve as a strategy in the design of appropriate drug delivery systems are elaborated in the first chapter. The next two chapters deal with the design of peroral and parenteral dosage forms with prolonged action in terms of both the kinetics of the release of such forms and generalities of manufacturing. Enough information is provided for practical application without actually giving laboratory procedures. Two chapters are devoted to the design of topical drugs in a thoroughly comprehensive and clear manner.

The remaining four chapters deal with specialized subjects. A survey of sunscreen preparations covers uv radiation and its effect on human skin, the various types of filters and a number of formulations and evaluations of these preparations.

A discussion of litholytic agents considers the factors influencing stone formation and the various means of dissolving the four chief types of stones found.

The design of biologically active nucleosides does not seem to belong in this volume but it certainly does belong in the series. This 74 page review has 733 bibliographic citations which is indicative of the mass of material considered. Over half is devoted to structural considerations and the remainder to biological effects.

The final chapter on the design of insecticides is included to demonstrate that the principles of drug design may be used in other areas.

A substantial author and subject index is included in this volume.

This book is on a par with the other volumes in this series in every respect.

BILL ELPERN, *9 Surrey Way, White Plains, New York 10607*

Quantitative Analysis with Electron Microprobes and Secondary Ion Mass Spectrometry. Edited by E. PREUSS. Zentralbibliothek der Kernforschungsanlage Jülich GmbH, Jülich, W. Germany, 1972. ii + 361 pp. Obtainable on request.

This soft-bound volume presents the proceedings of a meeting held in October 1972, at the German Institute for Nuclear Research (Kernforschungsanlage) in Jülich, W. Germany. The purpose of the meeting was to acquaint the scientific staff of the Institute, and the German-speaking scientific community, with recent progress in microprobe analysis, and with its relevance to the study of surfaces. Accordingly, the contributions are reviews of the state-of-art. Concerning the application to surface characterization, the emphasis is on parameters and limitations of the techniques rather than on specific problems. Twelve communications deal with diverse aspects of electron probe microanalysis, and 4 with secondary ion mass spectrometry; 10 are in German and 6 in English.

The selection of topics for this volume is very appropriate, and the reading material presented in it is very useful to those of us who are fluent in reading German. I find the contribution by E. R. Krefting and L. Reimer particularly valuable. These authors describe the complexity of the models used in the Monte-Carlo techniques for electron-·target interaction in more depth and detail than most publications in this field directed to the electron probe analyst. The papers on secondary ion mass spectrometry, which reflect the growing importance of ion probe microanalysis, are equally well written. The communication by A. Benninghoven and L. Wiedmann is particularly striking in view of its implication that secondary ion mass spectrometry can provide information at the molecular level.

The publication of proceedings of meetings does not remove the need for authoritative and balanced systematic treatises on instrumental microanalysis. Ironically, in spite of a steadily increasing flood of printed matter, such treatises are still sadly lacking. Perhaps the issuance of well-written proceedings such as the present one will presage, rather than deter, publications of a more lasting format.

<div style="text-align:right">

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Theory of Electric Polarization. Vol. 1. Dielectrics in Static Fields. By C. J. F. BÖTTCHER. 2nd revised ed. by O. C. VAN BELLE, P. BORDEWIJK AND A. RIP. American Elsevier, New York, 1973. xx + 377 pp. $38.50.

This volume is the first part of the second completely revised edition of the famous book of Professor Böttcher. The book contains the theory of the static behavior of dielectrics or low-frequency fields. It not only maintains the beautiful features, as giving a comprehensive treatment of the classical theory of dielectrics, of the original edition but also introduces much up-to-date materials from the new developments during the course of 20 yr advancing (since the first publication appeared in 1952) in this particular field. It is purposely to be used as a handbook as well as a textbook.

The book consists of one section of historical introduction and seven chapters. In addition, the mathematical definitions and derivations used in the book are reviewed concisely but clearly in the three appendices. These appendices involve some applications of vector and tensor calculus; the solution of Laplace's and Poisson's equations; some properties of the Legendre polynomials; etc. Important symbols; relevant references; and indexes of author, subject, and chemical name are also provided for the reader to use the text easily and effectively.

Specifically, the excellent section of historical introduction gives a general and concise review on the historical and conceptual developments of the theory of electric polarization. Briefly, such important developments included the first successful construction of a condensor (named as Leyden jar) by Cunaeus and Musschenbroek in 1745 and the announcement of Faraday's definition of dielectric constant or permittivity (it was initially called specific inductive capacity) in 1837. In the middle of 1860s, Maxwell published the theory of electromagnetic phenomena and the Maxwell relation, $\epsilon = n^2$ (where ϵ = dielectric constant, n = refractive index). While the derivations of the Clausius-Mossotti equation and the Lorenz-Lorentz equation correlating the dielectric constant with the microscopic structure of matter were derived in the middle of 19th century. The extension of the Clausius-Mossotti equation to the Debye equation for describing the dependence of dielectric constant on the molecular polarizability and the permanent moment of the molecule was presented by Debye in 1912. In 1936, Onsager modified Debye's approach to the so called Onsager equation, which gives the relation

between the dielectric constant and the molecular dipole moment. Meanwhile, the derivations of Kirwood's theory of short-range specific interactions for interpreting of associating liquids was contributed by Kirkwood in 1939, and so forth.

Chapter 1 deals with the electric dipoles and multipoles. The materials presented include electric moment and electric dipoles; the electric field of an ideal dipole *in vacuo*; nonideal dipoles — the description of potentials with the aid of Legendre functions; axial multipoles; and general multipoles. However, particular emphases have been placed on the demonstration of the fundamental concepts of the phenomenological theory of the electric polarization with the help of Maxwell's theory. Moreover, the stepwise introduction and application of the Legendre functions to the solution of the sophisticated electrostatical and potential problems (for example, Laplace's equation for no electric charges region) have also been extensively treated.

In Chapter 2, some concepts and problems of electrostatics such as the vector fields E (the electric field strength) and D (the dielectric displacement); the electric polarization (P); the relation between E and P; some electrostatic problems; and the polarizability α are comprehensively described with the aids of the clear and exact mathematical definitions and derivations. It is suggested that the behavior of the molecule can be characterized with the help of a scalar or tensorial polarizability. However, the polarizability is a function of a macroscopic quantity, the deielectric constant, of the polarizable body; so that the internal dielectric constant cannot be applied to give a complete characterization of the behavior of a molecule in an external electric field.

Chapter 3 is concerned with polarization and energy. It gives the direct relations among the potential energy, electric work, and the thermodynamic quantity. The materials cover the relation between potential and energy; the work required to assemble a charge distribution; the work of assembly as a thermodynamic quantity; the energy of a dielectric in an external field; the energy of an induced dipole in an external field; the electrostatic interaction of two particles. In particular, the interaction energy of two unpolarizable particles or molecules and characterized by their permanent multipole moments and dipole polarizability of the particles have been carefully and adequately considered.

Chapter 4 considers the reaction field (R) of a nonpolarizable and of a polarizable point dipole with more attention being paid to the case of an ellipsoidal cavity. Some critics of Martin and Bell's method are mentioned. The investigations of the energy of a charge distribution in its R and the average $\bar{R}$ of an arbitrary charge distribution in an ellipsoidal cavity are described. Furthermore, the uses of R to the calculation of the energy of an ideal dipole in its own R and to the computation of the contribution of the permanent dipoles to the cohesion energy of a liquid are also critically demonstrated.

In Chapter 5, the dielectric constant in the continuum approach to the environment of the molecule is comprehensively treated. First, it deals with the dependence of P on the internal and the directing field for both nonpolar and polar dielectrics. Then, the derivations and the discussion of the applicability and inadequacy of the Onsager equation; the generalization of the Onsager equation for ellipsoidal molecules are presented. Some critical reviews on the derivations of the Debye equation; the correction to the Clausius-Mossotti equation for ellipsoidal molecules; and the treatment of mixtures of nonpolar compounds have also been briefly mentioned.

The second method for calculation of the polarization from molecular parameters (which considers the specific molecular interactions) by the use of statistical mechanics is given in Chapter 6. (The continuum approach to the environment of the molecule as described in Chapter 5 is the first method). In the statistical-mechanical theories of the dielectric constant simplified models are often used for the molecules and the intermolecular forces to make the calculations tractable. Therefore, on the bases of

simplified models with approximate expressions or series expansions, a formalism of the $3N$-dimensional vectors and tensors for evaluation of statistical-mechanical averages in which a number of derivations can be made conveniently is first provided. Then, its applications to the treatment of the Clausius-Mossotti expression as a power series in the polarizability and in the density for the dielectric virial coefficients of both polar and nonpolar gases are effectively derived. Finally, the developments and applications of Kirkwood-Fröhlich equation to polymers and to associating liquids are critically discussed and illustrated in detail.

The last chapter presents the materials on the new developments in the field of nonlinear dielectrics. This nonlinear effect is characterized by the quantity $\Delta\epsilon/E^2$. Five special cases such as normal and anomalous saturation; anisotropic polarizability and hyperpolarizabilities; and electrostriction are primarily concerned.

In summary, this excellent volume presents the comprehensive collection and compilation of the well developed theory of electric polarization which appeared in the century. The mathematical definitions and derivations as well as the physical descriptions and interpretations for each specific theory and/or equation are exact, clear and understandable. It is definitely sure that this second revised volume will be accorded a favorable reception similar to or over that given to the first original edition. This volume is, therefore, highly recommended to the theoretical scientists, physical chemists, physicists, and experimental workers as an extremely valuable reference book or handbook, and to the undergraduate and graduate students as the best textbook in this particular field.

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Bacteremia: Clinical and Laboratory Aspects. Edited by ALEX C. SONNEN-WIRTH. Thomas, Springfield, IL, 1973. 106 pp. $7.95.

This book is a compilation of six well-documented papers presented at an American Society for Microbiology (ASM) seminar held in May, 1971, plus an author and subject index. Among the important aspects of the subject discussed are: diagnostic methods in present use, possible acceleration and improvement in accuracy of methods, kinds of microorganisms detected, and hazards of contaminated intravenous solutions. The last paper is epidemiological in nature; the other papers focus on laboratory aspects. Pathological and therapeutic aspects are not included.

The book is attractively and accurately printed, but the cost is considerably higher than would have been the case if the brief papers could have been included in an ongoing ASM publication. If the latter solution is not possible for future seminars, a reasonable alternative would be to use paperback rather than hard cover vehicles.

EUGENE D. WEINBERG, *Microbiology & Medical Sciences,*
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Organic Syntheses with Noble Metal Catalysts. By PAUL N. RYLANDER, Academic Press, New York, 1973. viii + 331 pp. $22.50.

This is Volume 28 in the continuing series of monographs on Organic Chemistry under the general editorship of Blomquist and Wasserman.

The nine chapter headings are: Dehydrogenation, Homogeneous Hydrogenation, Oxidation, Osmium and Ruthenium Tetroxides as Oxidation Catalysts, Isomerization,

Oligomerizations, Telomerizations and Condensations, Carbonylation and Hydroformylation, Decarbonylation and Desulfonylation and lastly Silicon Chemistry.

Each of the chapters includes an abundance of clearly presented reaction equations in the text and references at the end. Little or no attempt is made to define the mechanisms, but there are descriptions of what happens or can be expected to happen under specific conditions. Much of this can be used as a laboratory manual. Yields are given where possible and the effect of alternate conditions are frequently introduced.

The subject index is essentially a list of the compounds cited in the text. Coupled with an extensive author index this book is both easy and rewarding to use.

The expansion of the use of noble metal catalysts during the past decade makes this a timely review and worthy addition to this series.

BILL ELPERN, *9 Surrey Way, White Plains, New York 10607*

Quantitative Thin Layer Chromatography. Edited by JOSEPH C. TOUCHSTONE. Wiley (Interscience), New York, 1973. xiv + 330 pp. $14.95.

This book presents the results of two symposia on quantitative thin layer chromatography — "Quantitative Thin Layer Chromatography," Philadelphia, PA, Dec. 1970 and one sponsored by The American Oil Chemists Society Nov. 1971. This volume reviews recent advances in the quantitative aspects of TLC with special emphasis on spectrodensitometry. The quantitation of a variety of compounds separated on TLC by *in situ* spectrodensitometry demonstrates the ease and reliability of this technique.

After an introductory chapter on spectrodensitometry the book presents quantitative aspects of a wide variety of compounds. The 16 chapters include the determination of specific activity of isotopic materials, polymer molecular weight distribution, lipid classes, carbohydrates, fluorodensitometry of mycotoxins, amino acid abnormalities, steroids, urinary purines, steroid hormones, polynucleotides, pharmaceutical quality control, gangliosides, chlorpromazine and its metabolites, pesticides, and air pollution measurements.

The recent advent of reliable spectrodensitometers has established *in situ* measurements of TLC plates as a valuable technique. The wide variety of compounds covered in this volume demonstrates the modes of methodology involved. The book presents the techniques necessary for accurate quantitation as well as a valuable up-to-date reference source. This well written and excellently illustrated edition will be welcomed by analytical chemists, biochemists, molecular biologists, and medicinal and pharmaceutical chemists.

DAVID F. TOMKINS, *Hoffman-La Roche Inc., Nutley, New Jersey 07110*

Encyclopedia of Industrial Chemical Analysis. Vol. 18. Edited by F. D. SNELL AND L. S. ETTRE. Wiley (Interscience), New York, 1973, xiv + 543 pp. $40.00 ($35.00 by subscription).

With Volume 19 this major source of ready-to-use information on applied chemical analysis spans the chemical alphabet of products and product classes from silicon (and inorganic compounds) to thiophene. Intervening articles include Silicon Compounds, Organic; Silver (and compounds); Soaps; Sodium (and compounds); Steel; Strontium (and compounds); Styrene; Styrene Polymers; Sugar; Sulfur (and inorganic compounds); Tantalum (and compounds); Tea; and Thallium.

Authors of articles in this volume include three from U.S. firms, one from an African

institution; and two from a British college or university. F. A. Lowenheim authored eight of the articles representing about 262 pages, or about 48% of the entire text. Dr. Lowenheim also was responsible for about 44% of the text of Volume 16 and 53% of Volume 17. It is evident that the diversity of contributors achieved with earlier volumes of the work has not been maintained. However, the series has not been affected adversely; Dr. Lowenheim's contributions are well-researched and well-balanced.

The 108-page article on organic silicon compounds by C. R. Thrush and G. Beall of Union Carbide Corp. provides an excellent practical introduction to the characterization and analysis of chlorosilanes, silane esters, silicones, etc. Some of the procedures and information presented were previously available largely through Union Carbide customer service bulletins. This article includes about 25 infrared spectra and over 10 gas chromatograms.

The 63-page article (251 references) by P. J. Hilton of the Tea Research Foundation of Central Africa, located in Malawi, proved intriguing to the reviewer. Tea is manufactured from the short tips of the tea plant, which has been cultivated for over 2000 yr. During the past 20 yr, as this article attests, much analytical information has been amassed on tea and many procedures have been developed for its analysis and the evaluation of its quality. However, the majority of the world's tea is still sold by auction and the buyers still rely almost exclusively upon the assessment of quality by professional tea tasters. It would appear that one taste can still be worth more than many laboratory determinations!

For the specialist in applied microchemistry or microanalysis, whether inorganic or organic, this volume and earlier ones can be recommended. Analysts in industry should check that their company library is securing this encyclopedia in its entirety.

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Instrumentation in Analytical Chemistry. Edited by ALAN J. SENZEL, American Chemical Society, Washington, DC, 1973. ix + 428 pp. Hardcover $7.95; Paperback $4.50.

Chemists who use analytical instruments do so for one of two reasons. Either they are analyzing for a particular substance or they are "instrumentalists" — more concerned about *how* the instrument does its job than in analytical methodology. This book, aimed at the latter group, comprises 43 instrumentation articles from issues of *Analytical Chemistry* over the period January 1969 to July 1972.

Almost half the book is devoted to spectrometry. Fourier transform techniques, currently in vogue, are treated in three separate articles. The treatment by Farrar is especially clear and well written. The remainder of the book is devoted to chromatography (3 articles), electrochemistry (4 articles) and various miscellaneous subjects. Some of the miscellaneous articles, such as McKee's "Modular Approach to Chemical Instrumentation," Enke's "Digital Domains" and Hieftje's "Signal to Noise Enhancement Through Instrumental Techniques" contain basic information which is applicable to many disciplines. Placing these articles at the beginning of the book rather than near the end would have benefited the novice instrumentalist or those unfamiliar with instrument jargon such as "white noise," "bandpass," "ground loop," and "Bode plot."

One very enjoyable feature of the book is the many commentaries by the late Ralph H. Muller. His remarks provide an interesting change of pace to the survey nature of the articles, and his intimate knowledge of a wide variety of analytical techniques is truly enviable.

This book is attractively priced and will appeal to those who would like to know more about measurement principles as well as those who find it difficult to keep up with the new techniques and evolving disciplines.

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Modern Methods of Steroid Analysis. Edited by ERICH HEFTMANN. Academic Press, New York, 1973. xx + 523 pp. $37.50.

This reference text discusses the application of some current physicochemical techniques to the specialized field of steroid chemistry. The chromatography section deals with liquid column chromatography of hormonal steroids, application of high-pressure liquid chromatography to the separation of insect molting hormones, gradient elution and thin-layer chromatography in the analysis of corticosteroids and 17-ketosteroids, gas chromatography of steroid hormones, and qualitative and quantitative analysis of plant sterols by gas–liquid chromatography. Mass spectrometry topics include some aspects of mass spectrometry in steroid analysis, and derivatization and gas chromatography in the mass spectrometry of steroids. The infrared and raman spectroscopy section covers a computerized method for rapid comparison and retrieval of infrared spectral data, and the Raman spectroscopy of steroids. There is an introduction to nuclear magnetic resonance and applications of lanthanide shift reagents in the nuclear magnetic resonance section. X-Ray diffraction analysis subjects include X-ray analysis of steroid structures and the automated diffractometer, application of direct methods of X-ray structure analysis to steroids, and the *Faltmolekül* method and other Patterson search techniques in structure analysis. Recent optical rotatory dispersion and circular dichroism studies in the steroid field, Cotton effects and allylic-homoallylic chirality of steroidal olefins and conjugated dienes and enones, and Cotton effects of acid derivatives, aromatic steroids, and nitrogen-, sulfur-, and halogen-containing steroids encompass the optical rotatory dispersion and circular dichroism topics. The last section deals with radioisotope methods and includes estrogen analysis by the double isotope derivative method, corticosteroid analysis of competitive protein binding, enzymic isotope displacement assay of digitalis glycosides, radioimmunoassay of plasma steroid hormones, and radioimmunoassay of plasma aldosterone.

The list of contributors to the various chapters is impressive: Burgstahler, Crabbé, Nakanishi, Schooley, etc.; researchers who have been the leaders in developing these useful techniques. For analytical laboratories and research groups dealing with qualitative and quantitative analyses of known steroids or structure elucidation of new ones this text, with its rather exhaustive discussions of topic material and useful bibliographies, is invaluable. Judging from the price, the text was probably planned for and is here recommended for purchase by the above groups as well as science libraries.

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Announcement

21st Canadian Spectroscopy Symposium
Ottawa, October 7–9, 1974

Contact: Mr. J. L. Dalton, Secretary
21st Canadian Spectroscopy Symposium
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