RESULTS AND DISCUSSION

The reactor–gas chromatographic technique was applied to known mixtures of amino acids (Figure 2). The amino acids were qualitatively identified by the retention time of the corresponding aldehydes. Known mixtures of aldehydes, passed through the system under identical conditions, gave analogous chromatograms.

The quantitative determination of the amino acids is based on measurement of the peak areas, correction for the number of carbons of each aldehyde, and normalization. Because each peak is really due to methane, no calibrations are necessary. Individual aldehydes passed through the system give peak areas which are a linear function of their concentration.

The results of the analysis of two synthetic mixtures of amino acids, are illustrated in Table I. The error for the values of each amino acid, with the exception of norleucine, is less than 5%. This is of the same order of magnitude as the error involved in measuring the areas of the respective peaks by the weight method. Also, when the values for total amino acid are considered, the error becomes even smaller, suggesting a cancellation of experimental random errors.

To demonstrate that the amino acids had reacted completely with ninhydrin, 15 μl. of water or a saturated aqueous ninhydrin solution were injected into the reactor after each amino acid run. The chromatograms showed no evidence of unreacted amino acids at the operating temperature of 140° C. At lower temperatures (100° C.) small amounts of amino acids remained unreacted.

An analysis of a casein hydrolyzate solution by this method showed four main peaks on the chromatogram corresponding to alanine, valine, leucine, and isoleucine in the approximate relative composition to be expected from casein. The other amino acids present in the mixture did not produce any other detectable volatile products with the exception of carbon dioxide. The carbon dioxide peak, although considerably enlarged, was sufficiently separated from the alanine peak to allow the measurement of this amino acid.

As the amino acid norvaline (also norleucine and α-amino-*n*-butyric acid) is not normally present in proteins, it should be possible to use it as a marker for the analysis of the other amino acids. This would involve diluting a small amount of norvaline solution with the protein hydrolyzate solution and injecting the mixture into the chromatographic system. Knowing the exact molarity of the norvaline solution and the dilution factor, the absolute concentration of each one of the aliphatic amino acids present in a protein hydrolyzate could be calculated. This has the added advantage that there is no need to measure the sample size of the mixture of amino acids to be injected if the response of the norvaline solution has been carefully determined beforehand.

One microgram of an amino acid can be detected using this technique. With the application of new detection systems (*9, 10*), it should be possible to increase the sensitivity so that as little as 0.001 γ will be detected.

The approach presented here is direct, sensitive, and rapid for analyzing amino acids which produce volatile aldehydes on oxidation with ninhydrin. In its present form the method can be useful in certain specific applications, such as the analysis of the per cent composition of strictly aliphatic amino acids of proteins, and determination of the leucine-isoleucine ratios of certain proteins or peptides. The method can also be important in the confirmation of results obtained by radio tracer techniques on the incorporation of analogs of certain aliphatic amino acids into proteins.

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Gas-Liquid Chromatography of Pyridines Using a New Solid Support

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▶ **A solid support has been developed for the gas-liquid chromatography of pyridines, superior to Chromosorb or Celite 545; symmetric peaks were obtained when nonpolar substrates were used. The solid support was prepared from a commercial detergent by heating it and then extracting it with petroleum ether. The porous residue was used as a liquid substrate carrier. The new solid support was used to study the selectivity of several nonpolar and slightly polar liquid substrates. Decided differences in the selectivities of the liquid substrates permit a wide choice in the use of these substrates for separations of pyridines. The study on the selectivity of the liquid substrates was used to select two columns employed in series to separate a test mixture of 14 pyridines.**

ONE phase of the Bureau of Mines investigation on the characterization of shale oil is determination of the pyridines produced in retorting oil shale. Attempts to apply conventional gas-liquid chromatographic techniques using Chromosorb and Celite 545 to the analysis of shale-oil tar bases were hampered by asymmetry of the peaks obtained.

Symmetric peaks are obtained when strongly polar liquid substrates are used on either Chromosorb or Celite. However, a solid support which gives symmetric peaks only when strongly polar

substrates are used restricts the choice of liquid substrates.

Nonpolar substrates on acid- and base-washed Chromosorb gave extremely asymmetric peaks for pyridines. Celite 545, washed with alcoholic sodium hydroxide, has been used with some success by others for the gas-liquid chromatography of pyridines and amines (*1, 6–8, 10, 11*). However, there was still sufficient adsorption to make the peaks markedly asymmetric (*10*) when the solid was used with nonpolar or slightly polar liquid substrates. Celite 545 gave resolution of peaks, but there was still appreciable tailing. This tailing obscures small peaks and interferes in the separation of a pure constituent by freeze-out techniques.

Close-boiling pyridines have been separated by gas-liquid chromatography by exploiting structural and polarity differences by the use of selective liquid substrates. The techniques developed utilized a series arrangement of monohydroxyethyltrihydroxypropylethylenediamine and diphenyl phthalate on a new solid support prepared from a commercial detergent by heating to remove the volatiles and then extracting with petroleum ether to remove soluble material. This solid gave symmetric peaks; so it is superior to Chromosorb or Celite 545 for the determination of pyridines.

Retention data were obtained for 15 pyridines using 10 liquid substrates on the new solid support. The 10 liquid substrates were classified into two groups, based on their selectivities for pyridines. Four of them were nonselective for pyridines, separating them on a boiling point basis. The remaining liquid substrates gave separations which depend upon different activities of the pyridines in the liquid substrate.

EXPERIMENTAL

Apparatus. The gas-liquid chromatographic apparatus was assembled in this laboratory and consisted of a thermostatically controlled, stirred air bath which contained a thermistor-type detector and the chromatographic column. The column was maintained at 130° ± 0.5° C. for all of the retention data determinations. Helium, regulated to a flow rate of 60.0 ± 0.1 ml. per minute, was used as the carrier gas. The flow rate was measured at the column exit, using a soap-bubbler flowmeter (*9*).

The column for obtaining the retention data consisted of 2 meters of $^1/_4$-inch aluminum tubing. Longer columns (3.5 meters) were used to obtain the separation data for the pyridines. The columns were filled with the packing by introducing small portions into the top while tapping the column continuously with a small wooden mallet. Glass wool was placed in each end of the column, which was coiled and fitted to the detector.

Table I. Materials Studied as Stationary Liquid Substrates

Substrate	Character of Material	Source
Squalane	Branched-chain C_{30} alkane	Distillation Products Industries
Mineral oil	High molecular weight alkanes and cycloalkanes	E. R. Squibb and Sons
Apiezon-L	Saturated hydrocarbon lubricant	James G. Biddle Co.
Silicone high-vacuum grease	Silicone lubricant	Dow Corning Corp.
Silicone oil, D.C. 703	Phenylsilicones, mol. wt. 570	Distillation Products Industries
Octoil	Di-2-ethylhexyl phthalate	Consolidated Vacuum Corp.
Octoil-S	Di-2-ethylhexyl sebacate	Consolidated Vacuum Corp.
Tri-*m*-acresyl phosphate	...	Distillation Products Industries
Diphenyl phthalate	...	Distillation Products Industries
Monohydroxyethyltrihydroxypropylethylenediamine[a]	...	Visco Products Co., Inc.
Tide[b]	Commercial detergent containing alkylaryl sulfonate	Procter and Gamble

[a] Abbreviated as MTE.
[b] Investigated by using the dried commercial detergent as a column packing (*3*).

Solid Supports. CHROMOSORB. Chromosorb, a chromatographic grade of calcined diatomaceous earth manufactured by Johns - Manville, was washed with hot concentrated hydrochloric acid. The acid - washed Chromosorb was rinsed several times with distilled water and then given a hot aqueous sodium hydroxide wash to reduce the acidity of the surface. The Chromosorb was washed with distilled water until the wash water had a pH of 8. The acid- and base-treated material was dried in an oven at 185° C. for 10 hours.

CELITE 545. Celite 545 was size-graded to a 60- to 100-mesh fraction and then treated in one of two ways before use. In the first treatment, it was acid- and base-washed as described for the Chromosorb. In the second treatment, it was washed with methanolic sodium hydroxide as suggested by James, Martin, and Smith (*10*).

SOLID SUPPORT PREPARED FROM COMMERCIAL DETERGENT. A commercial detergent (Tide) was crushed on a screen with a porcelain pestle, by using a gentle rotary motion. The material was sieved and the 40- to 60-mesh fraction retained. This fraction was spread in a thin layer and dried in an oven at 185° C. for 24 hours. The materials removed in the drying procedure were about 14 weight % of the detergent. The dried material was sieved and the 40- to 60-mesh fraction extracted with petroleum ether (30° to 60° C.) in a Soxhlet extractor. The soluble material removed in the extraction was about 14 weight % of the detergent. The porous material remaining in the thimble was used as the solid support. Approximately 40% of the detergent as purchased was lost as fines in the crushing, drying, and extraction steps.

Liquid Substrates. The liquid substrates studied are listed in Table I. All of these materials were obtained commercially and have vapor pressures for satisfactory use at the temperature employed. The first 10 organic materials were utilized by dispersing them on the appropriate solid support. Tide was investigated by using the dried commercial detergent as a column packing as suggested by Desty and Harbourn (*3*).

Preparation of Packings. The new solid support was used as a carrier for each of the liquid substrates studied.

Packings for the determination of retention data were prepared to give a ratio of approximately 15 grams of liquid per 100 grams of solid. Those for the separation of a 14-component test mixture were prepared to give a ratio of 10 grams of liquid per 100 grams of solid. The liquid substrate was dissolved in a quantity of petroleum ether or acetone equivalent to the bulk volume of the solid used. The solid was added to the solution. Most of the solvent evaporated while the mixture was gently stirred. The packing was heated at 110° C. for 2 hours for final removal of solvent. The packing prepared in this way was a granular, free-flowing material that appeared dry. The weight of the liquid substrate per 100 grams of solid was calculated from the weight of the dried packing. The packing was sieved, and the 40- to 60-mesh fraction was retained. This material was used to pack each column.

Packings were prepared from each of the liquids as described above, using acid- and base-treated Chromosorb as the solid support. Celite 545, treated in two ways, was used as the solid support for Dow Corning 703 silicone oil. The ratio of liquid to solid was 15 grams of liquid per 100 grams of solid. The packings were sieved, and the 40- to 60-mesh fraction was used to pack each column.

Pyridines. Fifteen pyridines, listed in Table II, were used in this study. The purity of 14 of the pyridines was 97% or higher, as estimated by gas-liquid chromatography, and the identity of each was confirmed by infrared analysis. The sample of 2,4-dimethylpyridine was approximately 60% pure. Its major impurities were identified as 2,3- and 2,5-dimethylpyridine by gas-liquid chromatography.

Table II. Pyridines Used in This Study

Compound	Source
Pyridine	American Petroleum Institute Research Project 52
2-Methylpyridine	American Petroleum Institute Research Project 52
3-Methylpyridine	American Petroleum Institute Research Project 52
4-Methylpyridine	American Petroleum Institute Research Project 52
2,3-Dimethylpyridine	Matheson Coleman & Bell Div., Matheson Co.
2,4-Dimethylpyridine	Distillation Products Industries
2,5-Dimethylpyridine	Reilly Tar & Chemical Corp.
2,6-Dimethylpyridine	Paragon Testing Laboratories
2-Ethylpyridine	Reilly Tar & Chemical Corp.
4-Ethylpyridine	Reilly Tar & Chemical Corp.
2,4,6-Trimethylpyridine	Reilly Tar & Chemical Corp.
2-Methyl-5-ethylpyridine	L. Light & Co., Ltd.
3-Ethyl-4-methylpyridine	Aldrich Chemical Co., Inc.
4-Isopropylpyridine	Chemicals Procurement Co.
4-*n*-Propylpyridine	Reilly Tar & Chemical Corp.

Table III. V_g Values of 2,5-Dimethylpyridine for Various Liquid Substrates

Substrate	V_g, Ml./G.
Tide[a]	89
Silicone high-vacuum grease	139
Apiezon-L	150
Silicone oil, D.C. 703	202
Squalane	217
Mineral oil	228
Diphenyl phthalate	290
Monohydroxyethyltrihydroxypropylethylenediamine[b]	296
Tri-*m*-cresyl phosphate	301
Octoil-S	317
Octoil	334

[a] Investigated by using the dried commercial detergent as a column packing (*3*).
[b] MTE.

Table IV. Composition of Tide Detergent[a]

Constituent	Weight %
Lauryl sulfate[b]	12.0
Alkyl aryl sulfonate[b]	5.0
Sodium sulfate	15.0
Higher molecular phosphates	45.0
Silicates	9.0
Lauryl alcohol	1.5
Water	11.0
Carboxymethylcellulose[b]	1.0
Optical dyes	...

[a] Data obtained for a 1951 sample of Tide as reported by Stüpel (*12*).
[b] Although not reported as such by Stüpel, these are presumably the sodium salts.

A 14-component mixture of pyridines with normal boiling points to 195° C. was used as a test mixture. The mixture was composed of all the pyridines listed in Table II except 2,4-dimethylpyridine.

Retention Data. Retention data for the 15 pyridines were obtained for each of the liquid substrates on the new solid support. For this purpose several mixtures of pyridines were prepared so that the identification of each component was unequivocal. 2,5-Dimethylpyridine, with a normal boiling point midway in the series of pyridines, was used as the reference compound.

The flow rate was corrected to dry helium at 25° C. and atmospheric pressure (585 mm. of mercury). The limiting retention volume, V_R°, was calculated from the inlet and outlet pressures (*2*). The corrected retention volume per gram of liquid phase, V_g, was calculated for 2,5-dimethylpyridine by the relationship: V_R° divided by the weight of liquid substrate in the column. These V_g values are shown in Table III. The retention values of the other pyridines were calculated relative to the reference compound. The reference compound was run frequently to calibrate for minor changes in the operating variables of the apparatus.

The boiling point of each pyridine was determined at a pressure of 760 mm. of mercury, using a Cottrell-type ebulliometer and a platinum resistance thermometer (*5*). Each pyridine was distilled from calcium hydride to ensure a dry sample for the determination of boiling point.

The retention data for Tide were obtained for the particular sample on hand. This commercial detergent is variable in composition and the retention data are also dependent upon the method of preparing the detergent for use as a column packing.

DISCUSSION AND RESULTS

New Solid Support. The commercial detergent used in the preparation of the new solid support is manufactured by a spray-drying process. Thus, surfactants such as sodium alkylbenzene sulfonate and sodium alkyl sulfate are intimately mixed with the inorganic constituents. Table IV presents the composition reported by Stüpel for a particular sample of Tide (*12*). This table is included only to show the types of constituents and the approximate percentages present. Removal of the petroleum ether–soluble material in the extraction step gives a porous mainly inorganic residue which is used as the solid support. Viewed under a microscope, the pores appear as large craters in the solid.

Preparation of the solid support from a spray-dried material produces a mixture of inorganic salts in a form capable of holding the quantity of liquid substrate needed in gas-liquid chromatography. The solid will hold 70 grams of liquid substrate to 100 grams of solid before appearing wet and losing its free-flowing properties. By contrast, crystalline sodium sulfate (60- to 100-mesh) appears very wet when it is coated with 2 grams of liquid substrate to 100 grams of solid.

Column packings prepared from the new solid support are used in exactly the same manner as those prepared from Chromosorb. The columns are convenient to prepare and exhibit a column pressure drop of the same order of magnitude as that obtained using Chromosorb as the solid support. For example, a 2-meter column of the solid support (40- to 60-mesh) required an inlet pressure of 5.1 p.s.i.g. to give a flow rate of 60 ml. per minute of helium through the column, while a similar column of Chromosorb required an inlet pressure of 4.1 p.s.i.g. to give the same flow rate.

The new solid support is somewhat more fragile than either Chromosorb or Celite 545. The column packing is resieved after addition of the liquid substrate to the solid in order to remove the fines in the preparation of the packing.

The commercial detergent was heated and dried under several conditions, in order to compare the retention data obtained when using solid supports prepared under a variety of conditions. Table V lists the results of the heating and petroleum ether extractions of Tide. Solid by Method A was not used as a liquid substrate carrier because the extraction with petroleum ether did not remove enough of the organic material. It was necessary to heat the detergent above 100° C. to permit removal of the organic material by petroleum ether extraction.

Solids by Methods B, C, and D were used to prepare column packings with each of two liquid substrates: Dow Corning 703 silicone oil and monohydroxyethyltrihydroxypropylethylenediamine (MTE). The relative retention values for the 15 pyridines were determined on these six columns. The results obtained indicated that the small amounts of residual surfactants in these three solids (Table V) did not cause appreciable differences in the retention values.

The MTE is a strongly polar compound. It was the only liquid substrate studied that gave symmetric peaks for pyridines when dispersed on Chromosorb. Retention data obtained for

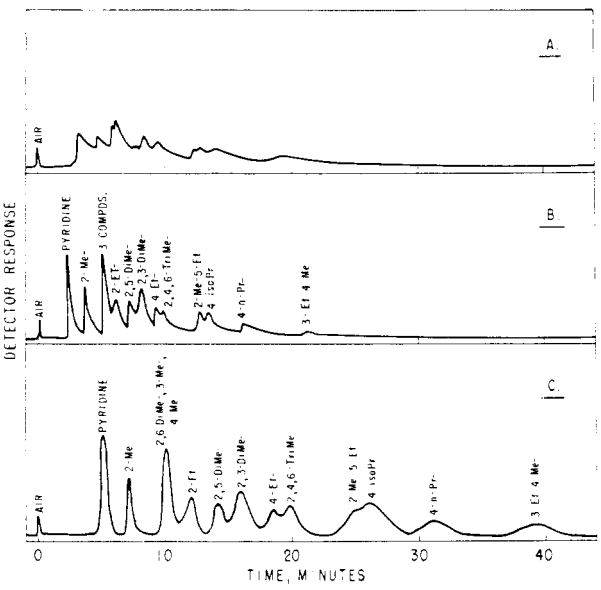


Figure 1. Separation of 14-component pyridine mixture on 15% Dow Corning 703 silicone oil on three solid supports

A. Acid- and base-washed Chromosorb
B. Acid- and base-washed Celite 545
C. Solid support prepared from Tide

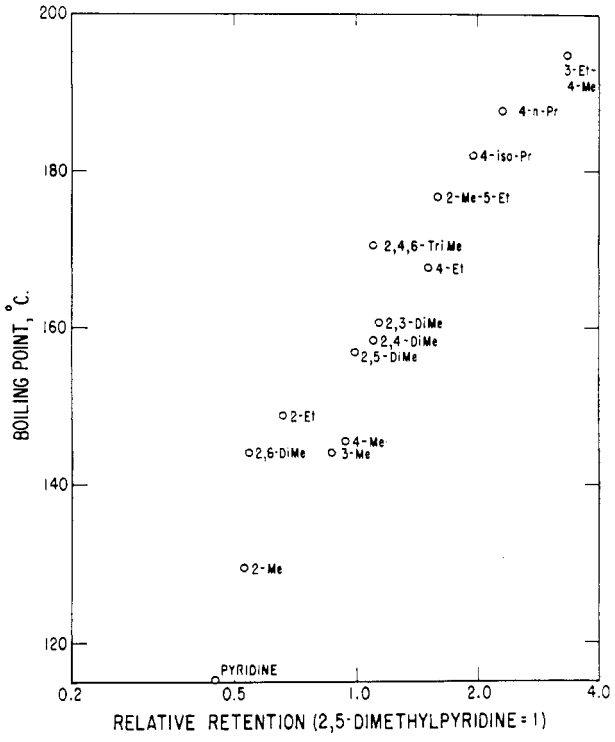
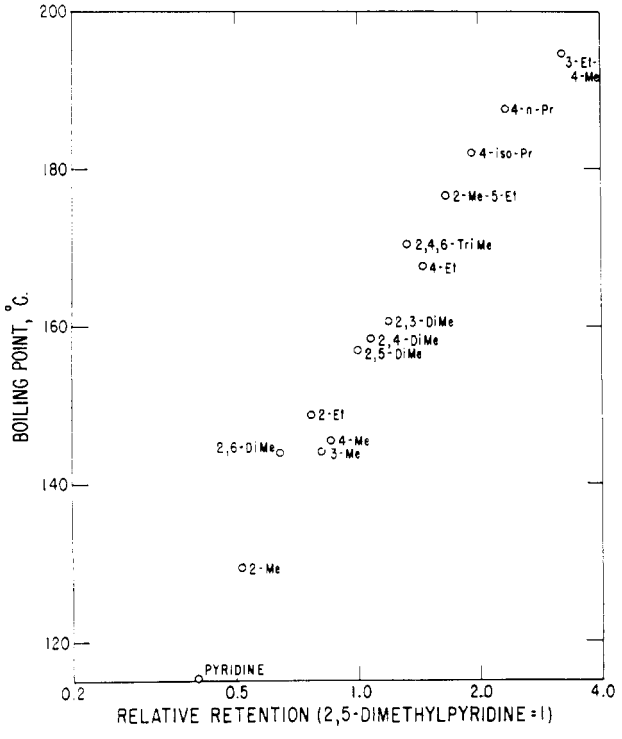


Figure 2. Selectivity of diphenyl phthalate for pyridines

Figure 3. Selectivity of monohydroxyethyltrihydroxypropylethylenediamine



MTE on acid- and base-washed Chromosorb were in close agreement with those obtained for solids B, C, and D. This shows that the amounts of surfactants remaining in these solids, as shown in Table V, did not significantly affect the retention data.

Comparison of Solid Supports. The column prepared with strongly polar MTE supported on Chromosorb separated the 14-component mixture of pyridines into nine peaks. The peaks obtained were symmetrical, but the MTE was not selective enough to separate the components of the mixture completely; hence, complete resolution required the use of an additional liquid substrate. However, skewed and unresolved peaks were obtained when the 14-component mixture was passed over nine available liquid substrates supported on Chromosorb. The chromatogram shown in Figure 1, *A*, which was obtained for the test mixture over Dow Corning 703 silicone oil, is typical of those given by the nine substrates.

Figure 1, *B*, shows a chromatogram of the same test mixture run over Dow Corning 703 silicone oil on acid- and base-washed Celite 545. Considerable improvement in peak symmetry and resolution is observed, but there is still appreciable tailing.

Symmetrical peaks were obtained when each of the substrates was supported on the new solid. The chromatogram obtained using Dow Corning 703 silicone oil is shown in Figure 1, *C*. The resolution and symmetry of the peaks in this figure as compared with those in Figure 1, *A* and *B*, show the superiority of the new solid support.

The skewed peaks obtained using Chromosorb or Celite 545 may be due to absorption of the pyridines by this solid support. To compare the adsorptivities of acid- and base-washed Chromosorb, acid- and base-washed Celite 545, and the new support, a 2-meter by $^1/_4$-inch column was filled with each bare solid. The columns were maintained at 130° C. and a flow rate of 60 ml. per minute of helium was used. Chromatograms obtained with the new support showed short emergence times for the pyridines and peaks with only slight tailing. On the other hand, the chromatograms for Chromosorb showed long emergence times and peaks with pronounced tailing. The chromatograms for Celite 545 showed considerably less tailing than those for Chromosorb, but more than for the new solid support. These results showed the greater adsorptivity of Chromosorb

and Celite 545 for pyridines as compared with the new support.

Selectivity of Liquid Substrate. Table VI lists the observed boiling points and the retention data for the 15 pyridines on the various liquid substrates. The decided differences in the selectivity of the liquid substrates permit a wide choice in their use for separations of pyridines. On the basis of the data the substrates were classified into two major groups: those that gave a nonselective separation based on boiling points, and those that gave a selective separation based on activity differences in the liquid substrate.

The first group of substrates consisted of Dow Corning 703 silicone oil, Dow Corning high-vacuum silicone grease, Octoil, and Octoil-S. Plots of the log of the relative retention *vs.* normal boiling points for pyridines using these substrates gave straight lines.

The second major group of substrates —those that gave selective separations of pyridines based on differences of the activities of the pyridines in the liquid substrate—were classified into two subgroups:

Plots of the data for squalane, mineral oil, and Apiezon-L gave points that deviated from a straight line. In general for compounds in a limited boiling range, the more basic pyridines had a greater retention than the less basic compounds. Thus, for squalane the relative retention value for 3-methylpyridine was 0.64 and for 2,6-dimethylpyridine was 0.73.

Plots of the data for tri-*m*-cresyl phosphate, Tide, MTE, and diphenyl phthalate also gave points that deviated from a straight line. However, in this case for compounds in a limited boiling range, the pyridine of greater basicity had a smaller retention than the less basic compound—for example, on tri-*m*-cresyl phosphate the relative retention value for 3-methylpyridine was 0.81 and for 2,6-dimethylpyridine was 0.68. The deviation was the greatest for MTE and diphenyl phthalate—for example, on MTE the relative retention value for 3-methylpyridine was 0.88 and for 2,6-dimethylpyridine was 0.55.

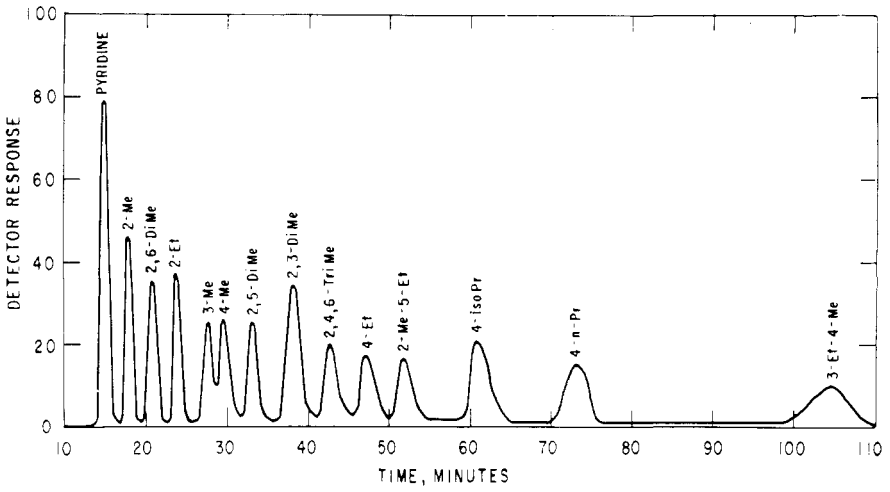


Figure 4. Separation of 14 pyridines using the series arrangement of columns

Application of Data. Examination of the selectivity of the liquid substrates permitted a choice of a series arrangement of columns that gave a separation of the 14 pyridines of the test mixture. Figure 2 shows the selectivity of diphenyl phthalate and Figure 3 the selectivity of MTE. Examination of these figures shows that the two liquid substrates are complementary in their selectivities for the compounds in the test mixture.

Table V. Results of Heating and Petroleum Ether Extraction of a Commercial Detergent

	Heating				Extraction	
	Conditions			Quantity volatilized,	Quantity extracted,	Surfactants unextracted,
Method	° C.	Mm. Hg	Hr.	wt. %	wt. %[a]	wt. %[b]
A	100	100	20	6.5	6.1	17.1
B	140	100	20	7.7	24.3	3.0
C	185	100	20	9.3	23.4	1.7
D	185	Atmos.	24	14.3	13.8	3.4

[a] Calculation based on original detergent.
[b] Figures obtained by Lawrence A. Gemel, Purex Corp., South Gate, Calif. Percentages are based on an assumed average molecular weight of 348, and were obtained on heated and extracted Tide, using a modification of Epton's method (4).

Table VI. Relative Retention of 15 Pyridines for Various Liquid Substrates

		Relative Retentions (2,5-Dimethylpyridine = 1)										
Pyridine	Boiling Point, ° C., 760 Mm.	Squalane	Mineral oil	Apiezon-L	Silicone high-vacuum grease	Silicone oil D.C. 703	Octoil	Octoil-S	TCP[a]	Tide	DPP[b]	MTE[c]
Pyridine	115.3	0.30	0.29	0.33	0.40	0.35	0.34	0.34	0.40	0.44	0.40	0.45
2-Methyl-	129.4	0.47	0.46	0.51	0.53	0.50	0.48	0.50	0.53	0.51	0.52	0.53
2,6-Dimethyl-	144.0	0.73	0.72	0.73	0.66	0.70	0.67	0.67	0.68	0.69	0.65	0.55
3-Methyl-	144.1	0.64	0.64	0.69	0.74	0.71	0.71	0.71	0.81	0.86	0.82	0.88
4-Methyl-	145.4	0.64	0.64	0.71	0.71	0.71	0.74	0.69	0.83	0.86	0.86	0.95
2-Ethyl-	148.9	0.81	0.81	0.80	0.82	0.83	0.79	0.79	0.78	0.76	0.77	0.66
2,5-Dimethyl-	157.0	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
2,4-Dimethyl-	158.3	1.00	1.00	1.05	0.96	1.01	1.02	1.01	1.07	1.05	1.08	1.11
2,3-Dimethyl-	160.7	1.09	1.08	1.14	1.09	1.11	1.10	1.11	1.21	1.13	1.19	1.15
4-Ethyl-	167.8	1.19	1.18	1.26	1.22	1.30	1.33	1.27	1.43	1.47	1.46	1.51
2,4,6-Trimethyl-	170.3	1.50	1.51	1.49	1.30	1.36	1.35	1.42	1.42	1.35	1.33	1.11
2-Methyl-5-ethyl-	176.8	1.79	1.80	1.82	1.57	1.74	1.75	1.72	1.70	1.49	1.65	1.60
4-Isopropyl-	182.0	1.77	1.76	1.81	1.71	1.87	1.92	1.83	1.94	1.93	1.92	1.96
4-*n*-Propyl-	187.8	2.07	2.09	2.15	2.07	2.15	2.23	2.19	2.40	2.28	2.32	2.31
3-Ethyl-4-methyl-	194.9	2.55	2.61	2.77	2.55	2.77	3.02	2.75	3.08	2.77	3.24	3.37

[a] Tri-*m*-cresyl phosphate (tri-*m*-tolyl phosphate).
[b] Diphenyl phthalate.
[c] Monohydroxyethyltrihydroxypropylethylenediamine.

Accordingly, a 3.5-meter column of 1/4-inch aluminum tubing was filled with a packing prepared using a ratio of 10 grams of diphenyl phthalate to 100 grams of solid. This column was connected in a series to 3.5 meters of 1/4-inch aluminum tubing filled with a packing prepared using a ratio of 10 grams of MTE to 100 grams of solid. The ratio of 10 grams of substrate to 100 grams of solid for each column was chosen to give sharper peaks than those obtained for columns using a greater substrate-solid ratio.

The columns were maintained at 130° ± 0.5° C. and a flow rate of helium of 90 ml. per minute was used.

Under these conditions, the 14 components of the test mixture were completely resolved, as shown in Figure 4. The series arrangement of columns had an efficiency of 1890 theoretical plates measured on the 2,6-dimethylpyridine peak.

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Factors Affecting the Use of Gas-Liquid Chromatography for the Separation of Large Samples

Sample Inlet System, Distribution Coefficient of Solute, and Amount of Liquid in Stationary Phase

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▶ Column efficiency decreases as the sample volume is increased. The effects of a number of factors on this decrease have been studied both theoretically and experimentally. Highest column efficiencies are obtained for large samples by introducing the sample in the form of a concentrated plug, by choosing a stationary phase in which the distribution coefficient of the solute is small, and by using relatively large amounts of liquid in the stationary phase.

GAS-LIQUID chromatography has established itself as a powerful technique for the separation of small (milligram) amounts of volatile chemical compounds. The extension of the technique to handle larger (gram) quantities of material offers a number of attractive possibilities. The components of a mixture could be isolated in sufficient quantities to enable auxiliary methods of analysis to be used to identify each component. Pure samples of volatile compounds which are required for numerous physicochemical studies could be obtained at relatively low cost from impure mixtures.

Although large-scale chromatography is being used at present (*1*, *2*, *10*), no systematic study of the optimum conditions under which such columns should be operated appears to have been undertaken. This is the purpose of the present investigation.

APPARATUS

A schematic diagram of the apparatus is shown in Figures 1 and 2. Columns were constructed from lengths of straight glass tubing and were packed with a mixture of Celite 545 and dibutylphthalate (30% of the liquid phase) to a density of 0.53 gram per ml., unless otherwise stated. The packing was held in position by plugs of glass wool, *J*, one of which, J_1, served to promote rapid evaporation of the liquid samples. The columns were supported on pressed asbestos rings, *K*, in a length of glass tubing (75-mm. inner diameter) around which two sections of resistance wire were wound—one serving to heat the packed portion of the column and the other being used as a preheater at the column inlet. The temperature of each heated section could be controlled independently and measured on thermometers, *I*.

The flow rate of the nitrogen carrier gas was measured on a rotameter, *A*. A manometer (not shown) was used to measure the gas pressure at the column inlet. After passing through an electrically heated furnace, *F*, packed with granular copper oxide to remove traces of oxygen, the nitrogen was preheated to a suitable temperature in the electrical heater, *B*. All heated portions of the apparatus were lagged with asbestos, *D*, to minimize heat loss.

Liquid samples were placed in a vessel, *H*, and were swept into the column preheater by bypassing the nitrogen through the stopcock, *G*. The nonreturn valve, E_2, consisted of a small metal ball fitted onto a ground seat and prevented the escape of carrier gas while *H* was open. Because of the rapid evaporation of the sample, it was

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