CHANGES OCCURRING WITH THE IMMOBILE LIQUID PHASE IN GAS–LIQUID CHROMATOGRAPHY

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The liquid selected for the immobile phase of a gas–liquid chromatogram should be chemically stable and virtually nonvolatile at the operating temperature of the column. Definitions of stability and volatility have taken various forms. HARVEY AND CHALKEY[1] recommended that the maximum operating temperature of the column should be at least 100° below the normal boiling point of the immobile liquid. May and Baker Ltd.[2], KEULEMANS[3] and KNIGHT[4] listed a number of popular partitioning liquids along with their maximum operating temperature if the column is to be used a number of times, although this temperature may be exceeded by 20–25° for one or two analyses. Another approach has been to specify the limiting vapor pressure of the immobile liquid at the temperature of the column, *e.g.*, 0.01 or at the most 0.1 mm Hg[5]. This is not completely satisfactory, *e.g.*, dinonyl phthalate has a vapor pressure of 0.03 mm of Hg at 140° which qualifies it for use, whereas in practice, it is rapidly decomposed by the catalytic activity of Celite at this temperature. On the other hand, polyethylene glycol 400 has a vapor pressure of 0.15 mm of Hg at 100°, which is outside the acceptable limit, yet columns of this material have been operated continuously for 400 h at 1 l/h flowrate with little loss of chromatographic efficiency[6]. The life of a column may be judged by whether a significant change in the weight of the stationary phase occurs with use. "Significant" has been interpreted by defining the limit of temperature as that where the column loses 50 % of its stationary phase in 1000 h of continuous operation at 15 ml/min flowrate[2]. This last publication gives a test for the determination of volatility. The partitioner and support are mixed and packed in a 10 × 3 cm glass column capable of direct weighing, placed in an oven, and preheated nitrogen passed over it at 15 ml/min. The loss of weight is determined every hour for three hours. No attention is paid to purification of the nitrogen. Loss of immobile phase results in shorter elution times and decreased resolution as exemplified by the work of TAYLOR AND DUNLOP[7] with dimethylformamide as the stationary liquid. SCOTT[8] remarked that an early criterion of the Gas Chromatography Committee on Nomenclature was that for a given column, retention volumes should not vary by more than 2 % in 3 months of continual use. This generalization should be qualified by the remark that the precision of retention volumes is also a function of the solute and flowrate. Hypersensitive detectors are very responsive to the appearance of the partitioner in the effluent stream which reduces the upper limit of temperature below that established by thermal conductivity cells. Attempts have been made to define the limit of solvent loss on the basis of detector behavior. The paper of GERRARD, HAWKES AND MOONEY[9] is particularly relevant to this topic. COOPER *et al.*[10] preconditioned columns by isothermal heating while passing gas through them. The columns were then attached to the analyzer and the machine adjusted to give a zero base line at room temperature. The temperature of the column was increased and the maximum allowable operating temperature, MAOT, defined as that where the base line showed a deflection of 0.5 % of full scale at maximum sensitivity. This temperature increased with the extent of preconditioning. Apiezon L grease required heating at 250° for four days per meter length of column to reach a MAOT of 245°. High temperature gas–liquid chromatography compounds the problem of stability and loss of partitioner while temperature programmed techniques involve a continuously changing loss with increasing temperature. The partitioning liquid may contaminate substances separated on preparative columns[9].

KEPPLER, DIJKSTRA AND SCHOLS[11] found that polythenes, halogenated ethylene polymer, silicones, and Apiezon M grease were lost at the rate of 3–8 mg/h at 210–250° and 15 ml/min flowrate. Paraffin oil and wax were unsuitable above 150–170°. HAWKES[12] operated a column supporting Apiezon L vacuum grease for the equivalent of two months at 290–295° with no noticeable change in peak areas and a darkening of the partitioner did not impair efficiency. No attention was paid to peak position. A similar darkening unaccompanied by changes in chromatographic behavior was noted by WISEMAN[13]. TAYLOR AND DUNLOP[7] found that calibration factors for peak areas and heights did not differ significantly for old and new columns whereas there was a difference in peak positions. Opposed to this, MIYAKE[14] advised that peak area *vs.* quantity standardizations should be performed at least once per month. Prolonged use of his column led to results higher than the true values for low boiling compounds and low analytical results for high boiling compounds. DIJKSTRA[15] attributed the oxidation of Apiezon greases, polythene, and probably silicones at high temperatures to oxygen in the carrier gas. GUILD, BINGHAM AND AUL[16] reported that commercial helium contains 200 p.p.m. of water vapor, nitrogen, oxygen, and methane. RAY[17] remarked that 5 p.p.m. of oxygen is sufficient to cause darkening of polymeric hydrocarbon phases which apparently form peroxides and then ketones. Presumably careful purification of carrier gas as suggested by GLUECKAUF[18] would do much to reduce chemical change of the partitioner. In spite of purification of the helium and conditioning of a column holding Apiezon L grease by passing the gas through it for 14 h at 100°, GUILD, BINGHAM AND AUL[16] reported a loss of immobile phase when the temperature of the column was increased. Repeated heating of the column gradually reduced this loss. They expressed concern that frequent heating of the column near or above the recommended temperature limit would eventually reduce the amount of immobile partitioner and seriously alter resolution and retention volumes. They also advised vacuum treatment of the stationary phase to remove volatile contaminants. BLOM[19] measured a loss of 0.1 mg of hydrocarbons per minute from an Apiezon L preparative column at 200°.

DREW[20] reported aromatics from paraffin oil at 150°. WHITMAN[21] felt decomposition to be the origin of benzene in the effluent from silicone columns above 150°. Such silicone columns, operated for four months, showed no change in resolution of the compounds analyzed. DIJKSTRA[15] measured a loss of 50 mg of material in the first 8 h from a column holding 3 g of a silicone (temperature not indicated). KWANTES[22] observed such a loss above 180°. CASON AND MILLER[23] found considerable variation of retention times and band widths for certain compounds between chromatograms performed on different samples of commercial high vacuum stopcock greases. These greases gave more constant results and improved resolution when heated 5–8 days and as long as 12 days after application to Chromosorb at 325–335° in a glass tube through which nitrogen was passed. A volatile, low melting solid and a mobile liquid were evolved during the curing. The cured partitioner was stable under 275° but bled a little at 300° until the column had been used 1000 h or more. Retention times were smaller than those found with the uncured packing. The cured material gave constant retention times and unimpaired resolution for several thousand hours at 275°, but near 300° there was a slow decrease in retention times and loss of resolution after 2000 h. Monocetyl phthalate, octadecyl stearate, paraffin wax, polyethylene succinate, tricresyl phosphate, and silicone fluid charred or slowly distilled at 250°[24]. *o*-Phthalic-ethylene glycol polyester showed limited bleeding at 225°[25]. Polypropylene sebacate underwent thermal condensation at 150° to release water which had an adverse effect on the detector[9]. Loss of volatile components decreased with preheating. Silicone oil was continually lost to a stream of nitrogen at 260° over 200 h; the rate of loss decreased with time. These same authors found dinonyl phthalate to escape at a rate of 0.017 mg/l of carrier at 56° and 5.0 mg/l at 181° from a 10 % immobile phase–Celite column at 8 ml/min flowrate. ADLARD[6] in his survey of polyethylene glycol polymers, found that although higher molecular weight compounds were less volatile, they were undesirable because of a greater thermal instability and were less selective from a separations point of view because of decreased polarity which arose from a reduction in the number of free hydroxyl groups. The material was also hygroscopic containing 0.19–0.70% water depending upon the molecular weight.

Much interest has been shown in the preparation of polyesters[26] and their use as partitioners. HORNING, MOSCATELLI AND SWEELEY[27] commented that there has been considerable variation reported between polyester columns when prepared in what appeared to be similar fashions. Polyesters also showed considerable thermal degradation between 180° and 220°. Degradation and bleeding were reduced, the conditioning period shortened, a more uniform distribution of the liquid obtained, and a greater reproducibility experienced between packings employing the same ester if the acid washed Celite support was first coated with a silicone before application of the polyester. HOWARD AND MARTIN[28] used this procedure to wet the support with organic phases in reversed phase liquid–liquid chromatography. It is likely that the same phenomenon occurs here although this would not explain the decreased degradation, however. We may suppose that the support catalyzes a reaction of the ester and that this is reduced by the silicone. CORSE AND TERANISHI[29] blamed the instability of polyesters on the presence of acid impurities which they removed by an ion exchanger. This further elucidates the catalytic effect of the support since OTTENSTEIN[30] has reported both acidic and basic sites in diatomaceous earth materials.

High temperature gas chromatography offers a method of studying immobile phases, *e.g.*, DAL NOGARE AND SAFRANSKI[31] chromatographed a silicone oil which gave 16 peaks attributed to homologs and evidence of volatile degradation products. This suggests the chromatography of the effluent from a column by a technique similar to that used in catalytic studies[32].

The remarks of TUEY[33] are most interesting. In using a particular silicone, there was no relation between the change in retention properties and the loss or chemical change of the stationary phase. During the first eight hours of operation at 200°, retention times and column efficiencies dropped by 30–40 % with no measurable loss of immobile phase. The physical properties of the silicone were unchanged on extended heating with Celite in a test tube inside the column vapor jacket. After the initial heating operation, the column appeared quite constant for an indefinite period. This observation poses a puzzle. Tuey found a change in retention times and efficiencies with no observable change in the partitioner while Hawkes[12], Wiseman[13], and Whitman[21] reported changes in the immobile phase but none in chromatographic behavior. Unfortunately, few workers have addressed themselves directly to the problem. They also felt that there must be some redistribution of the partitioner on the support. Knight[4] remarked that most of the loss will come from the first part of the column which may become exhausted of liquid leading to an effect on retention times and peak shapes. Moderate losses will reduce retention times but the relative times will be unchanged. Solvent bleeding is reduced for columns of low solvent concentration because of the reduction of the volatility of a thin film by adsorption on the support[4]. The rate of loss of silicone oil E-301 from a liquid film is about three times that of a film adsorbed on Celite[34]. The long column life of dimethylformamide supported by alumina and operated at room temperature was attributed to such adsorption[35]. In contrast to this, Friedrich[36] found gradual shifts in band positions and loss of resolution of propane–butane mixtures over a 630 h period using silica gel coated with 3 % dioctyl sebacate as a tailing reducer operated at 50 %. Calculations indicate that this difference in evaporative rates is inexplicable on the basis of evaporation of a liquid held in small pores by capillary forces[11].

Maintenance of the solvent level on the column may be accomplished by saturating the carrier with solvent before entering the column as was done by Kwantes and Rijnders[37] in their determination of activity coefficients. Van der Craats[38] did not subscribe to this procedure, claiming that evaporation would still occur farther along the column where there was a decrease in total pressure. Drew[39] noted that some workers increase the column life by packing the outlet end with dry support which removes the immobile phase from the effluent gas. Unless the support is proven inert with respect to the solutes being separated, this is certain to affect results.

Debbrecht[40] of F. and M. Scientific Corp. studied bleeding from columns of 20 % silicone gum rubber (SGR) and diisodecyl phthalate (DIDP) supported by 60–80 mesh Chromosorb at temperatures where bleeding was significant. The bleeding rate increased with flowrate for a column of fixed length for both partitioners for three different lengths of columns. Debbrecht adopted the thesis that for a fixed temperature and flowrate, the bleeding rate should increase with column length until finally a sufficient length was reached where the effluent was saturated with partitioner and would remain constant for longer columns. When tested, he found that for flowrates of 75, 50, and 30 ml/min and columns up to 6 ft. in length, there was no indication of closeness to equilibrium vapor pressure conditions. Plots of bleeding rate of DIDP *vs.* column length were linear with a constant positive slope and showed no trend to an asymptotic value. This result is contrary to the evidence we present. A similar study of the SGR column gave the surprising result that bleeding *vs.* column length was not linear but curved upward very rapidly at lengths in excess of 4 ft., the curvature being opposite to that expected on the basis of the original thesis. Carrying these results one step further, DEBBRECHT defined the column life as the time required to deplete the partitioner from 20 to 15 % and calculated that the column life of DIDP was constant with respect to column length but that for SGR, a one foot column would have a life of 1200 min while a 6 ft. column would have one of slightly over 200 min. The result is not unique in that the author refers to a similar observation, by another worker, made with linear polypropylene. The author speculated that this phenomenon might be due to some chemical reaction or degradation. GIDDINGS[41] has suggested some autocatalytic effect where the degradation products in the gas stream, which increase in concentration as the carrier proceeds through the column, cause the greatest reaction near the outlet end than at the inlet. Degradation also increases as the flowrate decreases for a fixed column length.

Another of DEBBRECHT'S observations is pertinent to column conditioning. Two columns of SGR were conditioned at 400° for two hours; one with no carrier gas passing through it and the other at a flowrate of 75 ml/min. After this process, the "no flow" column performed better than the other and contained more partitioner. The "no flow" column was further conditioned at 400° and 75 ml/min for two hours after which its performance was still superior to the column conditioned with carrier flow. The author attributed this to the opportunity given the SGR to stabilize by cross-linking before depletion of the partitioner under "no flow" conditions.

The foregoing information permits some conclusions relevant to chromatographic behavior. The immobile phase may contain volatile contaminants of residual solvent from the solution used to apply the immobile phase, or, if the partitioner is hygroscopic, water adsorbed from the atmosphere. If these are not removed before packing the column then, as carrier gas passes down the tube, the impurities will be removed at the inlet but not farther along the column if, by this time, the carrier is saturated. The result is a non-constant distribution of immobile phase on the support and perhaps different retention properties of the partitioner along the column depending upon the effect of the impurities. In addition, the amount of liquid on the column is unknown. Volatile impurities in the original liquid may be removed by vacuum distillation of the immobile phase before application to the support followed by storage of the material under vacuum or by heating after application to remove contaminants introduced by this operation. This problem may prove to be quite stubborn. KIESER AND SISSONS[42] found it necessary to heat polyethylene glycol at 100° and 0.01 mm Hg pressure for 16 h to remove formaldehyde and formic acid which were responsible for anomalous behavior. On the other hand, WILLIAMS[43] claimed the removal of the solvent, used to apply the partitioner, within minutes by use of a rotating vacuum evaporator which gave materials requiring little further conditioning. In the work reported here, one packing material was preheated before loading the column. Results were not satisfactory. The immobile phase may undergo chemical conversion from the material deliberately selected for its separation properties to something new and unknown. Such changes may predominate with a fresh column and decrease with use to give something approximately constant. After conditioning, one is unsure of the identity of the solvent or its quantity. If contaminants in the carrier are responsible for the change, careful purification of the gas will retain the identity of the solvent. If the reaction is one of thermal condensation, a catalyzed reaction due to nonvolatile impurities in the partitioner or due to the support, carrier purification will not solve the problem and the reaction will proceed throughout the life of the column to a greater or lesser extent. One may attempt purification of the partitioner and deactivation or masking of the support. All of these effects may be accompanied by a redistribution of solvent on the column, an effect which has been investigated in this laboratory. Chemical changes are not necessarily undesirable. GERRARD, HAWKES AND MOONEY[9] found decomposed polyoxypropylene to be more effective in some separations than the original material. As the experience with dinonyl phthalate has shown, it is not wise to draw conclusions from the behavior of the immobile phase separated from its support. Finally, the partitioning liquid may be boiled off of the support and this will predominate at the column inlet. This may affect the detector and chromatographic results. Presently, there is little agreement in the literature as to which of these should be the basis of a definition of minimum allowable solvent loss. Also, there is no definition of a properly "conditioned" column nor of "column lifetime". It is hoped that those groups concerned with establishing standards for gas chromatography will devote some attention to these problems. Those workers using gas chromatography to determine physical properties of solutes should be cognizant of these phenomena.

Although we have concerned ourselves with the macroscopic redistribution of the immobile phase of the column with use, which has implications for the conditioning process, and presume these changes to be the result of volatility or chemical change, it seems appropriate to make some remarks concerning changes which may occur with non-reactive, nonvolatile partitioners during conditioning. In general, the common solid supports used in gas chromatography are porous. The excellent data of BAKER, LEE AND WALL[44] indicate that on application of the partitioning liquid, the smallest pores are filled first. Presuming that the liquid wets the solid, we picture a particle of the packing as a series of cavities of various sizes, containing liquid connected by a film as shown in Fig. 1. The thermodynamic activity of the liquid, as reflected by the surface free energy and vapor pressure above each cavity, will depend upon the geometry of the cavity and the liquid in it. If the immobile phase has been deposited in such a manner that the activities of the liquid in the pores are not all equal, then a potential for a microscopic redistribution will exist, and such a redistribution will continue until the activity of the liquid is the same all over the support. We envision this as occurring in two ways. The fluid may flow from one cavity to another, or immobile phase may evaporate from one cavity and condense in another. Presumably, these processes will be very slow but will be accelerated by the increase in temperature employed during conditioning. Liquid may also redistribute at the contact points

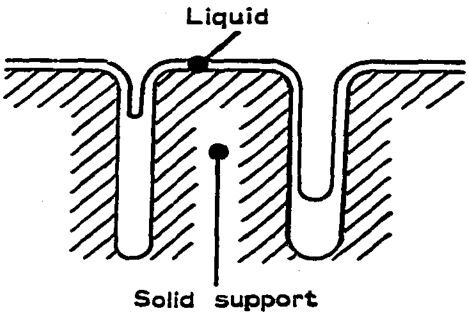

Fig. 1. Distribution of the immobile phase on a porous support.

between particles where there is a very small radius of curvature of the liquid film. Although an equilibrium distribution of fluid in the pores may be approached by a very slow deposition of immobile phase, the redistribution at the contact points can only occur after the column is packed. We know of no investigations concerned with this effect.

APPARATUS AND PROCEDURE

Analyzer

Separations were made with a Cenco No. 70130 Vapor Phase Analyzer (Central Scientific Co., Chicago, Ill.) equipped with a katharometer detector employing tungsten filaments. Column, katharometer, and sample injection unit were all at the same temperature. Elution diagrams were recorded on a Leeds and Northrup Speedomax Model S, variable range, variable sensitivity recorder of 1 sec response time and 30 in./h chart speed. Sample introduction was by a 0.25 ml hypodermic syringe. Driving pressure was measured by a mercury manometer placed at the source of the helium carrier and flowrates were measured at the outlet by means of a soap-film flowmeter[3] and stopwatch.

Partitioning agents

The partitioning agents, already applied to Chromosorb, were dinonyl phthalate (DNP), tricresyl phosphate (TCP), polyethylene glycol (PG), and *n*-hexadecane (*n*-Hex.), and were obtained from Burrell Corporation (Pittsburgh, Penn.). The columns were packed without any pretreatment with the exception of Column 5 where the packing was heated for 27 h at 75° in a drying oven. The amount of immobile phase present was determined by extracting a weighed amount of packing with a Soxhlet extractor and the appropriate solvent, or, in the case of more volatile liquids, by heating them in an oven at 125–130° until two successive weighings differed by no more than 0.2 mg. Where applicable, the latter method was preferred because some mechanical disintegration occurred with the extractor. Table I gives the data relevant to the columns and partitioners.

TABLE I

COLUMN CHARACTERISTICS

Column	*Immobile phase*	*Per cent immobile phase*	*Column length (m)*	*Weight of packing (g)*	*Advised max. operating temperature (°C)*
1	DNP	30.9	3.0	31.45	125–140
2	TCP	31.4	3.0	32.80	120
3	PG	31.6	3.0	33.24	100
4	PG	31.6	1.5	16.45	100
5	PG	2.80	1.5	11.80	100
6	*n*-Hex.	31.1	3.0	34.47	40
7	TCP	31.4	1.5	18.23	120
8	Firebrick	0.0	0.15	0.12	
	TCP	31.4	1.35	16.45	120

Columns

Columns were of coiled 0.25 in. copper tubing. Column 8 was a composite of uncoated firebrick, heated for 10 days at 125–130°, at the inlet followed by partitioner laden firebrick. See Table I.

Solutes

The solutes chromatographed were the C_1 to C_7 normal aliphatic alcohols and the iso-alcohols from C_3 to C_6. With the exception of isohexanol, which we synthesized ourselves from isobutyl chloride and ethylene oxide (WAGNER AND ZOOK[45]), all other alcohols were obtained from commercial suppliers and were used without further purification. Impurities, which evidenced themselves as minor peaks, were ignored and the major peak taken as the named component. The solutes were chromatographed singly and as mixtures. To avoid overlap of the peaks, the mixtures were even carbon *n*-alcohols, odd carbon *n*-alcohols, even carbon iso-alcohols, and odd carbon iso-alcohols. The composition of the mixtures was adjusted to give peaks of approximately equal height.

Method of reporting data

We report retention volumes, $V°$, relative to the air peak, as milliliters of dry gas at 1 atm. pressure and 0° corrected for the pressure drop across the column. This quantity is not a specific retention volume since we feel this parameter has little meaning where there is a nonuniform distribution of partitioner in the column. Operating temperatures are the observed temperatures and their maximum deviation. Flowrates are in milliliters of dry gas at standard temperature and pressure per minute and were measured every 5–10 min for short duration chromatograms and every 30 min. for longer chromatograms. Average flowrates were used when the individual values showed a random variation about the mean. If, however, there was a trend in the flowrate due to a drop in the driving pressure, a least squares line was fitted to a plot of flowrate *vs.* time, and the retention volume determined from an empirical equation. Where this was done, the average driving pressure was used in correcting for the pressure drop across the column. Uncertainties in the retention volumes are based on average deviations from the mean or average deviations from the least squares line. We base the per cent differences in $V°$ on the difference in the extreme values of $V°$ divided by the mean value of $V°$ for all of the data points of the set in question.

LOSS OF WEIGHT ON IGNITION

Perhaps the simplest and most naive approach to determining the rate of loss of immobile phase from the support is to simply heat a weighed amount of the packing in an oven. This we have done. Fig. 2 shows the loss of weight of TCP, DNP, and PG at various temperatures *vs.* time. TCP shows the least loss. DNP shows an apparent change in the rate of loss at 80 h. PG shows a strange behavior. There is a very rapid loss of partitioner in the early stages at all three temperatures. At 75° and 100°, the quantity of partitioner remains constant after 30 h whereas all of it is lost after 30 h at 128°. Such a discontinuous change is certainly unexpected. One would expect the plateau at 100° to lie between the plateaus at 75° and 128°. Our guess is that the glycol is quite inhomogeneous and that different amounts of volatile material are removed at different temperatures. A sample of PG stored over phosphorus pentoxide for three months at room temperature showed a loss of weight of 19.33 % which indicates evolution of some rather volatile material. This is much larger than the 0.19–0.70 % water reported by ADLARD[6]. Fig. 3 gives the rate of loss of *n*-Hex. The curves are what one would expect of a homogeneous material, *i.e.*, the rate of loss is fairly constant until all of the material has evaporated off.

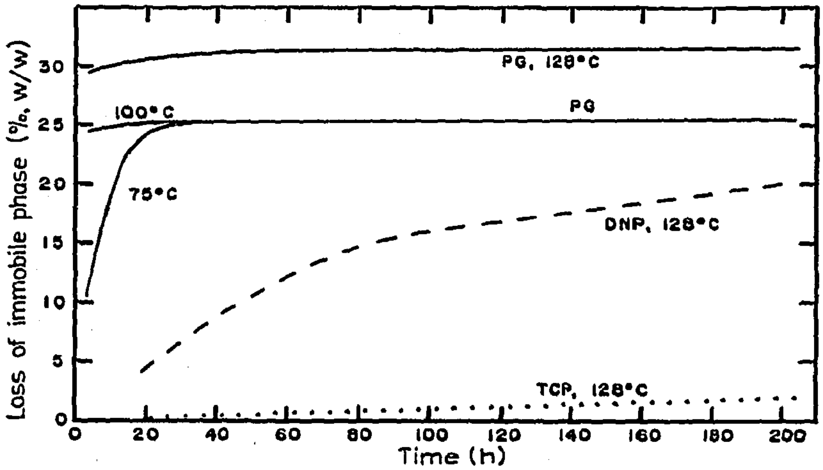

Fig. 2. The loss on ignition of TCP, DNP, and PG from the support with time.

This procedure is far from ideal. Oxidative changes can occur in the atmosphere of air in the oven. Also the loss of partitioner is not uniform from the bed of packing materials in that the top layers dry first. The rate of loss from a small quantity of material is much faster than from a large amount if the surface of the bulk is the same in both cases. Agitation of the material between weighings would also increase the apparent loss. Because of this, we have refrained from emphasizing the change in the slope of the curve for DNP and for *n*-Hex. near its asymptotic value. Nonetheless, the results are indicative.

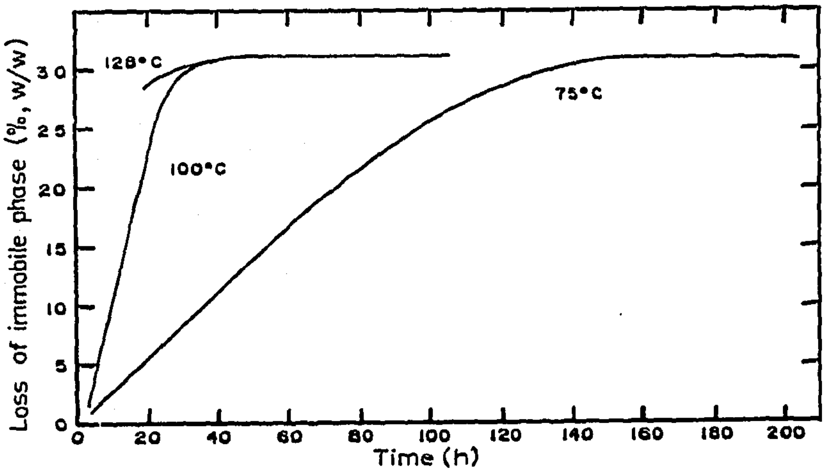

Fig. 3. The loss on ignition of *n*-Hex. from the support with time.

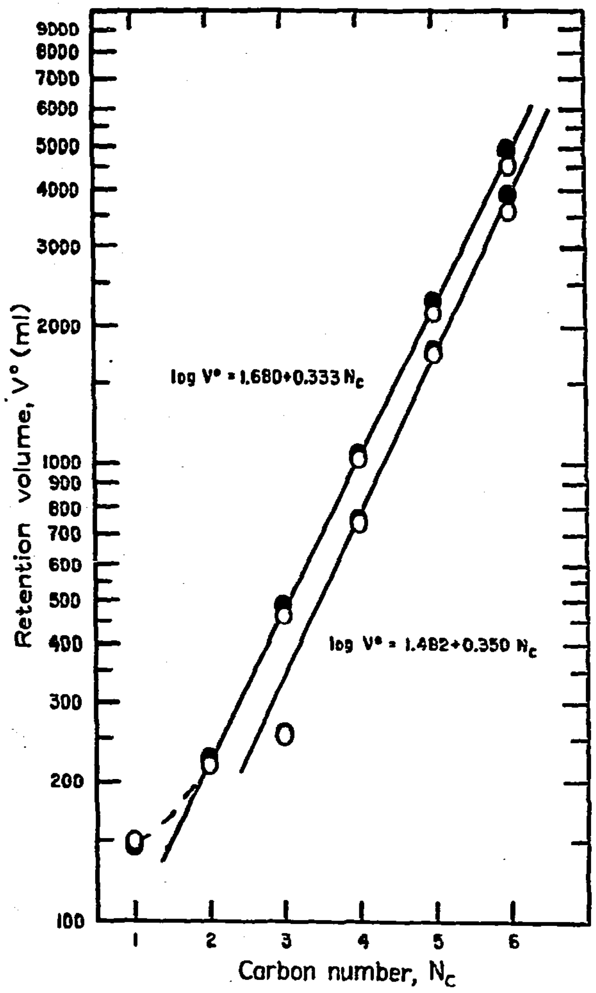

Fig. 4. Retention volumes of the *n*- and *iso*-aliphatic alcohols on TCP as a function of the number of carbon atoms in the molecule (Column 2).

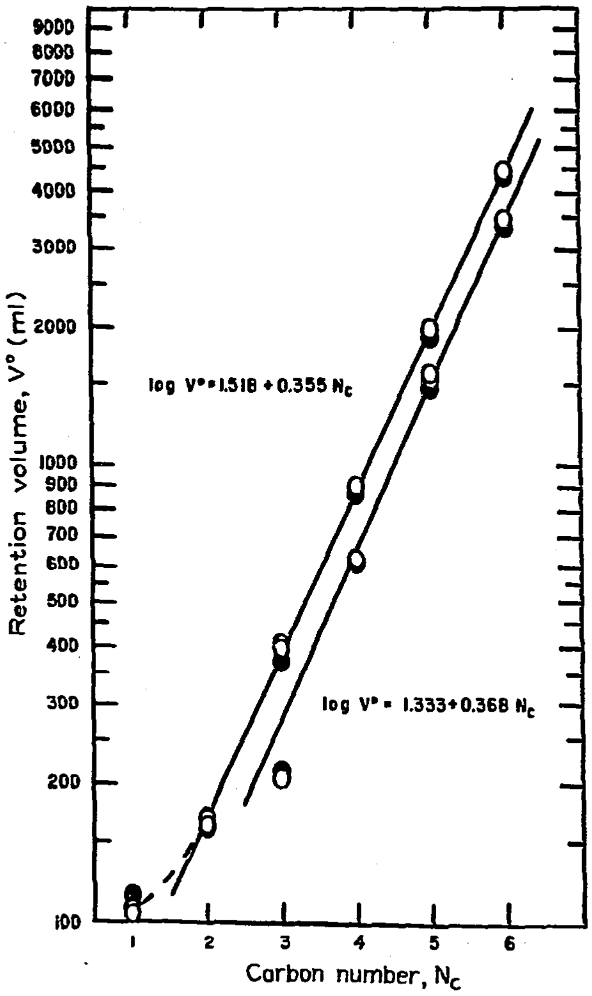

Fig. 5. Retention volumes of the *n*- and *iso*-aliphatic alcohols on DNP as a function of the number of carbon atoms in the molecule (Column 1).

CHROMATOGRAPHIC RESULTS

In the course of determining the temperature dependence of the retention volumes of the alcohols on various partitioners, a sequence of chromatograms was performed at increasingly higher temperatures and finally repeated at the original lowest temperature. In some cases, there was reproducibility between the first and the last series while with others there was not. On completion of the chromatographic study, the columns were cut into short sections and the amount of immobile phase determined in each section by extraction or by heating. Thus we characterize the redistribution of the partitioner not by the extent of use, the temperature to which it was subjected or the total amount of condensable material in the effluent, but by the actual final distribution of the immobile phase on the column.

Tricresyl phosphate and dinonyl phthalate columns

Determinations were made at 100° (series a), 125, 150 and 175° before repeating the work at 100° (series b). Figs. 4 and 5 show semi-logarithmic plots of V° *vs.* the number of carbon atoms in the molecule, N_C, for the normal (upper line) and iso-aliphatic alcohols (lower line) on TCP and DNP respectively. The open circles are the data points for the initial chromatograms at 100° while the solid circles are the data points at this temperature after operation at the higher temperatures. The least squares lines for these points are shown on the figures. In computing these lines, the data points for methanol and isopropanol were omitted because of their deviation from the line. Such a deviation for methanol is not remarkable since it seems to be a property of the lower members of any homologous series. Isopropanol is probably better charac-

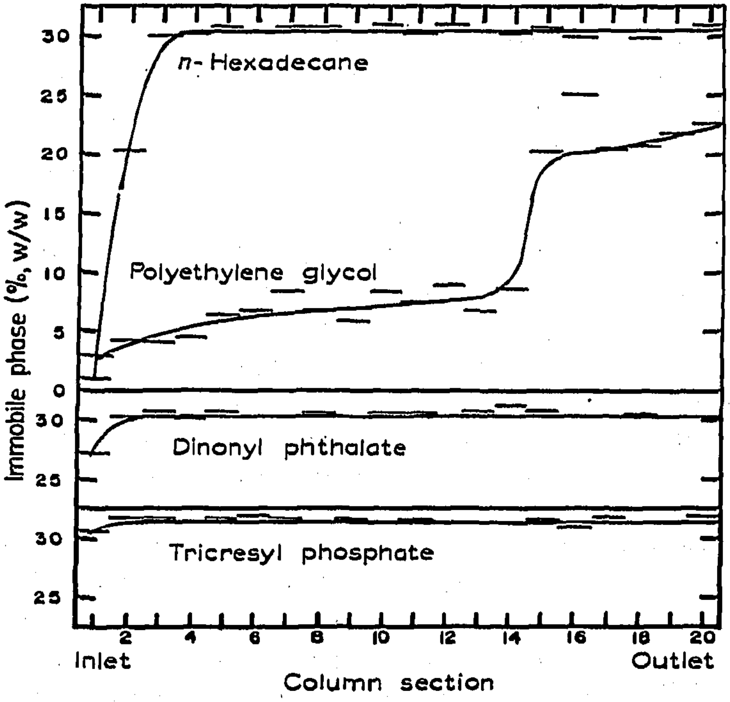

Fig. 6. Distribution of the liquid partitioner on columns supporting *n*-Hex., PG, DNP, and TCP after extended use.

terized as a secondary alcohol and not as a member of the homologous series of iso-alcohols. Fig. 6 shows the distribution of the immobile phase on the support. The short horizontal lines represent the experimentally determined amount of immobile phase present in each section of packing while the smooth curve is a likely fit to these values. The remark of KNIGHT[4] is substantiated in that solvent loss occurs in the first part of the column. DNP lost more than the TCP column which is reasonable from the rate of loss on ignition. Both columns recovered their original values in the second section. Evaporation farther down the column could not be detected (VAN DER CRAATS[38]). These curves can be explained by assuming that the carrier gas is rapidly saturated with partitioner in the first portion of the column and that further evaporation does not occur. This is contrary to the conclusion of DEBBRECHT, however.

The problem now is to decide if the loss in the first section affected the reproducibility of V° values. This was approached in two ways. The average per cent difference in V° between series a and b for the TCP column is 4.0 %, while for the DNP column it is 4.5 %. Although not very different, both exceed the criterion of 2 % mentioned by SCOTT[8]. On the DNP column, duplicate determinations made before the column was used at higher temperatures with methanol, ethanol, *n*-propanol, and isopentanol give an average difference of V° of 2.1 %, very close to SCOTT's value. From this, one could claim that this small redistribution has affected the reproducibility of the retention volumes. Arguing against this, however, is the observation that with DNP the V° values after heating at the higher temperatures are predominately less than the initial values at 100° while the reverse is true with TCP as can be seen in Figs. 4 and 5. Since this is in disagreement with the remark that retention volumes should decrease with loss of partitioner[2,7], we attribute the variation to experimental fluctuations. Experience with our equipment leads us to conclude that routine reproducibility is about 4–5 %. For the purposes of later statistical argument, we would like to pool the data points of series a and b for the TCP column and for the DNP column and assume that in each case, they came from the same population, *i.e.*, that use at high temperatures did not affect chromatographic results. This was also done in fitting the least squares lines and is the foundation of our statistical hypothesis.

If one considers the least squares line to be a moving average[46], then one may compute an unbiased variance, s^2, and a standard deviation, s, of a single value of log V° if two degrees of freedom are deducted from the sample size. This we have done for each of the straight lines of Figs. 4 and 5. The variances and standard deviations for the two families of alcohols on the two columns may then be combined[47] and result in values of $2.63 \cdot 10^{-4}$ and $1.62 \cdot 10^{-2}$ respectively. We may now state our statistical hypothesis in a more definite manner: Assuming that the variation of the points about the least squares line is independent of the nature of the immobile phase, the family of alcohols, and the history of the column, one may expect the unbiased variance of log V° to be $2.63 \cdot 10^{-4}$, the unbiased standard deviation of log V° to be $1.62 \cdot 10^{-2}$, and the average difference in V° to be 4.3 %. This establishes our standard for comparison.

Polyethylene glycol column

In all cases freshly prepared columns lost material to the effluent stream as evidenced by a drifting base line and condensation of material in the outlet tube from the apparatus. This agrees with the loss of weight when the packing was stored over phosphorus pentoxide. An appreciable conditioning period was always required before a reliable base line could be established. We have no idea of the redistribution which may have occurred during this period. Column 3 was conditioned at 101° and a series of chromatograms executed at 101, 125, 100, 150, 175, and 100°. The third and sixth series at 100° furnished the data reported here. Fig. 6 shows the redistribution of partitioner on the column. Notice that no section contains the original 31.6 % partitioner. Also, the distribution does not correspond to a homogeneous liquid. We feel that during the initial conditioning process and low temperature separations, easily volatilized materials were driven off the column to leave about 22 % of the liquid on the support. At higher temperatures, another group of volatiles were removed, which, if they had been completely driven off, would have left about 8 %. Evidently we terminated our work before this occurred so that we have a discontinuity of the column at the 14th section. The first section of the column only contains 3 % partitioner. This hypothesis is supported by the data from loss on ignition. Fig. 7 shows the chromatographic results. Line A is for the early chromatograms at 100° but after conditioning and operation at 101 and 125° while line B is for the series run after operation of the column at higher temperatures. In both cases the iso-alcohols lie below the normal alcohols. It is obvious that operation of the column at high temperatures has produced a marked change in the retention volumes. Omitting methanol and isopropanol and the line for the iso-alcohols on the initial column (2 data points), the combined unbiased variance for the three lines is $3.14 \cdot 10^{-4}$

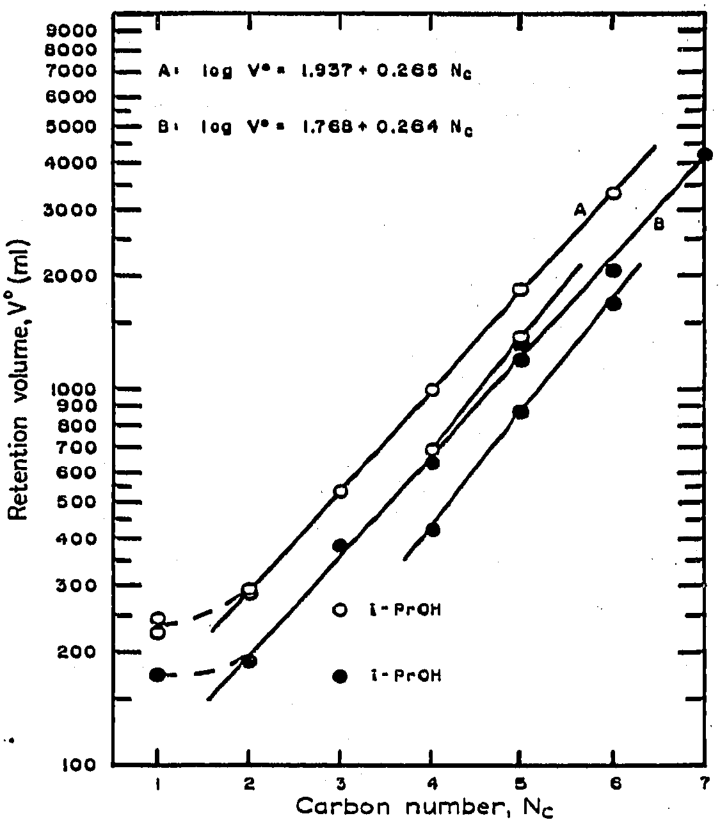

Fig. 7. Retention volumes of the *n*- and *iso*-aliphatic alcohols on PG as a function of the number of carbon atoms in the molecule (Column 3).

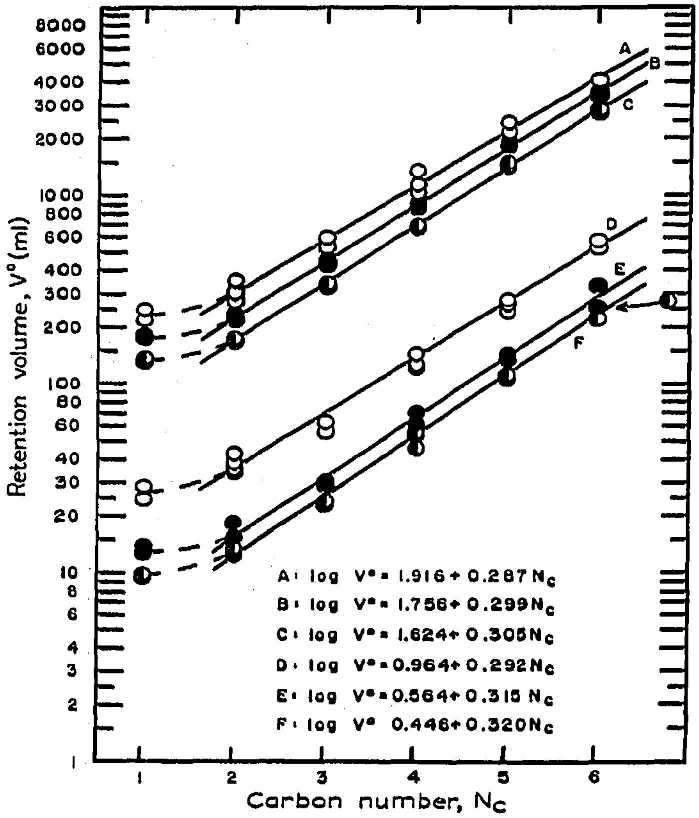

Fig. 8. Retention volumes of the *n*-aliphatic alcohols on PG as a function of the number of carbon atoms in the molecule and the history of the column (Columns 4 and 5).

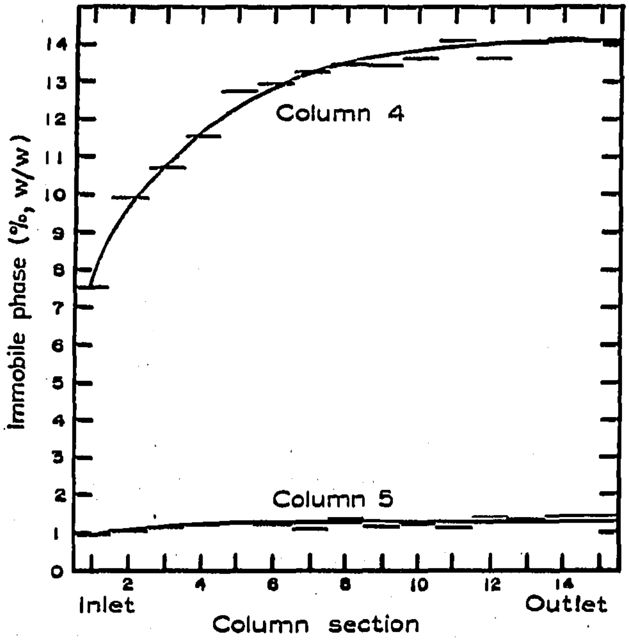

Fig. 9. Distribution of PG on Columns 4 and 5 after extended use.

and the standard deviation is $1.77 \cdot 10^{-2}$. The points are slightly more scattered about the line for PG than for the "good columns" of TCP and DNP. We are not inclined to place a great deal of faith in these data since there are few duplicates for any one alcohol, we have no idea of the extent of conditioning introduced by the initial determinations at 101 and 125°, and the population sampled is small. The variance of line A, Fig. 7, is $0.13 \cdot 10^{-4}$ which is superior to the TCP and DNP columns. However, this is based on the results of a single separation of each of two mixtures. It was felt the results were fortuitous and did not represent the variation introduced by random experimental errors. The combined variance is probably more reliable.

One remarkable conclusion from Fig. 7 is that apparently the intercept is changed by the drastic redistribution but not the slope. The unbiased variance of the slope[48] of line B is $4.05 \cdot 10^{-5}$ and the unbiased standard deviation is 0.006. The slopes are well within one standard deviation of one another. The average difference in $V°$ between the two series is 41.5 %.

TABLE II

STATISTICAL PARAMETERS FOR COLUMNS 4 AND 5

Line	*Unbiased variance of log $V° \times 10^4$*	*Unbiased standard deviation of log $V° \times 10^2$*	*Average % diff. in $V°$*
	Untreated packing, Column 4		
A	22.62	4.76	18.9
B	3.87	1.97	4.8
C	1.57	1.25	2.8
	Preheated packing, Column 5		
D	22.65	4.76	15.4
E	21.99	4.69	13.3
F	12.43	3.53	8.2

In order to determine if indeed the slope remained constant during redistribution, Column 4 was prepared from the PG material, heated at 75° until the base line was constant, and a series of chromatograms of the *n*-alcohols performed at this temperature. The column was subsequently heated at 125° for a period of time and another series of chromatograms performed with the same samples, again at 75°. This procedure was repeated once more. This same procedure was followed in Column 5 containing the preheated packing. Fig. 8 is a graphical representation of the performance of both columns. Fig. 9 demonstrates the distribution of liquid on the columns as determined from 10-cm sections. Later chromatograms in the same series always showed a lower retention volume, *e.g.*, *n*-propanol on the fresh column had a retention volume of 602 ml as the second chromatogram and a retention volume of 530 ml as the fourth determination which is a change of 13 %. This trend was observed for all of the solutes. We feel that both Columns 4 and 5 were undergoing alteration during the early determinations. Table II gives the parameters used to judge the "goodness" of the various columns. If one bases the standard for a "good" column on the combined data for the TCP and DNP columns, then using the variance and the percentage difference in $V°$, Column 4 was satisfactory after the second heating (line C) and

Column 5 was not satisfactory anywhere. Notice that here the per cent difference in $V°$ was based on data for a single series and was not used to compare two series interrupted by heating at a different temperature as was done previously. The preheated, low loaded column showed no improvement as far as conditioning was concerned. This was a surprising result since, at least for this material, it would indicate that conditioning should be performed with the packing in the column. Before this can be accepted as generally true, much more experience is required with other partitioners at other loadings.

One may compare the slopes of two lines by dividing the difference between the slopes by the square root of the variance of the difference and applying the Student's test with $(n_1 + n_2 - 4)$ degrees of freedom (n_1 and n_2 are the sample sizes)[48]. Table III shows the lines of Fig. 8 compared, the value of t computed in the described manner, and the tabulated value of t at the 5 % significance level. We conclude that lines A, B, C and D do not differ in slope at the 5 % significance level but lines E and F have slopes different from the others but not from each other. Thus it appears that, as far as the slope of the line is concerned, loss or redistribution on a low-loaded column is more significant than with a higher loaded column but we refrain from ascribing this to any definite effect such as retention by the support. We hope to consider this question in a future publication.

TABLE III

COMPARISON OF SLOPES OF THE LINES OF FIG. 8

Lines compared	*Calculated t*	*Tabulated t at 5 % significance level*[48]
A, B	0.80	2.15
B, C	0.48	2.09
A, C	0.72	2.07
D, E	2.16	2.11
E, F	1.05	2.11
A, D	0.36	2.08

n-Hexadecane column

n-Hexadecane is a volatile hydrocarbon and has an advised maximum operating temperature of 40° (Table I). Nonetheless, Column 6 was operated at 50, 70, 90, 110 and 50°. The data of the first and last series were compared. As a further insult to the recommendations of the literature, polar alcohols were chromatographed on this non-polar hydrocarbon. ORMEROD AND SCOTT[49] and FUKUDA[50] indicated that one should anticipate adsorption of polar solutes on the support when using a non-polar liquid. Indeed, our data substantiated the occurrence of nonlinear retention. Peaks showed very sharp fronts and pronounced tailing. The retention volume also depended on the sample size as can be seen from ethanol (Table IV) where two consecutive chromatograms at identical conditions, except sample size, showed a difference in $V°$ of 162 ml (measured from the peak maxima). Fig. 6 shows the distribution of the liquid on the column. The first section of the column was essentially bare support.

The small reduction on extraction can be ascribed to mechanical loss. Again, in contrast to DEBBRECHT's remarks, the carrier seemed to become saturated with partitioner in the early sections of the column. The curve can be explained by the evaporation of a homogeneous liquid from the support as was the case with TCP and DNP but opposed to PG.

Table IV records our data for the first and last series at 50°. As can be seen, there was very poor reproducibility. Some of this can no doubt be attributed to minor differences in sample size although every effort was made to keep these as near equal as possible. We would like to focus attention on the number of minor peaks appearing after use of the column at high temperatures. The principal peak is designated by M. Such artifact peaks were called "peaks" if they passed through a definite maximum which permitted calculation of a $V°$. If, however, the record showed an increase to a plateau, devoid of any maximum from which a $V°$ could be determined, and which extended into the principal peak, it was termed a "foot". Such "feet" resembled the concentration profiles obtained in frontal analysis or displacement development[51]. It will be noticed that (Table IV): (1) multiple zones and "feet" predominately occurred with the column after use at high temperature, and (2) the minor peak or "foot" preceded the principal peak. In their review of multiple zoning as it occurs in liquid–liquid chromatography, KELLER AND GIDDINGS[52] pointed out that a discontinuity in the immobile phase can be the source of more than one zone for a single solute. We believe that the discontinuity of passing from bare firebrick to solvent laden support was the source of the artifact peaks observed here. We also recognized two types of discontinuities. One was a discontinuity in the concentration of the partitioner as exemplified by the PG column, and the other was a discontinuity in the nature of the partitioner, *e.g.*, passage from adsorption to partition, as perhaps exemplified by the *n*-Hex. column. Our idea is that a fraction of a solute was strongly adsorbed as it passed over the exposed support so that only a part of it entered the

TABLE IV

OPERATING CONDITIONS AND RETENTION VOLUMES FOR COLUMN 6 (*n*-HEXADECANE)

*Solute**	*Sample size (ml)*	*Temperature (°C)*	*Flowrate (ml/min)*	*Inlet pressure (mm Hg)*	*Outlet pressure (mm Hg)*	*$V°$** (ml)*
C_1 a	0.01	50.1 ± 0.1	40.8 ± 0.2	27.2	69.8	298 ± 2
b	0.01	50.1 ± 0.1	41.4 ± 0.3	27.2	70.0	183 ± 2
C_2 a	0.02	49.9 ± 0.1	41.8 ± 0.2	27.4	70.1	342 ± 2
a	0.01	49.9 ± 0.1	41.8 ± 0.2	27.4	70.1	504 ± 2
b	0.01	50.1 ± 0.1	41.2 ± 0	27.1	70.0	400
						632 M
n-C_3 a	0.02	50.2 ± 0	40.7 ± 0.1	27.0	69.8	955 ± 2
b	0.02	50.0 ± 0.1	41.2 ± 0.4	26.9	70.0	706 ± 7
						890 ± 8 M
n-C_4 a†	0.02	49.9 ± 0.3	42.1 ± 0.3	27.4	70.1	2936 ± 21
b†	0.02	50.0 ± 0.1	41.2 ± 0.3	26.8	70.0	2529 ± 18
i-C_3 a	0.02	50.2 ± 0.1	40.4 ± 0.3	26.9	69.8	529 ± 4
b†	0.02	50.2 ± 0.3	42.9 ± 0.3	28.0	70.1	458 ± 3

* a: Initial results; b: Results after use at high temperatures; † Peak preceded by a "foot".
** M: Principal peak.

column holding liquid. When this solute was followed by a later sample of a different material, the latter displaced the first from the support, and, since the first was the more volatile, it proceeded through the column in advance of its displacer to appear as an artifact peak or "foot". The concentration profile was a combination of displacement and elution effects. Redistribution of immobile liquid as it gives rise to this kind of discontinuity could lead to spurious results and erroneous conclusions as to sample composition. Columns 7 and 8 were prepared to test this hypothesis. TCP was chosen as the partitioning liquid since it does not redistribute on the column at the conditions employed here and would not alter the discontinuity deliberately introduced in the construction of Column 8 where the first section consisted of preheated, unladen firebrick. Fig. 10a represents the record of methanol and ethanol separated as individuals on Column 7 where there was no discontinuity. As can be seen, there were no artifact peaks or "feet". Fig. 10b represents the profiles obtained on Column 8. The dotted line is for pure methanol while the solid line represents an immediately following sample of pure ethanol. The last peak was preceded by a "foot". It should be noted that (1) the alcohols are chromatographically pure with respect to TCP as shown by Fig. 10a, and (2) the discontinuity alone will not give rise to a "foot" as shown by its absence in the curve for methanol in Fig. 10b, but must be preceded by another solute. The size of the sample of methanol necessary to produce a "foot" on the following ethanol peak had to be rather large.

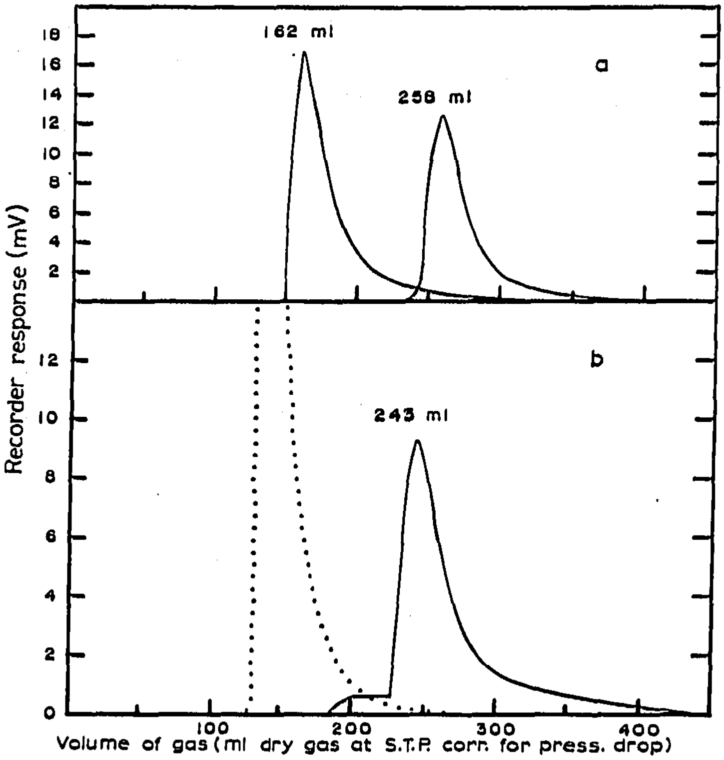

Fig. 10. Concentration profiles of methanol and ethanol on: (a) TCP column, and (b) TCP column having a discontinuity in the nature of the partitioner. Temperature: 75°.

CONCLUSIONS

The immobile liquid phase may undergo chemical changes during the chromatographic process or during the conditioning period. These may be due to: (1) impurities in the carrier gas, particularly oxygen, (2) nonvolatile impurities in the partitioning liquid, *e.g.*, hydrogen ion, (3) catalytic reaction with the support, (4) catalytic reaction with degradation products arising from the liquid, or, (5) further condensation of a polymeric material. Any or all of these may introduce material into the effluent stream. Volatiles may also be introduced into the gas stream which may arise from: (1) volatile impurities in the original partitioner, (2) solvent introduced if the partitioner is deposited from a solution, (3) water adsorbed from the atmosphere, or (4) evaporation of the partitioner. Both chemical change and physical evaporation produce a redistribution of the material on the support which is generally most marked at the inlet end of the column where the entering gas is unsaturated with volatiles or carries unreacted impurities. The distribution may show a gradual change as one proceeds along the column or it may be discontinuous. There may be a discontinuity in concentration where there is a sudden change from a low-loaded section to one of higher loading. In such a case, the retention volume is reduced and the log V° *vs.* carbon number line has a lower intercept but apparently the same slope. This was found to be true for a 20–30 % polyethylene glycol column tested statistically at the 5 % significance level. Low loaded columns show a change in slope for this test, however. Another discontinuity may be one involving a sudden change in the retention process. For example, a change from adsorption to solution can lead to multiple zones. Such a discontinuity can result from evaporation of the liquid from the support at the inlet.

Small losses in the early sections of the column do not appreciably affect chromatographic behavior.

Our study indicates that each partitioning liquid, in combination with its support, is an individual problem since a variety of changes can occur. A series of tests should be devised to determine the suitability of a particular packing and definite standardized procedures for preparation of the material and its conditioning are needed. A satisfactory definition of a suitable column and of the life-time of a column are required. This is likely to be difficult since our work indicates that redistribution and bleeding change continually during the use of a column, yet we are asking that a definite decision be made at some point during the history of a column as to whether it is properly conditioned and when its lifetime has been exceeded. Such tests and procedures should also be simple if they are to gain popular adoption. Until this is done, reported partition coefficients, specific retention volumes, etc., are suspect.

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SUMMARY

Changes which the liquid partitioner of a gas–liquid chromatographic column may undergo are reviewed. The redistribution of liquid within the column may introduce a discontinuity in the concentration of the liquid or a discontinuity in the retention mechanism by exposure of the adsorptive support. Experiments show that the former changes the intercept of the semi-logarithmic plot of retention volumes *vs.* the carbon number for a homologous series of alkyl alcohols but not the slope. The second type of discontinuity may give spurious peaks.

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