GAS–LIQUID CHROMATOGRAPHY IN QUALITATIVE ANALYSIS

PART VIII. THE EVALUATION OF POLYMERIC STATIONARY PHASES WITH SPECIAL REFERENCE TO THE POLYOXYETHYLENE GLYCOLS*

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SUMMARY

A scheme for the evaluation of polymeric liquid phases is proposed and this used to show that polyoxyethylene glycols are eminently suitable for qualitative gas chromatographic analysis.

INTRODUCTION

In gas–liquid chromatography the stationary liquid phase plays a most important role, since it is upon solution properties that the successful resolution of the components of complex mixtures rests. Throughout the short history of gas chromatography there has been an almost continuous quest for more efficient and rapid separations, and as a consequence there is now available commercially a bewildering number of stationary phases. These fall into two broad types, chemically discrete compounds and mixtures, the latter often being chemically ill-defined. Whereas the solution properties of the former have been studied in some detail[1-5], few attempts have been made to understand the factors which determine the retention characteristics of the latter. As polymeric mixtures are extensively used as liquid phases, largely because of the temperature limitations of the former type[6,7], it was decided to investigate their solution properties, in order to determine whether or not meaningful retention data could be obtained using them.

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* See also Ref. 12.

EXPERIMENTAL

Apparatus and procedures

Chromatograms were obtained using an apparatus consisting of glass columns (5 ft. long and 4 mm I.D.) with a modified flame ionisation detector[8]. The columns were packed with 20% w/w mixtures of stationary phase and 60–70 B.S. mesh Celite (J.J's Chromatography Ltd., Kings Lynn, Norfolk) except where stated. The stationary phases used included:

     polyoxyethylene glycol-200 (Shell Chemicals),
     polyoxyethylene glycol-400 (Merck, Shell and Union Carbide),
     polyoxyethylene glycol-1000 (Shell),
     polyoxyethylene glycol-4000 (Merck),
     polyoxyethylene glycol-20 M (Applied Science).

The columns were heated by means of vapour jackets; methylal 42°, methanol 65°, water 100°, *n*-pentanol 138° and 2-ethylhexanol 183°. A mixture of hydrogen and nitrogen (3:1, by volume) was used as carrier gas; this was dried by passage through a tube containing Linde molecular sieve (13X pellets supplied by B.D.H.) situated between the gas cylinder and sample injection port. Samples for analysis ($\sim$ 10γ) were introduced onto the column as dilute solutions in either acetone, dichloromethane or *n*-pentane by means of stainless steel capillary pipettes[9]. All the solutes and *n*-alkanes used in this investigation were obtained commercially.

Measurement of values for retention

Relative retentions in KOVATS' retention index units[10] were determined from chromatograms of mixtures of the solutes of interest and the appropriate *n*-alkane internal standards. The adjusted retention distances were measured between the individual peak maxima and that of methane, which was used as column dead volume marker[11], and values for retention index calculated according to the expression:

$$I = 100\ N + 100\ n \left(\frac{\log R_x - \log R_N}{\log R_{N+n} - \log R_N} \right)$$

where R_x, R_N and R_{N+n} are the adjusted retentions of the solute and *n*-alkanes possessing N and $N+n$ carbon atoms respectively.

DISCUSSION AND RESULTS

A consideration of the requirements of gas chromatographic liquid phases and a scheme for their evaluation

In order that columns of high intrinsic efficiency and resolving power may be prepared, the materials intended for use as stationary liquid phases should be:
     (i) easily available and reproducible chemicals which are preferably definite compounds;
     (ii) internally mobile over a wide range of temperature below the decomposition temperature;
     (iii) thermally stable up to the temperatures required to elute solutes in a reasonable period of time;

(iv) chemically unreactive towards the materials being examined;

(v) unreactive towards oxygen and moisture, and

(vi) they should possess good solvent properties for a wide range of chemical types.

Partition coefficients, specific retention volumes, and simple relative retention ratios, used in the previous studies of liquid phase behaviour[1-5], have been found to be dependent to a greater or lesser degree upon the instrumental operating conditions[12]. Therefore it is probable that with these units slight changes in the retention characteristics of the column packing might be obscured by shifts of retention arising from fluctuations of the instrumental operating conditions. Such units are consequently much less suitable for the evaluation of liquid phases than are the scales of relative retention (with the n-alkanes as internal standards) which have been found to be largely independent of instrumental variables yet sensitive to slight differences of solute–solvent interaction. Of these scales, KOVATs' retention index was considered to be the most suitable for our investigation of stationary phase behaviour.

The principal requirement of any liquid phase required for use in qualitative analysis is that it should be capable of giving reproducible values for retention, not only for one particular column on different occasions, but also different columns. Therefore, before a stationary phase can be recommended for use in analysis, the constancy of its retention characteristics should be checked over the range of temperatures it is proposed to use the material. Also the dependence of its solvent properties upon its origin should be determined, because slight differences in the techniques of synthesis and purification could affect the chemical composition of the material. With polymeric mixtures it is possible that the overall retention behaviour may be influenced by the difference in the polarities of the end groups and repeat units, the molecular weight distribution, stereoregularity, and sequence distribution in the case of copolymers. Therefore any evaluation of stationary phases should include a consideration of the above factors, whenever possible bearing in mind the somewhat primitive nature of the currently available methods of polymer characterisation. Also, the effect of moisture and oxygen should be determined, particularly with hydrophilic and oxidisable materials, as these are common contaminants of gas chromatographic columns.

Based upon these arguments, a scheme for the evaluation of gas chromatographic liquid phases has been drawn up involving the following steps:

(i) determination of the constancy of retention data, in KOVATs' retention indices, with column usage over the range of temperatures proposed for its use;

(ii) the determination of the dependence of retention data upon the origin of the material, and where possible an investigation of batch variability;

(iii) where appropriate, the determination of the influence upon retention data of polymer end group concentration, and molecular weight distribution;

(iv) study of the influence of the tacticity of stereoregular polymers, and heterogeneity of copolymers upon retention data;

(v) investigation of the effect of the absorption of moisture and oxygen upon the retention characteristics of a column.

In order to illustrate the application of this scheme it has been applied to the evaluation of the ubiquitous polyoxyethylene glycols.

The evaluation of liquid phases in terms of the constancy of retention data with column usage

The constancy of the retention characteristics of a range of polyoxyethylene glycols was tested by a method* involving the following steps:

(i) preparation of the column;

(ii) determination of the maximum operating temperature;

(iii) preparation of suitable calibration solutions involving solutes of a wide range of chemical types and *n*-alkane internal standards;

(iv) chromatography of the calibration solutions whilst the column temperature is alternated between a convenient temperature in the range 60–100°, and the maximum operating temperature;

(v) calculation of retention indices and graphical analysis of the data.

In each case elevated temperatures were avoided during the preparation of the liquid phase, support mixtures by the normal slurry technique. Also, the carrier gas was allowed to flow continuously throughout each investigation in order to avoid anomalous results due to thermal degradation or the absorption of moisture owing to back diffusion.

TABLE I

THE MAXIMUM OPERATING TEMPERATURE OF POLYOXYETHYLENE GLYCOL LIQUID PHASES AS INDICATED BY THE CONSTANCY OF RETENTION INDEX DATA

Celite columns, 5 ft. × 4 mm, 20 % polyoxyethylene glycol.

Average molecular weight	Maximum temperature (°C)	P_I[b]
400	100	0.7
1000	138	0.5
4000	183	0.6
20,000	225[a]	0.6

[a] Work carried out using a Pye Panchromatograph in this case.

[b] Average value for root mean square deviation in retention index for the seven solutes used to test the reproducibility of the retention characteristics.

Typical results being as shown in Table II.

Facts arising from this investigation of the solution behaviour of polyoxyethylene glycols (see results in Tables I and II) are as follows:

(i) the maximum operating temperature increases with the average molecular weight of the polyoxyethylene glycols, as would be expected;

(ii) provided these temperatures are not exceeded retention data can be obtained which is comparable in accuracy to that given by dinonyl phthalate and squalane columns[13].

During the initial stages of this work some difficulty was experienced in obtaining repeatable relative retentions, particularly with *n*-alkanes as internal standards. As this variability was associated with "leading" chromatographic peaks

* For more completed experimental details see ref. 26.

TABLE II

A TYPICAL EXAMPLE OF THE REPRODUCIBILITY OF RETENTION INDEX DATA WITH POLYOXYETHYLENE GLYCOL LIQUID PHASES

20 % polyethylene glycol-4000, 183°.

Solute	Mean retention index	P_I
n-Nonyl alcohol	1652.3	0.4
Nitrobenzene	1801.7	0.8
2-Phenylethanol	1927.6	0.8
N,N-Di-n-butylaniline	1937.5	0.5
Pentadecan-8-one	1956.0	0.6
1-Chlorohexadecane	2102.0	0.6
Methyl palmitate	2227.1	0.5

and as it was eliminated by the injection of samples as dilute solutions in dichloromethane (see Table III) it was presumed to be due to inefficient volatilisation of solutes and standards. Accordingly dilute solutions (~ 0.2 % with respect to each component) were used throughout the remainder of our work on liquid phase evaluation.

The dependence of the retention characteristics of polyoxyethylene glycol liquid phases upon their origin

With pure chemically discrete stationary phases the retention characteristics of columns prepared using different batches of liquid phase should be identical, provided values for retention are not perturbed by adsorption effects[14,15]. However, this need not be the case with polymeric materials, particularly when the end groups are chemically dissimilar to the repeat units, as in the case of the polyoxyethylene glycols. In an attempt to determine the magnitude of the changes of retention which could arise and recognise the factors which are responsible for these differences, three columns were prepared using samples of polyoxyethylene glycol-400 from three different manufacturers, and the retention indices of a range of solutes determined (see Table IV).

Polyoxyethylene glycols, which are prepared either by the reaction between α:ω-dichloro oxyethylenes and the monosodium salt of ethylene glycol[16], or by the polymerisation of ethylene oxide[17], are linear polymers[18] having the general formula $HO(CH_2CH_2O)_nCH_2CH_2OH$. The materials used in gas chromatography are almost invariably prepared by polymerisation of ethylene oxide. The products of these polymerisations, which are polydisperse in terms of molecular weight, are separated into series of fractions having nominal average molecular weights of 200, 300, 400 etc. (calculated from the chemically determined hydroxyl concentration). For a particular polyoxyethylene glycol fraction these average molecular weights are not all the same, for instance with polyoxyethylene glycol-400 a tolerance of ± 5 % is generally accepted. Therefore the differences of retention reported in Table IV could arise from differences of hydroxyl content or different molecular weight distributions. In connection with the latter it is interesting to note that on the basis of a gas chromatographic analysis of the methylation products of two samples of polyoxyethylene glycol-400 CELADES AND PAQUOT[19] found evidence of all the individual species

TABLE III

THE EFFECT OF INEFFICIENT VAPORISATION OF SAMPLES UPON RELATIVE RETENTIONS OBTAINED WITH POLAR LIQUID PHASES

Celite columns, 5 ft. × 4 mm, 20% polyoxyethylene glycol-400, at 100°. Samples injected as dilute solutions in dichloromethane at the column temperature. n-Pentadecane and n-heptadecane as internal standards. Sample volume 0.1 μl in each case.

Substance	Concentration (%w/w)	Retention index	P_{skew}[a]
Benzaldehyde	10	1618.0	+0.42
	5	1619.5	+0.29
	2	1623.4	+0.08
	1	1623.6	−0.02
	0.5	1623.7	−0.04
2-Ethylhexan-1-ol	10	1609.6	−0.50
	5	1612.4	+0.32
	2	1617.0	+0.09
	1	1618.3	+0.02
	0.5	1618.4	−0.01
Hexadec-1-ene	10	1652.9	+0.62
	5	1654.6	+0.40
	2	1655.2	+0.10
	1	1656.0	+0.04
	0.5	1655.8	+0.02

[a] P_{skew} is the coefficient of peak skewness defined by the expression:

$$P_{skew} = \frac{\sigma_2 - \sigma_1}{\sigma_1 + \sigma_2}$$

where σ_1 and σ_2 are as shown in the diagram:
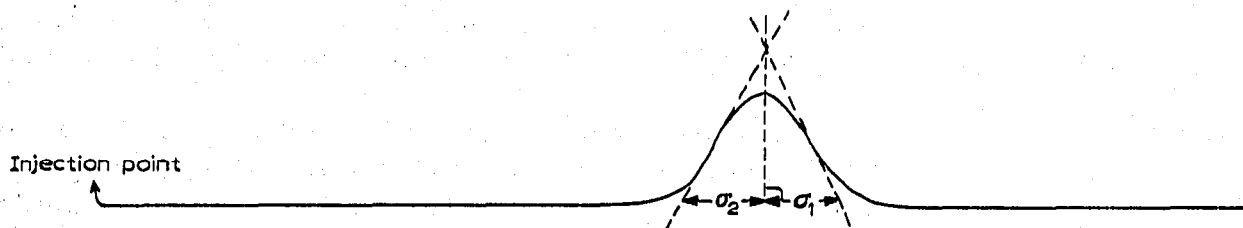
Thus positive values for P_{skew} reflect peak asymmetry typical of leading peaks (generally due to inefficient volatilisation) whilst negative values are characteristic of tailing peaks (usually associated with some form of adsorption phenomenon).

between dioxyethylene glycol and tetradecyloxyethylene glycol, with the octa- and nona- derivatives as the major components.

    Alternatively the differences of retention could be due to either slight changes of column temperature, differences of the adsorptivity of the support, or the presence of ionic impurities such as catalyst residues. Fortunately all three possibilities can be ruled out. The first, because the values for b (the slope of the n-alkane log. plot) are virtually the same for the three columns, the second because a further Merck column prepared using the same batch of support gave retention index values within ± 1.5

TABLE IV

THE BATCH DEPENDENCE OF THE RETENTION CHARACTERISTICS OF POLYOXYETHYLENE GLYCOL LIQUID PHASES

Celite columns, 5 ft. × 4 mm, 20 % w/w polyoxyethylene glycol-400, 65°. Samples of liquid phase obtained from Merck (West Germany), Shell Chemicals (U.K.) and Union Carbide (U.S.A.), the same batch of Celite being used throughout.

| | Values for retention index | | |
	Merck	Union Carbide	Shell
Benzene	994.6	998.6	1000.6
Pentan-2-one	1062.1	1066.1	1069.0
Dihydromyrcene	1133.7	1136.6	1137.2
Diethyl carbonate	1175.6	1181.4	1183.0
Isopropylbenzene	1219.8	1225.0	1226.3
Heptan-3-one	1229.5	1234.7	1237.5
Pentan-2-ol	1250.5	1255.7	1261.3
Ethyl 4-methylpentanoate	1253.3	1258.8	1260.7
Butan-1-ol	1279.5	1285.8	1292.4
1-Chloro-octane	1282.8	1287.8	1289.8
Methyl n-octyl ether	1298.6	1302.6	1304.5
Diisopropyl disulphide	1299.0	1304.3	1305.5
2-Methylhexan-2-ol	1327.5	1333.4	1339.3
b	0.286	0.288	0.287

index units of those shown in Table IV. Lastly, conductance measurements revealed the absence of any significant quantity of ionic material in either of the three samples.

As polyoxyethylene glycols are hydrophilic, another possible cause of the observed variations in retention is changes in the retention characteristics of the column resulting from the absorption of moisture. This and other possibilities mentioned earlier will now be considered in more detail.

The influence of moisture upon the retention characteristics of polyoxyethylene glycol columns

Whereas the retention characteristics of hydrophobic liquid phases would not be expected to be affected by the presence of moisture, those of hydrophilic liquid phases might be expected to change. Since the presence of moisture was thought to be a possible cause of the batch variations shown in Table IV, and as polyoxyethylene glycols are used extensively for the analysis of aqueous solutions, such as blood[20], wines and spirits[21], urine[22] and pharmaceutical preparations[23], it was decided to start with this possibility.

One of the columns used in the previous experiment was saturated by the passage of moist carrier gas at room temperature. After 24 h the saturator was removed and the column temperature raised as quickly as possible to 65° and chromatograms of suitable calibration solutions recorded. The temperature was then increased to 100° and further chromatograms obtained. In contrast to our normal procedure the mean carrier gas flow rate was determined, by means of a soap film bubble meter, at both operating temperatures. The adjusted retentions were measured and values for retention index and specific retention volume[24] calculated (see Table V). The latter units were included because a preliminary experiment revealed that both

TABLE V

THE EFFECT OF MOISTURE UPON THE RETENTION BEHAVIOUR OF POLYOXYETHYLENE GLYCOL COLUMNS

Celite columns, 5 ft. × 4 mm, 20% w/w polyoxyethylene glycol-400 (Union Carbide).

Substance	Temperature (°C)	Original column		Wet column		After drying	
		Vg	I	Vg	I	Vg	I
Methyl n-heptyl ether	65	199	1093.6	119	1091.3	203	1092.0
Allyl tert.-butyl sulphide	65	267	1135.0	157	1133.5	269	1133.3
1-Chloroheptane	65	354	1178.5	199	1176.2	359	1177.9
Methyl n-hexoate	65	552	1245.2	313	1244.7	560	1244.3
Dodec-1-ene	65	574	1251.6	325	1250.8	582	1251.8
2,4-Dimethylpentan-3-ol	65	741	1292.2	424	1290.8	752	1290.2
b		0.287		0.285		0.287	
Percentage standard deviation in Vg				42.5		1.5	
Equivalent percentage standard deviation in retention index				1.1		0.8	
n-Propylbenzene	100	143	1270.6	77	1268.3	147	1269.8
n-Valeronitrile	100	171	1304.7	92	1301.6	175	1302.2
1,3-Dimethylbut-2-enol	100	279	1394.9	151	1392.0	283	1392.9
n-Nonyl mercaptan	100	503	1504.9	274	1504.6	518	1504.2
trans-2-Methylpent-2-enol	100	571	1529.5	309	1526.5	584	1528.1
1-Bromodecane	100	878	1609.6	479	1608.4	910	1609.1
b		0.233		0.232		0.232	
Percentage standard deviation in Vg				44.3		2.8	
Equivalent percentage standard deviation in retention index				1.3		0.8	

the solutes and n-alkane standards were more rapidly eluted from a "wet" column than the original, even though nominally the same carrier gas flow rate was employed in both cases.

As can be seen from the data in Table V, contamination of the column with moisture caused almost insignificant changes in the values for retention index but dramatic changes in the corresponding values for absolute retention; the latter changes being virtually independent of the chemical nature of the solutes, for those examined. However, the specific retention volumes were restored to their former values, within the limits of experimental error, by drying the column by the passage of dry carrier gas for a period of 30 h at 100°.

As the operating variables were maintained constant throughout the above investigation, the reductions of absolute retention accompanying the introduction of moisture must arise from a reduction of the weight of liquid phase available for partitioning purposes. Hydrolysis, or other permanent chemical changes, can be discounted because the passage of dry carrier gas restored the column to its original condition. On the other hand, formation of a tightly bound hydrogen-bonded cross-linked network involving the polyoxyethylene and water molecules, with the latter acting as the cross-links, could account for the observed changes of retention. Such a network would be expected to exclude all but the most intensely polar solute molecules, which might possibly disrupt the network. Thus both moderately polar solutes

and *n*-alkane standards would be expected to travel more rapidly through the wet column, thereby giving rise to lower values for specific retention volume; calculated assuming no change in the effective weight of liquid phase had occurred. As the solutes and standards are equally affected, the relative retention ratios would be expected to exhibit no change, consistent with observation.

If the above explanation is correct, the retentions of strongly hydrophilic substances would be expected to increase relative to those of hydrophobic solutes, when a polyoxyethylene glycol column is contaminated with water.

A fresh column was prepared and the retention indices of a number of hydrophilic materials determined from chromatograms recorded at 42°. The column was then saturated with moisture at room temperature, the temperature again raised to 42°, and further chromatograms obtained. Apart from the introduction of moisture, the experimental conditions were kept constant.

As can be seen from the experimental results in Table VI the behaviour of hydrophilic solutes is clearly consistant with our hypothesis.

TABLE VI

CHANGES IN THE RELATIVE RETENTION RATIOS OF HYDROPHILIC SOLUTES CAUSED BY THE CONTAMINATION OF A POLYOXYETHYLENE GLYCOL COLUMN WITH MOISTURE

Celite columns, 20 % polyoxyethylene glycol-400 (Union Carbide), at 42°.

Substance	Values for retention index		δI
	Original column	Wet column	
Acetone	900	1041	+ 141
Methyl ethyl ketone	979	1087	+ 108
Methyl alcohol	1044	1242	+ 198
Dec-1-ene	1052	1054	+ 2
Acrylonitrile	1062	1120	+ 58
Acetonitrile	1082	1242	+ 160
Ethyl alcohol	1084	1275	+ 191
n-Propyl alcohol	1190	1246	+ 56
b	0.330	0.328	

Similar, albeit smaller, shifts of retention were noticed after the injection of several samples of a dilute aqueous solution of acetone and ethanol (shifts became apparent after the introduction of 10–20 injections of 5 μl volume) into the same column at 42°. Since polyoxyethylene glycol columns are used for the analysis of blood alcohol[25], such shifts of retention could have interesting legal ramifications. Therefore, the use of polyoxyethylene glycol columns for the analysis of aqueous solutions would seem to be less desirable than the use of hydrophobic solvents, such as silicone oil, supported on inert solids. If, however, adsorptive supports such as firebrick are used, changes of retention will accompany the introduction of moisture, but for a different reason. For instance, with a silicone fluid MS 550, firebrick column it was found that the retentions of polar solutes decreased with the introduction of moisture. As these reductions of retention were accompanied by reductions of peak asymmetry, it is almost certain that the shifts are due to the deactivation of the active sites upon the support surface by adsorbed water molecules.

The dependence of the retention characteristics of polyoxyethylene glycol liquid phases upon their hydroxyl contents

As stated earlier, commercial preparations of polyoxyethylene glycol of nominally the same average molecular weight contain slightly different hydroxyl contents. Since the hydroxyl group would be expected to play a more important role than the ether group in solute–solvent interactions, it is possible that the differences in retention reported in Table IV are due to the presence of slightly different quantities of hydroxyl in the three samples of polyoxyethylene glycol examined.

In order to obtain a clearer picture of the dependence of the retention characteristics of polyoxyethylene glycol columns upon hydroxyl content, columns were prepared using materials with nominal average molecular weights of 200, 400, 1000 and 4000. Celite from the same batch was used throughout in an attempt to avoid complications due to variability of support adsorptivity. After drying, chromatograms of suitable calibration solutions were recorded and values for retention index calculated. Unfortunately in this experiment it was found necessary to use a higher temperature than that used to determine the batch variability of polyoxyethylene glycol-400, because the material with an average molecular weight of 4000 gave rise to anomalous shaped peaks at 65°, presumably due to a combination of normal partition and gas–solid adsorption chromatography on the partially molten stationary phase.

For all the solutes examined the values for retention exhibited a marked dependence upon molecular weight, and by virtue of this hydroxyl content of the liquid phase. As can be seen from the data in Table VII the largest changes of retention occurred with those solutes possessing either hydrogen bond acceptor or donator properties or strong permanent dipoles. In fact, those compounds which would be expected to interact most strongly with the terminal hydroxyl groups of the stationary

TABLE VII

THE DEPENDENCE OF RETENTION INDICES UPON THE AVERAGE MOLECULAR WEIGHTS OF POLYOXYETHYLENE GLYCOL STATIONARY PHASES

Celite columns, 5 ft. × 4 mm, 20% w/w stationary phase, at 100°.

Stationary phase	Values for retention index			
	PEG-200	PEG-400	PEG-1000	PEG-4000
1-Bromoheptane	1349.5	1311.9	1287.5	1274.2
n-Butylbenzene	1418.0	1374.8	1346.1	1332.7
Di-n-hexyl ether	1434.9	1398.5	1374.5	1363.1
Tetradec-1-ene	1462.3	1457.1	1450.6	1448.3
Isoamyl alcohol	1482.1	1342.8	1254.4	1207.9
Crotyl alcohol	1505.3	1358.7	1268.0	1219.8
n-Amyl cyanide	1506.0	1410.0	1349.2	1316.5
Phenetole	1530.1	1464.4	1420.7	1398.8
Methyl n-octanoate	1535.5	1466.1	1423.4	1400.3
6-Methylhept-5-en-2-one	1539.8	1442.9	1381.9	1350.1
Iodobenzene	1646.0	1585.6	1541.5	1521.8
N,N-Dimethylaniline	1695.4	1622.2	1572.3	1547.4
Benzaldehyde	1728.5	1626.4	1562.3	1528.0
2-Ethylhexan-1-ol	1741.8	1622.3	1539.4	1495.5
b	0.219	0.232	0.242	0.246

phase. This fact suggests that the solution behaviour of the polyoxyethylene glycols is determined to a large extent by interactions between the terminal hydroxyl groups and the solute molecules.

Another interesting feature of the experimental results is the linear relationship between the values for retention index and the hydroxyl contents of the liquid phases; calculated assuming that the actual molecular weights are 200, 400, 1000 and 4000, respectively. Typical examples are shown in Fig. 1.
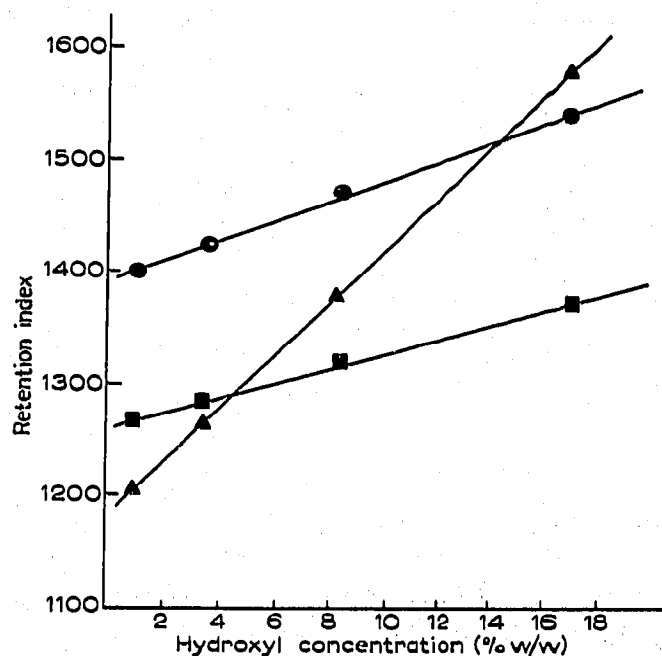

Fig. 1. The relationship between retention index and the hydroxyl content of polyoxyethylene glycol stationary phases for isoamyl alcohol ▲, methyl n-octanoate ● and 1-bromoheptane ■.

The origin of this linear dependence of retention index upon hydroxyl content is somewhat obscure. It could be due to a genuine linear dependence of free energy of solution upon hydroxyl content; in the same sense as the free energies of n-alkanes are a linear function of their chemical composition. On the other hand, as the hydroxyl groups of polyoxyethylene glycol are known to be adsorbed upon the active sites of diatomaceous earths[15], the linearity may be fortuitous, arising from the interplay of several factors. Notwithstanding this, the linear plots have proved useful, enabling the determination of the hydroxyl contents of the three samples of polyoxyethylene glycol-400 examined previously, by extrapolation of the straight lines drawn through the points for polyoxyethylene glycol 1000 and 4000. The data obtained (see Table VIII) is on the whole consistent with the differences between the three samples being due to differences of hydroxyl content. However, there are a number of notable exceptions, in particular iodobenzene and n-butylbenzene, which suggest the contribution of other factors.

Thus far the possibility that the differences between the retention behaviour of the three samples of polyoxyethylene glycol-400 are due to the presence of organic impurities had not been considered. Accordingly the absorption spectra were obtained in the infrared and ultraviolet regions. These revealed the absence in all three samples of aromatic and conjugated impurities ($< 0.01 \%$) and the absence ($< 1.0 \%$) of the

TABLE VIII

COMPARISON OF THE HYDROXYL CONTENTS OF SAMPLES OF POLYOXYETHYLENE GLYCOL GIVEN BY RETENTION MEASUREMENTS

Celite columns, 5 ft. × 4 mm, 20 % polyoxyethylene glycol-400, at 100°.

	Values for retention index			Hydroxyl content (% w/w) *		
	A**	B	C	A	B	C
1-Bromoheptane	1318.7	1311.9	1317.2	9.3	8.1	9.0
n-Butylbenzene	1381.1	1374.8	1380.0	10.0	8.7	9.7
Di-n-hexyl ether	1401.1	1398.5	1401.4	9.4	8.7	9.4
Isoamyl alcohol	1352.8	1342.8	1346.8	8.8	8.2	8.5
Crotyl alcohol	1369.7	1358.7	1364.4	8.8	8.2	8.5
n-Amyl cyanide	1419.7	1410.0	1416.8	8.9	8.1	8.7
Phenetole	1472.6	1464.4	1471.1	9.4	8.5	9.2
Methyl n-octanoate	1475.1	1466.1	1474.0	9.1	8.1	9.0
6-Methylhept-5-en-2-one	1452.0	1442.9	1449.8	9.0	8.3	8.9
Iodobenzene	1595.0	1585.6	1591.9	10.3	9.1	10.0
N,N-Dimethylaniline	1633.5	1622.2	1631.4	9.7	8.5	9.3
Benzaldehyde	1638.0	1626.4	1635.4	9.0	8.2	8.8
2-Ethylhexan-1-ol	1635.0	1622.3	1628.3	8.5	8.2	8.6
Mean values for % OH				9.2	8.4	9.1

* Hydroxyl content of PEG.400 8.5 % w/w.
** A = PEG.400 (Shell); B = PEG.400 (Merck); C = PEG.400 (Union Carbide).

likely impurity polyoxypropylene glycol. On the other hand the spectra revealed the absence of carbonyl impurities (< 0.1 %) in the Shell and Merck products but up to 2 % w/w of a saturated open chain ketone (assumed to have a molecular weight of 400) in the Union Carbide material. It is possible that this carbonyl impurity may account in some degree for the large scatter in the values for hydroxyl content obtained for the Union Carbide polyoxyethylene glycol-400 shown in Table VIII.

The dependence of the retention characteristics of polyoxyethylene glycol stationary phases upon their molecular weight distributions

CELADES AND PAQUOT[19] have shown that commercial samples of polyoxyethylene glycol-400 are poly disperse in terms of molecular weight. As this is likely to apply to the samples used in our investigation of the batch dependence of polyoxyethylene glycols, it is possible that the differences in retention reported in Table IV are in part due to differences of molecular weight distribution. Rather than repeat CELADES AND PAQUOT's analysis, it was decided to investigate the effect of gross differences of molecular weight distribution by comparing the retention characteristics of the Merck polyoxyethylene glycol-400 column with that prepared from a mixture of polyoxyethylene glycol-200 and 4000 containing the equivalent concentration of hydroxyl groups.

The experimental results (see Table IX) immediately suggest that differences of molecular weight distribution have but an insignificant effect upon the retention characteristics of polyoxyethylene glycol columns. In fact, it is possible that the differences which do exist between the two columns are due to the preferential loss of polyoxyethylene glycol-200 from the mixed column, particularly as repeat deter-

TABLE IX

THE EFFECT OF DIFFERENCES OF MOLECULAR WEIGHT DISTRIBUTION UPON THE RETENTION
CHARACTERISTICS OF POLYOXYETHYLENE GLYCOL COLUMNS

Celite columns, 20 % polyoxyethylene glycol-400, at 100°.

Substance*	Values for retention index		δI
	Normal	Mixed	
Isoamyl alcohol	1342.8	1330.6	−12.2
n-Amyl cyanide	1410.0	1400.6	− 9.4
Crotyl alcohol	1358.7	1346.0	−12.7
6-Methylhept-5-en-2-one	1442.9	1431.7	−11.2
n-Butylbenzene	1374.8	1367.5	− 7.3
Phenetole	1464.4	1453.8	−10.6
1-Bromoheptane	1311.9	1304.8	− 7.1
Methyl n-octanoate	1466.1	1455.9	−10.8
Iodobenzene	1585.6	1572.5	−13.1
N,N-Dimethylaniline	1622.2	1608.9	−13.3
Di-n-hexyl ether	1398.5	1392.7	− 5.8
2-Ethylhexan-1-ol	1622.3	1600.7	−21.6
Benzaldehyde	1626.4	1600.5	−25.9

* Solutes listed according to the sequence of experimental determination.

TABLE X

THE EFFECT OF DIFFERENCES OF MOLECULAR WEIGHT DISTRIBUTION UPON THE RETENTION
CHARACTERISTICS OF POLYOXYETHYLENE GLYCOL COLUMNS

Celite columns, 20 % polyoxyethylene glycol-400, at 65°.

Substance	Values for retention index*		δI
	Normal	Mixed	
Pentan-2-ol	1250.5	1248.5	−2.0
Benzene	994.6	992.0	−2.6
Pentan-2-one	1062.1	1059.9	−2.2
2-Methylhexan-2-ol	1327.5	1324.3	−3.2
Ethyl 4-methylpentanoate	1253.3	1248.4	−4.9
1-Chloro-octane	1282.8	1277.5	−5.3
Butan-1-ol	1279.5	1276.9	−2.6
Diethyl carbonate	1175.6	1171.2	−4.4

* Standard deviation 3.3 index units.

minations of the retention indices of the alcohols gave values which decreased progressively with column usage. A view which is substantiated by the results of a second experiment carried out at 65° (see Table X). These clearly reveal that the retention characteristics of polyoxyethylene glycols are virtually independent of their molecular weight distributions. Therefore, it should be possible to prepare polyoxyethylene glycol mixtures of any desired retention characteristics by the appropriate blending. Obviously this would enable the preparation of chromatographically standardised polyoxyethylene glycol liquid phases.

Our work has revealed that meaningful retention data can be obtained with homogeneous polar polymers such as the polyoxyethylene glycols. Further work,

however, revealed that this was no longer the case with copolymers where the two monomers differ widely in polarity. For instance, with the nitrilesilicones a linear relation between retention index and nitrile content was observed but the retention characteristics of blends were found to be completely anomalous. In each case the retention indices obtained with the mixed columns were much lower than would have been expected for the amount of nitrile present. As yet we are not in a position to put forward what we consider to be an adequate explanation for these observations. No doubt with further work, which is now in progress, one will be forthcoming. In view of its incompleteness this aspect of our work will not be discussed further at this stage. The above comments are merely included to warn the reader that not all polar polymeric stationary phases are as straightforward as polyoxyethylene glycol.

CONCLUSIONS

A scheme for the evaluation of gas chromatographic stationary phases has been formulated, involving the following steps:

(i) Determination of the constancy of retention data, in KOVATS' retention indices, with column usage over the range of temperature it is intended to use the liquid phase;

(ii) the determination of the dependence of retention data upon the origin of the material, and where possible an investigation of batch variability;

(iii) where appropriate, determination of the influence upon retention data of polymer end group concentration and molecular weight distribution;

(iv) study of the effect of the tacticity of stereo-regular polymers and heterogeneity of copolymers, where pertinent, upon retention data;

(v) the determination of the effect of the absorption of moisture and oxygen upon the retention characteristics of a column.

The application of the proposed scheme is illustrated by an assessment of the suitability of polyoxyethylene glycols for qualitative analysis.

As a result of this work it has been discovered that meaningful retention data can be obtained with polyoxyethylene glycol columns, and that the principal factors determining the retention characteristics of these columns, apart from the adsorptivity of the support, are the presence of moisture, the concentration of the hydroxyl end groups, and to a very much smaller degree the molecular weight distribution of the liquid phase.

The presence of moisture has been found to cause large changes of absolute retention which may be positive or negative depending on whether the solutes are hydrophilic or hydrophobic, respectively. With the latter the decreases of retention are almost uniform, so that values for relative retention are unaffected. The explanation afforded for these observations is that the introduction of moisture causes the formation of a hydrogen bonded cross-linked network, involving the water molecules as cross-links between the polyoxyethylene glycol molecules, which excludes all but the most strongly hydrophilic solutes.

The most important single factor upon which the retention characteristics of polyoxyethylene glycol liquid phases is dependent has been found to be the concentration of hydroxyl groups available for partitioning purposes. With celite as the support, phase values for retention index have been found to increase linearly with the hy-

droxyl concentration; the selectivity of the liquid phase decreasing with its molecular weight.

Molecular weight distribution on the other hand has been found to have an insignificant effect upon retention data. Therefore, it should be possible to produce liquid phases of any desired retention behaviour by the appropriate blending of different polyoxyethylene glycols, provided the overall molecular weight range is sufficiently small to preclude errors arising from the volatilisation of the lower molecular weight species during the preparation and use of the column.

REFERENCES

1 A. B. LITTLEWOOD, *J. Gas Chromatog.*, 1, No. 11 (1963) 16.
2 A. B. LITTLEWOOD, *Anal. Chem.*, 36 (1964) 1441.
3 A. B. LITTLEWOOD AND F. W. WILLMOTT, *Anal. Chem.*, 38 (1966) 1031.
4 A. B. LITTLEWOOD, *Gas Chromatography*, Academic Press, London and New York, 1962.
5 J. H. PURNELL, *Gas Chromatography*, John Wiley and Sons, London, 1962.
6 W. GERRARD, S. J. HAWKES AND E. F. MOONEY, in R. P. W. SCOTT (Editor), *Gas Chromatography 1960*, Butterworths, London, 1960, p. 263.
7 S. T. PRESTON, JR., *J. Gas Chromatog.*, 1, No. 3 (1963) 8.
8 J. F. SMITH, in R. P. W. SCOTT (Editor), *Gas Chromatography 1960*, Butterworths, London, 1960, p. 114.
9 R. P. W. SCOTT, in D. H. DESTY (Editor), *Vapour Phase Chromatography 1956*, Butterworths, London, 1956, p. 131.
10 E. KOVATS, *Helv. Chim. Acta*, 41 (1958) 1915.
11 R. FEINLAND, A. J. ANDREATCH AND D. P. COTRUPE, *Anal. Chem.*, 33 (1961) 991.
12 M. B. EVANS, *Ph. D. Thesis*, London University, 1967.
13 M. B. EVANS AND J. F. SMITH, *J. Chromatog.*, 6 (1961) 293.
14 M. B. EVANS AND J. F. SMITH, *J. Chromatog.*, 28 (1967) 277.
15 M. B. EVANS AND J. F. SMITH, *J. Chromatog.*, 30 (1967) 325.
16 S. Z. PERRY AND H. HIBBERT, *Can. J. Research*, 14B (1936) 77.
17 A. WURTZ, *Compt. Rend.*, 83 (1876) 1141.
18 S. Z. PERRY AND H. HIBBERT, *J. Am. Chem. Soc.*, 62 (1940) 2599.
19 C. CELADES AND C. PAQUOT, *Rev. Franc. Corps Gras.*, 9 (1962) 149.
20 J. E. FOX, *Proc. Soc. Expt. Biol. Med.*, 97 (1958) 236.
21 D. D. SINGER, *Analyst*, 91 (1966) 127.
22 R. BONNICHSEN AND M. LINTURI, *Acta Chem. Scand.*, 16 (1962) 1289.
23 L. BREALEY, D. A. ELVIDGE AND K. A. PROCTOR, *Analyst*, 84 (1959) 221.
24 A. B. LITTLEWOOD, C. S. G. PHILLIPS AND D. T. PRICE, *J. Chem. Soc.*, (1955) 1480.
25 A. S. CURRY, G. W. WALKER AND G. S. SIMPSON, *Analyst*, 91 (1966) 1088.
26 M. B. EVANS, *J. Chromatog.*, 11 (1963) 401.