GAS CHROMATOGRAPHY IN QUALITATIVE ANALYSIS

IV. AN INVESTIGATION OF THE CHANGES IN RELATIVE RETENTION DATA ACCOMPANYING THE OXIDATION OF APOLAR STATIONARY PHASES

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INTRODUCTION

The results of correlation trials conducted by the Gas Chromatography Discussion Group[1,2] have revealed that the reproducibility of relative retention data obtained by workers in different laboratories is markedly inferior to that of data obtained by an individual worker[3,4]. Possible reasons for the greater scatter of the former results are, slight differences in the adsorptivity of the support materials used (Celite in the first trial, Chromosorb G in the second), non-uniformity of column temperature, and with the apolar stationary phases perturbation of retention data as the result of oxidation. In order to determine the significance of these three effects in qualitative analysis, the roles of the support, column temperature, and stationary phase oxidation in retention measurements have been determined; the latter being the subject of this communication (the other phenomena will be discussed in subsequent papers).

EXPERIMENTAL

Apparatus

Chromatograms were obtained using an apparatus consisting of Pyrex glass columns (5 ft. long, 4 mm I.D.) with a modified flame ionisation detector[5]. The columns were packed with 20 % w/w mixtures of stationary phase (for details see text) and acid washed (1 % v/v phosphoric acid) 60–72 mesh Celite (J.J's Chromatography Ltd., King's Lynn, Norfolk). The column temperature was regulated by a vapour jacket consisting of boiling water (100°). A mixture of hydrogen and nitrogen (3:1 by volume) was used as carrier gas, except in the oxidation experiments. Mixtures of the solutes of interest and suitable *n*-alkane standards in either *n*-pentane or *n*-heptane solution (~ 5 % w/v) were introduced onto the column by means of stainless steel capillary pipettes[6].

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Measurement of relative retention data

Relative retentions, in KOVATS' retention index units[7], were obtained from chromatograms of suitable mixtures containing *n*-alkanes as internal standards. Retention distances, measured between point of injection and peak maxima, were corrected for the column gas hold-up volume by means of the retention of methane[8]. The adjusted retentions were then used to calculate values for retention index by means of the following expression:

$$I = 100\,N + 100\,n\left\{\frac{\log_{10}R_x - \log_{10}R_N}{\log_{10}R_{N+n} - \log_{10}R_N}\right\}$$

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

DISCUSSION AND RESULTS

Previous work carried out in the authors' laboratory has revealed that the apparent polarity, as indicated by values for retention, of apolar stationary phases frequently increases with column usage, particularly at elevated temperatures. In so far as it was found that this effect could be largely eliminated if care was taken to exclude air from the column it was tentatively ascribed to the oxidation of the liquid phase. In order to substantiate this hypothesis, squalane–Celite columns have been deliberately oxidised and the changes in polarity followed by retention measurements.

Selection of solutes suitable for use as physico-chemical probes

In order to select solutes which would reveal the chemical changes induced by oxidation, the retentions of compounds of a wide range of chemical types were determined using squalane containing known quantities of combined oxygen. Although the possibility of oxidising squalane to known extents was considered, this method was not used owing to the likelihood of the oxygenated groups being lost or modified during the preparation of the liquid phase–support mixtures. Instead, known mixtures of squalane and involatile, thermally stable, aliphatic oxygenated substances (namely, *n*-octadecanol and di-*n*-octyl ketone) were prepared by the following procedure:

(i) Appropriate quantities of squalane and *n*-octadecanol or di-*n*-octyl ketone (total 4.0 g in each case) were weighed into a 250 ml round bottomed flask, 30 ml of redistilled dichloromethane added and the solution warmed until the alcohol or ketone had dissolved fully.

(ii) Sixteen grams of Celite were then poured into the solution and the solvent carefully evaporated under a water pump vacuum; the flask being periodically immersed in warm water (approximately 50°) in order to compensate for the heat losses accompanying evaporation.

The columns were packed in the normal way and the final traces of solvent removed by the passage of carrier gas at 100°; the columns being deemed ready for use once a stable base-line had been obtained.

The retentions, in KOVATS' retention index units, of all the solutes examined were found to increase with the oxygen content of the mixed stationary phases, as shown in Table I. In each case the shifts of retention were found to vary linearly with
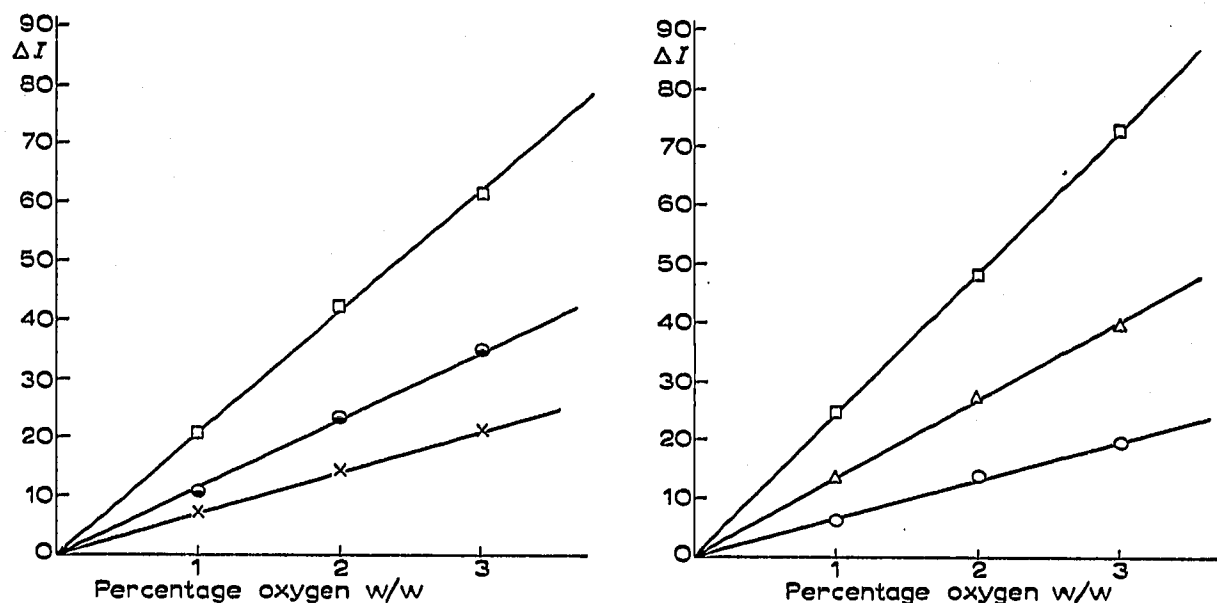
Fig. 1. Change of retention index with introduction of oxygen into a squalane column (as *n*-octadecanol). (□) = Nitrobenzene; (◉) = phenetole; (×) = *n*-butylbenzene.

Fig. 2. Change of retention index with introduction of oxygen into a squalane column (as di-*n*-octyl ketone). (□) = Nitrobenzene; (△) = 1,2-dibromoethane; (O) = toluene.

oxygen content, as illustrated by the typical examples in Figs. 1 and 2, indicating the possibility of determining the extent of oxidation of an "aged" apolar column from retention data. On this basis, the results for operator G, in the Gas Chromatography Discussion Group retention data correlation trial (see Table II in ref. 1), would suggest the presence of approximately 3 % of combined oxygen in the squalane used, provided that the groups formed on oxidation have similar retentive properties to alcoholic hydroxyl and ketonic carbonyl respectively, and that the contribution of the

TABLE I

CHANGES IN VALUES FOR RETENTION INDEX ACCOMPANYING THE INTRODUCTION OF OXYGEN, AS EITHER HYDROXYL OR CARBONYL GROUPS, INTO AN APOLAR STATIONARY PHASE

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

	Oxygen as *n*-octadecanol (% w/w)				Oxygen as di-*n*-octyl ketone (% w/w)		
	0	1.0	2.0	3.0	1.0	2.0	3.0
Toluene	756.4	764.5	771.4	779.0	762.4	770.0	775.8
1,2-Dibromoethane	783.9	798.9	811.3	821.9	797.2	811.2	823.3
Chlorobenzene	826.3	836.1	845.3	854.3	836.2	846.4	856.6
Allyl *tert.*-butyl sulphide	855.7	866.3	876.4	884.7	861.4	869.3	877.8
Benzonitrile	905.5	927.4	955.2	977.8	928.8	953.4	978.5
Camphene	947.4	949.7	952.4	955.6	948.2	949.7	950.3
Phenetole	953.7	964.3	976.9	988.5	961.0	972.6	982.4
trans-2,6-Dimethyl-octa-2,6-diene	978.4	982.1	985.6	988.3	980.1	982.0	985.4
Nitrobenzene	1008.0	1028.6	1050.2	1069.3	1032.4	1056.1	1080.8
1-Iodohexane	1011.8	1019.8	1028.0	1034.8	1019.2	1026.3	1035.4
n-Butylbenzene	1035.2	1042.8	1049.5	1056.0	1040.6	1047.6	1054.5
Di-*n*-butyl sulphide	1060.0	1069.5	1078.4	1085.6	1064.8	1071.4	1078.0

support to retention is negligible (see Table II). The inclusion, in this investigation, of materials containing strongly polar functional groups, such as alcohols, ketones, and esters, was precluded by severe peak tailing; presumably due to an adsorption phenomenon*.

On the basis of the results given in Table I, toluene, 1,2-dibromoethane, benzonitrile, phenotole, nitrobenzene, and allyl *tert.*-butyl sulphide were chosen as physico-chemical probes for the oxidation experiments. The sulphide was later withdrawn from this list because it was found to be oxidised, presumably to either the corresponding sulphoxide or sulphone, by oxidised squalane.

Changes of retention induced by the deliberate oxidation of a squalane–Celite column

A fresh squalane–Celite column was prepared and the retention indices of the compounds chosen as physico-chemical probes determined at 100°, by the established procedures. The column was then oxidised, as follows:

(i) The cracked ammonia carrier gas supply (H_2–N_2, 3:1) was replaced by oxygen by the appropriate adjustment of a three-way tap (connecting the column, and the oxygen and cracked ammonia cylinders, respectively).

(ii) The oxygen was allowed to flow for approximately 10 min, during which time the rate of flow was monitored continuously by means of a soap film bubble meter (in order that the volume of oxygen passing through the column could be determined).

(iii) The oxygen flow was then replaced by the hydrogen–nitrogen mixture, and the chromatograms of suitable calibration solutions of the solutes shown in Table III and suitable *n*-alkane internal standards were obtained.

(iv) The oxygen flow was resumed and the cycle repeated.

Diatomaceous earths, which are widely employed as gas chromatographic supports, have relatively high surface areas[9] (Celite ~ 1 sq.m/g) so that the average film thickness of the liquid phase in a normal packed column is only about 0.2 μ. It is therefore probable that, in the presence of oxygen at elevated temperatures, apolar stationary phases would undergo oxidation to yield oxygenated groups, which in the bulk liquid phase would give rise to increased forces of solute–solvent inter-

TABLE II

DETERMINATION OF THE EXTENT OF OXIDATION OF THE SQUALANE COLUMN USED BY OPERATOR G, IN THE G.C.D.G. CORRELATION TRIAL[a], FROM VALUES FOR RETENTION INDEX
Column: 5 ft. × 4 mm 20% w/w stationary phase, Celite at 100°.

	Values for retention index			Oxygen content (% w/w)[a]	
	Operator G	Operators C.E.H. (mean value)	δI	$> C = O$	—OH
Toluene	776	756	20	2.45	3.10
Chlorobenzene	855	827	28	3.05	2.90
Di-*n*-butyl sulphide	1079	1059	20	2.05	3.25

[a] The oxygen contents were determined by interpolation on the appropriate plots of retention index change *versus* percentage combined oxygen.

* Subsequent work (to be described in Part VII of this series) revealed that meaningful chromatograms could be obtained for apolar stationary phases provided low surface area, chemically inactive supports, such as Chromosorb G, were used.

action, and in turn increased values for retention index. However, when oxygen was passed through a squalane–Celite column at 100° the retention indices of all the solutes, with the exception of toluene and 1,2-dibromoethane, were found to decrease, pass through a minimum value, and then increase*. In each case, the decreases in retention were accompanied by improvements of peak symmetry, the peaks being symmetrical at the minimum and beyond. The changes in the values for retention index induced by oxidation are summarised in Table III and illustrated by the typical examples shown in Fig. 3.
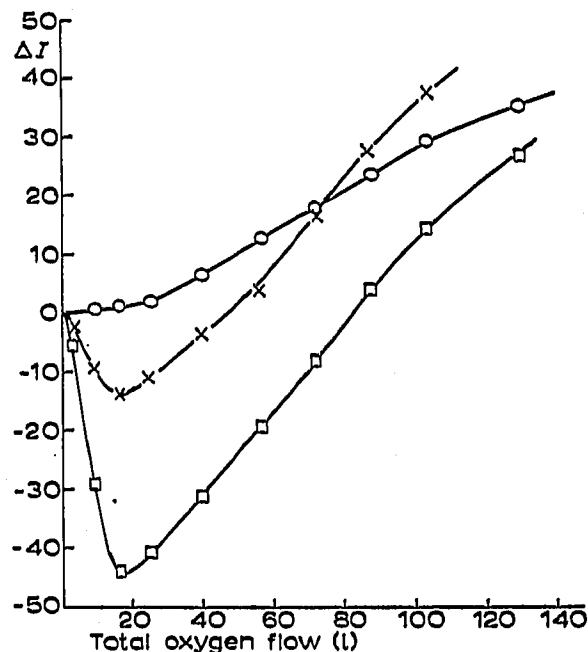

Fig. 3. Changes of retention index observed during the oxidation of a squalane column at 100°. (O) = 1,2-Dibromoethane; (□) = benzonitrile; (×) = nitrobenzene.

A likely explanation of these changes of retention is that oxidation of squalane, or impurities in the material supplied for use in gas chromatography, gives rise to strongly polar groups which are preferentially adsorbed on to the support surface, thus precluding the adsorption of the solute molecules. The gradual formation of a coherent protective film would thus give rise to the observed peak symmetry and retention changes; it being assumed that the peak asymmetry (tailing) observed in the case of the squalane column prior to oxidation was a consequence of adsorption of solute molecules upon active sites on the support surface. Once the adsorptive sites are saturated, the excess oxygenated groups would then be available for partitioning purposes, in the bulk liquid phase, thus increasing the apparent polarity of the liquid phase, consistent with the observed values for retention. On this evidence the minimum of the curve may be regarded as the retention index characteristic of the simple partitioning of the solute molecules between the mobile gas phase and pure squalane. Whilst the increases to the left of the minimum may be regarded as the contributions

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* This characteristic behaviour was not detected in the case of the squalane–n-octadecanol and squalane–di-n-octyl ketone mixtures, presumably because of the relatively high proportion of oxygenated material in the mixtures. However, later work has revealed that minima do occur on the plots of retention index versus oxygen content provided the concentration of combined oxygen is in the region of 0.1 to 0.3 %[10].

TABLE III

CHANGES OF RETENTION ACCOMPANYING THE OXIDATION OF A SQUALANE–CELITE COLUMN AT 100°

Substance	Values for retention index												
Toluene	756.4*	756.1**	757.3	756.6	756.7	756.8	760.0	762.3	764.7	767.5	771.0	774.2	778.3
1,2-Dibromoethane	783.9	783.1	782.9	783.7	784.4	784.9	789.7	796.1	800.7	806.6	812.4	818.5	825.7
Benzonitrile	905.5	935.8	930.6	906.0	891.8	895.1	904.5	916.4	917.8	939.9	950.1	962.8	979.8
Phenetole	953.7	952.7	952.1	950.4	949.4	951.0	954.8	957.9	964.3	969.3	974.0	979.7	987.1
Nitrobenzene	1008.0	1017.5	1015.2	1008.2	1003.7	1006.8	1014.0	1021.0	1034.7	1045.1	1055.0	1066.2	1081.3
Volume of oxygen passed (l)	Nil		3.4	9.8	16.2	24.9	39.8	56.5	72.2	87.7	104.0	130.3	166.8

* Original column used in calibration work.
** New squalane–Celite column prepared using a fresh batch of Celite.
   The differences between these two sets of retention data are almost certainly due to presence of more active sites on the surface of the second batch of Celite.

to the overall retention due to the adsorption of solute molecules at the liquid–solid interface. Adsorption at the gas–solid interface may be discarded, in this context, since two 10 % squalane–Celite columns, one prepared by the normal slurry method and the other by mixing appropriate quantities of Celite and a 20 % squalane–Celite mixture, gave identical retention and peak symmetry characteristics for a wide range of solutes, thereby revealing squalane to be sufficiently mobile to completely cover the support surface.

TABLE IV

COMPARISON OF THE RETENTION CHARACTERISTICS OF A NORMAL SQUALANE–CELITE COLUMN WITH ONE CONTAMINATED WITH SILICA GEL

Column A: 20 % w/w squalane–Celite at 100°.
Column B: 20 % w/w squalane–0.1 % w/w silica gel–Celite at 100°.

	Values for retention index			
	Column A		Column B	
	Initial	Minimum	Initial	Minimum
Toluene	756	—*	758	756
1,2-Dibromoethane	783	—*	786	784
Benzonitrile	936	891	956	894
Phenetole	953	949	960	950
Nitrobenzene	1017	1002	1028	1004

* No minimum detected.

If the above arguments were correct, contamination of Celite with a highly adsorptive material, such as silica gel, would be expected to give rise to increased initial values for retention index for polar solutes, but not weakly polar solutes, but with the same minimum values on the plots of retention index *versus* oxygen flow, as a normal squalane–Celite column. Oxidation of a squalane column prepared using Celite deliberately contaminated with silica gel gave rise to this predicted behaviour, as illustrated by the data in Table IV, thus confirming our hypothesis. Accordingly there appears to be little doubt that in the case of apolar liquid phases adsorption of solute molecules at the liquid–solid interface can contribute significantly to the overall values for retention. This phenomenon will be explored further in a future paper in the present series.

SUMMARY

Oxidation of an apolar stationary phase has been shown to lead to large changes of values for retention index, which can be either negative or positive depending upon the extent of oxidation, the chemical nature of the solutes, and the adsorptivity of the support. Therefore it would seem necessary to deoxygenate apolar phases, by conventional column chromatography, prior to preparing liquid phase–support mixtures and ensure the exclusion of oxygen during the use of the column, in order to obtain reproducible retention data. Furthermore, it would seem necessary to use supports of low adsorptivity, which as far as possible are batch invariant.

The results of this work also reveal that adsorption of solutes at the liquid–solid interface can lead to increased values for retention index with concomitant peak asymmetry.

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