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Gas chromatography in qualitative analysis

XX. The deactivation of diatomaceous supports by batyl alcohol

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ABSTRACT

Treatment of diatomaceous supports by the naturally occurring 1,2-diol batyl alcohol is shown to prevent problems due to the adsorption of polar analytes, at the liquid–solid interface, in packed column gas–liquid chromatography. The addition of small quantities of batyl alcohol is found to deactivate diatomaceous supports effectively, as judged by peak symmetry measurements and a study of the dehydration of tertiary aliphatic alcohols by stop-flow chromatography. The protection provided by batyl alcohol is shown to be equivalent to that afforded by diglycerol, although not as efficient at elevated temperatures. Batyl alcohol is found to be more effective as a deactivator than the equivalent *n*-alkanol behenyl alcohol. The relative merits of *n*-alkanols, alkane-1,2-diols and polyoxyethylene glycols are considered and the deactivation process discussed.

INTRODUCTION

In gas–liquid chromatography the adsorption of solute molecules at the liquid–solid interface can lead to shifts of retention [1], with concomitant peak asymmetry, and in extreme cases solute isomerism or decomposition [2]. The detrimental effects of this phenomenon upon the reliability of analytical results has long been appreciated and a number of methods have been developed for the deactivation of diatomaceous support materials, including: (i) acid [3] or base [4] washing; (ii) coating with carbon [5], a noble metal [6] or an inert polymer [7]; (iii) partial sintering at elevated temperatures [8]; (iv) chemical deactivation of the active sites by conversion to apolar silyl ethers [9]; (v) saturation of the surface adsorptivity by a surfactant [10]; and (vi) deposition of a non-extractable film of Carbowax 20M [11].

When carried out under carefully controlled conditions each of these methods is capable of preventing solute adsorption. However, on a routine laboratory scale it has been our experience that the use of surfactants is capable of reliable results more consistently than the other methods. In particular, outstanding results have been achieved with the use of polyols, such as diglycerol [12].

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Batyl alcohol, DL-3-octadecyloxy-1,2-propanediol, is a contaminant of naturally occurring squalene, which is the precursor of the commonly used apolar phase squalane. During the hydrogenation of squalene, and the subsequent cleanup of the product, the batyl alcohol is completely removed [13]. Batyl alcohol is structurally related to diglycerol, thus it would seem that a potential tail-reducer provided by nature is inadvertently lost. In order to test this hypothesis the work, now to be described, was performed.

EXPERIMENTAL

Equipment

Gas chromatography was performed using a Series 204 gas chromatograph equipped with glass columns and flame-ionization detection (Philips Analytical, Cambridge, U.K.). The columns (2 m × 4 mm I.D.) were packed with 20% (w/w) mixtures of stationary phase (for details see text) and non-acid-washed 60–80 BS mesh Chromosorb P (Phase Separations, Deeside, Clwyd, U.K.). The packings were prepared by a two-stage slurry technique [12] and purged at 20°C with nitrogen prior to use. The following operating conditions were employed: column temperature, 100°C (except where stated); injection temperature, 120°C; detector temperature, 150°C; and carrier gas nitrogen, at a flow-rate of 40 ml/min. Mixtures of test solutes and *n*-alkane standards, dissolved in an inert solvent, were introduced by means of SGE microsyringes (Scientific Glass Engineering, Milton Keynes, UK).

Stop-flow chromatography

In order to monitor the dehydration of *tert.*-alkanols the chromatograph was modified by the introduction of a simple Hoke toggle valve into the carrier gas pipework, between the nitrogen cylinder regulator and the pressure control module. Mixtures of *tert.*-alkanols and *n*-alkane standards were introduced into the chromatograph and the carrier gas flow stopped, by closure of the toggle valve, once the solutes were judged to be half-way down the column.

After a predetermined time the flow of nitrogen was resumed and chromatograms were recorded in the normal way. Generally it was found to be necessary to re-ignite the hydrogen flame of the detector on resumption of the carrier gas flow.

Materials

The stationary phases used were obtained from Jones Chromatography (Hengoed, U.K.). The test solutes and *n*-alkanes were obtained from BDH (Poole, U.K.) or Aldrich Chemicals (Gillingham, U.K.) and were used as supplied. The batyl and benenyl alcohols, used as deactivators, were obtained from Aldrich Chemicals, neither were further purified before use.

RESULTS AND DISCUSSION

Previously it has been suggested that the deactivator molecule should be capable of directing polar functional groups towards the active sites on the support surface whilst presenting an apolar surface towards the liquid phase [12], as illustrated in Fig. 1.

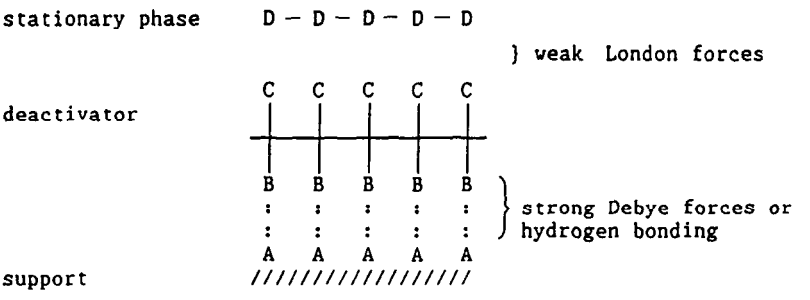

Fig. 1. Direction of polar functional groups. A = Silanol, SiOH, active sites on the surface of diatomaceous supports; B = pendant polar groups (amino, carboxyl, hydroxyl, nitrile, nitro) strongly bonded to active sites; and C = apolar groups (methyl, methylene, phenyl), weak association with stationary phase (D).

n-Alkanols and alkane-1,2-diols would be expected to yield protective films due to strong hydrogen bonding between the hydroxyl groups and the acidic silanol groups on the support surface (Fig. 2).

Diglycerol has been found to be particularly effective as a support deactivator, however, excess diol can cause significant shifts of retention, particularly for hydroxylic analytes. Batyl alcohol, which possesses a terminal 1,2-diol group together with a long alkyl chain, might be expected to be as effective as diglycerol as a deactivator but with less effect upon the overall selectivity of the column.

In order to assess the tail-reducing properties of batyl alcohol a series of experiments were performed as follows: (i) measurement of peak asymmetry values for

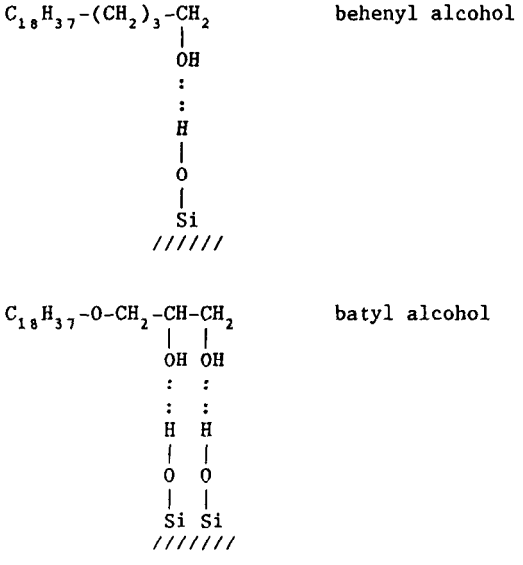

Fig. 2. Hydrogen bonding between hydroxyl groups of behenyl and batyl alcohol, respectively, and silanol groups of the solid support.

a range of polar solutes as a test of support adsorptivity; (ii) determination of the degree of decomposition of compounds, which readily undergo acid-catalysed elimination reactions, by stop-flow chromatography; (iii) study of the thermal stability of the protective film; and (iv) investigation of the retention characteristics of the deactivated columns, in particular variations of retention with changes of deactivator concentration.

Study of peak symmetry

The adsorption of solute molecules at the liquid–solid interface results in the distortion of the normally symmetrical peaks typical of linear non-ideal chromatography. When the liquid–solid adsorption is characterised by a convex isotherm, as is normally the case, a distinctive tailing peak is observed. The degree of peak distortion may be defined quantitatively by the peak asymmetry factor [14], *viz.*,

$$P_{\text{asym.}} = \frac{W_1 - W_2}{W_1 + W_2}$$

where W_1 and W_2 are the component peak widths at base (in order of increasing retention) measured either side of a perpendicular through the peak maximum. Thus a $P_{\text{asym.}}$ value of zero corresponds to a symmetrical peak, a positive value to a leading peak and a negative value to a tailing peak. Furthermore, the greater the numerical value the more severe is the peak distortion.

Of the commonly available diatomaceous supports the firebrick type are the most adsorptive [15]. Accordingly, squalane columns were prepared using Chromosorb P, deactivated by pretreatment with either batyl alcohol or behenyl alcohol, and $P_{\text{asym.}}$ values measured for solutes possessing a range of polar functional groups. In order to achieve a meaningful comparison of the adsorptivity of the various columns, steps were taken to ensure the introduction of the test solutes at a constant on-column sample size.

As expected the column prepared using untreated Chromosorb P gave highly distorted peaks for all solutes with the exception of *n*-alkanes. On the other hand, that treated with batyl alcohol gave substantially symmetrical peaks, in all cases, indicating that the surface silanols had been effectively deactivated. In contrast the more polar solutes gave rise to tailing peaks with the equivalent behenyl alcohol column, as illustrated by the peak asymmetry data in Table I. As can be seen, even when the concentration of behenyl alcohol was increased to give the equivalent hydroxyl content to that of the batyl alcohol treated column the peak tailing persisted. At first sight the results for 2-methylpentan-2-ol appear to be inconsistent. This we believe could be associated with partial on-column decomposition occurring on the behenyl alcohol column leading to perturbation of the $P_{\text{asym.}}$ values.

On the evidence of the peak symmetry experiments it would seem that batyl alcohol is a more efficient deactivator than behenyl alcohol. Presumably because it is able to cope more effectively with vicinal silanol groups.

Study of on-column reactions

The adsorption of solute molecules upon the support surface can lead to solute isomerisation or decomposition. This phenomenon has been used to compare the

TABLE I

PEAK ASYMMETRY VALUES FOR BATYL ALCOHOL AND BEHENYL ALCOHOL TREATED SQUALANE–CHROMOSORB P COLUMNS

Columns: I = 20% squalane, 1% batyl alcohol, Chromosorb P; II = 20% squalane, 1% behenyl alcohol, Chromosorb P; III = 20% squalane, 2% behenyl alcohol, Chromosorb P.

Solute	$P_{asym.}$		
	I	II	III
Anisole	0.00	−0.05	0.00
n-Butyl acetate	−0.07	−0.44	−0.11
n-Butyl cyanide	0.00	−0.67	−0.30
Hexane-2-one	0.00	−0.55	−0.08
Isopropylbenzene	0.00	−0.07	−0.03
2-Methylpentan-2-ol	+0.09	−0.18	−0.23
Pentan-1-ol	−0.08	−0.48	−0.09

adsorptivity of support materials, and the effectiveness of deactivation procedures through: (i) detection of additional peaks due to volatile decomposition products together with measurements of peak distortion [16]; (ii) measurements of changes of peak areas, relative to those of *n*-alkanes, with elevation of column temperature [15]; (iii) monitoring the on-column decomposition of compounds, susceptible to acid-catalysed reactions, by stop-flow chromatography; and (iv) spectroscopic analysis of the solute before and after chromatography [2].

In our experience the third of these methods is the most reliable and is applicable equally well to gas chromatography, normal-phase liquid chromatography and packed-column supercritical fluid chromatography [17]. Accordingly the respective efficiency of batyl alcohol and behenyl alcohol deactivation was tested by a study of the acid-catalysed dehydration of 2-methylpentan-2-ol and 3-methylpentan-3-ol, which proceeds via an initial protonation followed by the elimination of water to yield alkene mixtures [18].

Test mixtures of the tertiary alcohols and *n*-octane as internal standard were chromatographed and relative peak areas measured for a range of column residence times. With the untreated squalane, Chromosorb P column the alcohol peaks disappeared rapidly with residence time, due to on-column reaction. In contrast the *tert.*-alkanols remained unchanged on the batyl alcohol column for residence times up to 60 min, as shown by the results in Table II. With the behenyl alcohol column evidence of on-column decomposition was discernible after only 10 min, even with the 2% loading.

As expected peak widths increased with column residence time as indicated by the relative plate values shown in Table III. Whereas with open-tubular columns peak dispersion during stop-flow chromatography can be a problem [19], in the case of packed-columns the peak dispersion is much less than might have been expected for a gaseous mobile phase. For this reason packed-column gas chromatography would appear to be an ideal vehicle for the study of a wide range of chemical reactions by the stop-flow technique.

TABLE II

INVESTIGATION OF THE EFFICIENCY OF BATYL ALCOHOL AND BEHENYL ALCOHOL PROTECTIVE FILMS BY THE STOP-FLOW CHROMATOGRAPHY OF *tert.*-ALKANOLS

Columns: I = 20% squalane, 1% batyl alcohol, Chromosorb P; II = 20% squalane, 1% behenyl alcohol, Chromosorb P; III = 20% squalane, 2% behenyl alcohol, Chromosorb P.

Solute	Residence time[a] (min)	Peak area ratio[b]		
		I	II	III
2-Methylpentan-2-ol	0	1.00	1.00	1.00
	10	1.00	0.60	0.94
	20	1.01	0.40	0.84
	30	1.02	0.24	0.77
	40	1.01	0.19	0.66
	50	1.01	–	0.61
	60	1.00	–	0.52
3-Methylpentan-3-ol	0	1.00	1.00	1.00
	10	1.02	0.53	0.89
	20	0.99	0.38	0.80
	30	1.00	0.17	0.76
	40	1.00	0.12	0.68
	50	1.00	–	0.61
	60	1.01	–	0.57

[a] Residence time correspond to the period of time during which the carrier gas flow was stopped.
[b] *n*-Octane used is internal standard. Peak area ratios expressed relative to the value for zero residence time.

TABLE III

DEPENDENCE OF COLUMN PLATE VALUE UPON RESIDENCE TIME IN STOP-FLOW GAS CHROMATOGRAPHY

Columns: I = 20% squalane, 1% batyl alcohol, Chromosorb P; II = 20% squalane, 2% behenyl alcohol, Chromosorb P.

Solute	Residence time[a] (min)	Relative plate number[b]	
		I	II
n-Octane	0	1.00	1.00
	10	0.81	0.79
	20	0.75	0.76
	30	0.67	0.70
	40	0.59	0.61
	50	0.52	0.54
	60	0.43	0.48

[a] Residence time corresponds to the period of time during which the carrier gas flow was stopped.
[b] Relative plate numbers expressed relative to the value for zero residence time — initial plate numbers 1971 and 1652, respectively.

Thermal stability of the protective film

The thermal stability of a gas chromatographic liquid phase may be determined by thermogravimetry [20], measurement of detector standing current [21] or relative retention measurements [22]. Each of the methods was used to determine the stability of diglycerol deactivated supports [12] and it was concluded that the third was the most appropriate. Accordingly the respective stabilities of the batyl alcohol and behenyl alcohol protective films were determined by retention index measurements [22], using a thermally stable silicone fluid as stationary phase.

As described previously [12], the columns were cycled daily between 100 and 200°C and the retention characteristics monitored by recording chromatograms of appropriate calibration mixtures at 100°C. In contrast to diglycerol deactivated columns, both the batyl alcohol and behenyl alcohol treated columns displayed shifts of retention indicative of the loss of the protective film.

At 150°C, however, the batyl alcohol deactivated column gave satisfactorily stable columns, as illustrated by the data in Table IV. The thermal stability of the behenyl alcohol protected column was somewhat inferior. With the hydroxylic solutes the values for retention index were found to decrease, pass through a minimum value and then increase. Since these increases of retention index were accompanied by peak tailing it would seem that the loss of behenyl alcohol was sufficient to give analyte molecules access to surface silanols.

TABLE IV

INVESTIGATION OF THE THERMAL STABILITY OF BATYL ALCOHOL AND BEHENYL ALCOHOL SURFACE COATINGS

Temperature: 150°C. Columns: I = 17% OV-17, 3% batyl alcohol, Chromosorb P; II = 17% OV-17, 3% behenyl alcohol, Chromosorb P.

Solute	Column	Retention index[a]						
		0 h	5 h	10 h	15 h	20 h	25 h	30 h
Isopropyl acetate	I	721	721	721	720	720	720	721
2-Methylpropan-1-ol	I	721	725	725	724	725	724	722
3-Methylbutan-2-one	I	748	747	746	746	746	746	745
Butan-1-ol	I	771	772	769	770	771	766	770
Pentan-1-ol	I	882	882	882	884	885	885	884
n-Butyl cyanide	I	906	906	906	905	905	905	904
Isopropyl acetate	II	721	720	719	722	723	723	725
2-Methylpropan-1-ol	II	714	712	711	714	717	720	724
3-Methylbutan-2-one	II	748	745	745	746	748	748	749
Butan-1-ol	II	763	759	762	768	776	783	792
Pentan-1-ol	II	872	867	869	874	884	889	898
n-Butyl cyanide	II	901	900	900	902	904	906	909

[a] Retention index calculated using the expression:

$$I = 100\,N + 100\,n \cdot \frac{\log R_x - \log R_N}{\log R_{N+n} - \log R_N}$$

where R_x, R_N and R_{N+n} are the adjusted retentions, obtained using methane as void volume marker [23], of the analyte and *n*-alkane standards containing N and $N + n$ carbon atoms, respectively.

TABLE V

THE EFFECT OF BATYL ALCOHOL DEACTIVATOR CONCENTRATION UPON THE RETENTION BEHAVIOUR OF SQUALANE COLUMNS

Columns: I = 18% squalane, 2% batyl alcohol, Chromosorb P; II = 16% squalane, 4% batyl alcohol, Chromosorb P.

Solute	Retention index			
	I	II	δI^a	$\delta I'^b$
Toluene	763	769	6	1
4-Methylpent-3-en-2-one	765	784	19	4
1,2-Dibromethane	800	811	11	3
Chlorobenzene	837	846	9	1
3-Bromopropan-1-ol	900	954	54	54
Benzonitrile	927	951	24	10
Phenetole	963	974	11	1

[a] $\delta I = I_{4\%} - I_{2\%}$.
[b] $\delta I'$ = change in retention index for equivalent diglycerol columns.

The differences between the thermal stability of the diglycerol, batyl alcohol and behenyl alcohol protected columns may be explained in terms of the potential points of attachment between deactivator and support. With diglycerol both diol groups may act as ligands, so that to remove the protective film up to four hydrogen bonds need to be broken, whereas with batyl alcohol and behenyl alcohol only two hydrogen bonds and a single hydrogen bond, respectively, need to be cleaved.

Effect of batyl alcohol pretreatment upon the retention behaviour of apolar columns

On the evidence of previous work [1] batyl alcohol pretreatment might be expected to affect the retention behaviour of apolar columns in either of two ways, depending upon the activity of the support and the deactivator concentration, firstly, by preventing the adsorption of solute molecules and secondly by contributing to the partition process.

In order to determine the magnitude of the latter effect, which from an analytical standpoint is the more important, squalane columns were prepared using batches of Chromosorb P coated with 2 and 4% (w/w), respectively, of batyl alcohol. After careful conditioning the retention behaviour of each column was determined by measuring the values for the retention index of a range of solutes. The results obtained, which are shown in Table V, indicate that the addition of batyl alcohol has little effect upon phase selectivity, except towards hydroxylic solutes. In general the shifts of retention are slightly greater than those observed with diglycerol but less than those for amine antioxidant deactivated columns [24]. These observations suggest that batyl alcohol approaches the behaviour of the ideal deactivator by providing a compact polar layer on the support surface with an apolar overlayer in contact with the stationary phase.

CONCLUSIONS

Pretreatment of diatomaceous supports with hydroxylic compounds has been found to lead to apolar columns equally suitable for the analysis of both apolar and polar solutes. In general alkane-1,2-diols, such as batyl alcohol, appear to be more effective than monohydroxyl compounds, presumably because the former are able to chelate more effectively the surface silanols. On this evidence it is interesting to speculate about the apparent efficiency of polyoxyethylene glycols [25] as support deactivators. Because of their low hydroxyl content, interactions in addition to those involving the terminal hydroxyl groups need to invoked. One possibility is the interaction between segments of the polyoxyethylene chain, oriented in the form of a crown ether, and alkali metal ions held electrostatically on the support surface. Work is in progress to test this hypothesis and the results will be presented in a later communication.

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