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GAS CHROMATOGRAPHY IN QUALITATIVE ANALYSIS

XV. DEACTIVATION OF DIATOMACEOUS SUPPORTS BY AMINE ANTI-OXIDANTS

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SUMMARY

Errors in qualitative gas chromatographic analysis due to the oxidation of apolar stationary phases and the adsorption of polar solutes are reduced by pre-treatment of diatomaceous supports with amine antioxidants. Addition of small quantities of N,N'-disubstituted *p*-phenylenediamines to column packings is shown to prevent peak tailing, due to the adsorption of polar solute molecules at the liquid–solid interface, and solute decomposition. The protective film is shown to have a slightly superior thermal stability to that provided by diglycerol but to have a greater influence upon retention data.

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 or base washing, (ii) coating with carbon[3], a noble metal[4] or an inert polymer[5], (iii) partial sintering at elevated temperatures[6], (iv) chemical deactivation of the active sites by conversion to apolar silyl ethers[7], (v) saturation of the surface adsorptivity by a surfactant[8].

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, outstandingly successful results have been obtained using polyols, such as diglycerol[9].

A further complication that can arise with apolar columns is oxidative degradation of hydrocarbon liquid phases. Oxidation leads to the introduction of polar functional groups and evolution of volatile scission products, which lead to shifts of retention and detector standing current[10]. The deleterious effects of oxidation can be prevented by either the total exclusion of oxygen from the column or more simply by addition of an antioxidant. Recently it has been demonstrated that N,N'-disubstituted *p*-phenylenediamines afford excellent protection, even for readily oxidised liquid phases such as squalene[11].

The gas chromatography of amines is generally associated with severe peak tailing owing to their ready adsorption onto the support active sites. Therefore, the pretreatment of diatomaceous supports by an amine antioxidant might be expected to preclude solute adsorption in addition to preventing errors due to oxidation. The tail-reducing properties of typical amine antioxidants has been investigated and the results of this work are now reported.

EXPERIMENTAL

Gas chromatograms were obtained by means of a Philips PV 4000 gas chromatograph equipped with glass columns and flame ionization detection. The columns (3 m × 4 mm I.D.) were packed with a 10% w/w mixture of stationary phase (for details see text) and either non acid-washed Chromosorb W or hydrochloric acid-washed Diatomite S. The packings were prepared by a two-stage slurry technique[9] and purged continuously with oxygen-free nitrogen carrier gas until required for use. Mixtures of test solutes and *n*-alkane standards, dissolved in an inert solvent, were introduced by means of SGE microsyringes.

Relative retentions, in Kováts' retention index units, were obtained from chromatograms of calibration mixtures containing *n*-alkanes as internal standards[12]. Adjusted retentions were measured between individual peak maxima and that of methane, added as void volume marker[13], and values for I and b calculated by means of a PDP-10 computer using a simple programme in BASIC[14].

The antioxidants used in this work, which were kindly supplied by I.C.I. Ltd. (Organics Division), were purified by recrystallisation.

RESULTS AND DISCUSSION

In order that oxidation-resistant apolar columns of high intrinsic efficiency and reproducible retention characteristics may be prepared, irrespective of the adsorptivity of the support phase, the materials intended for use as deactivators should be:

(i) efficient oxidation inhibitors which are readily available in a reasonable state of purity,

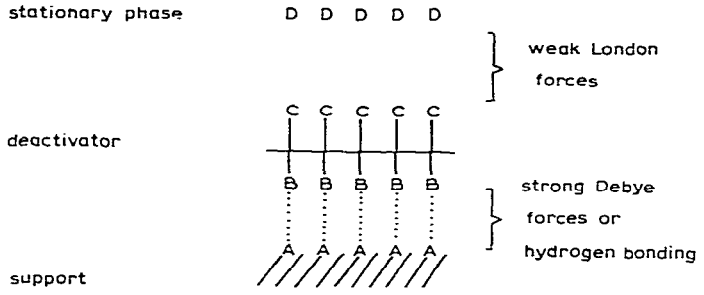
(ii) polar liquids, or low-melting solids, with a high affinity for the active sites of the support and sufficient mobility to form a coherent protective film,

(iii) thermally stable up to the limiting operating temperature of the stationary phase, and

(iv) inert towards the stationary phase and the solutes under test; likewise

the products of oxidation of the antioxidant should not interfere with the elution process.

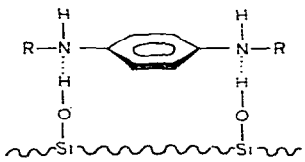
Previously it has been suggested that the deactivator molecule should be capable of directing polar functional groups towards the active sites on the support surface whilst presenting an apolar surface towards the liquid phase[9], as illustrated by the following diagram:



where

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.
C = apolar groups (methyl, methylene, phenyl), weak association with stationary phase.

Substituted *p*-phenylenediamine antioxidants would be expected to yield protective films due to strong hydrogen bonding between the basic amino groups and the acidic silanol groups, *viz.*,



Furthermore as these substances are readily available in a reasonable state of purity, inexpensive, and thermally stable they would seem to be admirably suited to the dual role of oxidation inhibitor and support deactivator. However, whereas the antioxidant efficiency under gas chromatographic conditions of these materials has been demonstrated[11], their efficiency as support deactivators and possible effects upon retention characteristics of apolar stationary phases have not been investigated. Accordingly typical N,N'-disubstituted *p*-phenylenediamines have been evaluated using a general test procedure involving the following steps:

(i) measurement of peak asymmetry of a range of polar solutes as a test of support activity,

(ii) determination of the degree of decomposition of test solutes which readily undergo acid-catalysed elimination reactions,

(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[15], *viz.*,

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

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 $P_{asym.}$ 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 is the most adsorptive[16]. Accordingly squalane columns were prepared using Diatomite S and $P_{asym.}$ values determined for solutes possessing a range of polar functional groups. In order to achieve a meaningful comparison of the various columns a constant sample volume was used throughout the investigation. As expected the untreated column gave severely distorted peaks. Unexpectedly the squalane column prepared using Diatomite S treated with 1% w/w of N-isopropyl-N′-phenyl-*p*-phenylenediamine (IPPD) also gave highly distorted peaks. As IPPD is a solid at room temperature, a possible explanation of this observation is poor coverage of the support surface during the first step of the slurry procedure. Therefore two further columns were prepared, one where the mixture of IPPD and support was heated under nitrogen to a temperature above the melting point of the antioxidant before coating with squalane, the other where the IPPD was replaced by N,N′-bis-1-methylheptyl-*p*-phenylenediamine (DOPD), which is a liquid at room temperature. Again asymmetric peaks were observed. However, columns prepared using Chromosorb W in place of Diatomite showed marked improvements of peak symmetry when the support was treated with either IPPD or DOPD. The results of the above experiments are summarised in Table I.

The brown colour of the firebrick-type supports is generally associated with the presence of iron oxide[17], which may be removed by acid washing. Marriott and Freeborn[18] have found that the presence of iron in rubber vulcanizates seriously affects the antioxidant efficiency of N,N′-bis-2-naphthyl-*p*-phenylenediamine. This antagonism was ascribed to the formation of a coordination compound between the antioxidant and iron compounds. It is possible, therefore, that the apparent lack of deactivation observed in our preliminary experiments is due to a similar phenomenon.

TABLE I

PEAK ASYMMETRY VALUES FOR AMINE ANTIOXIDANT TREATED SQUALANE COLUMNS

I = 10% squalane, Diatomite S, 100°; II = 10% squalane, 1% IPPD, Diatomite S, 100°; III = 10% squalane, 1% IPPD, Diatomite S, preheated, 100°; IV = 10% squalane, 1% DOPD, Diatomite S, 100°; V = 10% squalane, Chromosorb W, 100°; VI = 10% squalane, 1% IPPD, Chromosorb W, 100°.

Solute	$P_{asym.}$					
	I	*II*	*III*	*IV*	*V*	*VI*
n-Butyl acetate	−0.96	−0.74	−0.64	−0.84	−0.35	−0.02
n-Butyl cyanide	−0.96	−0.63	−0.65	−0.78	−0.42	−0.02
Hexan-2-one	−0.94	−0.86	−0.72	−0.80	−0.30	−0.03
Isopropylbenzene	−0.11	−0.06	−0.04	−0.06	−0.04	+0.01
Pentan-1-ol	−0.97	−0.80	−0.85	−0.84	−0.48	−0.04

Additional squalane–antioxidant columns were prepared using Diatomite S which had been continuously washed with concentrated hydrochloric acid for four days in a Soxhlet apparatus. Now both IPPD and DOPD were found to prevent the adsorption of solute molecules. Subsequent chemical and spectroscopic analysis of the support, before and after acid washing, revealed that only 40% of the iron originally present had been removed by the Soxhlet extraction. This observation is consistent with the results of a recent study by Aue *et al.*[19], which revealed that the total removal of iron required reaction with hydrogen chloride at 800°. Furthermore, we have found that the mixing of anhydrous iron(III) chloride and IPPD in methylene chloride solution gives rise to a deep blue colour, $\lambda_{max.} = 602$ nm, due to the formation of a 1:1 complex[20]. It may be concluded, therefore, that the more loosely held iron compounds on the support surface can coordinate with N,N′-disubstituted *p*-phenylenediamines to form products which are insufficiently polar to deactivate the acidic sites. Peak asymmetry values obtained using the columns based upon acid-washed supports are shown in Table II.

Consistent with the above conclusion, independent experiments revealed that induction periods for IPPD-inhibited oxidations, monitored by inverse gas chromatography, are markedly reduced by the introduction of iron(III) salts.

TABLE II

PEAK ASYMMETRY VALUES FOR SQUALANE COLUMNS PREPARED USING ACID-WASHED DIATOMITE S

I = 10% squalane, acid-washed Diatomite S, 100°; II = 10% squalane, 1% IPPD, acid-washed Diatomite S, 100°; III = 10% squalane, 1% DOPD, acid-washed Diatomite S, 100°.

Solute	$P_{asym.}$		
	I	*II*	*III*
n-Butyl acetate	−0.75	−0.03	−0.02
n-Butyl cyanide	−0.95	−0.04	−0.05
Hexan-2-one	−0.90	−0.05	−0.02
Isopropylbenzene	−0.10	+0.10	+0.01
Pentan-1-ol	−0.88	−0.04	−0.03

Study of the decomposition of labile solute molecules

The adsorption of solute molecules upon the support surface can lead to solute isomerisation or decomposition. This phenomenon has been used to compare the adsorptivity of support materials, and effectiveness of deactivation procedures, through:

(i) detection of additional peaks due to volatile decomposition products together with measurements of peak distortion[21],

(ii) measurements of changes of peak areas, relative to those of *n*-alkane standards, with elevation of column temperature[22], and

(iii) spectroscopic analysis of the solute before and after chromatography[2].

In our experience the second of the above methods is the most reliable. Accordingly the efficiency of the IPPD treatment was tested by means of a study of the elution behaviour of two *tert.*-alkanols, which readily undergo acid-catalysed dehydration on acidic gas chromatographic columns[2]. Test mixtures of alkanol and a suitable *n*-alkane internal standard were chromatographed over a range of temperatures using columns prepared from acid-washed Diatomite S. Consistent with the peak symmetry experiments the amine-treated columns were found to preclude the adsorption of polar solutes. Whereas the peak area ratios of alkanol to alkane obtained with the unprotected column decreased with increase of temperature, consistent with a greater degree of decomposition, the antioxidant-treated columns gave constant values indicating that no dehydration had occurred. The results are summarised in Table III.

TABLE III

INVESTIGATION OF THE EFFICIENCY OF AMINE ANTIOXIDANT PROTECTIVE FILMS BY MEANS OF PEAK AREA MEASUREMENTS

I = 10% squalane, acid-washed Diatomite S; II = 10% squalane, 1% IPPD, acid-washed Diatomite S; III = 10% squalane, 1% DOPD, acid-washed Diatomite S.

Solute	*Temperature (°C)*	*Peak area ratio**		
		I	*II*	*III*
2-Methylpentan-2-ol	70	1.00	1.00	1.00
	90	0.69	0.99	0.98
	100	0.40	1.01	0.99
3-Methylpentan-3-ol	70	1.00	1.00	1.00
	90	0.74	0.98	0.99
	100	0.42	1.01	1.00

* *n*-Nonane used as internal standard. Peak area ratios expressed relative to the value at 70° being unity.

Subsequent work revealed that successful results also could be obtained with non acid-washed supports, provided that sufficient antioxidant was added to compensate for that lost due to coordination. With Diatomite S, 2% by weight was found to be adequate. Therefore, it was decided to test the thermal stability of the protective films and to study the retention behaviour of amine-deactivated columns.

Thermal stability of the protective film

The thermal stability of a gas chromatographic liquid phase may be determined by thermogravimetry[23], measurement of detector standing current[24], or relative retention measurements[25]. Each of these methods was used to determine the stability of diglycerol deactivated supports[9] and it was concluded that the latter was the most appropriate. Accordingly the stability of IPPD protective films was determined from retention index measurements using a thermally stable silicone oil as stationary phase.

As described previously[9], the column was cycled daily between 100° and 200° and the retention characteristics monitored by recording chromatograms of appropriate calibration mixtures at 100°. The results obtained, which are summarised in Table IV, indicate that once the column had stabilised virtually constant retention behaviour was maintained at a temperature well in excess of the vaporization temperature of the antioxidant. Clearly on this evidence IPPD would appear to be a satisfactory support deactivator for columns operating up to at least 180°.

TABLE IV

INVESTIGATION OF THE THERMAL STABILITY OF IPPD SURFACE COATINGS

Column packing: 17% silicone MS550 3% IPPD on non acid-washed Diatomite S.

Solute	*Retention index**						
	0 h	*10 h*	*20 h*	*30 h*	*40 h*	*50 h***	*70 h*
Isopropyl acetate	735	702	703	703	702	704	702
3-Methylbutan-2-one	759	724	722	721	721	723	722
Pentan-1-ol	827	804	803	800	800	802	803
n-Butyl cyanide	918	869	869	868	869	870	868
Chlorobenzene	933	911	911	912	913	911	912
Isopropylbenzene	989	971	970	972	969	971	970
*b****	0.309	0.306	0.300	0.297	0.299	0.300	0.298

* Retention index values measured at 100°.

** Beyond 50 h at 200° diglycerol columns were found to deteriorate rapidly.

*** Mean values for *b* obtained during the calculation of *I*.

In an attempt to afford prolonged protection at temperatures in excess of 200° columns were prepared using N,N'-bis-2-naphthyl-*p*-phenylenediamine, which is markedly less volatile than IPPD[26]. In this case, however, it proved to be impossible to get efficient support deactivation, presumably due to a reduction of the hydrogen bond acceptor properties of the phenylenediamine due to steric hindrance by the bulky substituents.

Effect of IPPD pretreatment upon the retention behaviour of apolar columns

On the evidence of previous work[1] amine 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 contribution to the partition process.

In order to determine the magnitude of the latter effect, which from an analytical standpoint is the more important, two squalane columns were prepared using batches of Diatomite S coated with 2 and 4% w/w, respectively, of IPPD. After careful conditioning the retention behaviour of each column was determined by measurement of values for retention index for a range of solutes. In each case, care was taken to ensure constancy of retention data throughout the period of investigation. The results obtained, which are shown in Table V, indicate that IPPD has a greater effect upon the retention character of apolar columns than diglycerol. Nevertheless, provided care is taken to regulate deactivator concentration it is possible to obtain reproducible data.

TABLE V

THE EFFECT OF IPPD DEACTIVATOR CONCENTRATION UPON THE RETENTION CHARACTERISTICS OF SQUALANE COLUMNS

I = 18% squalane, 2% IPPD, Diatomite S at 100°; II = 16% squalane, 4% IPPD, Diatomite S at 100°.

Solute	*Retention index*		δI^*	$\delta I'^{**}$
	I	*II*		
Toluene	767	779	12	1
4-Methyl-pent-3-en-2-one	781	813	32	4
1,2-Dibromoethane	810	832	22	3
Chlorobenzene	842	857	15	1
3-Bromopropanol	928	959	31	54
Benzonitrile	943	991	48	10
Phenetole	970	992	22	1
Nitrobenzene	1045	1086	41	7

* $\delta I = I_{4\%} - I_{2\%}$.

** $\delta I'$ = change in retention index for equivalent diglycerol columns.

CONCLUSIONS

Pretreatment of diatomaceous supports with amine antioxidants has been found to lead to apolar columns with increased stability, which are suitable for the analysis of both apolar and strongly polar solutes. Since the retentions of polar solutes are significantly less on hydrocarbon phases than on the polar phases normally used, our deactivated columns could prove to be invaluable for the rapid analysis of these materials.

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