Gas Chromatography in Qualitative Analysis

Part II: A Study of the Retention Characteristics of Diglycerol Deactivated Gas-Liquid Chromatographic Columns

Gas-Chromatographie in der qualitativen Analyse

Teil II: Untersuchung der Retentionseigenschaften von mit Diglycerin inaktivierten Säulen für die Gas-Flüssig-Chromatographie

La chromatographie en phase gazeuse au service de l'analyse qualitative

Partie II: Etude des caractéristiques de rétention de colonnes pour chromatographie gaz-liquide renfermant des supports désactivées par le diglycérol

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Summary: Treatment of gas chromatographic supports with diglycerol has been found to be an effective means of eliminating errors in qualitative analysis due to the adsorption of polar solutes at the liquid-solid interface. The requirements of a gas chromatographic "tail-reducer" are discussed and an evaluation procedure proposed, and this used to show that diglycerol is an efficient "tail-reducer" up to column temperatures of at least 160 °C. An investigation of the retention characteristics of squalane columns prepared using pretreated C-22 firebrick has produced further evidence to support the explanation previously proposed for changes of retention accompanying the oxidation of apolar liquid phases [1].

Zusammenfassung: Die Behandlung von gas-chromatographischem Trägermaterial mit Diglycerin hat sich als ein wirksames Mittel zur Ausschaltung von Fehlern in der qualitativen Analyse erwiesen, die auf Adsorption von polaren Gelösten an der flüssig-festen Grenzschicht zurückzuführen sind.

Die Erfordernisse eines gas-chromatographischen "Tailing-Reduzieres" werden diskutiert und ein Auswertungsverfahren vorgeschlagen, mit dessen Hilfe gezeigt wird, daß Diglycerin ein wirksamer "Tailing-Reduzierer" bis zu Säulentemperaturen von mindestens 160 °C ist.

Eine Untersuchung der Retentionseigenschaften von Squalan-Säulen, die unter Verwendung von mit Diglycerin behandeltem Firebrick C-22 hergestellt wurden, unterstützte erneut die schon vorgeschlagene Erklärung für Retentionszeitänderungen, die bei der Oxydation von unpolaren flüssigen Phasen auftreten [1].

Resume: Le traitement par du diglycérol des supports utilisés en chromatographie en phase gazeuse s'est révélé efficace pour éliminer certaines erreurs en analyse qualitative, erreurs dûes à l'adsorption des solutés polaires à l'interface liquide-solide.

On étudie ce qui est exigé d'un "réducteur de dissymétrie de pics" en chromatographie en phase gazeuse et propose un procédé d'évaluation de l'efficacité; ce dernier est employé pour montrer que le diglycérol est un "réducteur efficace de dissymétrie de pics" jusqu'à des températures de colonne d'au moins 160 °C.

Une étude des caractéristiques de rétention de colonnes de squalane préparées en utilisant de la brique siliceuse C-22 traitée par du diglycérol confirme l'explication déjà proposée pour les modifications de temps de rétention observés par suite de l'oxydation des phases liquides non polaires [1].

In gas-liquid chromatography the adsorption of solute molecules at the liquid-solid interface can lead to shifts of retention, with concomitant peak asymmetry, and in extreme cases solute isomerisation or degradation [2]. The detrimental effects of these phenomena upon the reliability of g.l.c. analyses has long been appreciated, and a variety of methods have been developed for the deactivation of diatomaceous support materials, including:

i) Acid or base washing, [3, 4]

ii) coating with carbon, [5] a metal [6] or an inert polymer, [7, 8]

iii) partial sintering at elevated temperatures, [9]

iv) chemical deactivation of the active sites by conversion to apolar silyl ether groups, [10, 11] and,

v) saturation of the surface adsorptivity with anionic, cationic or non-ionic surfactants [12, 13, 14].

When carried out under optimum conditions most of these methods are successful in preventing adsorption artefacts. However, on the routine laboratory scale, it has been the author's experience that the use of surfactants is capable of yielding reliable results more consistently than the other methods. Nevertheless, errors in qualitative analysis can still arise due to the ill-defined chemical nature of many of the materials available for use in this context, and the lack of knowledge of the effect of the surfactant upon the retention characteristics of a column. During a recent investigation of the isomerisation of olefins on adsorptive squalane, C-22 firebrick columns it was discovered that pretreatment of the support with diglycerol precluded reaction at the liquid-solid interface [15]. Accordingly the efficiency of the deactivation of adsorptive supports by diglycerol, and the effect of diglycerol concentration upon the retention behavious of apolar columns has been investigated. The results of this work are now reported.

Experimental

Gas chromatograms were obtained using a Philips PV 4000 Gas Chromatograph equipped with glass columns (2 or 3 m length, 4 mm I. D.) and a flame ionization detector. Nitrogen was used as carrier gas. Mixtures of solutes of interest and the appropriate n-alkane standards were introduced by means of Hamilton microsyringes.

In part of the work a Pye-Unicam Research Chromatograph (model R) equipped with glass columns, flame ionization detector and manual preparative attachment was used.

Diglycerol deactivated column packings were prepared by a two-stage slurry technique in which the support (C-22 firebrick, supplied by J.J's Chromatography Ltd. King's Lynn) was first coated with a thin film of diglycerol from a mixture of acetone and methylene chloride (1:1 by volume). After removal of the mixed solvent (under a water pump vacuum) the stationary phase (squalane, supplied by B. D. H. Ltd.) was introduced from solution in 30–40° petroleum ether. Preliminary experiments having shown that it was necessary to use a solvent which is insufficiently polar to displace the film of diglycerol from the support surface.

Relative retentions, in Kovats' retention index units [16], 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 [17]. The adjusted

retentions were then used to calculate values for retention index by means of the expression recommended by the Gas Chromatography Discussion Group [18]:

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

where R_x, R_N and R_{N+n} are the adjusted retentions of the solute and n-alkanes containing N and N + n carbon atoms, respectively.
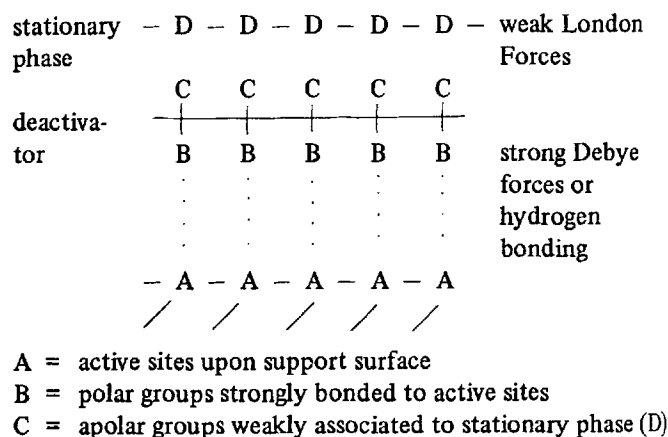
Results and Discussion

In order that 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 (tail-reducer) should be:

i) easily available and reproducible substances, preferably definite compounds,

ii) polar liquids with a high affinity for the active sites and sufficiently mobile 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.

The principal requirement of a tail-reducer is that it should be capable of preventing the adsorption of solute molecules at the liquid-solid interface without affecting the retention characteristics of the stationary phase.
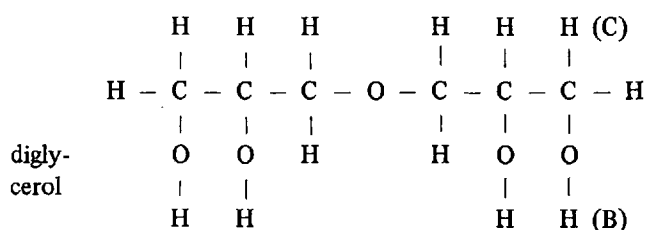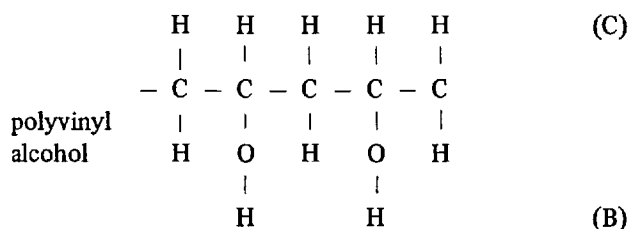
Obviously in order in fulfill this requirement the material should be capable of directing polar functional groups towards the active sites on the support surface, whilst presenting an apolar surface towards the liquid phase, as illustrated by the following diagram:

stationary – D – D – D – D – D – weak London
phase   Forces

deactiva-
tor

A = active sites upon support surface
B = polar groups strongly bonded to active sites
C = apolar groups weakly associated to stationary phase (D)

Ideally the functional groups in contact with the stationary phase should be apolar, preferably methyl or methylene, so that solute molecules would show little tendency to be selectively retained at the stationary phase – tail reducer interface. The identity of the groups (B) adsorbed onto the support will be determined by the chemical characteristics of the active sites (A). For diatomaceous materials

there is strong evidence that the active sites are acidic in nature, with the prinicpal groups being silanol, Si-OH, groups [19, 20]. These groups are known to form strong hydrogen bonds with molecules possessing proton donor or acceptor properties, such as alcohols, amines and carboxylic acids. Furthermore, owing to its strong permanent dipole, the silanol group undergoes strong interaction with other dipolar groups, such as nitro and nitrile, through Debye forces. On these grounds, the ideal deactivator molecule would appear to be a linear hydrocarbon with pendant amino, carboxyl, hydroxyl, nitrile or nitro groups. The present publication is concerned with an investigation of hydroxylic tail-reducers using a test procedure which should be of general application.

A survey of readily available hydroxylic materials suggested the use of either polyvinyl alcohol or diglycerol, both of which might be expected to be oriented at the support surface in the same manner as the ideal deactivator discussed earlier, namely:

polyvinyl
alcohol

$$
\begin{array}{ccccc}
H & H & H & H & H \quad (C)\\
| & | & | & | & |\\
-C & -C & -C & -C & -C\\
| & | & | & | & |\\
H & O & H & O & H\\
& | & & |\\
& H & & H \quad (B)
\end{array}
$$

digly-
cerol

$$
\begin{array}{ccccccc}
H & H & H & & H & H & H \;(C)\\
| & | & | & & | & | & |\\
H-C & -C & -C & -O- & C & -C & -C-H\\
| & | & | & & | & | & |\\
O & O & H & & H & O & O\\
| & | & & & & | & |\\
H & H & & & & H & H \;(B)
\end{array}
$$

Preliminary experiments revealed that pretreatment of C-22 firebrick with polyvinyl alcohol was significantly less effective than diglycerol in preventing the tailing of alcohols with squalane as liquid phase [21]. A possible explanation of this behaviour is that the polymer is insufficiently mobile to form a coherent film. Diglycerol was therefore selected for further study using a general test procedure involving the following steps:

i)   study of the peak symmetry of a wide range of compounds possessing, either hydrogen bond, acceptor or donor properties, strong dipoles, or readily polarisable groups,

ii)   determination of the degree of decomposition of test compounds 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.

Study of peak symmetry

Adsorption of solute molecules at the liquid-solid interface results in the distortion of the normally symmetrical peaks 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 by a peak asymmetry factor [22], determined from peak width measurements as illustrated in the following diagram:
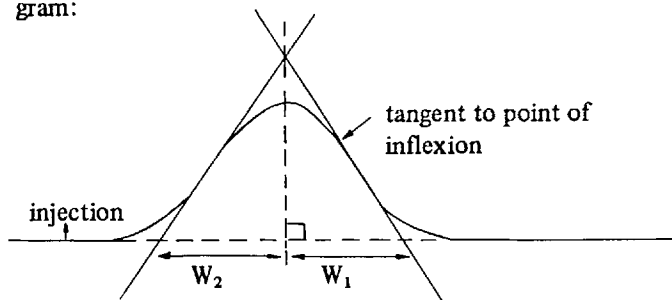
$$
P_{asym} = \frac{W_2 - W_1}{W_1 + W_2}
$$

Thus a P_{asym} value of zero is characteristic of a symmetrical peak, a positive value a leading peak and a negative value a tailing peak, the numerical value increasing with peak distortion. Typical values for squalane, C-22 firebrick columns shown in Table 1 clearly indicate the effectiveness of a small concentration of diglycerol in eliminating the adsorption of solute molecules.

Alternatively peak asymmetry may be reported visually by means of typical chromatograms. Here again the efficiency of diglycerol as a tail-reducer is clearly apparent (see Fig. 1).
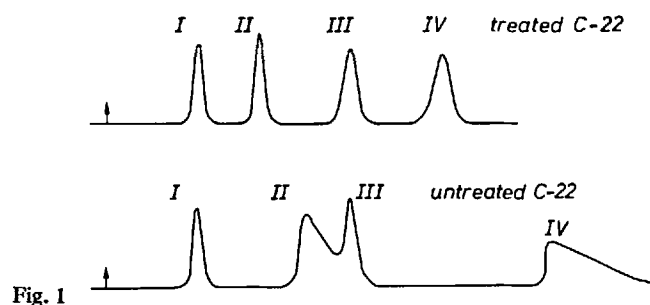

Fig. 1

● Improvement of peak symmetry for hexan-2-one and 2-methyl pentan-1-ol on precoating C-22 firebrick with diglycerol.
I = n-heptane, II = hexan-2-one, III = n-octane, IV = 2-methyl pentan-1-ol.
Composition of column packings, 19 % squalane, 1 % diglycerol, 80 % C-22 and 20 % squalane, 80 % C-22. Temperature: 100 °C.

● Verbesserung der Peak-Symmetrie für Hexan-2-on und 2-Methyl-pentan-1-ol bei Vorbehandlung von Firebrick C-22 mit Diglycerin.
I = n-Heptan, II = Hexan-2-on, III = n-Octan, IV = 2-Methyl-pentan-1-ol.
Säulenfüllmaterial: 19 % Squalan, 1 % Diglycerin, 80 % C-22 bzw. 20 % Squalan, 80 % C-22. Temperatur: 100 °C.

● Réduction de la dissymétrie des pics pour l'hexane-2-one et le 2-méthyl-pentane-1-ol après traitement de la brique siliceuse C-22 par le diglycérol.
I = n-heptane, II = hexane-2-one, III = n-octane, IV = 2-méthyl-pentane-1-ol.
Composition du matériel de remplissage: soit 19 % de squalane, 1 % de diglycérol, 80 % de brique C-22 ou 20 % de squalane, 80 % de brique C-22. Température: 100 °C.

Decomposition or isomerisation of solute molecules

The adsorption of solute molecules upon the support surface is frequently the precursor of isomerisation or decomposition. This phenomenon has been used to compare the adsorptivity of support materials through the detection of additional peaks due to volatile decomposition products [23], relative peak area measurements with n-alkanes as internal standards [24], and spectroscopic analysis of the solute subsequent to its elution [15].

Table 1. Comparison of peak asymmetry values for squalane, C-22 firebrick columns before and after treatment with diglycerol

Solute	Pasym	
	untreated	treated*
Allyl benzene	− 0.17	+ 0.02
Benzonitrile	− 0.63	− 0.03
3-Bromopropan-1-ol	− 0.87	0.0
Diethyl disulphide	− 0.19	0.0
Hexan-2-one	− 0.45	+ 0.03
1-Iodohexane	− 0.17	0.0
2-Methyl pentan-1-ol	− 0.83	− 0.03
3-Methyl pentan-3-thiol	− 0.19	+ 0.03

Chromatograms recorded at 100°, solutes injected as dilute solutions (2 % v/v) in dichloromethane

* Composition of packing, 19 % squalane, 1 % diglycerol, 80 % C-22; the untreated column contained 20 % squalane 80 % C-22, 2 m column.

Two of the above methods have been used in the evaluation of diglycerol pretreated supports. The decomposition of a tertiary alcohol, through the acid catalysed elimination of water, as determined by peak area measurements, whilst, on the other hand, the extent of the isomerisation of Δ-1 olefins was determined by spectroscopic measurements. The results summarised in Tables 2 and 3 again clearly show the effectiveness of diglycerol pretreatment.

Furthermore, the data for the alcohol and mercaptan afford further evidence of the validity of the previously postulated mechanisms [15] for solute decomposition involving initial protonation of the solute molecule. A subsequent examination of dimethylacetals and di-n-propylmercaptals gave consistent results [25].

Thermal stability of diglycerol films

The thermal stability of a gas chromatographic liquid phase may be determined by thermogravimetric analysis [26], measurement of detector standing current [27], or relative retention measurements [28].

Preliminary thermal analysis experiments with C-22 firebrick coated with 1 % w/w of diglycerol indicated that the deactivator volatilised at a temperature between 210 and 230°. A study of detector response gave a similar overall result but indicated a significant rate of vaporisation at lower temperature than detected by the thermobalance. A possible criticism of the above methods is that

Table 2. Investigation of the efficiency of diglycerol protective films by means of peak area measurements

Solute	Temperature	Peak Area Ratio untreated column	Solute / n-Alkane treated column*
3-Methyl pentan-3-ol	70°	1.00	1.00
	90°	0.75	1.02
	100°	0.42	0.98
3-Methyl pentan-3-thiol	70°	1.00	1.00
	90°	0.96	1.01
	100°	0.98	0.98

+ Column packings as shown in Table 1, a 2 m column was used.
* n-Nonane used as internal standard. Peak area ratios expressed relative to the value at 70° being unity

Table 3. The isomerisation of olefins on squalane, C-22 firebrick columns

	Percentage purity +	
	2-methyl oct-1-ene	dl-limonene
Original mixture	70.0	85.0
Recovered from squalane, C-22 firebrick column at 140° *	17.4	39.5
Recovered from squalane, diglycerol, firebrick column at 140°	70.8	85.5

+ Based upon infra-red analysis using the out-of-plane C-H deformation vibration band at 890 cm^{-1}
* Column packings as shown in Table 1, a 3 m column was used.

they do not necessarily test the thermal stability of the diglycerol film under the conditions prevailing in an analytical g.l.c. column. Accordingly, a further test was carried out in which a column packed with a mixture of silicone fluid MS 550, diglycerol and C-22 firebrick was cycled daily between 100° and 200° for 7 hour periods. The retention characteristics of the column were tested by recording chromatograms of suitable calibration mixtures at 100 °C. A previous independent experiment had shown that silicone fluid MS 550 was chromatographically stable at 200°, provided low molecular weight siloxanes were first removed by heating 'in vacuo'.

The results of this investigation of the constancy of relative retention data with column usuage at elevated temperatures are shown in Table 4. The most significant fact that emerges from this data is that prolonged heating has virtually no effect upon solutes other than those possessing strong dipoles and hydrogen bonding capabilities. As the retention characteristics of the silicone liquid phase did not appear to have changed, as judged by the constancy of the slope of the n-alkane log plot (b), it may be concluded that these changes reflect a redistribution or

Table 4. Investigation of the thermal stability of diglycerol surface coatings by means of retention measurements

Solute	Retention Indices[+]				δ 1
	0	14 hr	28 hr	42 hr	for 50 hr at 200°
Nitroethane	752.0	747.0	746.0	744.2	9.2
2-Methyl pent-1-ene-4-ol	826.2	812.7	810.1	807.8	19.6
n-Valeronitrile	871.1	865.4	864.2	863.1	8.9
Di-n-butyl ether	902.1	901.6	901.4	901.2	1.2
n-Heptaldehyde	963.5	960.7	959.8	959.2	4.5
l-Iodopentane	987.3	986.5	986.1	985.8	1.8
Diallyl carbonate	1 023.0	1 020.5	1 019.6	1 019.2	4.3
1-Ethyl 2-methyl pent-2-ene-1-ol	1 038.8	1 032.0	1 028.4	1 025.8	14.6
N, N-Dimethylaniline	1 176.1	1 174.7	1 173.8	1 173.2	3.3
b*	0.293	0.292	0.293	0.291	

+ Retention indices calculated from retention distances on chromatograms recorded at 100 °C
  Column packing: 17 % silicone MS 550 3 % diglycerol 80 % C-22 firebrick

* Mean values for b obtained during the calculation of retention index

Table 5. The effect of diglycerol concentration upon the retention characteristics of squalane, C-22 firebrick columns.

Solute	Values for Retention Index at 100 °C				
	I	II	III	IV	V
Hexan-2-one	770*	728.6	729.0	731.0	733.9
Mesityl oxide	795*	741.8	742.4	745.8	750.1
Toluene	765	756.6	756.6	757.2	758.2
2-Methyl pentan-1-ol	–	774.4	774.6	782.8	790.6
1,2-Dibromoethane	794	785.4	785.3	787.8	792.0
3-Bromo propan-1-ol	–	820.9	834.3	889.3	942.5
Chlorobenzene	831.7	826.9	827.2	827.9	830.0
2-Methyl oct-2-ene	888.6	887.9	887.5	888.1	888.2
Diethyl disulphide	902*	891.9	892.0	892.4	893.6
Benzonitrile	980*	897.4	898.5	906.8	918.0
Ethyl 4-methyl pentanoate	925*	902.6	902.8	903.5	904.5
Allylbenzene	932	921.2	920.0	921.4	922.6
n-Octaldehyde	985*	945.2	945.3	945.7	947.3
Phenetole	980*	950.4	950.6	951.1	952.8
Benzyl alcohol	–	967.4	978.0	1 020.7	1 066.7
Nitrobenzene	1 121*	1 006.8	1 007.6	1 011.6	1 022.3
b+	0.3267	0.3269	0.3280	0.3268	0.3274

I   20   % Squalane,   80 % C-22

II   19.5 % Squalane,   0.5 % Diglycerol, 80 % C-22

III   19.0 % Squalane,   1.0 % Diglycerol, 80 % C-22

IV   17.0 % Squalane,   3.0 % Diglycerol, 80 % C-22

V   15.0 % Squalane,   5.0 % Diglycerol, 80 % C-22

* Approximate values, peaks distorted due to absorption in some cases no meaningful values could be obtained.

+ Calculated according to the method of *Evans* and *Smith* [29]

loss of diglycerol. A later investigation of the effect of diglycerol concentration upon the retention behaviour of apolar columns gave results consistent with this conclusion.

A further experiment in which a fresh silicone, diglycerol, C-22 firebrick column was cycled between 100° and 175° revealed no significant retention changes for alcohols over a period of 50 hours. It may therefore be concluded that diglycerol pretreated columns should be suitable for prolonged routine analytical work up to at least 160 °C.

Effect of diglycerol pretreatment upon the retention behaviour of apolar columns

On the evidence of previous work [1], diglycerol pretreatment might be expected to affect the retention behaviour of apolar columns in either of two ways, depending upon the concentration of diglycerol and the chemical and physical nature of the support surface. Firstly, be prevention of the adsorption of solute molecules at the liquid-solid interface, and secondly, by contribution to the overall partitioning process.

Thus as a protective film is built up, with increase of diglycerol concentration, the retention indices of polar solutes would be expected to fall owing to reduction of the contribution of liquid-solid adsorption to the overall retention. On completion of a coherent film of diglycerol the retention indices would be expected to pass through a minimum value to rise with further increase of concentration, due to a contribution to the overall partitioning process by the excess diglycerol.

In order to test this hypothesis a series of squalane columns were prepared using freshly chromatographed squalane and C-22 firebrick pretreated with different quantities of diglycerol. Retention data obtained using these columns, shown in Table 5, reveal apolar solutes to be unaffected by the presence of diglycerol, whereas polar solutes display a marked and characteristic dependence, as illustrated by Fig. 2.

As expected the retention of polar solutes initially decreased with increase of diglycerol concentration, with concomitant improvement of peak symmetry, to pass through a minimum value. This minimum value is believed to correpond to completion of the protective layer and to be characteristic of a purely gas-liquid partitioning process. Beyond the minimum, increase of the diglycerol concentration gave rise to pronounced shifts of retention, particularly for hydroxylic solutes, indicative of a significant contribution by diglycerol to partition.

The similarity of the minimum values for retention index obtained in this work and during the investigation of the oxidation of apolar liquid phases, as shown in Table 6, tends to confirm the validity of the explanations put forward to account for the changes of retention with oxidation and also with the addition of diglycerol.
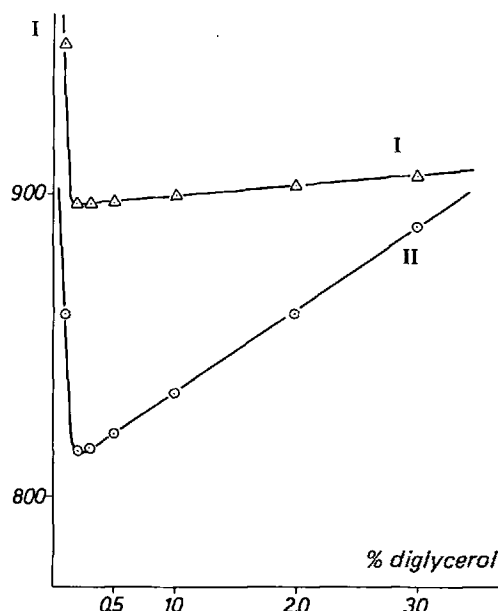
Fig. 2

● Dependence of the retention characteristics of polar solutes upon the diglycerol concentration of precoated C-22, squalane columns at 100 °C
I = Benzonitrile, II = 3-bromo propanol-1-ol

● Abhängigkeit der Retentionseigenschaften von polaren Gelösten von der Diglycerin-Konzentration bei vorbehandeltem C-22 – Squalan-Säulen bei 100 °C.
I = Benzonitril, II = 3-Brompropanol-1-ol

● Relation entre les caractéristiques de rétention de solutés polaires et la concentration de diglycérol dans la brique C-22, colonnes: phase fixe squalane, à 100 °C.
I = benzonitrile, II = 3-bromo-propanol-1-ol

Conclusions

A scheme for the evaluation of gas chromatographic tail-reducers has been formulated, involving the following steps:

i)   study of the peak symmetry of a wide range of compounds possessing either, hydrogen bond acceptor or donor properties, strong dipoles, or readily polarisable groups,

ii) determination of the degree of decomposition of test compounds 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.

The application of the proposed scheme is illustrated by an assessment of the suitability of diglycerol pretreated supports for analysis.

As a result of this work it has been found that diglycerol pretreatment of diatomaceous supports prevents errors in quantitative gas chromatographic analysis due to the adsorption of polar solute molecules at the liquid-solid interface. On the other hand, these columns have not been found to be altogether suitable for qualitative analysis

Table 6. Comparison of the minimum values for retention index obtained with diglycerol pretreated columns with those obtained in the study of the oxidation of squalane columns [1]

| | Values for Retention Index at 100° | | |
	Benzo-nitrile	Nitro-benzene	Phene-tole
Oxidation of squalane, celite column	891	1 002	949
Oxidation of squalane, silica gel, celite column.	894	1 004	950
Squalane, diglycerol, C-22 firebrick columns	897	1 007	950

based upon retention coincidence. However, they are perfectly satisfactory for qualitative analysis using spectroscopic measurements, provided an operating temperature of 160° is not exceeded.

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