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Gas–liquid chromatography in qualitative analysis

XIX. The use of antioxidants to delay the oxidation of polyoxyethylene glycol stationary phases

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ABSTRACT

The sensitivity that polyoxyethylene glycols (PEG) show towards oxidative degradation at elevated temperatures is well known. Experiments have been conducted to investigate the possible use of antioxidants as stationary phase additives to prevent this oxidation. It has been shown that significant differences in antioxidant efficiency exist, depending on whether the activity is determined under static or dynamic conditions. This difference has been shown to be due to the volatility of the antioxidants in the carrier gas stream.

In view of the above findings, a novel antioxidant based on the PEG 400 molecule has been synthesized and evaluated. This compound is markedly less volatile than the conventional antioxidants and inhibits significantly the oxidation of PEG phases.

INTRODUCTION

The polyoxyethylene glycols (PEGs) are a ubiquitous group of compounds which, among other uses, have been employed for many years as polar stationary phases in gas–liquid chromatography [1]. Applications of these phases are legion and include the separation of alcohols [2], aldehydes [3], ketones [4] and fatty acids [5]. PEGs are extremely sensitive to aerial oxidation, especially at elevated temperatures and this can lead to serious difficulties in analysis, due to stationary phase degradation.

The oxidation of polyoxyethylene glycols has been studied by various workers who have identified several compounds as degradation products, which include formic acid [6], formaldehyde [7] and acetaldehyde [8]. In contrast, litte work is published of the non-volatile products arising from aerial oxidation. However, it is known that when PEGs are oxidised as thick films or blocks, shorter chain PEGs are formed as non-volatile products [9]. Previous studies have also suggested that the degradation proceeds, at least in part, by a free radical mechanism [10].

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Free-radial scavengers are commonly used to protect foodstuffs [11], rubbers and plastics [12] from the deleterious effects of aerial oxidation. Accordingly, it was decided to test the efficiency of such compounds as oxidation inhibitors in PEG stationary phases. The results of this work are now reported.

MATERIALS AND METHODS

Instrumentation

The gas chromatograph used was a Pye Model 104 (Pye-Unicam, Cambridge, UK), equipped with glass columns (6 ft. × 0.25 in. I.D.) and flame ionisation detection.

The column packing comprised of 10% PEG 400 or PEG 20M supported on 60–80 mesh Chromosorb W AW prepared and packed in the conventional manner [13].

The output of the gas chromatograph was interfaced with a Spectra-Physics (Hemel Hempstead, UK) System 4 integrator to yield retention data.

Sylvester apparatus

The apparatus was constructed in-house and is shown in Fig. 1. The compound under test (0.1 g) was dissolved in the appropriate stationary phase, generally PEG 400 (10 ml). The mixture was transferred to a 100-ml conical flask fitted with a B14 ground glass joint and the adaptor tube fitted. The assembly was filled with pure oxygen gas and the rubber tube connection to the manometer quickly fitted to the adaptor. The completed apparatus was placed, as indicated in Fig. 1, in an oil bath which was maintained at 100°C. The oxygen uptake was measured by the movement of the float.

A typical trace of oxygen uptake *versus* time is shown in Fig. 2. Generally, an induction period is observed, even for pure materials, followed by a rapid exponential increase. Eventually, an upper limit is approached, at which the rate of uptake decreases to zero.

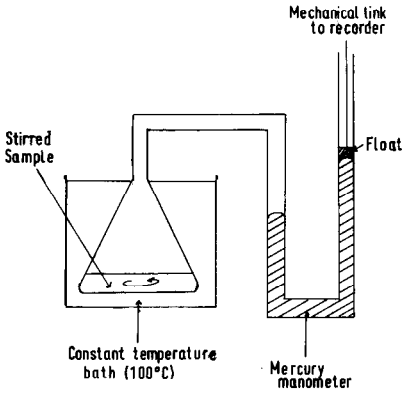


Fig. 1. Sylvester apparatus used in oxygen uptake experiments.

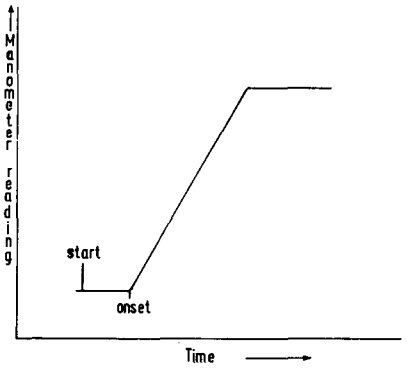


Fig. 2. Typical trace from Sylvester apparatus.

Materials

The support and stationary phase materials used were supplied by Greyhound Chemicals (Liverpool, UK). All other chemicals were purchased from BDH (Poole, UK) except the Ionex 220, which was obtained from Shell Industries (Sittingbourne, UK). All chemicals were analytical grade (A.R.) or the purest available, and all were used without further purification.

Hydrogen and oxygen-free nitrogen were supplied by British Oxygen (London, UK) and the helium by Air Products (London, UK). The compressed air was supplied from an in-house compressor, freed from oil droplets by a simple trap and passed through a carbon filter before use.

The following compounds were used to determine the Kováts indices of the stationary phases: iodobutane, dioxane, 1-butanol, pyridine, toluene, octan-2-yne, chlorobenzene, hexan-2-one and 2,4-dimethylpentan-3-ol.

Preparation of the novel antioxidant based on PEG 400

3,5-Di-*tert.*-butyl-4-hydroxybenzoic acid (1 g, 0.004 mol) was heated under reflux with thionyl chloride (5 ml, 0.005 mol) using an oil bath for 1 h at 120°C. After this time, the reaction had moderated and the excess thionyl chloride was distilled to waste.

The crude acid chloride was an orange oil at the temperature of the oil bath; no attempt was made to characterise the product. Polyoxyethylene glycol 400 (5 ml, 0.08 mol) was then added to the hot acid chloride via the reflux condenser. Initially, two layers were formed but there followed a rapid evolution of acidic gases and the contents of the flask became homogeneous.

The solution was heated at 120°C for a further 30 min, then allowed to cool. Chloroform (30 ml) was added to the flask and the resultant extracted with saturated sodium hydrogen carbonate solution (3 × 20 ml) and then washed with water (3 × 20 ml). The organic phase was separated, dried over anhydrous sodium sulphate and the chloroform removed by evaporation under nitrogen to yield 2.0 g of PEG 3,5-di-*tert.*-4-hydroxybenzoate. Details of the purification and characterisation of the product are given elsewhere [14].

RESULTS AND DISCUSSION

A range of conventional antioxidants, listed in Table I, was investigated for their ability to delay the oxidation of PEGs under static conditions, using the Sylvester apparatus and PEG 400 as the test compound. A concentration of 1% was chosen and, as the results, shown as Table I, indicate, all the compounds were effective in delaying the uptake of oxygen, and hence the onset of oxidation, for at least 2 h, whilst the control sample of PEG 400 started to degrade after about 30 min and that of the PEG 20M within 1 h.

The experiment was then repeated under dynamic conditions, by incorporating each antioxidant at a 1% concentration into a series of 10% PEG 400, Chromosorb W AW column packings. Air was used as the carrier gas at 150°C at a flow-rate of 50 ml/min and the oxidation monitored by the periodic chromatography of the test compounds. Appropriate hydrocarbons were included in the calibration solutions to enable the Kováts retention indices of the test compounds to be determined as a test of phase polarity [15,16]. The results, a representative example of which is shown as Fig. 3, indicate that little or no protection against aerial oxidation was afforded by any of the antioxidants under the conditions investigated.

As may be seen from Fig. 3, the k' values showed a rapid decrease, indicating that the mass of the stationary phase within the column was decreasing. There was, however, no concomitant change in the retention index (I) values for the test compounds, as shown in Table II, suggesting that the chemical structure of the remaining stationary phase was little changed during oxidation, in agreement with evidence based on high-performance liquid chromatographic studies [14]. A similar result was found when PEG 20M was substituted for PEG 400, but, as might be expected, the rate of degradation was somewhat slower.

During these experiments, it was noticed that the antioxidant ethoxyquin has a

TABLE I

THE EFFECTIVENESS OF CERTAIN ANTIOXIDANTS, INCLUDING A NOVEL PEG BASED ANTIOXIDANT, IN DELAYING THE ONSET OF OXIDATION OF PEG 400 AND PEG 20M, UNDER STATIC CONDITIONS

Antioxidant	Time before onset of oxidation (h)	
	PEG 400	PEG 20M
Control	0.5	1.0
BHT[a]	2.0	2.0
BHA[b]	2.5	2.8
Ionex 220[c]	10.0	10.5
4-Hydroxy-3,5-di-*tert.*-butylphenol	18.0	20.0
4-Hydroxy-3,5-di-*tert.*-benzoic acid	5.5	6.0
Ethoxyquin[d]	11.0	12.5
PEG based antioxidant	>26.0	>26.0

[a] BHT = butylated hydroxytoluene or 4-methyl-2,6-di-*tert.*-butylphenol.
[b] BHA = butylated hydroxyanisole or 4-methoxy-2-*tert.*-butylphenol.
[c] Ionex 220 = 4,4'-methylene-bis-di-*tert*-butylphenol.
[d] Ethoxyquin = 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline.

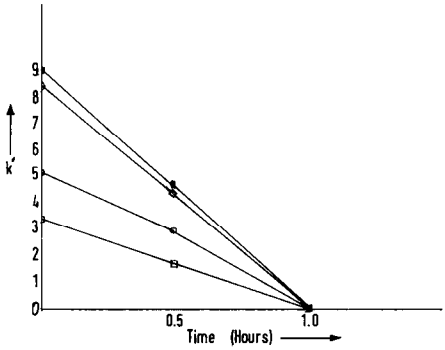


Fig. 3. The effect of oxidation upon a column packed with 10% PEG 400 with 1% BHA coated on Chromosorb W AW. The plot shows the variation of capacity factor (k') with time. Carrier gas: air at 150°C at a flow-rate of 50 ml/min. □ = Octan-2-yne; ○ = dioxane; ◇ = 1-butanol; ■ = chlorobenzene.

native fluorophore when illuminated with radiation at 380 nm. By examining a column packed with PEG 400 and ethoxyquin on Chromosorb W AW under such conditions at various times during the oxidation, it was found that the fluorescence was rapidly lost from the column, with the loss starting at the injector end and moving steadily through the column. This observation strongly supports the hypothesis that the antioxidants investigated were rapidly lost from the column under dynamic conditions, thereby explaining their apparent lack of protection. A similar observation also has been reported by Evans and Newton [17] during an investigation of hindered phenols as antioxidants by inverse chromatography with squalene as the stationary phase.

These findings suggest that the conventional antioxidants investigated were of little practical value under dynamic conditions due to their volatility. Attempts were therefore made to prepare an antioxidant compatible with PEGs that might be expected to be less volatile than the materials so far examined. After a consideration of a number of possibilities, it was decided to use PEG 400 as the base material and to introduce antioxidant groups by esterification of the terminal hydroxyls by a hindered phenol, namely 3,5-di-*tert*.-4-hydroxybenzoic acid.

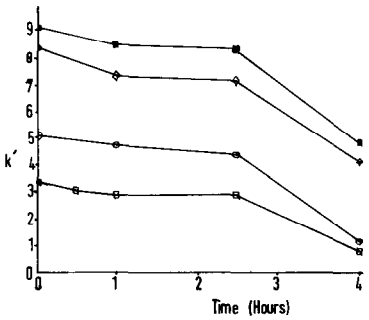
The PEG 400 ester antioxidant proved to be relatively simple to prepare, as

TABLE II

THE EFFECT OF OXIDATION UPON A COLUMN PACKED WITH 10% PEG 400, INCORPORATING 1% BHA COATED ON CHROMOSORB W AW

Variation in retention index measurements. Air as carrier gas 150°C and a flow-rate of 50 ml/min.

Compound	Time (h)		
	0	0.5	1.0
Octan-2-yne	1062	1058	1057
Dioxane	1140	1138	1145
1-Butanol	1234	1236	1238



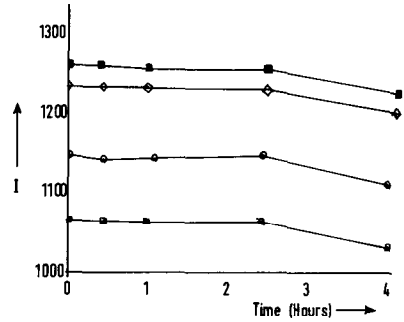


Fig. 4. The effect of oxidation upon a column packed with 10% PEG 400 and 1% PEG based antioxidant on Chromosorb W AW. The plot shows the variation of capacity factor with time. Carrier gas: air at 150°C at a flow-rate of 50 ml/min. □ = Octan-2-yne; ○ = dioxane; ◇ = 1-butanol; ■ = chlorobenzene.

Fig. 5. The effect of oxidation upon a column packed with 10% PEG 400 and 1% PEG based antioxidant on Chromosorb W AW. The plot shows the variation of retention index with time. Conditions and symbols as in Fig. 4.

described earlier. In addition to structural characterisation, the novel compound was subjected to a COSHH hazard assessment, which included an Ames test for potential mutagenicity [18]. This revealed the material to be a very weak mutagen and reasonably safe.

The PEG 400 antioxidant was evaluated under static conditions using the Sylvester apparatus and found to be very efficient in delaying the onset of oxidation of PEG 400 and PEG 20M, the results being shown in Table I. These results show the compound to be as efficient as the other antioxidants investigated.

The evaluation under the dynamic conditions showed it to be extremely good at delaying the oxidation of PEG 400 at 150°C, again with air as the carrier gas with a flow-rate of 50 ml/min, as demonstrated by the data shown in the oxidation curves in Figs. 4 and 5.

The k' and I values were found to be fairly constant for about 150 min, compared with the BHT control, where the k' values showed a decrease after 30 min. After the delay period, the rate of degradation, as indicated by the change in the k' values, was similar for both the PEG antioxidant and the control columns.

When the experiment was repeated at 175°C, the novel antioxidant delayed the onset of oxidation for PEG 400 for about 10 min. These results should be compared with the other antioxidants, where no protection was observed and degradation started immediately. Similar results were obtained when PEG 20M was used as the stationary phase, suggesting that the antioxidant was not affected by the molecular weight of the stationary phase.

CONCLUSIONS

The potential use of antioxidants to prevent, or at least delay, the onset of oxidation of PEG stationary phases has been investigated. It has been shown that, under static conditions, the antioxidants investigated do delay the onset of oxidation but this effect is not found under the dynamic conditions of gas chromatography. The

difference between static and dynamic systems has been shown to be related to the volatility of the antioxidant in the carrier gas stream.

In view of this finding, a novel antioxidant based on the PEG 400 molecule has been synthesised and the compound tested. The results of static and dynamic tests show that this novel antioxidant delays the oxidation of PEG stationary phases for significant periods of time and offers a significant advantage over conventional antioxidants at moderate operating temperatures. Further work is in progress designed to yield PEG based antioxidants with improved thermal stability.

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