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Journal of Chromatography A, 800 (1998) 231–238

JOURNAL OF CHROMATOGRAPHY A

# Determination of poly(ethylene glycol)s by both normal-phase and reversed-phase modes of high-performance liquid chromatography

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Received 28 July 1997; received in revised form 7 November 1997; accepted 12 November 1997

**Abstract**

A normal-phase HPLC system using an amino column has been developed to characterise oligomers of poly(ethylene glycol)s (PEGs) of average $M_r$ 400 to 2000 with derivatisation by dinitrobenzoate. Normal-phase HPLC with gradient elution using ternary solvents of hexane, dichloromethane and methanol has produced a baseline resolution for oligomers of PEG 400, 600 and 1000, while PEG 1000 and 2000 were analysed by using binary solvents of acetonitrile and water. Mixtures of PEGs have been determined by these HPLC systems. PEG 400 in a textile finish has also been determined with satisfactory recovery. It has been found that the hydroxyl group of solvents in normal-phase HPLC plays an important role in resolution and retention of PEG oligomers. Derivatisation efficiency for PEGs by dinitrobenzoyl chloride and quantitative determination of derivatised PEGs by HPLC have been studied. A reversed-phase (RP) mode of HPLC was examined for determination of PEG 400 oligomers. The normal-phase system provided greater resolution for oligomers of PEGs. © 1998 Elsevier Science B.V.

*Keywords:* Poly(ethylene glycol); Polymers

## 1. Introduction

Poly(ethylene glycol)s (PEGs) and their derivatives are widely used in the textile industry [1,2] as spin finishes, emulsifiers and wetting agents. They are also applied extensively in the pharmaceutical, cosmetic and food industries. In particular, low-molecular-mass PEGs have been widely used in clinical research [3]. There are, therefore, many requirements for accurate quality control and product characterisation, particularly for clinical study.

Since oligomer distribution of PEGs is an important factor in quality control, chromatography is the preferred method for analysing such compounds. Other methods such as Fourier transform IR [4], colorimetry [5] and turbidimetry [6] could provide only bulk information for PEGs and are subject to much interference. Paper chromatography [7] has been used for the determination of PEGs, but is limited by its insensitivity and low repeatability. Supercritical fluid chromatography has been applied to determine PEGs [8], but it is not a popular analytical instrument. Recently, capillary gel electrophoresis [9] has been successfully applied to the separation of PEG oligomers, but it has drawbacks such as column fragility and cost.

Several high-performance liquid chromatographic methods [10–13] have been developed to determine PEGs of $M_r$ 200 to 2000. Most of these methods

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0021-9673/98/$19.00 
*PII* S0021-9673(97)01150-3

have focused on application of reversed-phase high-performance liquid chromatography (RP-HPLC). Some of them met with difficulty of resolution of oligomers for PEGs [12,13]. Okada [14] has used a cation-exchange resin for separation of PEG 400 oligomers according to the difference in the complexion with $K^+$. Since PEGs lack an easily detected moiety, the development of detection methods is usually an important aspect in most literature. Derivatisation techniques [11,15,16] have been used to enable the use of a conventional UV detector and are still the preferred detection method due to the popularity of the UV detector. The derivatisation also allows for fluorescent detection [11] to be used to improve the detection limits and to avoid interference. Without derivatisation, PEGs have been determined by using low-wavelength UV with the addition of sodium azide to equalise solvent absorbances of mobile phase components [8,17]. However, baseline drift was still a problem. Refractive index (RI) detection [12,13,18] has also been used, although its sensitivity is low and its application is limited to isocratic elution and qualitative analysis. Evaporation and light scattering detection (ELSD) has been successfully applied in the determination of PEGs without derivatisation [19]. Mass spectrometry (MS) has been also used as detector in HPLC for analysis of PEGs [20–22]. However, these types of detectors are not available for most laboratories.

Several reagents have been applied for derivatisation of PEGs to render their oligomers detectable. Chromophore-bearing reagents such as 3,5-dinitrobenzoyl chloride (DNBC), phenylisocyanate and 1-anthroylnitrile enable UV detection, while 1-/2-naphthoyl chloride and 5-dimethylamino-1-naphthalene-sulfonyl chloride allow fluorescence detection of the PEGs in some complex matrices.

In this investigation, normal-phase HPLC using an amino column has been successfully applied to determine dinitrobenzoate PEGs by their oligomers with baseline resolution. The reliability of quantitative derivatisation of PEGs by dinitrobenzoyl chloride and analysis by HPLC has also been evaluated. This method has been expanded to determine PEG 400 in a spin finish formulation. RP-HPLC using a $C_8$ column has also been used to resolve the oligomers of PEG 400.

## 2. Experimental

PEG 400 and a textile finish consisting of PEG 400 and PEG ester were supplied by Benjamin R. Vickers and Sons, UK. PEG 600, 1000 and 2000 were purchased from Aldrich, UK. Hexaethylene glycol (HEG) was purchased from Fluka, UK. DNBC was purchased from Lancaster Synthesis, UK. All solvents used as mobile phases were HPLC grade. All samples for HPLC analysis were prepared by dissolving the analytes in dichloromethane (DCM).

The HPLC system consisted of Model 510 pumps, a Model 660 solvent programmer, a Rheodyne 7725i manual injector, a Model 455 UV absorbance detector operated at 276 nm and a Model 740 integrator (Waters, UK). A 5 μm Spherisorb $NH_2$ column and a 5 μm Columbus $C_8$ column (250×4.6 mm; Phenomonex, UK) were used. Samples (5 μl) were injected into the HPLC column for all the analyses. All mobile phase solvents were ultrasonically degassed. The flow-rate was 1 ml $min^{-1}$.

The derivation reaction was similar to those reported previously [23]. An intimate mixture of DNBC (0.15 μm) and PEG (0.05 μm) was placed in a 25-ml round bottom flask. After 2 ml DCM and a few magnesium shavings were added, the flask was set for reflux on a water bath whose temperature was set to rise from 40°C to 80°C in 30 min. The product was cooled, transferred into a separatory funnel with 10 ml DCM and shaken with 2% sodium carbonate solution (10 ml). The organic phase was dried with anhydrous sodium sulphate (2 g) and then transferred to a 10-ml volumetric flask. 10, 20,...50 mg PEG 400 with DNBC (1:3 mol ratio) were examined for quantitative derivatisation.

## 3. Results and discussion

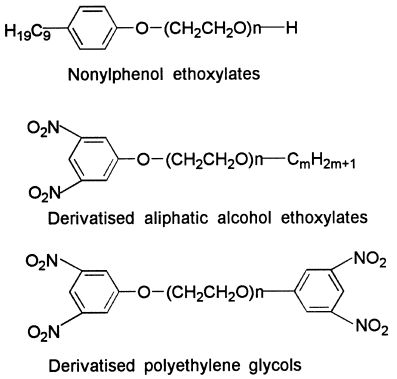
### *3.1. Resolution of PEG oligomers by normal-phase HPLC*

Although application of normal-phase HPLC has rarely been reported for determination of PEGs and their derivatives [24], there are many reports involving the use of normal-phase HPLC to determine

nonylphenol ethoxylates (NPEOs) [25,26] and aliphatic alcohol ethoxylates (AEs) [23,27,28]. Derivatised PEGs are chemically similar to NPEOs and derivatised AEs. The chemical structures (Fig. 1) indicate that the polarity of dinitrobenzoate PEGs should be close to that of NPEOs and aliphatic AEs.

Since normal-phase HPLC using an amino stationary phase has been successfully applied to determine NPEOs and AEs in this laboratory [23,25], it was selected to examine its ability to resolve PEG oligomers. Like AEs, PEGs have no UV detectable chromophore and must be derivatised in order to employ a UV detector. Since most PEGs have a broad oligomer distribution, a gradient elution is necessary for resolution of their oligomers.

PEG 400 was examined using an amino stationary phase and a ternary solvent system consisting of hexane as a base solvent, methanol as a strong solvent and DCM as a co-solvent. Baseline resolution of PEG 400 oligomers was achieved, Fig. 2A. By modifying the ratio of solvents in this ternary mixture, PEG 600 and PEG 1000 were also well separated into their oligomers (Fig. 2B, Fig. 2C). However, the oligomers of derivatised PEG 2000 could not be well resolved using this solvent system. Reasons for the poor resolution of PEG 2000 might be the insoluble nature of the derivatised PEG 2000 in the initial solvent (hexane as the base solvent) or some physicochemical interaction such as the formation of an emulsion of PEG 2000. DCM was tested




where n=0,1,2,3,… and m should be the number between 10,11,…16.

Fig. 1. Structures of three types of poly(ethylene oxide) compounds

as a base solvent for the analysis of PEG 2000, but without success. Using acetonitrile (ACN) as a base solvent [25] with water as strong solvent, the oligomers of PEG 2000 have been well resolved using the gradient programme given in Table 1 (Fig. 2D). This solvent system could also be modified for analysis of PEG 1000 (Fig. 2E). Moreover, it was found that methanol could replace water as the strong solvent in the binary solvent system (Fig. 2F).

The retention mechanism for PEGs on this HPLC system illustrates again that the hydroxyl group plays a key role in the separation of poly(ethylene oxide) [25]. If the retention mechanism was based on the polarity of the mobile phase, pure ACN should be more polar than methanol–DCM (1:4). However, ACN could be used as a base solvent only in the resolution of PEG oligomers. It was interesting to note that PEG 1000 was resolved by three different mobile phases with different polarities. In Fig. 2D and Fig. 2E methanol shows the same eluting ability as water. It is believed that hydroxyl groups in water and methanol interact with uncapped silicon oxide and/or amino groups in the stationary phases. The interaction not only reduces the uncapped silicon oxide available to absorb the PEGs, but also replaces the analytes in the stationary phase.

By comparison with most of the RP-HPLC methods reported, this normal-phase HPLC provided a better resolution for PEGs, particularly for PEG 400 to PEG 1000. It is believed that other modified silica stationary phases such as nitrile and diol could also be applicable in the resolution of PEG oligomers.

### *3.2. Quantitative derivatization and chromatographic analysis*

Although it is commonly accepted that PEGs can be quantitatively derivatised by DNBC, there are many differences in the mole ratio of PEGs to DNBC and in reaction conditions to be found in the literature [11,29,30], e.g., the molar ratio between reactive reagent and PEGs varies from equimolar (2:1) to high excess (>10:1) and different catalysers such as pyridine and magnesium shavings.

In this study, the reaction conditions and the ratio of PEGs to DNBC were investigated. The results demonstrated that the mole ratio of DNBC to PEGs

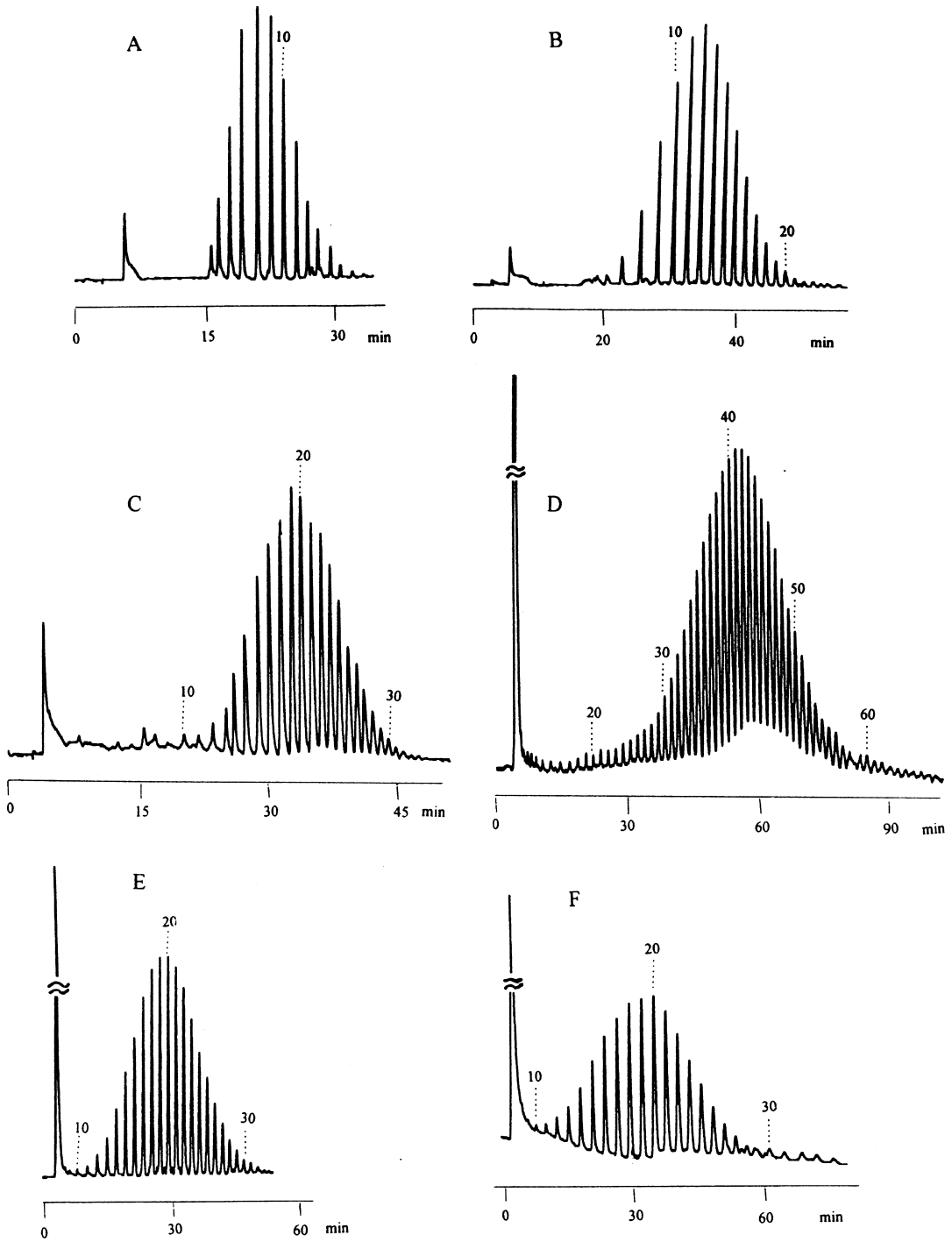



Fig. 2. Chromatograms of 3,5-nitrobenzoyl derivatives of PEGs by HPLC. Conditions: column, 5 μm Spherisorb $NH_2$ (250×4.6 mm I.D.); detection, UV at 276 nm and solvent programmes as described in Table 1. (A) PEG 400, (B) PEG 600, (C) PEG 1000, (D) PEG 2000 (E) and (F) PEG 1000.

Table 1
Solvent programmes for separation of derivatised PEG oligomers by HPLC

| PEGs | Solvent A | Solvent B | Gradient programmes |
|---|---|---|---|
| PEG 400 (Fig. 2A) | Hexane | DCM–methanol (4:1) | 10% B to 40% B in 30 min |
| PEG 600 (Fig. 2B) | Hexane | DCM–methanol (4:1) | 15% B to 50% B in 40 min |
| PEG 1000 (Fig. 2C) | Hexane | DCM–methanol (4:1) | 20% B to 60% B in 50 min |
| PEG 2000 (Fig. 2D) | ACN | ACN–water (4:1) | 20% B to 80% B in 90 min |
| PEG 1000 (Fig. 2E) | ACN | ACN–water (4:1) | 5% B to 50% B in 50 min |
| PEG 1000 (Fig. 2F) | ACN | ACN–methanol (4:1) | 10% B to 90% B in 60 min |
| PEG 400 and 1000 (Fig. 4B) | Hexane | DCM–methanol (4:1) | 10% B to 65% B in 65 min |
| PEG 1000 and 2000 (Fig. 4C) | ACN | ACN–water (4:1) | 10% B to 90% B in 110 min |

should be over 2.5:1. The excess of DNBC produced little interference in the determination of the oligomers of PEGs. Less DNBC in the reaction mixture resulted in the production of mono-dinitrobenzaote PEGs as illustrated in Fig. 3, making it difficult to determine the oligomers of PEGs quantitatively. The results of this investigation seem to recommend that the mole ratio of DNBC to PEGs should be no less than 2.5:1. In addition, it was found that magnesium shavings as the catalyser for removing HCl produced better results and a product which could be easily handled, in comparison to pyridine.

The validity of the derivatising process and the HPLC analysis was tested by constructing a calibration curve of peak area against concentration of derivatised PEGs i.e., increasing amounts of PEG were derivatised and a constant sample size (5 μl) was injected into the HPLC system. The straight line correlation obtained was described by $y=3.2\cdot10^{3}x+67$ ($\gamma=0.995$), where $y$=peak area and $x$=PEG concentration.

It was further tested by conducting a second calibration using HEG. Progressively larger samples of derivatised HEG (of the same concentration) were injected into the HPLC system i.e., 1, 2, 5, 10 and 20 μl. This also produced a linear relationship with $\gamma=0.999$.

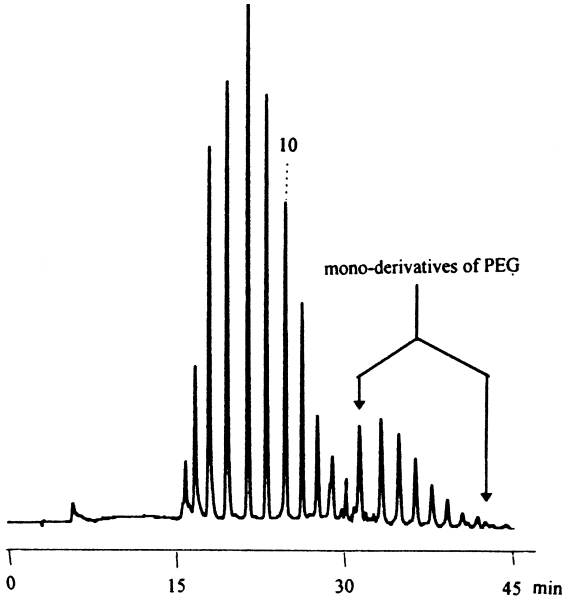



Fig. 3. Chromatogram of mixture of mono- and di-3,5 nitrobenzoyl derivatives of PEG 400. Conditions as described in Fig. 2A.

### 3.3. Oligomer distribution of PEGs

HEG was applied as an internal standard in the analysis of oligomers of PEG 400 (Fig. 4A). The chromatogram clearly demonstrated that octaethylene glycol was the major peak in this PEG 400. The oligomers of the other PEGs were deduced by co-analysis, e.g., PEG 1000 was analysed with PEG 400 and PEG 2000 with PEG 1000 (Fig. 4B, Fig. 4C).

Quantification of derivatised PEGs was examined by determination of the response factors for HEG, PEG 400, PEG 600 and PEG 1000. The response factors were $2.19\cdot10^{-4}$, $3.12\cdot10^{-4}$, $4.77\cdot10^{-4}$ and $7.51\cdot10^{-4}$ ppm for HEG, PEG 400, PEG 600 and PEG 1000, respectively. If expressed in term of micromoles, the response factors were $7.75\cdot10^{-10}$, $7.80\cdot10^{-10}$, $7.95\cdot10^{-10}$ and $7.51\cdot10^{-10}$ i.e., almost identical. This illustrates that the ethylene glycol chain has very little influence on the absorbance of derivatised PEGs at 276 nm and that each individual oligomer of the derivatised PEGs can be quantita-

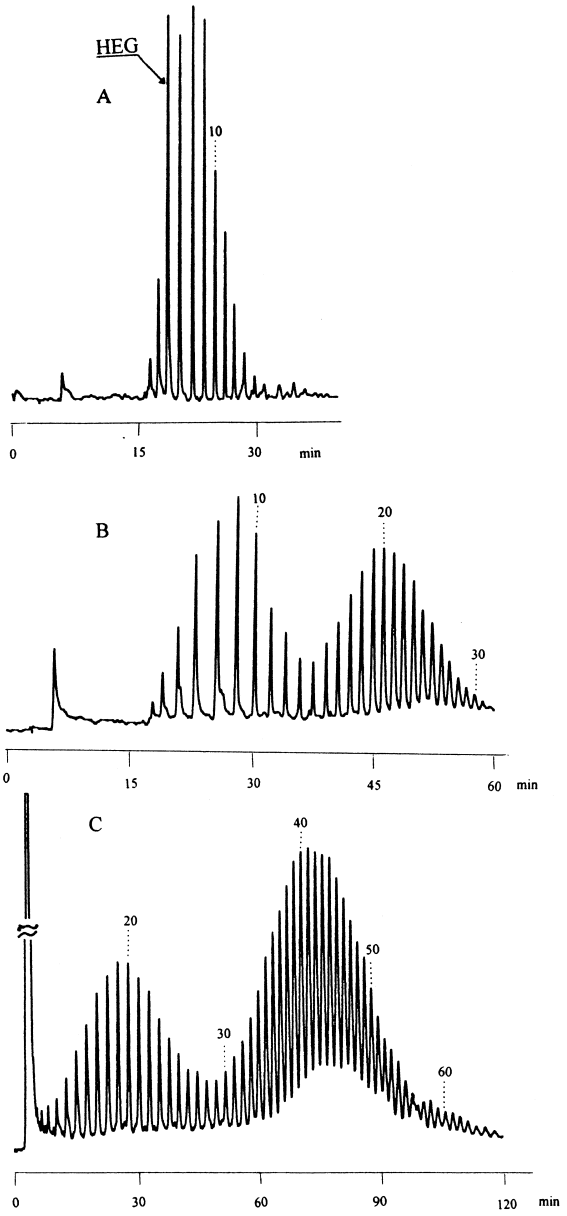



Fig. 4. Chromatograms of mixtures of PEGs. Conditions as described in Fig. 2 and Table 1. (A) PEG 400 and HEG, (B) PEG 400 and 1000 and (C) PEG 1000 and 2000.

tively determined. The detection limit for HEG was calculated as 0.041 ng for qualitative detection ($S/N=3$) and as 0.14 ng for quantitative determination ($S/N=10$).

Fig. 5 gives the oligomer distributions of the

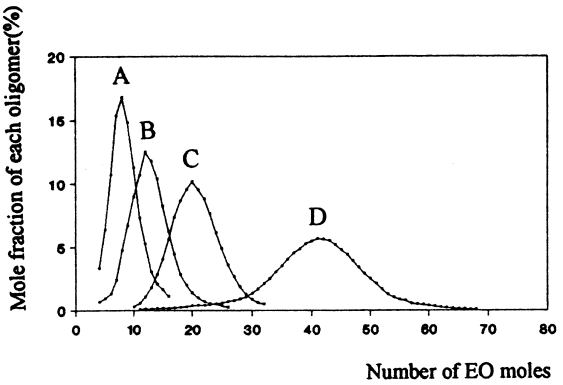



Fig. 5. Oligomer distributions of derivatised PEGs as determined by HPLC. (A) PEG 400, (B) PEG 600, (C) PEG 1000 and (D) PEG 2000.

PEGs, most of which show a Guassian distribution. The oligomer distribution of PEG 2000 was very broad (from EO11 to EO69). The average molecular masses of the PEGs have been calculated [31] as 392, 598, 919 and 1796 for PEG 400, 600, 1000 and 2000, respectively.

### *3.4. Determination of PEG 400 in a textile finish formulation*

Due to their biodegradability PEGs are now used both as emulsifiers and base components in textile finishes. A formulation consisting of 60% PEG 400 and 40% PEG ester was examined by the above HPLC system. Both the initial extraction followed by derivatisation method [23] and the direct derivatisation method as above were found to be suitable for HPLC analysis work. The chromatogram (Fig. 6) showed that there was virtually no background interference from PEG ester. The amount of PEG 400 determined in this formulation was $58.8\pm2.5\%$ demonstrating that this method is suitable for the quantitative determination of PEGs in textile finishes.

### *3.5. Determination of PEG 400 by RP-HPLC*

Although there are many reports [10–13] of the determination of PEGs using RP-HPLC, most of them are very complicated. In this study, a simple chromatographic system was developed. By using a

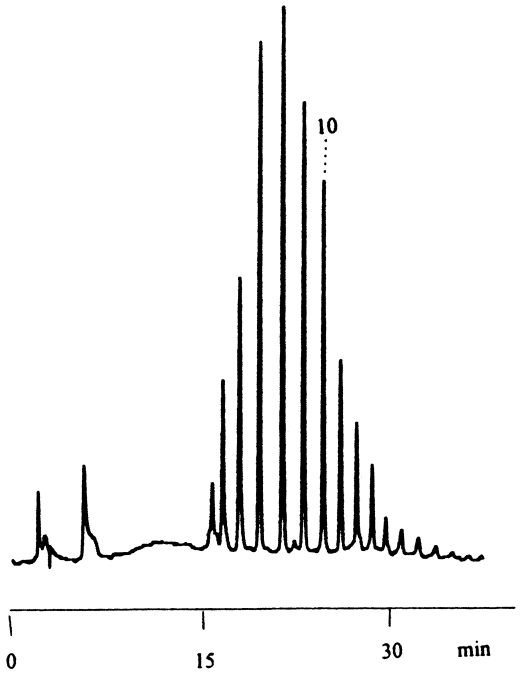



Fig. 6. Chromatogram of derivatised PEG 400 in spin finish by HPLC. Conditions as in Fig. 2A.

$C_8$ column with ACN and water as mobile phase, employing both gradient and isocratic elution, PEG 400 was satisfactorily separated into oligomers (Fig. 7). This system is suitable for the determination of oligomers of PEGs whose average molecular mass is less than 600. However, if the PEGs have high average molecular masses, the resolution of their oligomers was found to be low using this system. The determination of PEGs with high molecular masses is more satisfactorily achieved by using normal-phase HPLC.

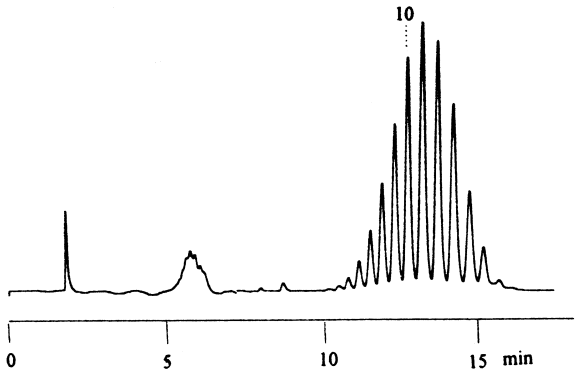



Fig. 7. Chromatogram of derivatised PEG 400 by RP-HPLC. Conditions: column, 5 μm Columbus (250×4.6 mm I.D.); detection, UV 276 nm and mobile phase, gradient elution [solvent A, ACN–water (40:60) and solvent B, ACN] from 20% B to 60% B in 30 min.

## 4. Conclusions

Normal-phase HPLC using an amino column could resolve oligomers of PEGs of average $M_r$ 400 to 2000. This HPLC system was also effective in analysing a mixture of PEGs, as well as PEGs in textile finishes.

The hydroxyl group of the solvent in the mobile phase was found to have an important influence on the retention of PEGs.

RP-HPLC with a $C_8$ column was found to be suitable for determination PEG 400, but it was difficult to resolve PEG oligomers with high molecular masses.

Overall, normal-phase HPLC provided a better resolution of PEG oligomers than RP-HPLC.

## Acknowledgements

The authors thank G. Redman of Benjamin R. Vickers and Sons for supplying the PEG 400 and spin finish sample.

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