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Application of capillary electrophoresis to the analysis of the oligomeric distribution of polydisperse polymers

John Bullock

Sterling-Winthrop Pharmaceuticals Research Division, Malvern, PA 19355 (USA)

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ABSTRACT

Capillary electrophoresis was investigated for characterizing the oligomeric distribution of some model ionic and non-ionic synthetic polymers. Ionic poly(alkyl oxide) oligomers in the molecular mass range from a few hundred to over 4000 were separated into as many as 60 individual oligomers and detected using an indirect UV approach. Neutral Triton X series oligomers from $n = 1$ to $n = 46$ were separated in under 20 min using a sodium dodecyl sulfate matrix with high levels of acetonitrile. Separations have also been obtained on neutral poly(ethylene glycol) polymers after derivatization with phthalic anhydride. In all cases the analyses were conducted in the open tube CE format without the aid of sieving media. Separations of individual oligomers were achieved by incorporating additives into the electrophoretic buffer to modulate either the charge on the surface of the capillary or the charge properties of the oligomeric analytes.

INTRODUCTION

The techniques typically used to characterize the molecular mass properties of synthetic polymers encompass a broad spectrum of physical, spectroscopic and separation methods. In the area of separations, size exclusion chromatography [1,2] and, to a lesser extent, different modes of sorption (reversed-phase, normal-phase and ion-exchange) chromatography [3–6] are used to determine average molecular mass values, molecular mass distributions and compositional heterogeneity in the case of copolymers. Except in the case of lower oligomers, size-exclusion chromatography is not able to completely resolve the various oligomers that might be found in a typical polydisperse polymer. Instead, molecular mass distribution analysis is achieved by calibrating the system with well characterized standards and deconvolution of the polymer sample chromatogram using appropriate data analysis software. Alternatively, molecular mass-sensitive detectors can be used to directly obtain this information from the chromatogram without relying on external calibration. Separation of individual oligomers in polymeric materials has been achieved, in a few instances, using normal- and reversed-phase HPLC [3–5], supercritical fluid chromatography [7], as well as ion-exchange chromatography [6].

Capillary electrophoresis (CE) has rapidly emerged as a fast high-resolution separation technique applicable to a wide variety of charged and uncharged compounds. Given the high resolution that has been demonstrated with CE, it would be anticipated that this technique would have utility for analyzing polydisperse polymers. While numerous reports exist demonstrating the application of CE to the analysis of various types of biopolymers including peptides [8], proteins [9], oligonucleotides [10] and DNA fragments [11], much less work has been reported in the area of CE analysis of other types of synthetic polymers. Poli and Schure [12] described the separation of polystyrene sulfonates using CE. Separation of polymers of different average molecular mass was achieved by incorporating a sieving medium into the buffer, although in-

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dividual oligomers were not resolved. Garcia and Henion [13] recently reported on the interfacing of capillary gel electrophoresis to an ion spray mass spectrometer. The authors demonstrated the separation of poly(acrylic acid) oligomers from $n = 7$ to $n = 14$ using this system. Amankwa *et al.* [14] examined the oligomeric distribution of poly(oxyalkylene) diamine polymers. Samples were derivatized with 2,3-naphthalenedialdehyde and detected by fluorescence detection. And Braud and Vert [15] used CE to resolve several low-molecular-mass oligomeric fragments of poly(malic acid).

In this work, CE in the open tube format with conventional UV detection was investigated for characterizing the oligomeric distribution of different types of polymers. Several different amine-terminated ionic poly(alkyl oxide) polymers were examined encompassing a molecular mass range from several hundred up to 4000. Fast high-resolution separations of individual oligomers were achieved using relatively simple buffer matrices. Triton oligomers from $n = 1$ to $n = 46$ corresponding to a mass range from 200 to 2000 were separated in under 20 min using a sodium dodecyl sulfate (SDS)–buffer matrix containing high levels of acetonitrile. And separations of oligomers of neutral poly(ethylene glycol) in the molecular mass range from about 1000 to over 3500 were also achieved. A different separation and detection strategy was required for the different types of polymers examined. Preliminary results demonstrate that CE will have utility for characterizing molecular mass and sample polydispersion for these polymeric materials. This work addresses some of the separation and detection strategies useful for analyzing these different polymers.

EXPERIMENTAL

Materials

Water was purified using a Milli-Q system (Millipore). Triton X series oligomers were purchased from Sigma. Poly(ethylene oxide) (PEO) molecular mass standards were from Polymer Labs. and poly(ethylene glycols) (PEGs) were purchased from Sigma. Jeffamine ED series polymers (polyoxypropylene–polyoxyethylene oxide diamine copolymers) were from Texaco Chemical. Poly(ethylene oxide) diamine of average molecular mass 3350 was from Sigma. Creatinine, phthalic anhydride and 1,3-diaminopropane were obtained from Aldrich and electrophoresis-grade SDS was from J.T. Baker. All other chemicals were of reagent grade and were obtained from J.T. Baker.

Derivatization of poly(ethylene glycols)

To 1.0 g of poly(ethylene glycol) polymer dissolved in 2 ml of acetonitrile was added an amount (g) of phthalic anhydride equivalent to 600/(average molecular mass of the polymer. The solution contained in a capped 5-ml glass vial was placed in a heating block at 100°C for 15 h. Samples were diluted 5 to 1000 with a mixture of acetonitrile–5 m*M* borate buffer (30:70) at pH 8.7 prior to analysis by capillary zone electrophoresis (CZE).

Instrumentation

A Spectra-Physics Model 1000 CZE instrument was used for this work. The instrument was controlled using an IBM model 70386 personal computer. Data were collected and processed with PENelson Access★Chrom software. All capillary tubing (50 μm I.D. × 375 μm O.D.) was from Polymicro Technologies. Capillaries were conditioned with 1 *M* NaOH for 15 min prior to use. All experiments were conducted in the constant voltage mode at 25°C. Hydrodynamic injections were accomplished using vacuum.

For the analyses of Tritons, a buffer consisting of 25 m*M* boric acid, 50 m*M* SDS adjusted to pH 8.6 in acetonitrile–water (35:65) was used. Samples were prepared in this buffer at concentrations from 0.5 to 3 mg/ml. Typical injection times were 1–2 s. Samples were separated in a 67 cm capillary (60 cm separation distance) at 25 kV (27 μA) and 25°C and detected at 200 nm.

Poly(alkyl oxide) diamines were analyzed in a 30 m*M* creatinine buffer adjusted to pH 4.8 with acetic acid. Various PEO molecular mass standards were added to the electrophoretic buffer at a level of 1 mg/ml to enhance separation of individual oligomers. Polymer samples were prepared in a 1 in 5 dilution of the run buffer at

concentrations from 0.5 to 2 mg/ml and injected for 5 s. Separations were performed in a 44 cm capillary (37 cm separation distance) at 25°C and 25 kV (13 μA). Indirect UV detection at 220 nm was used.

CE separations of phthalic anhydride derivatized samples of poly(ethylene glycol) were separated in a 44 cm (37 cm separation distance) × 50 μm I.D. capillary with a buffer of 57 m*M* boric acid +35 m*M* 1,3-diaminopropane, pH 9.7–acetonitrile (70:30). Samples were injected for 1 s and separated at 25°C and 25 kV (14 μA). Detection was at 205 nm.

RESULTS AND DISCUSSION

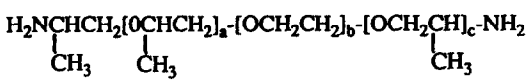
Synthetic polymers come in a wide variety of forms differing in shape (linear, branched, cross-linked, star-burst, etc.), sizes (from molecular masses of hundreds to over a million) and chemical characteristics (ionic, non-ionic, hydrophobic, hydrophilic, etc.). Due to this tremendous diversity in the properties of synthetic polymers, no one electrophoretic technique will be universally applicable to all polymers. Therefore, the particular approach used to analyze different types of polymers will often need to be uniquely tailored to the specific properties of the polymer. Additional considerations in relation to CE are the solubility properties of the polymer and mode of detection available. UV detection is by far the most prevalent detection mode available on commercial CE systems. Many polymers of interest are soluble only in non-aqueous solvents. Since many polymers exhibit very weak or no UV absorbance and show poor aqueous solubility, there are limitations on the use of CE for polymer analysis. The focus of this work is on the CE analysis of some common water-soluble ionic and non-ionic polymers and the different approaches useful for characterizing their oligomeric distributions.

CE analysis of Jeffamine ED series polymers

A series of different poly(alkyl oxide) diamine polymers were used as model compounds to examine the utility of CE for characterizing polymers terminated with ionic groups. This includes diamine homo and copolymers of polypropylene oxide and polyethylene oxide (refer to Fig. 1). The mixed poly(propylene oxide)–poly(ethylene oxide) diamine polymers (Jeffamine ED series) have been analyzed previously [14]. However, the procedure described in this reference entailed the use of a derivatizing agent (2,3-naphthalenedialdehyde) and the use of laser induced fluorescence detection. In the present work, a simpler approach was sought that would allow separation and detection without derivatization and without the need for fluorescence detection which is not available on most commercial instruments. In addition, derivatization would preclude the possibility of detecting monoamine oligomers in these samples, if present as by-products. Since these polymers have negligible native UV absorbance, an indirect UV approach was used as described by Foret *et al.* [16] for the analysis of rare earth metal ions. This entails the use of a UV absorbing back-

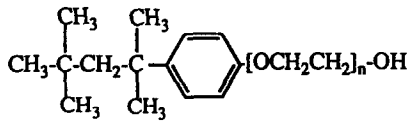
I. Polyoxyethylene/polyoxypropylene Diamines

(Jeffamine ED Series Polymers)



II. Polyethylene Oxide Diamines

III. Triton X Series Oligomers



IV. Poly(ethylene glycol) Derivatized With Phthalic Anhydride

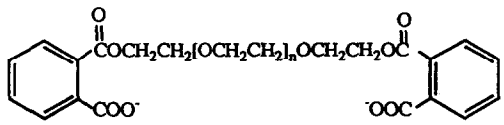


Fig. 1. Structures of polymeric compounds analyzed in this work.

ground electrolyte (creatinine) which carries a positive charge. Other types of UV-absorbing background electrolytes have also been demonstrated for indirect detection of cations [17,18]. The positively charged oligomers partially displace the creatinine in the separation capillary creating a negative UV signal as the analyte zones migrate through the detector. By reversing the leads form the detector, these negative signals produce positive peaks. Using this approach a very fast separation of three Jeffamine polymers of different average molecular mass was achieved in under 5 min as depicted in Fig. 2.

In an attempt to resolve the individual oligomers present in these polymers, several different buffer modifiers capable of reducing the electroosmotic flow were investigated. It was found that neutral PEO polymers were effective for this purpose. The effectiveness of a series of PEO additives of different average molecular mass was evaluated for enhancing the separation of the oligomers in the Jeffamine polymer of average molecular mass 600. The electropherograms in Fig. 3 shows that the higher the molecular mass of the PEO buffer additive, the greater is the reduction in the electroosmotic flow resulting in better resolution of individual oligomers. Optimal separations for the Jeffamine polymers of average molecular mass 600 and 900 are displayed in Fig. 4. The distinctive pattern of peaks observed in these electropherograms is indicative of the compositional distribution of the individual oligomers in terms of relative

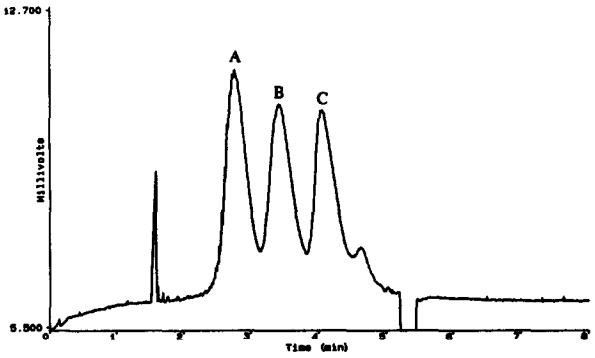


Fig. 2. CE separation obtained on a mixture of Jeffamine ED series polymers of average molecular mass (A) 900, (B) 2000 and (C) 4000. Conditions as given in the Experimental section using a buffer with no PEO additive.

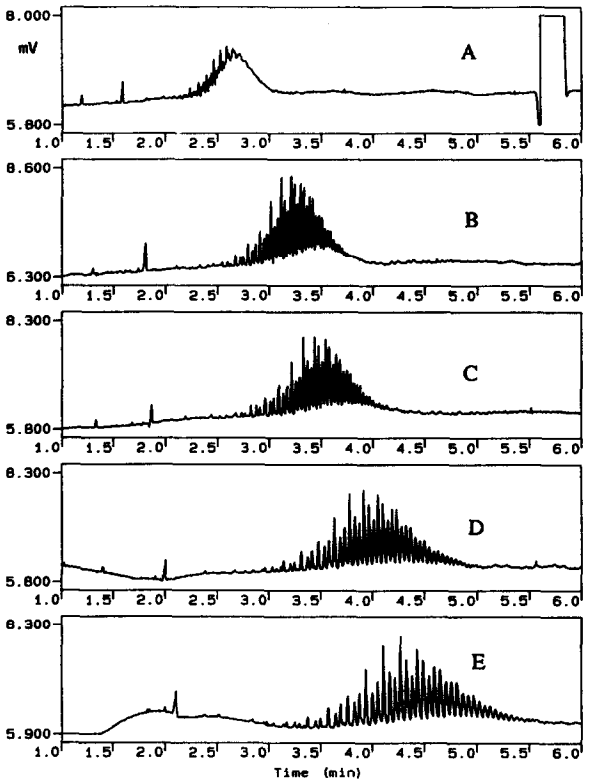


Fig. 3. CE separation of Jeffamine ED series polymer of average molecular mass 600 with (A) no buffer additive, (B) 1 mg/ml PEO-10 000, (C) 1 mg/ml PEO-20 000, (D) 1 mg/ml PEO-42 000 and (E) 1 mg/ml PEO-86 000 added to the buffer. Refer to Experimental section for additional conditions.

proportions of poly(propylene oxide) and poly(ethylene oxide) in each polymer chain.

To demonstrate that this enhancement in the resolution of Jeffamine oligomers with the addition of PEO to the buffer is due solely to reduced electroosmotic flow and not a sieving mechanism, the following experiment was performed. First, the capillary was equilibrated with buffer containing 1 mg/ml of the PEO additive. This buffer was then replaced with a similar buffer that did not have the PEO additive just prior to injecting the sample. The separation achieved in this experiment (data not shown) was virtually identical to that obtained when the PEO was present in the buffer. This is due to the fact that the PEO that was initially absorbed to the capillary surface is very slowly desorbed from the capillary. This maintains the significantly

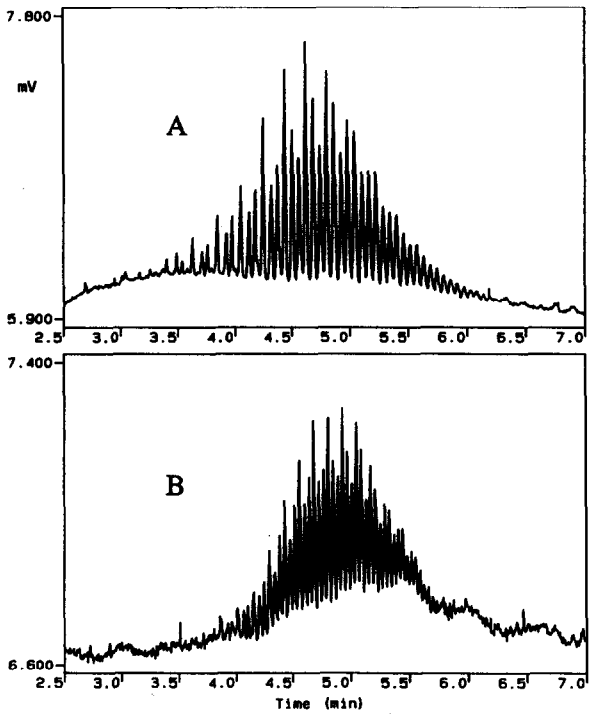


Fig. 4. Optimal CE separation for Jeffamine ED series polymers of average molecular mass (A) 600 and (B) 900. Conditions as given in the Experimental section with 1 mg/ml PEO-86 000 buffer additive.

reduced electroosmotic flow which produces the enhanced resolution of individual oligomers. It is conceivable that this dynamic coating of PEO also aids in reducing any interaction of the polymeric diamines with the surface of the capillary.

Poly(ethylene oxide) diamine polymers

A series of poly(ethylene oxide) diamine oligomers encompassing a molecular mass range from 1100 to 4000 were separated using a similar separation/detection protocol as depicted in the electropherogram in Fig. 5. Baseline resolution of oligomers from $n = 26$ to $n = 90$ were achieved in 16 min using a buffer containing 1 mg/ml PEO-86 000. Tentative assignment of oligomer numbers in this electropherogram is based on the peak average molecular mass values (determined by size-exclusion chromatography) for the three respective PEO–diamine polymers (molecular masses 1500, 2000 and 3350) composing this sample. The dependence of the migration time

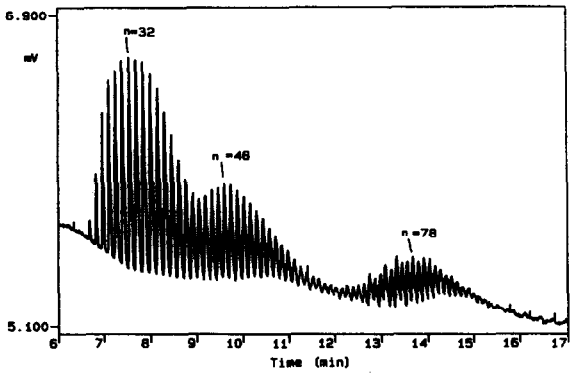


Fig. 5. CE separation of poly(ethylene oxide) diamine oligomers of molecular masses from 1100 to 4000. Conditions as given in the Experimental section using a buffer with 1 mg/ml PEO-86 000.

of the poly(ethylene oxide) diamine oligomers on their molecular mass is depicted in the plot in Fig. 6. The data (migration time *versus* log molecular mass) was fit to a third order polynomial resulting in an R^2 value of 1.000. Table I contains precision of migration time data for selected oligomers for replicate injections of this sample. R.S.D.s for migration times were all under 1.0%. This data suggests that this technique will be useful for molecular mass distribution analysis. Fig. 7 contains an electropherogram of a poly(ethylene oxide) diamine polymer of average molecular mass 1500. Using

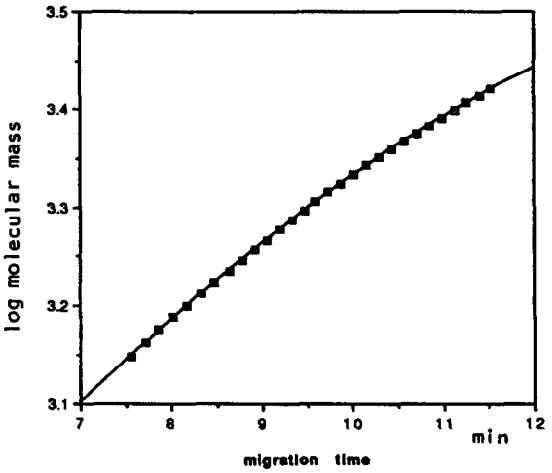


Fig. 6. Plot of the log of molecular mass of poly(ethylene oxide) diamine oligomers *versus* migration time. Data fit to a third-order polynomial. $y = 2.3534 + 0.11975x - 1.0690 \cdot 10^{-3}x^2 - 1.1161 \cdot 10^{-4}x^3$; $R^2 = 1.000$.

TABLE I

PRECISION OF MIGRATION TIMES FOR POLYETHYLENE OXIDE DIAMINE OLIGOMERS BY CE

CE conditions as given in Fig. 5. Mean, etc. are of 7 determinations.

	Migration times (min) for oligomers					
	$n = 27$	$n = 30$	$n = 33$	$n = 36$	$n = 39$	$n = 42$
Mean (min)	6.67	7.07	7.49	7.94	8.41	8.86
S.D. (min)	0.0430	0.0465	0.0522	0.0604	0.0641	0.0826
R.S.D. (%)	0.644	0.658	0.696	0.761	0.763	0.933

this technique, monoamine by-product oligomers present in this sample can be separated from the corresponding diamine oligomers.

CE analysis of Tritons

CE analysis of neutral polymers such as Triton X series oligomers which contain significant hydrophobic character is more problematic. Since they do not contain any charge, separation in the free solution mode is precluded. The related technique of micellar electrokinetic capillary chromatography is available but is of limited utility for these types of compounds due to the large size of the polymer and the strong interaction of the hydrophobic portions of the polymer with the SDS micelle. Separation of these types of polymers was achieved here using an alternative approach. By incorporating high concentrations of acetonitrile (>30%) into an SDS-

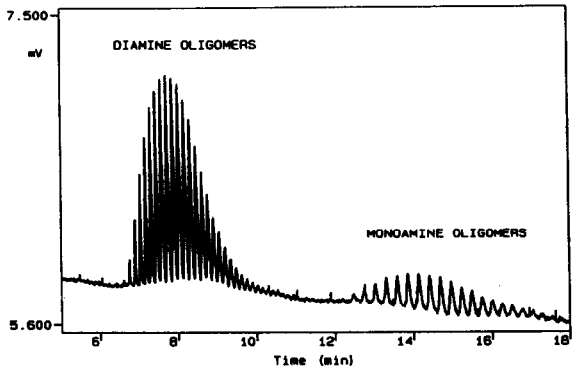


Fig. 7. CE separation of poly(ethylene oxide) diamine polymer of average molecular mass 1500 showing the separation of the monoamine oligomer by-products. Conditions as described in the Experimental section using 1 mg/ml PEO-86 000 in the buffer.

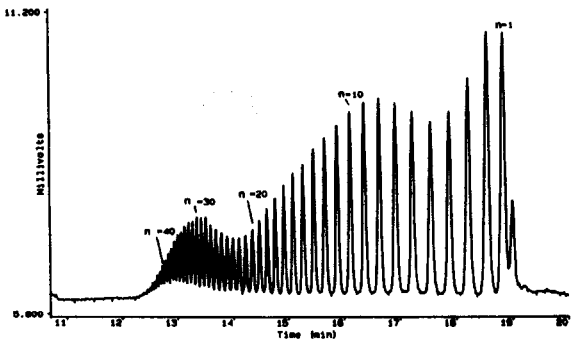


Fig. 8. CE separation of Triton X series oligomers from $n = 1$ to $n = 46$. Conditions as given in the Experimental section.

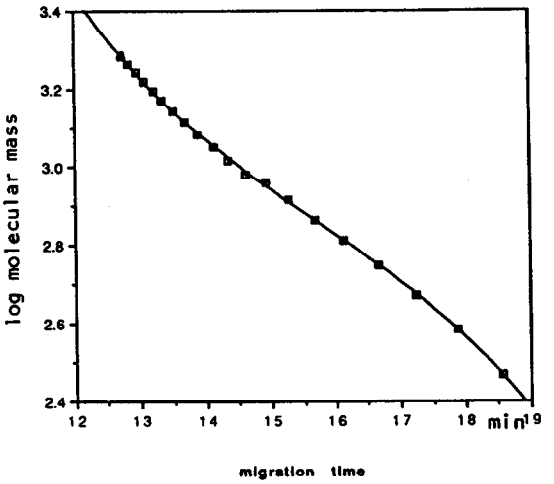


Fig. 9. Plot of the log of the molecular mass of Triton X series oligomers *versus* migration time. Data fit to a third-order polynomial. $y = 16.652 - 2.3997x + 0.14509x^2 - 3.0695 \cdot 10^{-3}x^3$; $R^2 = 1.000$.

containing buffer, the micelle structure is essentially broken down. The hydrophobic portions of individual SDS molecules can still associate with the hydrophobic portions of the Triton oligomers. This type of mechanism has been described as solvophobic association by Walbroehl and Jorgenson [19]. Individual Triton oligomers will, to a first approximation, associate with the same amount of SDS due to an apparent strong interaction with the alkylaryl portion of the molecule. Individual oligomers can then be separated based on differences in the lengths of the poly(ethylene oxide) chains. Fig. 8 shows the separation of Triton X series oligomers from $n = 1$ to $n = 46$. The dependence of the migration times on molecular mass is depicted in the plot of migration time *versus* log molecular mass shown in Fig. 9. Data were fit to a third-order polynomial resulting in an R^2 value of 1.000. The precision of the migration times for selected oligomers was determined for replicate injections. The data are compiled in Table II. The precision for absolute migration times achieved here is not as good as obtained for the poly(alky oxide) diamines, although the precision of relative migration times was quite good. It is anticipated that the precision of absolute migration times will be improved by incorporating an appropriate capillary regeneration protocol into the analysis. Fig. 10 shows the separation obtained on the oligomers in a sample of Triton X-100 surfactant which indicates about 20 oligomers.

To elucidate the mechanism of this separation (micellar or solvophobic association) conductivity measurements were obtained on solutions containing 25 m*M* boric acid pH 8.6, 35% acetonitrile with different amounts of SDS from 0 to 50 m*M*. A linear increase in conductivity (57.1 μS/m*M* SDS, $R^2 = 1.000$) was observed with increasing concentration of SDS. A similar experiment conducted in water without any acetonitrile revealed a sharp break in the conductivity relationship at about 7 m*M* which is consistent with the formation of micelles. These data indicate the absence of micelles in the presence of 35% acetonitrile and, therefore, a solvophobic association mechanism.

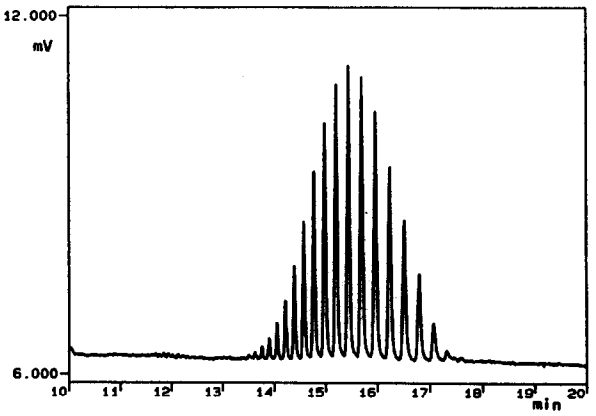


Fig. 10. CE separation of Triton X-100. Conditions as given in the Experimental section.

CE analysis of neutral poly(ethylene glycol) polymers

Neutral PEG polymers were not directly amenable to CE analysis due to a lack of charge as well as the absence of a UV chromophore. In order to impart these two characteristics to the PEG, a modification of the classical phthalic

TABLE II

PRECISION OF CE MIGRATION TIMES FOR TRITON X SERIES OLIGOMERS

Conditions as given in Fig. 8. Mean, etc. are of 7 determinations.

	Migration times (min) for oligomers					
	$n = 1$	$n = 5$	$n = 10$	$n = 20$	$n = 30$	$n = 40$
Mean (min)	18.40	17.18	15.83	14.13	13.17	12.56
S.D. (min)	0.4155	0.3517	0.2866	0.2182	0.1842	0.1646
R.S.D. (%)	2.26	2.05	1.80	1.54	1.40	1.31

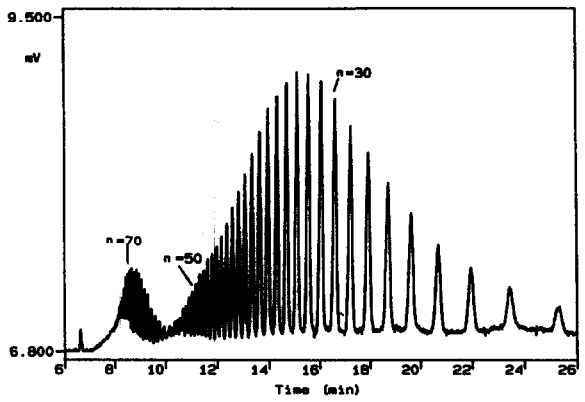


Fig. 11. CE separation of PEG oligomers derivatized with phthalic anhydride. Conditions as given in the Experimental section.

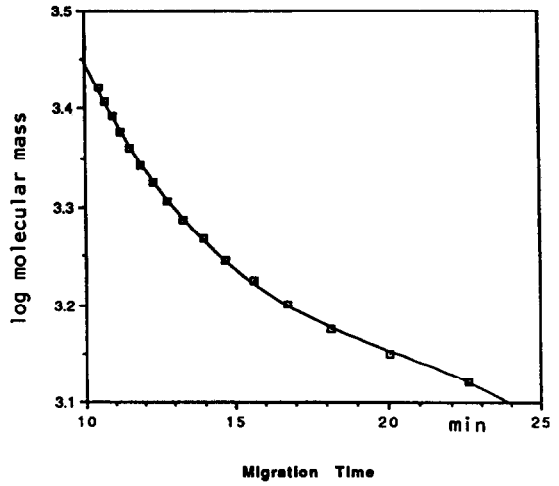


Fig. 12. Plot of the log of the molecular mass of PEG oligomers *versus* migration time. Data fit to a third-order polynomial. $y = 4.7042 - 0.20473x + 9.4356 \cdot 10^{-3}x^2 - 1.5388 \cdot 10^{-4}x^3$; $R^2 = 1.000$.

anhydride derivatization chemistry [20] was used. By reacting the terminal hydroxyl groups of the PEG with phthalic anhydride, a phthalate ester is tagged onto each end of the polymer giving it a chromophore (phenyl group) and ionic termini (carboxylic acids).

Fig. 11 shows a free solution CE separation of a mixture of a series of derivatized PEG oligomers with molecular masses from about 1000 to over 3500. In this case separation of individual oligomers was achieved by incorporating acetonitrile and 1,3-diaminopropane into the separation buffer. Both of these modifiers act to reduce the electroosmotic flow which correlated with improved resolution of individual oligomers. Fig. 12 shows a plot of the dependence of migration time on the molecular mass of the PEG oligomers. Again, the data were fit to a third-order polynomial resulting in an R^2 value of 1.000. To gauge the reproducibility of the separation, migration times for individual oligomers were measured for multiple injections. The data contained in Table III demonstrates R.S.D.s of under 1.0%.

CONCLUSIONS

This work has examined the utility of CE for characterizing the oligomeric distribution of different types of polydisperse polymers. Success has been achieved for polymers in which a difference in charge-to-size ratio for the oligomers exists (terminal ionic polymers) or where a hydrophobic tail is present at the ends of hydrophilic polymer chains (Tritons). For neutral

TABLE III

PRECISION OF CE MIGRATION TIMES FOR PEG PHTHALATES

Conditions as given in Fig. 11. Mean, etc. are of 7 determinations.

	Migration times (min) for oligomers					
	$n = 25$	$n = 30$	$n = 35$	$n = 40$	$n = 50$	$n = 65$
Mean (min)	20.02	16.15	13.94	12.51	10.79	8.88
S.D. (min)	0.1992	0.1343	0.1056	0.0877	0.0670	0.0486
R.S.D. (%)	0.995	0.831	0.758	0.701	0.621	0.547

PEG polymers, separation was achieved after derivatizing the polymer to impart charge and a UV chromophore. Each different type of polymer can present a different set of problems related to the separation and detection schemes needed for the analysis. Future plans include analysis of larger-sized polymers and the incorporation of sieving media to extend the utility of the technique to other types of polymers.

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