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Analysis of ethoxylated polymers by capillary electrophoresis in UV-transparent polymer networks and by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

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Abstract

This report describes the application of capillary electrophoresis (CE) in UV-transparent polymer solutions to the separation of poly(ethylene glycol)s and ethoxylated surfactants. Polymer networks of dextran and polyethylene oxide provide size selectivity in these separations. These ethoxylates were derivatized prior to CE analysis with 1,2,4-benzenetricarboxylic anhydride (BTA) to impart charge and detectability on the neutral polymer. The utilization of a polymer network with low UV absorbtion at 210 nm permits the detection of the ethoxylates with high sensitivity at this wavelength. Migration time was found to be linearly dependent on analyte molecular mass. The results obtained by capillary electrophoresis (CE) are compared with those obtained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and the off-line coupling of these techniques is described. © 1998 Elsevier Science B.V.

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1. Introduction.

Polymers with polyoxyethylene chains are an important class of compounds with extensive application in the consumer product industry. The preparation of these polymers usually involves the base-catalyzed reaction of ethylene oxide with a selected starting material. For example, ethoxylated surfactants are produced by reaction of ethylene oxide with a hydrophobe containing a reactive functionality. This process does not produce a discrete product but instead yields a polydisperse distribution of compounds. The dispersity and number-average molecular mass (M_n) of a distribution of polymers strongly influences its performance in a particular application. For this reason there has been considerable effort placed into the development of analytical methodology for the characterization of such polymer distributions [1,2].

Gel permeation chromatography (GPC) is the most commonly employed separation technique for determining the M_n and polydispersity of ethoxylated polymers [3]. High-performance liquid chromatography (HPLC) has also been used to obtain higher resolution separations of the components in polymer distributions [4–8]. More recently, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) [9–21] and, to a lesser extent, CE [22–24], are finding increased application in the field of synthetic polymer characterization. Absolute molecular masses can be often obtained with MALDI-TOF-MS. In addition, MALDI-TOF-MS typically demonstrates greater resolution of the components of a polymer distribution than chromatographic methods such as HPLC, especially when the degree of polymerization (D.P.) is relatively high

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(>20). MALDI-TOF-MS has the ability to rapidly measure the exact molecular mass and the relative contributions of the individual components in one or more distributions.

Capillary zone electrophoresis (CZE) and capillary gel electrophoresis (CGE) have been both applied to the separation and detection of ethoxylated surfactants and poly(ethylene glycol)s (PEGs) [22,23]. Bullock [22] demonstrated that CZE can resolve low-molecular-mass PEGs with D.P.s up to approximately 50. Recently, Wallingford utilized capillary gel electrophoresis (CGE) to resolve higher-molecular-mass PEG distributions (and ethoxylate surfactants) containing up to 120 ethylene oxide units (EO units) [23]. Despite the remarkable performance of CGE for ethoxylate separation, there are a number of problems associated with its use [23]:

1. Higher-molecular-mass ethoxylates suffer from both low sensitivity and lengthy analysis times. Cross-linked polyacrylamide absorbs appreciably below 230 nm and it was therefore not possible to improve sensitivity by utilizing a lower wavelength for ethoxylate detection [23]. Run-times are rather long, for example, the distribution associated with a PEG 3350 (PEG of $M_n \approx 3350$) is resolved in approximately 50 min [23].
2. Capillaries with cross-linked polyacrylamide gels are difficult to make reproducibly and performance may vary substantially [25]. They are also relatively expensive, fragile and have a limited lifetime.

As an alternative to cross-linked polyacrylamide, dilute solutions of polymers are often employed to supply the size discrimination required in CE separations [26]. Polymers such as polyvinylpyrrolidone have also demonstrated selectivity in separations which is not solely size dependent [27,28]. The appropriate polymer is dissolved in the running buffer and introduced by pressure into the capillary. The solution may be replaced after each run thereby increasing run-to-run reproducibility [29].

This paper reports the separation of ethoxylate polymers using CE in dilute solutions of dextran and polyethylene oxide. These solutions display low absorbance in the UV at 210 nm thus facilitating more sensitive detection of ethoxylates at this wavelength [30]. The ethoxylate polymers are derivatized with 1,2,4-benzenetricarboxylic anhydride (BTA) prior to the analysis (Fig. 1). This adds charge and a chromophore to the neutral polymer and permits their electrophoretic separation and UV detection. Previous studies utilized phthalic anhydride for this purpose [22,23]; however, the derivative formed with BTA is more charged at pH 8.3 resulting in substantially faster migration times and increased efficiency.

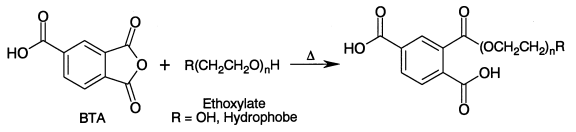

Fig. 1. Schematic of the derivatization of ethoxylates with BTA.

The second section of this paper compares results obtained by CE with those obtained by MALDI-TOF-MS. The latter technique can measure the absolute molecular mass of the polymer(s) more rapidly and without derivatization. CE, however, offers the possibility of quantitation and exhibits a different selectivity in the separation of ethoxylates. This difference may be used to separate distributions in certain polymer blends which are not easily resolvable by MS. The off-line coupling of these techniques is demonstrated.

2. Experimental

2.1. CE conditions

The separations were obtained on a Bio-Rad (Hercules, CA, USA) Biofocus 3000 CE system. Linear polyacrylamide (LPA)-coated silica capillaries (Bio-Rad Labs.) of 50 cm (effective length 45.6 cm)×75 μm I.D.×360 μm O.D. were utilized in these separations. The buffer consisted of 60 m*M* Tris–TAPS (Aldrich, Milwaukee, WI, USA) at pH 8.3. Dextran ($M_w \approx 2\,000\,000$) was obtained from Sigma (St. Louis, MO, USA). Stock solutions of dextran (3–5%) were made in the separation buffer (%, w/w=wt. polymer/vol. buffer×100). The resultant solution was introduced into the capillary by pressure injection. The voltage applied across the capillary (28 kV) generated a current of approximately 25 μA. Sample solution was introduced by pressure injection at the cathode end. The ethoxylates were detected at 210 nm. Fractions were

collected in receiver vials containing deionized water.

2.2. MALDI-TOF-MS

The mass spectra were measured by a Hewlett–Packard (HP) G2030A linear MALDI-TOF-MS (Palo Alto, CA, USA). The matrix utilized was prepared with 400 m*M* DHB (2,5-dihydroxybenzoic acid) in methanol. The matrix components were purchased from Aldrich. The samples were crystallized under a vacuum produced by the HP G2024A Sample Prep Accessory. The crystallization process is relatively rapid (<30 s).

2.3. Derivatization

The octylphenol ethoxylates were obtained from normal production lots at Rhone-Poulenc. PEG standards were purchased from Aldrich. Derivatization was performed by adding 2 ml of a 1 *M* solution of BTA in tetrahydrofuran (THF) to 0.1 g of polymer and heating the resultant solution at 100°C for 5 h. A schematic of the derivatization is shown in Fig. 1.

2.4. Sample preparation for CE analysis

The high relative concentration of derivatizing agent (BTA) and hydrolyzed BTA may interfere with the separation and detection of the analytes. Two approaches were successfully employed to remove the excess reagent. The first approach involved dialyzing the sample in disposable cellulose ester dialysis tubing with MWCO (molecular mass cut-off) of 1000 (Sigma). The tubing containing 1 ml of the sample was placed into a methanol–water (30:70) solution (1 l) and the solution stirred vigorously for 4 h. The sample was removed from the tubing and diluted 50-fold with deionized water prior to CE analysis. This approach, although successful in reducing low molecular mass interferences, is time consuming. The alternative method utilized solid-phase extraction. The sample (100 μl) was loaded onto a 3-ml, C_{18} stationary phase, disposable extraction column (J.T. Baker, Phillipsburg, NJ, USA). The excess reagent was removed by applying 10 ml of acetonitrile–water (10:90) solution. The ethoxylates were subsequently eluted in 1 ml of acetonitrile.

3. Results and discussion

3.1. Separation of PEGs by CE

Derivatization of PEGs with BTA provides four ionizable groups for each PEG molecule. Fig. 2a–c show the electropherograms obtained from three PEG samples ($M_n \approx 2000$, 3400 and 4700, respectively). Previous studies with cross-linked polyacrylamide gel columns demonstrated better resolution [23] but poor sensitivity and substantially longer run-times. The employment of a UV-transparent polymer network, such as dextran or polyethylene oxide [30], permits the detection of the polymer with greater sensitivity at a lower wavelength (210 nm). In addition, the derivative formed with BTA is more highly charged than that obtained with phthalic anhydride, thereby producing faster run-times and increased efficiency. The reaction time is substantially longer for BTA derivatization, however, when running multiple samples it is easy to multiplex the derivatization but not the separation. Separation efficiencies range from 250 000 to 450 000 theoretical plates. Migration time reproducibilities were between 0.7 and 1.0% R.S.D. ($n=30$, repeated analysis of a PEG 2000). Although the same polymer network may be used for multiple injections, reproducibility was superior when the polymer solution was replaced after each run. The two peaks eluting at approximately 3.6 and 4.5 min correspond to hydrolyzed BTA (faster migrating peak) and BTA itself.

Fig. 3 shows that there is a linear relationship between molecular mass and migration time ($R^2=0.99998$). The linearity of this relationship permits straight-forward calibration of PEG sizes. The plot was constructed with data obtained from an electropherogram of a sample containing a mixture of PEGs with D.P.s ranging from 30 to 120 (3% dextran solution, see Section 2.1 for CE conditions). The linearity of this plot also indicates that the separation mechanism involved with the polymer solution is similar to that observed with capillary gel electrophoresis [23].

Polyethylene oxide (PEO) solutions were found to

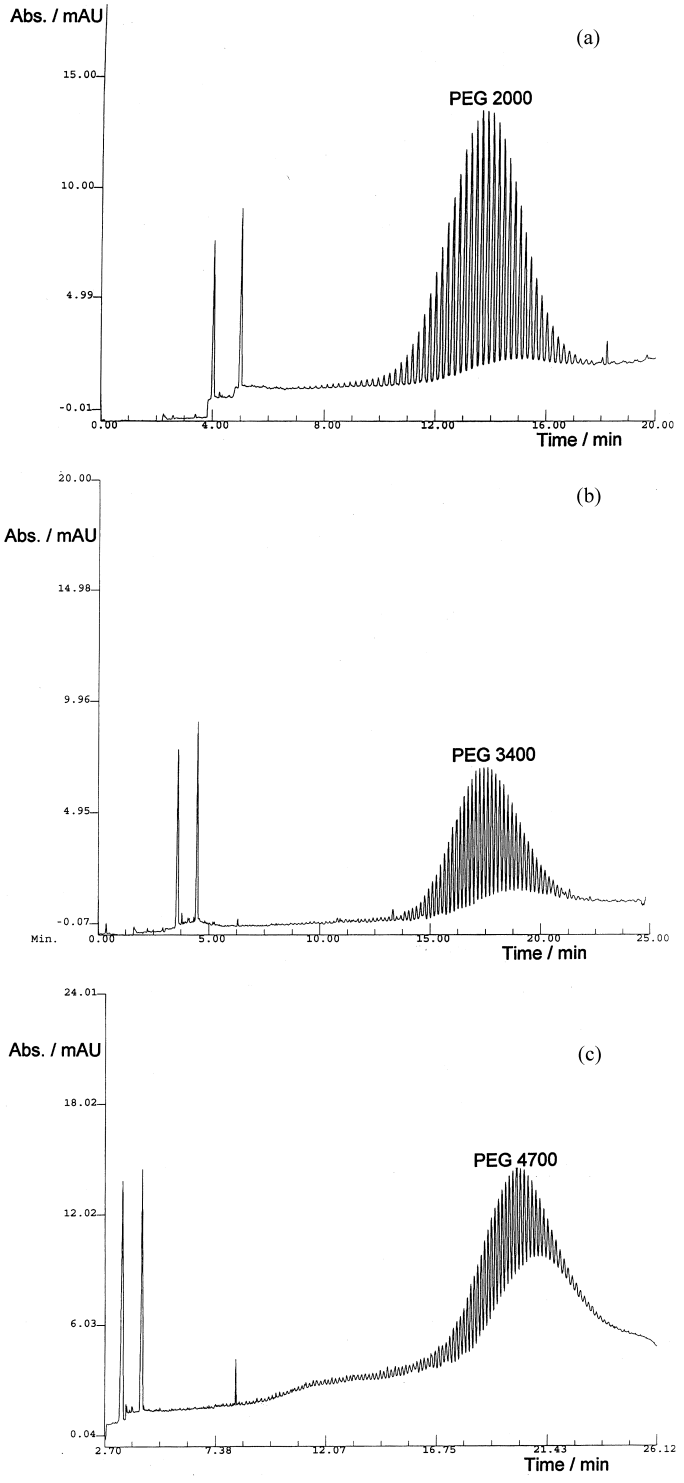

Fig. 2. Electropherogram of a PEG 2000 4% dextran solution. (b) Electropherogram of a PEG 3400 3% dextran solution. (c) Electropherogram of a PEG 4700 3% dextran solution.

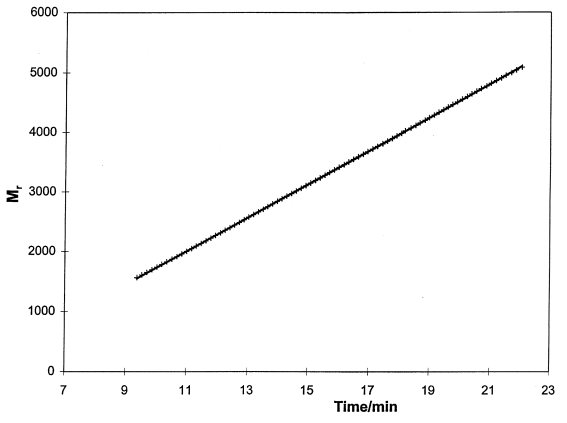

Fig. 3. Linear plot of M_r vs. migration time $R^2=0.99998$.

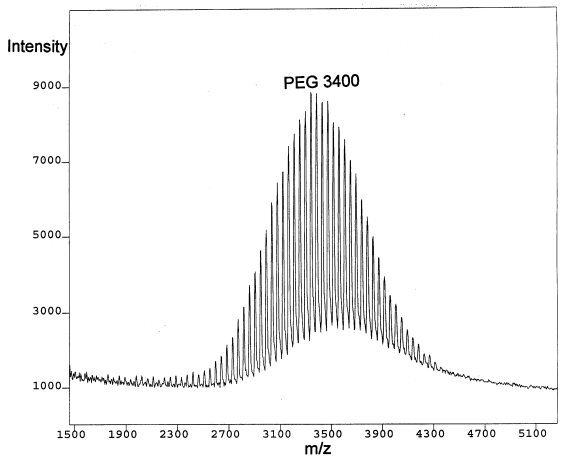

Fig. 4. MALDI-TOF-MS of a PEG 3400.

produce similar selectivity and efficiency to dextran and also enable low wavelength monitoring of the ethoxylates [30]. Batch-to-batch variation in migration times was lower for PEO solutions probably reflecting greater variation of dextran polymer distributions. The use of an internal calibrant is necessary to eliminate the potential problems of batch-to-batch reproducibility.

3.2. MALDI-TOF-MS separation and detection of PEGs

As mentioned previously, MALDI-TOF-MS is becoming increasingly popular for the analysis of synthetic polymers and has demonstrated conspicuous success for analysis of ethoxylated polymers. The advantages of MALDI-TOF-MS over other techniques are compelling: relatively high resolution separation, ease of sample preparation, speed of the analysis and the capability of measuring absolute molecular masses of components directly. CE, on the other hand, is less expensive, amenable to automation and offers the possibility of quantitative analysis—an important limitation of MALDI-TOF-MS. Fig. 2b Fig. 4 compare the separations of a PEG 3400 obtained by CE and MALDI-TOF-MS. The figures illustrate that CE can separate the components in PEG distributions with resolution which is at least comparable, if not superior, to the linear TOF instrument. Improvements by delayed extraction and reflectron technology, of course, enhance the resolution of the MS method.

3.3. Separations with CE and MALDI-TOF-MS

At first glance it appears that the separation selectivity of CE and MS are the same in this application — CE and MS both separate on the basis of mass (size)-to-charge ratio. Thus, coupling these techniques would not increase separation capability in the same way as coupling separating techniques with orthogonal selectivities. This, however, is only partially true. Because MALDI produces predominantly singly charged ions, the separation selectivity is more strictly based on mass whereas CE separates on the basis of size-to-charge ratio. An example of the use of this selectivity difference is described below.

PEG is a commonly produced by-product (resulting from non-anhydrous conditions) in the production of ethoxylated surfactants. The % of PEG in a surfactant batch is an important criterion of batch quality and performance. CE can separate PEGs and surfactants of similar mass more readily than MALDI-TOF-MS. The PEG oligomers carry twice the charge as the surfactant in the CE run (there are two hydroxyl groups derivatized on PEG as opposed to just one on the surfactant). Fig. 5a shows the MALDI-TOF-MS spectrum of a mixture of PEG ($M_n \approx 2000$) and an octylphenol ethoxylate surfactant ($M_n \approx 1600$). There are clearly two distributions observable in the spectrum; however, the distributions are not completely resolved making it difficult to determine M_n or the weight-average molecular

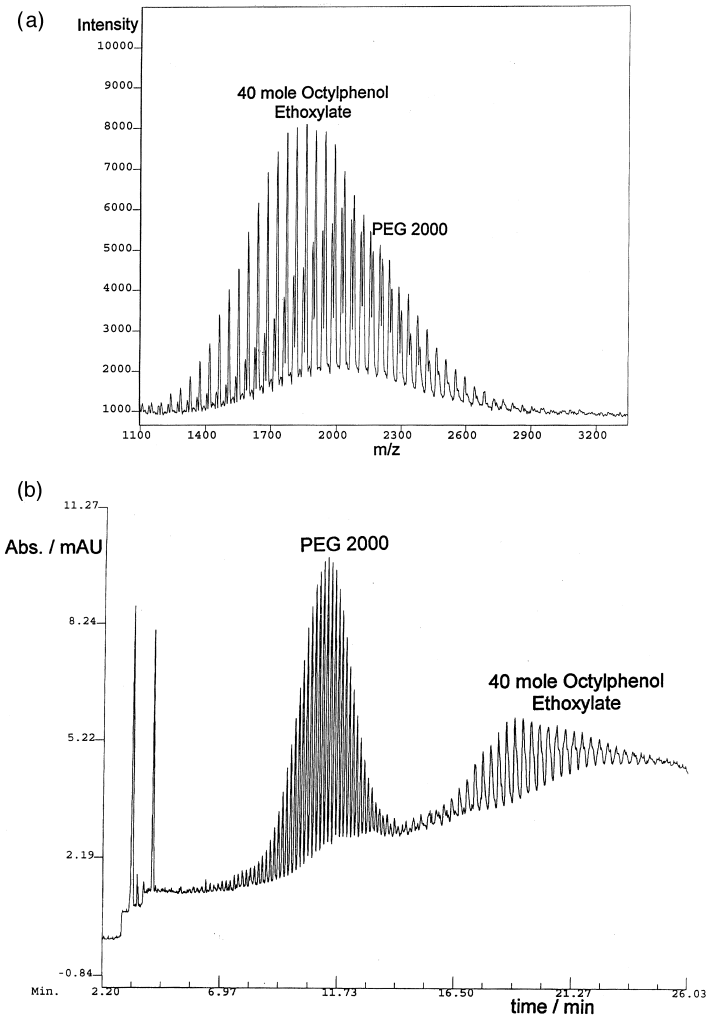

Fig. 5. (a) MALDI-TOF-MS of a blend of a PEG 2000 with a 40 mole octylphenol ethoxylate. (b) CE electropherogram of the blend of the PEG 2000 with a 40 mole octylphenol ethoxylate.

mass (M_w) from this spectrum. Fig. 5b shows the CE electropherogram of the derivatized sample. The PEG distribution migrates substantially faster than the surfactant and they are thus easily resolvable. (Note, MALDI also produces predominantly singly charged ions of the derivatives). It is possible to collect either distribution and subject the collected fractions to analysis by MALDI-TOF-MS if desired. High precision collection is not required since the distributions are wide (5–10 min) and individual distributions are well resolved. Fig. 6 shows the MALDI-TOF-MS of 50 fractions collected from 6–16 min. The distribution in the spectrum corresponds to the derivatized PEG. The appearance of satellite peaks in this spectrum is the result of the greater affinity the derivative has for metal cations (compared to the underivatized ethoxylate).

4. Conclusions

This report has demonstrated the application of CE and CE coupled to MALDI-TOF-MS to the analysis of ethoxylates. UV transparent networks of dextran

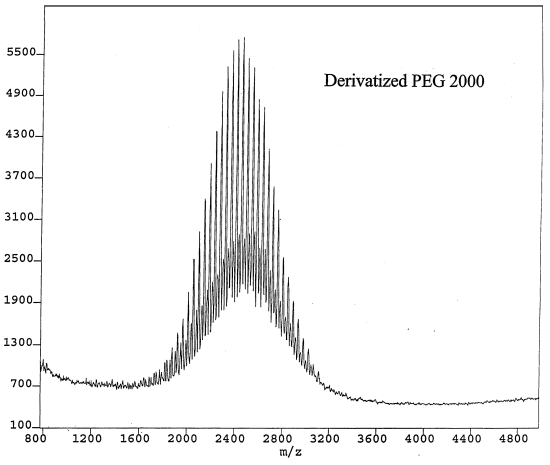

Fig. 6. MALDI-TOF-MS of fractions collected from 6 min to 16 min. The spectrum corresponds to the derivatized PEG 2000. Disodim cation adducts are also present in the spectrum.

and PEO provide size selectivity and permit relatively sensitive detection of the polymers at 210 nm. The major problem with the method described is the lengthy time required for derivatization.

The difference in separation selectivity between MALDI-TOF-MS and CE was also discussed and the application of this difference was illustrated in the separation of a PEG from a surfactant of similar mass.

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