Journal of Chromatography, 173 (1979) 357–363
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CHROM. 11,751

POST-COLUMN COMPLEXATION TECHNIQUE FOR THE SPECTROPHOTOMETRIC DETECTION OF POLY(OXY-1,2-ETHANEDIYL) OLIGOMERS IN STERIC EXCLUSION CHROMATOGRAPHY

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(Received January 24th, 1979)

SUMMARY

A solid-phase complexation column (SPCC), which consists of ammonium cobaltothiocyanate (ACTC) adsorbed on a solid support, has been developed as part of a selective detection technique for molecules bearing one or more poly(oxy-1,2-ethanediyl) oligomers (POEs). Due to the ion–dipole attraction between the ammonium ion and the negative dipoles of the polyether oxygen atoms, a quantity of ACTC is solubilized in the mobile phase as the POE–ACTC complex. The complexed ACTC is readily quantitated at 320 or 620 nm because of the strongly absorbing cobaltothiocyanate anion. The detection technique is approximately 100 times more sensitive than the differential refractive index detector for this class of compounds. This detector is used with steric exclusion chromatography on Sephadex LH-20 and μStyragel columns.

INTRODUCTION

Because of needs generated by the great variety of scientific and industrial applications of poly(oxy-1,2-ethanediyl) oligomers (POEs), many analytical methods have been developed. The procedures for POEs fall into two categories: procedures for specific substances that are well characterized structurally and techniques for the determination of the oxyethylene content of the sample. One example of the former is the thin-layer chromatographic (TLC)[1] procedure reported by Hodda[2]; the latter is exemplified by the hydrogen bromide cleavage of the polyoxyethylene ether linkages to yield one equivalent of 1,2-dibromoethane for each mole of ethylene oxide incorporated into the molecule[3].

Polysorbates, commonly used in food, have been quantitated by gas chromatographic analysis of the fatty acid moieties[4], TLC[1] and co-precipitation with barium phosphomolybdate[5]. Siggia *et al.*[6] developed a titrimetric procedure which is based on the reaction of POEs with hydriodic acid to yield iodine. Ehrenberger[7] used the hydriodic acid reaction and quantitated the resulting alkyl iodides by gas chromatography. In his review, Longman[8] discussed colorimetry and co-precipitation with heteropoly acids. Other methods for this class of compounds include high-performance

liquid chromatography[9–14] and nuclear magnetic resonance spectroscopy[15,16]. All of these techniques were considered in our search for a liquid chromatographic procedure for determining polyethylene glycols and polyethylene glycol sorbitan esters, such as polysorbates 20, 60, 65 and 80.

Steric exclusion chromatography (SEC) was selected as the method of choice, as the retention volumes of all sample components lie within a range limited by the interstitial volume and interstitial plus pore volumes of the column. Unfortunately, the previously published liquid chromatographic procedures rely upon differential refractive index (RI) or ultraviolet spectral (UV) detectors, neither of which is applicable because of the POEs and the sample matrices under consideration. POEs such as polysorbates, which are permitted as direct food additives[17], and polyethylene glycols do not absorb within the readily accessible region of the UV spectrum and, therefore, UV detection was not considered. As the ultimate goal of this work was to quantitate POEs in complex matrices, the RI detector would not be suitable because of its lack of selectivity and relative insensitivity. In view of these considerations, work was initiated on the development of a detector technique that would take advantage of a specific property common to these polyethers.

The propensity to form ion–dipole complexes with cations is characteristic of a variety of POEs. Because of optimal stereochemical and electronic factors, some crown ethers (cyclic POEs) react with potassium thiocyanate to form crystals of known stoichiometry with melting points higher than that of each of the components[18]. Recent work[19] has demonstrated that non-cyclic POEs will also form crystalline adducts. POEs that do not have the conformations required to form isolatable crystalline complexes can be shown to exhibit substantial ion–dipole complexation energies through phase transfer studies.

Phase transfer, in this context, refers to a marked change in the solubility or partion coefficient of a substance due to complexation. Most POEs will function as phase transfer agents for selected salts and this fact has been utilized in analytical method development. Of the large number of available colorimetric procedures based upon the phase transfer principle[8], the ammonium cobaltothiocyanate (ACTC) technique has emerged as the most generally useful[20]. The method employs a two-phase system consisting of an organic solvent, such as dichloromethane or toluene, and an aqueous solution of ACTC. In the absence of phase transfer agents, ACTC does not partition into the organic layer; however, the portion of the salt that is complexed by the POE in the sample will be transferred to the organic solvent after the two layers are thoroughly mixed to achieve equilibrium. Because of the strongly absorbing cobaltothiocyanate anion, spectrophotometry of the organic layer at 320 or 620 nm will, with suitable calibration, provide quantitation of the POE content.

As even hydrophilic POEs will solubilize certain salts in organic solvents if there is no aqueous layer present[21], we concluded that interaction of a POE with a solid phase source of ACTC, as depicted in Fig. 1, would ensure phase transfer of ACTC by the widest possible variety of POEs.

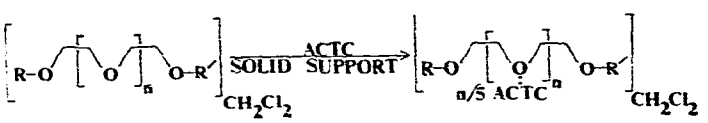

Fig. 1. Interaction of a POE with a solid-phase source of ACTC.

In this paper, the preparation and properties of a solid-phase complexation column (SPCC) are described. The SPCC in combination with a photometer has proved to be a very selective detector, producing linear responses. For POEs in the molecular weight range 200–1540 it is approximately two orders of magnitude more sensitive than the RI detector.

EXPERIMENTAL

Materials and apparatus

The following reagents were used as received: polysorbate 60 [polyoxyethylene (20) sorbitan monostearate] from Sigma (St. Louis, Mo., U.S.A.); polysorbate 80 [polyoxyethylene (20) sorbitan monooleate], Carbowax PEG 400, ammonium thiocyanate (ACS certified grade), and cobalt nitrate (ACS certified grade) from Fisher Scientific (Fair Lawn, N.J., U.S.A.); Brij 35 [polyoxyethylene (23) lauryl ether] from Aldrich (Milwaukee, Wisc., U.S.A.); Carbowax 20M permanently bonded packing, Super Pak 20M, from Analabs (New Haven, Conn., U.S.A.). All solvents were of Distilled-in-Glass quality (Burdick & Jackson Labs., Muskegon, Mich., U.S.A.). Sephadex LH-20 was purchased from Pharmacia (Piscataway, N.J., U.S.A.). The 500 Å μStyragel column was manufactured by Waters Assoc. (Milford, Mass., U.S.A.). Carbowax 400 distearate was purchased from Polysciences (Warrington, Pa., U.S.A.).

A Model 3500 (Spectra Physics, Santa Clara, Calif., U.S.A.) liquid chromatograph was used with a variety of injectors and detectors. A Model 725 automatic sample injector (Micromeritics, Norcross, Ga., U.S.A.) with a 10-μl sampling loop or a U6K injector (Waters Assoc.) was used. A Spectra Physics Model 8200 detector with the 312-nm filter as well as a Model SF/70 spectroflow monitor (Schoeffel, Westwood, N.J., U.S.A.) provided photometric detection. A "guard column", which consisted of a 60 mm × 6.4 mm O.D. (2.8 mm I.D.) stainless-steel tube, was equipped with 50-μm frits at each end (Whatman, Clifton, N.J., U.S.A.). The frits were secured with 1/4-to-1/16 in. reducing unions. Peak areas were determined with a Spectra Physics Minigrator. The LH-50 column was contained within a 60 cm × 11 mm O.D. glass column equipped with glass frits at each end.

Solid-phase complexation column

A 4.5-ml portion of a solution, prepared by dissolving 29.3 g of ammonium thiocyanate and 14.0 g of cobalt nitrate in 50 ml of deionized water, was added dropwise to 1 g of Super Pak 20M packing. The resulting slurry was shaken gently until all of the packing was thoroughly wetted by the solution. The slurry was transferred to a funnel with a glass frit. Dichloromethane was repeatedly forced through the packing bed until all obvious traces of water were removed. The resulting blue packing was exposed to the atmosphere until it was a free-flowing powder. The guard column was filled by simply pouring the ACTC–Super Pak 20M reagent into the column with a frit affixed to one end. The other frit was then positioned and secured. The SPCC was readied for use by connecting it to the chromatographic system and flushing with dichloromethane until no blue color was visible in the effluent. The outlet of the SPCC was then connected to the photometric detector.

Detector linearity studies

The relationship between peak area and amount injected was studied by injecting known amounts of each of the test compounds, Brij 35, Carbowax 400 and polysorbate 80, directly into the SPCC. The automatic sample injector was used with the Model 3500 liquid chromatograph. The Model 8200 detector was used with the 312-nm filter. Peak areas were determined with the Minigrator. Dichloromethane was used as the mobile phase at a flow-rate of 1.2 $ml \cdot min^{-1}$.

RESULTS AND DISCUSSION

In practice, the SPCC was used as shown diagrammatically in Fig. 2. Dichloromethane was selected as the mobile phase because there is no significant elution of ACTC from the SPCC in the absence of a phase transfer agent in the column effluent. However, as each POE emerges from the SEC column and passes through the SPCC, a portion of ACTC, which is complexed by ion–dipole interaction, is transferred to the mobile phase. The eluate from the SPCC is monitored at 320 or 620 nm to quantitate the strongly absorbing cobaltothiocyanate anion. The reaction (Fig. 1) is driven to completion because the eluate continuously comes in contact with additional ACTC as it proceeds through the SPCC. The ratio of one molecule of ACTC to five oxyethylene units is a weighted average derived from a review of the colorimetric reactions described in the literature[22].

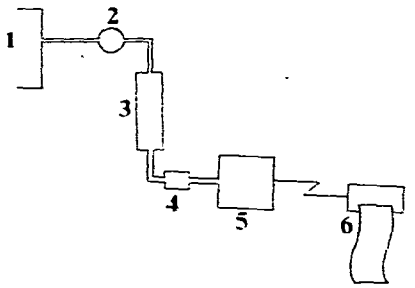

Fig. 2. Schematic representation of chromatography–detection system. 1 = Pump and dichloromethane reservoir; 2 = U6K injector; 3 = 60 cm X 11 mm I.D. LH-20 column; 4 = SPCC; 5 = UV/visible photometer; 6 = recorder.

The performance of the SPCC exceeded our expectations. The linearity is excellent over a concentration range of two orders of magnitude (a limitation imposed by the dynamic range of the photometer). The coefficient of correlation for the responses to the three substances given in Fig. 3 is greater than 0.999 for each substance. The data were obtained by direct introduction of the POE into the SPCC (see Experimental). The reproducibility is excellent, as indicated by Fig. 4. The use of the detector with a Sephadex LH-20 column produced the results shown in Fig. 5.

Figs. 6 and 7 illustrate the use of the SPCC–spectrophototometric detector at two different wavelengths with comparable data for the RI detector. With monitoring at 320 nm the SPCC is approximately two orders of magnitude more sensitive than the RI detector. The use of 620 nm for monitoring decreases the sensitivity of

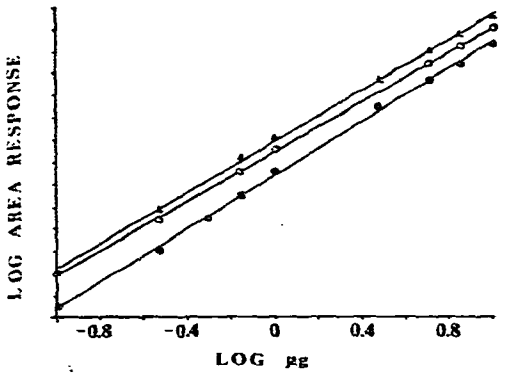

Fig. 3. Relationship of peak area response to amount injected directly on SPCC. △, Carbowax 400; ○, Brij 35; ●, polysorbate 80.

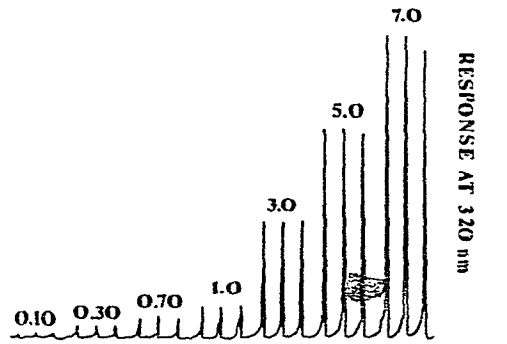

Fig. 4. Multiple injections of dichloromethane solution of Carbowax 400 introduced directly into the SPCC (see Experimental). The number above each set corresponds to micrograms of Carbowax 400.

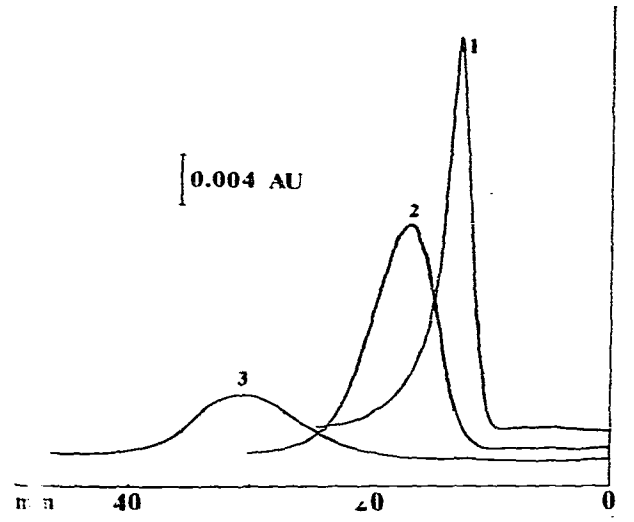

Fig. 5. Elution curves for 5.0 μg each of polysorbate 60 (1), Carbowax 600 (2) and Carbowax 200 (3). A system consisting of a 60 cm X 11 mm I.D. Sephadex LH-20 column with SPCC and photometric detection at 320 nm was used with dichloromethane as the mobile phase.

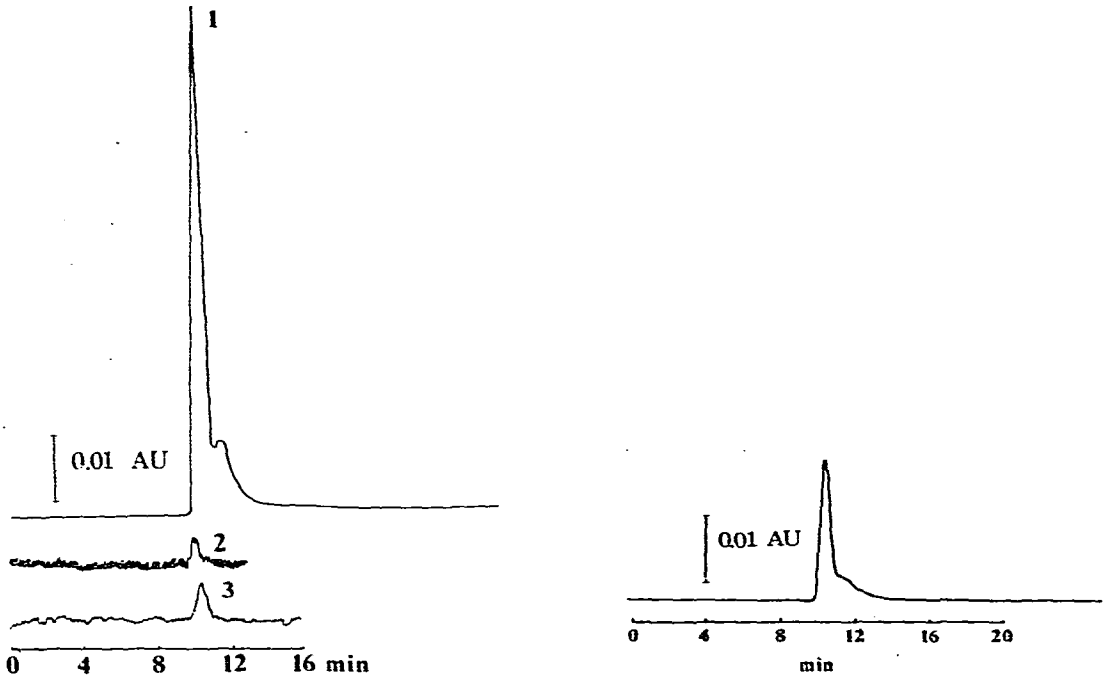

Fig. 6. Chromatography of Carbowax 400 distearate with a single 500 A μStyragel column and dichloromethane at 0.8 ml · min^{-1} as the mobile phase. A, 12 μg on-column with SPCC and spectrophotometric response at 320 nm (0.1 absorbance unit full scale); B, 12 μg on-column with the RI detector; C, 0.12 μg on-column with SPCC and spectrophotometric response at 320 nm (0.01 absorbance unit full scale).

Fig. 7. Chromatography of Carbowax 400 distearate with SPCC and spectrophotometric response at 620 nm; 12 μg on-column with same chromatographic conditions described in Fig. 6.

the SPCC–spectrophotometric technique by a factor of four but adds immeasurably to the selectivity.

CONCLUSION

The concept of a post-column reactor with an adsorbed reactant that will complex with selected components in the eluate has led to the development of a convenient detector. The sensitivity for this class of compounds exceeds that of the RI detector by two orders of magnitude. The SPCC is easily constructed and added to existing chromatographic systems. The technique is applicable, in combination with SEC, as a screening technique to detect intermediate molecular weight POEs in complex matrices such as food.

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