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Optimization of experimental parameters for separation of nonionic surfactants by supercritical fluid chromatography

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ABSTRACT

A synthetic polymer generally consists of a mixture of many compounds that share the same repeating unit but have different degrees of polymerization. Because of its ability to cope with a wide range of molecular weights, supercritical fluid chromatography (SFC) is a powerful technique for separating the individual components of polymers. However, the separation conditions for SFC are more sensitive than those of other types of chromatography, and many experimental parameters need to be carefully selected to achieve high resolutions. Methods for optimizing the experimental parameters for SFC of a polymeric surfactant were investigated by chemometric techniques. Increases or decreases in peak resolution and elution times were measured for the separation by SFC of the nonionic surfactant poly(ethylene glycol) nonylphenyl ether for various column temperatures, flow-rate gradients of modifier, and modifier compositions. To maximize the separation efficiency, the experimental results were analyzed by using the chromatographic response function (CRF). This is a function of the desired peak resolution, the maximum acceptable elution time, and an arbitrary weighting factor that is adjusted to achieve an optimal balance between the peak separation and the elution time. Because the numerical value of the CRF changes in response to the chromatographic performance, it provides a means of identifying the conditions for achieving optimal separation for a particular SFC system.

1. Introduction

In the analysis and fractionation of synthetic polymers, super-critical fluid chromatography (SFC) is almost the only technique that is capable of separating various n-mers from the corresponding $(n − 1)$mers or $(n + 1)$mers, because it displays greater resolution than other chromatographic techniques such as HPLC or size-exclusion chromatography. GC also has a high resolution, but it cannot be used to analyze high-molecular weight polymers, which are not sufficient volatile for GC separation. SFC is therefore uniquely suitable as a technique for separating polymers into individual fractions, each with a different degree of polymerization. However, it is difficult to optimize the experimental parameters for SFC of a particular sample. Although many studies have been made on the separation of synthetic polymers by SFC on capillary columns [1], these columns have recently been replaced by packed columns

for the purpose of fractionating polymers [2–8]. For example, Ute and co-workers succeeded in separating and fractionating samples of poly(methyl methacrylate), polystyrene, and several other polymers by using packed columns, and they analyzed the uniform oligomers separated by SFC [9–13].

Poly(ethylene glycol) has been separated and analyzed by SFC by applying modifier-gradient conditions [14–17]. Operating methods for SFC of organosoluble or water-soluble polymers on packed columns are known, but in the case of surfactants, which contain both hydrophilic moieties and hydrophobic moieties, methods for optimizing the SFC parameters have proved inadequate. Many polymeric surfactants are derived from poly(ethylene glycol) by modifying the end groups of the polymer. Such products have been widely used as detergents in cleaning and in industrial products, and they have been applied to biological materials [18,19]. There are, however, some concerns regarding the potential toxicity of nonionic surfactants. For example, the toxicity of 4-nonylphenol, which can be released by decomposition of poly(ethylene glycol) nonylphenyl ether, has become a concern and this substance is set to be controlled by various regulations [20,21]. For this reason, we selected poly(ethylene glycol) nonylphenyl ether as a material for

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separation by SFC. Over recent decades, many studies have been made on the separation by SFC of various surfactants derived from poly(ethylene glycol) by modifying the end groups of the polymer [22–26], but in no case has a systematic examination and optimization been attempted. The principal reason for this lack of adequate studies is the difficulty in detecting poly(ethylene glycol) derivatives.

Detection of analytes in SFC is more difficult than that in normal liquid chromatography, because the detector must be capable of operating at the high pressures required for SFC. In most cases, UV detectors are used for SFC, and these are generally capable of providing adequate quantification as mass detectors; however, they cannot be used under some conditions, for example, with modifier gradients or when UV absorbances are weak. Many poly(ethylene glycol)-derived surfactants do not absorb UV radiation adequately. To permit the detection of alcohol ethoxylates or propoxylates, Hoffman et al. [27,28] derivatized the materials by treating them with phenylated disilazane, which absorbs in the UV region. The addition of a UV-absorbing group to the alcohol ethoxylates and propoxylates permitted the detection of these analytes from their absorption peaks at 215 nm. Another approach to detecting samples that do not absorb UV radiation is to use an evaporative light-scattering detector (ELSD) [29–31]. The ELSD is especially useful as detector under modifier gradient conditions, because the mobile-phase components are vaporized before detection is performed. This permits changes to be made in many of the experimental parameters, such as the composition of the modifier or the flow rate of the mobile phase.

A wide range of conditions for SFC separation can be used in conjunction with an ELSD, and there are many reports on studies of the effects of various SFC parameters [32–41]. In this study, we optimized the experimental parameters for SFC for analysis of poly(ethylene glycol) nonylphenyl ether, including the column temperature, the modifier flow-rate gradient, and the composition of the modifier. Increases or decreases in the peak resolution and in the elution time in response to changes in the experimental parameters were measured and analyzed by chemometric techniques to permit maximization of the separation efficiency. The chromatographic response function (CRF) was applied to the experimental data as a function of the desired peak resolution and the maximum acceptable elution time. An arbitrary weighting factor between the resolution and the elution time was used to determine the optimal conditions for a given set of the parameters. The most-sensitive parameter for SFC separation of the surfactant was the composition of the modifier. Details of the relationship between the resolution and the composition of the modifier at various temperatures were elucidated.

2. Experimental

2.1. Samples

The test sample of nonionic surfactant consisted of poly(ethylene glycol) nonylphenyl ether purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). This surfactant consisted of various oligomers of structure $C_9H_{19}C_6H_4O-(CH_2CH_2O)_i-H$ with various degrees of polymerization i. The index i varied from 1 to about 20, and the weight-average molecular mass of the surfactant was about 700. The chemical structure of the surfactant was confirmed by using a matrix-assisted laser-desorption/ionization time-of-flight mass spectrometer (AXIMA CFR Plus; Shimadzu/Kratos, Kyoto, Japan) operated in the linear mode. The individual poly(ethylene glycol) nonylphenyl ether oligomers were observed as adducts cationized by Na^+ ions and a small number of K^+ ions. No other components
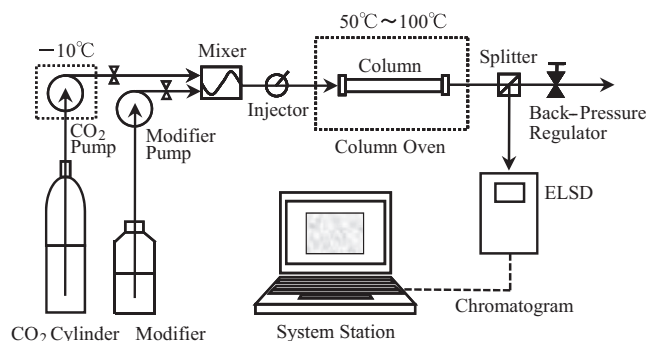

Fig. 1. Schematic representations of the SFC instruments.

were present [20], although the various isomers of the nonyl moiety, as well as *ortho-* and *para-*disubstituted phenyl groups, were detected in the surfactant sample [42].

For SFC, the modifier consisted of a mixture of methanol and extra-pure water. The methanol was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan) and the extra-pure water was produced by using a Milli-Q system (Nihon Millipore KK, Tokyo, Japan) fitted with 0.1-μm filters. The electric resistance of the extra-pure water was 18.2 MΩ cm and its total organic content was less than 6 ppb.

2.2. Supercritical fluid chromatograph

The instrument used for SFC was a SUPER-201 (JASCO Co., Tokyo, Japan). A 250-mm-long SFCpak SIL-5 silica gel column (JASCO) with a pore size of 6 nm, a particle size of 5 μm, and an internal diameter of 4.6 mm was used. A modifier gradient was used to separate the various oligomers of poly(ethylene glycol) nonylphenyl ether. The surfactant samples were dissolved in methanol to a concentration of 50 mg/mL, and the injection volume was 5 μL. The mobile phase consisted of a mixture of carbon dioxide (CO_2) and the aqueous methanol modifier in a ratio that was gradually changed to produce a gradient effect. The composition of the methanol–water modifier was varied between 1:0 and 9:1 (v/v at 25 °C, 0.1 MPa). Fig. 1 shows the settings of SFC instruments. The head of CO_2 pump was cooled at −10 °C to keep the flow rate of CO_2 constant in liquid state. In the same way, the head of modifier pump was kept at 25 °C to maintained constant flow rate of modifier liquid. The flow rate of the supercritical CO_2 was maintained at 2.0 mL/min. The slope of the flow-rate gradient of the modifier was changed from +0.01 mL/min² to +0.02 mL/min², and the initial flow rate was 0.20 mL/min. The column temperature was kept at a constant value of 50, 60, 80, or 100 °C. The pressure in the SFC system was maintained at a constant value of 19.6 MPa (200 kg/cm²) by means of a backpressure regulator.

The eluate from the SFC was passed from a three-way splitter to the ELSD through a 50-μm-bore tube of polyether ether ketone. The detector was an ELSD 2000 device (Alltech Associates, Deerfield, IL, USA) operated at a drift-tube temperature of 30 °C. The nebulizer gas for the ELSD was dust- and mist-free compressed air from an in-house system; its flow rate was maintained at 0.40 L/min. Impactors were not used in this case, because they cause deterioration in the quantitativeness achievable with the ELSD detector. The light source for the ELSD was a 5-mW laser diode with a wavelength of 670 nm, and the detector was a silicon photodiode.

2.3. Method for calculating the resolution of the separation of the polymer sample

In general, the resolution of a chromatogram is defined as the ratio of the depth of a valley to the height of its adjacent peaks, as