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Comprehensive two-dimensional separation for the analysis of alkylphenol ethoxylates employing hydrophilic interaction chromatography coupled with ion mobility-mass spectrometry

Qiang Ma[a,b,*], Guang-Cheng Xi[b], Chao Wang[b], Hua Bai[b], Qing Zhang[b], Hai-Wei Xi[b], Zi-Ming Wang[c], Liang-Hong Guo[a,*]

[a] State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China
[b] Chinese Academy of Inspection and Quarantine, Beijing 100123, China
[c] College of Chemistry, Jilin University, Changchun 130012, China

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ABSTRACT

A comprehensive two-dimensional system coupling hydrophilic interaction chromatography (HILIC) and ion mobility-mass spectrometry (IM-MS) has been developed for the separation and analysis of alkylphenol ethoxylates (APEOs). The first-dimensional HILIC was performed on porous silica stationary phase using acetonitrile–water gradient elution, which was readily compatible with electrospray ionization (ESI), and enabled good chromatographic separation of APEO oligomers on account of their differences in ethoxy chain length. Maintaining the fidelity of pre-ionization resolution in the first dimension, the second-dimensional IM-MS employed a hybrid quadrupole ion mobility time-of-flight mass spectrometer and added an orthogonal post-ionization separation for APEOs based on their size, shape and electric charge during a very short period of 13.0 ms. By virtue of the combination of HILIC and IM-MS, comprehensive resolution according to both hydrophobicity difference and mobility disparity has been achieved for APEO ethoxy homologues. The orthogonality of the developed two-dimensional system was evaluated with the correlation coefficient and peak spreading angle of 0.2191 and 77.34° for octylphenol ethoxylates (OPEOs), and 0.1490 and 81.43° for nonylphenol ethoxylates (NPEOs). A significant enhancement in peak capacity was achieved for the comprehensive two-dimensional plane with the actual peak capacity calculated to be approximately 7 and 122 times higher than that of the two dimensions used alone, respectively. The attractive potential for removing the effects of isobaric interference of APEOs by the rapid and solvent-free ion mobility approach was also highlighted.

1. Introduction

Alkylphenol ethoxylates (APEOs) make up one of the largest classes of surfactants, produced by reacting alkylphenols (APs) with ethylene or propylene oxide, and have been widely used as detergents, emulsifiers, solubilizers, wetting and dispersing agents in industrial, agricultural and household applications [1,2]. Among APEOs, octylphenol ethoxylates (OPEOs) and nonylphenol ethoxylates (NPEOs) are two of the most common non-ionic surfactants in the marketplace. As a result of the extensive usage, APEOs are incorporated into the environment by various ways, primarily through industrial and municipal wastewater discharges, manufacture of APEOs-containing products, etc. [3,4]. The broad environmental presence of APEOs (ambient air, surface water, wastewater, soil, sediments, biota, etc.) [5–7], is solely a consequence of anthropogenic activity. Biodegradation of APEOs can subsequently occur based on a mechanism of stepwise loss of ethoxy groups under both aerobic and anaerobic conditions, resulting in the formation of more toxic and more persistent metabolites than the parent compounds: short-chain alkylphenol mono- and diethoxylate (AP_1EO and AP_2EO), alkylphenoxy carboxylates (APECs), carboxyalkylphenol ethoxycarboxylates (CAPECs) and fully de-ethoxylated APs [8–10]. The main environmental concern about APEOs is usually not the toxicity of these compounds themselves, but rather the endocrine disruption potential of their decomposition products, which are suggested to mimic the natural hormones by interacting with the estrogen receptor [11–14]. The widespread use of APEOs, together with their estrogenic properties, has initiated an incitement for regulatory actions [15–19].

Great public concern about the effects of APEOs and their metabolites on wildlife and human health, and about their

* Corresponding authors at: State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China. Tel.: +86 10 62849685; fax: +86 10 62849685.
E-mail addresses: maqiang1129@yahoo.com.cn (Q. Ma), LHGuo@rcees.ac.cn (L.-H. Guo).

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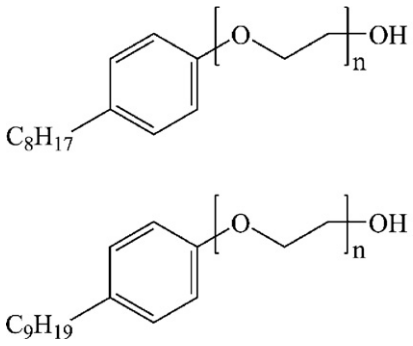

Octylphenol ethoxylates (OPEOs)

Nonylphenol ethoxylates (NPEOs)

Scheme 1. Chemical structures of OPEOs and NPEOs.

environmental fate, has led to the development of various analytical methods for the determination of these substances. APEOs are mixtures of homologues with different alkyl groups and oligomers with different numbers of ethylene oxide units. The considerable analytical challenge lies in the chemical complexity of APEOs, making the separation and characterization difficult. Given that their properties, toxicities, environmental occurrence and fate may depend on structural details, information about the exact oligomer distribution of APEOs is essential in assessing the associated environmental impact and risks. A sophisticated and specific method is therefore needed for their analysis.

Traditional methodologies of analysis are based on gas chromatography (GC) coupled with flame ionization detection (FID) [20], electron capture detection (ECD) [21] and low-resolution mass spectrometry (LRMS) [22,23] operating in electron ionization (EI) or chemical ionization (CI) mode. Nevertheless, the GC-based approaches are only appropriate for APEOs with a low number of ethylene oxide groups. Their applicability has been confined to short-chain ethoxylates due to volatilization issues. Further time-consuming derivatization procedures must be included to improve their volatility, which may possibly lead to potential analytical errors due to differences in reaction kinetics for individual oligomers and the formation of by-products during in-sample treatment. Alternatively, liquid chromatography (LC) hyphenated with ultraviolet detection [24,25] or fluorescence detection [26,27] has been applied for the routine analysis of APEOs. However, these optical detectors are becoming out of date when faced with MS detection, due mainly to the lack of specificity in analysis. In the past few years, growing attention has been paid to LC–MS methodologies for the identification and quantification of APEOs. Atmospheric pressure ionization (API) or matrix-assisted laser desorption ionization (MALDI) [28] sources, combined with various mass analyzers (single quadrupole [29], triple quadrupole [30,31], ion trap [32], and quadrupole/time-of-flight [33]) offer a broad range of instruments for use. Analytical advantages of superior sensitivity and specificity make LC–MS a better choice than titrimetric, colorimetric and spectroscopic detection.

A two-dimensional separation is generally considered to be advantageous over its one-dimensional counterpart for resolving complex mixtures because of extended peak capacity and higher resolution. It is recommended that the two dimensions be connected in an on-line manner, referred to as a comprehensive two-dimensional separation, rather than in an off-line or heart-cut manner, in order to approach the ideal multiplicative total of peak capacity. To date, a wide variety of separation techniques have been mutually interfaced to construct comprehensive two-dimensional separation systems [34–37]. Currently, LC in conjunction with ion mobility-mass spectrometry (IM-MS) is becoming increasingly popular for complex mixture analysis, due to complementary separation steps and elevated peak capacity. The applications include the analysis for environmental pollutants [38], rat urinary metabonome [39], human plasma proteome [40], peptide mixtures [41], pharmaceutical formulation [42], etc. The principle for IM-MS has been described in detail elsewhere [43]. Briefly, IM-MS is a gas phase electrophoretic technique that allows ionized target molecules to be separated on the basis of their mobility in the presence of an inert carrier gas and under the influence of a weak electric field. The system incorporating ion mobility separation in tandem with LC and MS enables the initial separation of components by LC, then transfers and disperses the ions generated in the ion source according to their differing mobility, and finally records their mass-to-charge ratio (m/z) by a time-of-flight (TOF) mass analyzer. In the current study, the separability afforded by different liquid chromatographic modes and ion mobility technique was explored to address the challenge derived from the compositional complexity of APEOs. Based on the experimental investigation, a comprehensive two-dimensional system hyphenating hydrophilic interaction chromatography (HILIC) and IM-MS (HILIC × IM-MS) was developed for the separation and analysis of APEOs.

2. Experimental

2.1. Materials

Technical oligometric standards of $OP_{10}EO$ (a mixture of OPEOs with an average of 10 ethylene oxide units) and $NP_{10}EO$ (a mixture of NPEOs with an average of 10 ethylene oxide units) were purchased from Tokyo Chemical Industry (Tokyo, Japan). Chemical structures of OPEOs and NPEOs are shown in Scheme 1. Sodium formate and leucine enkephalin were obtained from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile of LC–MS grade (J.T. Baker, Pittsburgh, PA, USA) and Milli-Q ultrapure water (Millipore, Bedford, MA, USA) were used as mobile phase constituents for HILIC separation.

2.2. Instrumentation

The HILIC separation of APEOs was performed on an ACQUITY LC system (Waters, Milford, MA, USA) equipped with a binary solvent manager, a sample manager and a column heater. A Waters Atlantis HILIC Silica analytical column (150 mm × 2.1 mm, 3 μm), packed with underivatized porous silica stationary phase was utilized. The chromatographic elution was conducted with binary mobile phase gradient consisting of an organic part – acetonitrile (A) and an aqueous part – water (B) at a flow rate of 0.25 mL/min. Initial gradient condition was set to 0% B before embracing a linear gradient increase to 35% B over 10 min and then held for 1 min. At 13 min, the gradient was programmed to the initial conditions. The column temperature was maintained constantly at 30 °C. The samples were kept at 10 °C in the thermostated sample manager compartment and a sampling volume of 10 μL was injected for each run.