Analytica Chimica Acta 808 (2014) 83–93

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Characterization of polysorbate 85, a nonionic surfactant, by liquid chromatography vs. ion mobility separation coupled with tandem mass spectrometry

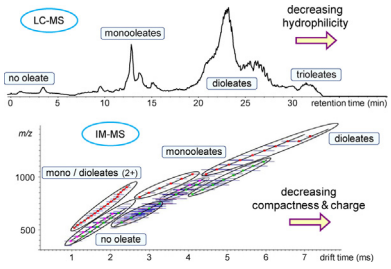
Nilüfer Solak Erdem [1], Nadrah Alawani, Chrys Wesdemiotis *

Department of Chemistry, The University of Akron, Akron, OH 44325-3601, United States

HIGHLIGHTS

- Liquid chromatography (LC) separates amphiphilic blends according to hydrophobicity.
- Ion mobility (IM) spectrometry separates these blends based on molecular size/shape.
- LC–MS provides the separation resolution needed for quantifying fatty acid content.
- IM–MS enables rapid, solvent-free separation and the detection of trace components.
- With either method, tandem MS allows to count the hydrophobic substituents.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 27 March 2013
Received in revised form 3 July 2013
Accepted 9 July 2013
Available online 15 July 2013

Keywords:
Reverse-phase chromatography
Ion mobility separation
Tandem mass spectrometry
Polysorbate 85
Nonionic surfactants
Isobar differentiation

ABSTRACT

Liquid chromatography (LC) and ion mobility (IM) separation have been coupled with mass spectrometry (MS) and tandem mass spectrometry (MS^2) to characterize a commercially important nonionic surfactant, polysorbate 85. The constituents of this amphiphilic blend contained a sorbitan or isosorbide core that was chain extended with poly(ethylene oxide) (PEO) and partially esterified at the PEO termini with oleic acid or, to a lesser extent, other fatty acids. Using interactive LC in reverse-phase mode, the oligomers of the surfactant were separated according to their hydrophobicity/hydrophilicity balance. On the other hand, IM spectrometry dispersed the surfactant oligomers by their charge and collision cross section (i.e. size/shape). With either separation method, an increased number of fatty ester groups and/or lack of the polar sorbitan (or isosorbide) core led to higher retention/drift times, enabling the separation of isobaric species or species with superimposed isotope patterns, so that their ester content could be conclusively identified by MS^2. LC–MS and IM–MS permitted the detection of several byproducts besides the major PEO-sorbitan oleate oligomers. LC–MS provides the separation resolution needed for quantitative determination of the degree of esterification. IM–MS, which minimizes analysis time and solvent use, is ideally suitable for a fast, qualitative survey of samples differing in their minor constituents or impurities.

1. Introduction

The invention of soft ionization techniques, in particular electrospray ionization (ESI) [1] and matrix assisted laser desorption ionization (MALDI) [2,3], has led to a steady growth of mass spectrometry (MS) applications to synthetic polymers [4–14]. Presently,

* Corresponding author. Tel.: +1 330 972 7699.
E-mail address: wesdemiotis@uakron.edu (C. Wesdemiotis).
[1] TÜBITAK Marmara Research Center, Kocaeli, Turkey.

0003-2670/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.aca.2013.07.026

MS is employed routinely to elucidate the composition, end groups, molar mass distributions, chain lengths, chemical heterogeneity and impurities in the sample under study [11–14]. In spite of its high sensitivity and speed and low detection limits, MS has limitations that need to be overcome for extending its application to complex mixtures, which may contain isobaric and isomeric components that cannot be distinguished by simple mass measurement, or many different constituents with similar structures that create complex, uninterpretable spectra, especially upon ESI where oligomer and functionality distributions are superimposed with charge distributions. In these cases, coupling MS to a separation technique such as size exclusion chromatography [15–17], liquid chromatography under interactive [18–20] or critical conditions [15,21,22] or ion mobility spectrometry [20,23–27] is called for, in order to separate and simplify the macromolecular mixture prior to MS analysis.

Interactive liquid chromatography (LC) [28] has high separation capacity, but it is not suitable for identifying unknown compounds with the commonly used LC detection systems, viz. refractive index, UV–vis and light scattering detectors. In contrast, MS provides compound-specific structural information, but is less efficient with complex mixtures. Consequently, LC and MS offer complementary advantages and interfacing them creates a powerful two-dimensional analytical tool with higher sensitivity and specificity than the individual methods, and capable of providing improved molecular structure information about complex oligomeric systems [28].

A different, more recently developed, hyphenated technique that couples separation with MS detection is ion mobility mass spectrometry (IM–MS) [29,30]. Here, ions produced by ionization or fragmentation are separated by their mobility through a buffer gas prior to mass analysis. In the IM dimension, the ions travel under the influence of an electric field through a drift tube (IM chamber) filled with a neutral buffer gas (drift gas); this process causes dispersion according to size (mass), charge and shape. After exiting the IM chamber, the separated ions enter the mass analyzer, where they are identified by their mass-to-charge ratio (m/z) [30]. The IM–MS technique has shown promise in separations of structural isomers [29], metabolites [31], chiral compounds [32], complex mixtures [33], polymeric isomers and conformers [20,23,27,34,35] and supramolecular compounds [24–26,36–39], whose unequivocal analysis would have been impossible using mass spectrometry alone.

Coupling LC–MS or IM–MS with tandem mass spectrometry (MS^2) can further facilitate the analysis of complex mixtures. Soft ionization techniques, such as ESI and MALDI, generally produce intact molecular ions, which provide compositional but no connectivity (sequence) insight. The latter information may be accessed by the fragments generated in MS^2 experiments [40]. MS^2 studies are essential for the identification of unknown compounds and the elucidation of fragmentation pathways [40]. The enhanced ability to ascertain the identity of polymer mixtures by interfacing LC or IM separation with tandem mass spectrometry will be demonstrated in this paper for nonionic surfactant blends.

Nonionic surfactants are usually blends of homologous amphiphilic molecules and often contain polymers, with poly(ethylene oxide), PEO, being one of the most common hydrophilic parts [41]. Polysorbates are a special class of nonionic surfactants that include fatty acid ester moieties attached to PEO chains condensed onto a sorbitan core; these star-branched macromolecules are used to improve the solubility of hydrophobic analytes, as they easily form micelles and stabilize emulsions [42]. Due its low toxicity, polysorbate 85, which is substituted with oleate esters, is widely employed as surfactant in pharmaceuticals and personal care products [43,44], textiles [45], detergents [46] and in food industry [47].

MALDI-MS studies have revealed that commercial polysorbate formulations are more complicated than suggested by their chemical names [48–50]. LC and LC–MS studies of surfactants containing fatty acid esters and fatty alcohol ethers of PEO ("ethoxylated" fatty acid esters and "ethoxylated" fatty alcohol ethers, respectively) have revealed that separation according to the fatty acid identity is possible with reverse-phase (RP) columns, while oligomers with a different number of ethylene oxide units can be separated by proper tuning of the mobile phase composition [18,51–53]. LC analysis with either UV [54] or MS detection [55] showed that polysorbate formulations are generally mixtures of esters (mono-, di-, tri-, tetra-) irrespective of the supplier's specified degree of esterification. With LC–MS, it is further possible to assess ester heterogeneity by performing in-source fragmentation [55], during which fatty acid esters of PEO yield dioxolanylium fragment ions at m/z values that are characteristic of the corresponding fatty acid alkyl chains [56,57]. More recently [58], the technique "atmospheric solids analysis probe" (ASAP) [59] was employed to determine individual fatty acid esters in various polysorbate surfactants. ASAP of ethoxylated fatty acid esters produces dioxolanylium ions which, as mentioned above, are indicative of the fatty acids. Based on the dioxolanylium ions detected in ASAP mass spectra, the relative fatty acid content in polysorbate blends could be estimated [58]. In the same study, MALDI-MS^2 coupled by IM separation was utilized to elucidate major and minor polysorbate constituents, including isobaric species which were resolved by their ion mobilities [58]. Here, we present the first in-depth characterization of polysorbate 85 by two different multidimensional techniques, viz. reverse-phase LC or IM separation, supported by online ESI-MS and MS^2. The strengths and shortcomings of either approach are also briefly evaluated.

2. Experimental

2.1. Materials

Polysorbate 85, which is supplied as "polyoxyethylene sorbitan trioleate," was acquired from Chem Service (West Chester, PA). HPLC grade methanol, water and THF were purchased from Sigma–Aldrich (St. Louis, MO). Sodium trifluoroacetate (NaTFA) and dithranol were obtained from Fluka (Buchs, Switzerland) and Alfa Aesar (Ward Hill, MA), respectively. All materials were used in the condition received from their supplier.

2.2. Chromatography

The chromatographic separation of polysorbate 85 was performed under reverse-phase conditions with an Agilent HP 1100 LC system on an Agilent Zorbax C-8 column (4.6 mm × 150 mm) filled with 5-μm particles (Agilent Technologies, Santa Clara, CA). Each run took 40 min with the adjusted flow rate of 1.0 mL min^{-1}. Gradient elution was employed with a water/methanol mixture as mobile phase. The mobile phase composition was gradually changed, from 70 to 80% methanol (v/v) within 10 min and from 90 to 100% methanol for the following 20 min; 100% methanol was run isocratically during the last 10 min. The flow rate of the effluent exiting the column was reduced with a microsplitter valve to 0.25 mL min^{-1} before introducing the eluate into the electrospray source.

2.3. Mass spectrometry

The Agilent HP 1100 LC system was coupled to a Bruker Esquire (Bruker Daltonics, Billerica, MA) quadrupole ion trap (QIT) equipped with an electrospray source. The QIT was operated in positive mode under the following, optimized conditions: nebulizer gas pressure, 10 psi; drying gas flow rate, 8.0 L min^{-1}, drying gas