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Isotachophoretic determination of carboxylic acids in biodegradation samples

Agnieszka Zgoła-Grześkowiak [a,*], Tomasz Grześkowiak [a], Joanna Zembrzuska [a], Magdalena Frańska [a], Rafał Frański [b], Zenon Łukaszewski [a]

[a] *Poznan University of Technology, Institute of Chemistry, Piotrowo 3, 60-965 Poznań, Poland*
[b] *Adam Mickiewicz University, Faculty of Chemistry, Grunwaldzka 6, 60-780 Poznań, Poland*

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Abstract

In the current study a method of isotachophoretic separation of selected carboxylic acids was developed. The method was used for the determination of carboxylated oligo(ethylene glycol)s and their degradation products in biodegradation tests of PEG 250 DA [a mixture of dicarboxylated oligo(ethylene glycol)s]. Two tests were performed in the studies: the Organization for Economic Cooperation and Development (OECD) screening test and the river water die-away test. Both the biodegradation tests proved relatively fast biodegradation of the studied compounds. In the OECD screening test the biodegradation was faster than in the river water die-away test which can be ascribed to a higher concentration of bacteria in the biodegradation liquor. The minimal sample pretreatment and relatively low cost of analysis by the isotachophoretic method used here make it a good alternative to existing methods of carboxylic acids analysis.

Keywords: Isotachophoresis; HPLC–MS; Carboxylic acids; Biodegradation; OECD

1. Introduction

Oligo(ethylene glycol)s (OEGs) are widely used in a number of human activities. They are constituents of the hydrophilic moiety of aliphatic alcohol ethoxylates (AEs), which are used in formulations of detergents [1]. AEs are rapidly biodegraded in water. A very probable pathway for AE biodegradation is central fission [2], which results in the formation of free fatty alcohol and oligo(ethylene glycol)s. OEGs biodegradation proceeds by successive depolymerisation of the ethoxy chain via a non-oxidative and oxidative pathway leading to mono- and dicarboxylated OEGs [3,4] as well as ethylene glycol. The generic structures of OEGs and their carboxylic derivatives are presented in Fig. 1. Further biodegradation of the ethoxylates gives low molecular mass acids: glycolic acid, glyoxylic acid, oxalic acid, acetic acid, formic acid and carbonic acid [5].

The biodegradation of OEGs has been studied extensively [6,7] and the formation of mono- and dicarboxylated OEGs confirmed [7]. However, carboxylated metabolites appear to be a class of environmental contaminants so far little explored. They were identified in biodegradation test liquors and sewage treatment plants influents and effluents [3]. However, no study has been performed concerning the biodegradation of dicarboxylated OEGs.

Analysis of low molecular weight carboxylic acids is often performed by well-established chromatographic techniques, such as gas chromatography (GC), high-performance liquid chromatography (HPLC) and ion chromatography (IC). The methods used are accurate, but there is still a demand for techniques that avoid time-consuming derivatization that is often necessary in chromatographic techniques. The volatility of carboxylic acids is usually too low for direct GC analysis. The use of HPLC is also problematic. Here, the detection of underivatized carboxylated OEGs with the most popular

* Corresponding author.
E-mail address: agnieszka.zgola@fct.put.poznan.pl (A. Zgoła-Grześkowiak).

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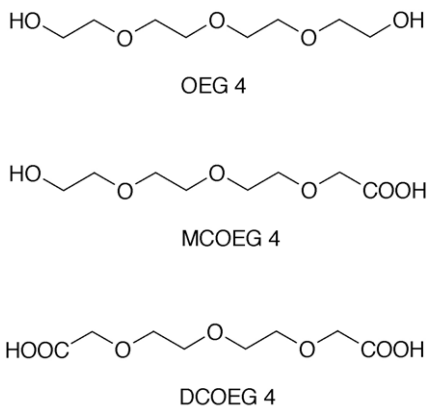

Fig. 1. Chemical structures of: OEG 4—oligo(ethylene glycol) with four ethoxy groups, MCOEG 4—monocarboxylated oligo(ethylene glycol) with four ethoxy groups and DCOEG 4—dicarboxylated oligo(ethylene glycol) with four ethoxy groups (the main constituent of PEG 250 DA studied in this paper).

UV absorbance detector is problematic due to lack of proper chromophores in their molecules, although direct analysis of carboxylic acid at wavelengths below 220 nm for high analyte concentration has been reported [8,9]. The use of derivatization gives the possibility of analysis of lower analyte concentrations with UV or fluorescent detection. Marcomini and Pojana [3] used HPLC with fluorescence detection for the analysis of carboxylated OEGs. However, the method reported requires a complicated and time-consuming isolation and derivatization procedure. A capillary isotachophoresis (ITP), which in this context can be considered as a relatively new technique, is a promising alternative for analysing this group of compounds.

ITP was extensively used for the determination of organic acids in different materials [10–15]. Sadecka and Polonsky [10] studied formic acid, acetic acid, oxalic acid as well as some other organic acids and inorganic ions in tobacco after their extraction with 0.5 mM L^{-1} sulphuric acid and subsequent dilution before ITP measurements. Kosobucki and Buszewski [11] analysed organic acids in compost. The compost samples were extracted with water before ITP analysis of: formic acid, lactic acid, acetic acid and propionic acid. Polonsky et al. [12] investigated formic acid, acetic acid, glycolic acid, oxalic acid as well as some other organic acids in steep waters arising in the production of maize starch. The samples were analysed directly without preconcentration. Also Barth [13], studied a number of carboxylic acids without any preconcentration. The paper contains a study on a number of carboxylic acids in water associated with oil-bearing formations in North Sea. The samples were analysed directly without any preconcentration. Koval et al. [14] studied saturated fatty acids having from 1 to 18 carbon atoms. The samples in hydrocarbon matrices were diluted with methanol before ITP measurement. Hutta et al. [15] also analysed fatty acids. However, they determined the acids in the water matrix. For sample enrichment they used solid-phase extraction (SPE) with a carbonaceous sorbent. Moreover, due to problems with low breakthrough volume, only butyric acid and higher fatty acids were quantitatively recovered from the sorbent. A review article written by Bocek et al. [16] presents more interesting examples of carboxylic acids analyses. However, despite the large number of papers, no isotachophoretic method for the assay of biodegradation products has yet been reported. The use of capillary isotachophoresis with a conductivity detector enables simple and fast analysis of dicarboxylic acids coming from the biodegradation process without derivatization.

In the current study, the isotachophoretic procedure is presented for identification and quantification of carboxylic acids in biodegradation samples. The samples for the tests were directly analysed by ITP, which facilitated fast analysis. The same samples were also analysed by HPLC–MS, which gave confirmation of the ITP measurements.

2. Experimental

2.1. Reagents and chemicals

The chemicals used were of analytical reagent grade. Hydrochloric acid was from Merck (Darmstadt, Germany) and propionic acid from Riedel-de Haën (Seelze, Germany). Tris(hydroxymethyl)aminomethane (Tris), 2-morpholinoethanesulfonic acid monohydrate (MES), histidine and β-alanine were all from Fluka (Buchs, Switzerland). Water used in the preparation of the electrolyte systems and of the solutions of the model mixtures was prepared by reverse osmosis in a Demiwa System from Watek (Ledec nad Sazavou, Czech Republic), followed by double distillation.

Poly(ethylene glycol)bis(carboxymethyl)ether (PEG 250 DA) from Aldrich (St. Louis, MO, USA), oxalic acid from POCh (Gliwice, Poland), glycolic acid from Fluka, acetic acid from Merck, formic acid from Riedel-de Haën were used as received. All reagents used for preparation of synthetic sewage were purchased from POCh.

2.2. Apparatus

2.2.1. Isotachopherograph

Isotachophoretic separations were performed using the Electrophoretic Analyser EA 100 (Villa Labeco, Spišská Nová Ves, Slovak Republic) equipped with a column coupling system consisting of two capillaries made of fluorinated ethylene-propylene copolymer. The first, preseparation capillary (90 mm × 0.8 mm I.D.) was connected to the analytical capillary (160 mm × 0.3 mm I.D.) via the bifurcation block. The analyser was equipped with a sample valve of 30 μL fixed volume and conductivity detectors placed on both columns 40 mm from the outlet ends. Separations were performed at an ambient temperature of 22–24 °C. The isotachopherograms were evaluated by a personal computer software package supplied with the analyser.