Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1068 (2005) 327–333
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

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*

Received 5 November 2004; received in revised form 3 January 2005; accepted 25 January 2005

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).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.01.092

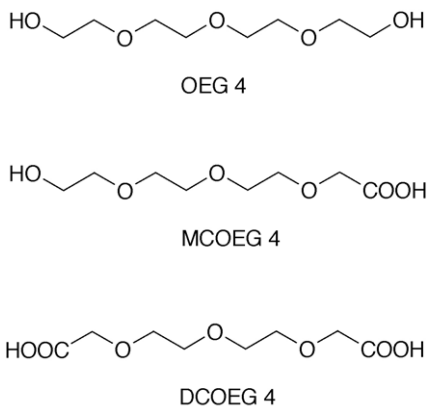

Fig. 1. Chemical structures of: OEG 4—oligo(ethylene glycol) with four ethoxy groups, MCOEG 4—monocarboxylated oligo(ethylene glycol) with four ethoxy groups and DCOEG 4—dicarboxylated oligo(ethylene glycol) with four ethoxy groups (the main constituent of PEG 250 DA studied in this paper).

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.

Two systems of isotachophoretic electrolytes were chosen in order to test separation of the analytes. In this way, separation of the analytes was checked in two modes—with higher and lower influence of their pK_a values on their effective mobilities.

In system A, the leading electrolyte was 10 mM L^{-1} hydrochloric acid, including 1% poly(vinylpyrrolidone) adjusted with β-alanine to pH 3.5. The terminating electrolyte was 10 mM L^{-1} propionic acid. The driving current in the preseparation capillary was 250 μA. The initial driving current in the analytical capillary was 30 μA. During detection, the current was reduced to 8 μA.

In system B, the leading electrolyte was 10 mM L^{-1} hydrochloric acid, including 1% poly(vinylpyrrolidone) adjusted with histidine to pH 6.0. The terminating electrolyte was 5 mM L^{-1} MES adjusted to pH 6.0 with Tris. The driving current in the preseparation capillary was 250 μA and in the analytical capillary, 30 μA.

2.2.2. Mass spectrometers

The HPLC–MS analyses were performed using a Waters/Micromass (Manchester, UK) ZQ mass spectrometer. The instrument was coupled to a Waters model 2690 HPLC pump (Milford, MA, USA). Using an autosampler, the sample solutions were injected into the Nova Pak C_{18} RP column (150 mm × 3.9 mm I.D., Waters). A gradient of acetonitrile and 5% formic acid in water–methanol (9:1, v/v) mixture was applied. The linear gradient started from 0% of acetonitrile reaching 95% of acetonitrile after 15 min and the latter concentration was maintained for 5 min. The electrospray (ESI) source potentials were: capillary 3 kV, lens 0.5 kV, extractor 4 V and cone voltage 30 V. The source temperature was 120 °C and desolvation temperature 300 °C. Nitrogen was used as the nebulizing and desolvation gas at flow rates of 100 and 300 L h^{-1}, respectively.

Liquid secondary ion mass spectrum of PEG 250 DA was obtained on an AMD 604 two sector mass spectrometer of reverse B/E geometry, made by AMD Intectra (Germany). A CsJ gun supplied the primary ion beam (12 keV, Cs^+). The secondary ion beam was accelerated to 8 kV.

2.3. Biodegradation tests

The methods used were the OECD screening test and river water die-away test. In both these tests the test compound is the sole source of organic carbon.

2.3.1. OECD screening test

In the OECD screening test an activated sludge is used as an *inoculum*. The activated sludge used in this experiment originated from the sewage treatment plant located in Szamotuły (near Poznan, Poland) which treats typical municipal sewage. The sludge was placed in the Husmann plant [17] for 1-week cleaning process. During this time synthetic sewage was delivered to the Husmann plant to clean up the sludge. The synthetic sewage consisted of: pepton, beef extract, urea, NaCl, $CaCl_2$, $MgSO_4$, K_2HPO_4, $NaHCO_3$ [18]. After the cleaning process was finished, the activated sludge concentration in water from the Husmann plant was measured. The OECD test was performed in bottles filled with 200 mL of medium consisting of water spiked with PEG 250 DA and an aliquot of activated sludge solution. The concentration of PEG 250 DA in the samples was 10 mg L^{-1}. The samples were left in open bottles protected against the dust and light at room temperature 21 ± 2 °C. All the bottles were shaken on a rotary shaker to provide oxygen. The samples were analysed on days 1, 2, 3, 4, 9 and 12 of the biodegradation test.

2.3.2. River water die-away test

The second biodegradation test was performed according to the river die-away method [18,19], which uses water sampled directly from the river. The river water for testing was sampled from the River Warta in Poznan (Queen Jadwiga Bridge) from 1 m depth. The biodegradation mixture was not aerated, but only stirred continuously; oxygen transfer occurred only through the surface. Incubation proceeded at laboratory temperature. The samples were analysed on days 1, 2, 8, 10 and 14 of the biodegradation test.

2.4. Sample preparation

The samples for isotachophoretic measurements were filtrated before analysis. A portion of every filtrate was also left for HPLC–MS analysis. These samples were diluted with methanol to stop the biodegradation process. The MS measurements were performed after the biodegradation tests were completed.

The SPE experiments with enrichment of the samples were made with two types of cartridge: a 500 mg quaternary amine from J.T. Baker (Deventer, The Netherlands) and a 250 mg EnviCarb (non-porous graphitised carbon black (GCB), 120–400 mesh, 100 m^2 g^{-1} surface area) from Supelco (Bellefonte, PA, USA).

The anion exchanger cartridge was conditioned by slowly sucking through 10 mL of 0.1 M NaOH and 10 mL of water. Without letting the cartridge become dry, 25 mL of water sample was applied at a speed of ca. 3 mL min^{-1}. The cartridge was then dried for 0.5 h and the analytes were eluted with a mixture of 0.2 M HCl–methanol (1:1, v/v), all at a drop-by-drop rate. The eluate was diluted to lower concentration of chlorides.

The GCB cartridge was conditioned with 5 mL of a mixture dichloromethane–methanol (8:2, v/v), 2 mL of methanol and 15 mL of water. Without letting the cartridge become dry, 40 mL of water sample was applied. Next, the cartridge was dried to remove traces of water. The analytes were eluted with 4–10 mL of methanol, or with a combination of 1 mL of methanol and 9 mL of a mixture dichloromethane–methanol (8:2, v/v). The mixture was used with or without the addition of 1 M HCl. The eluate was evaporated under a gentle stream of nitrogen. It was then reconstituted in 1 mL of water.

3. Results and discussion

3.1. Isotachophoretic analysis

Isotachophoresis is a convenient analytical technique for the separation of organic anions in various matrices. However, the ionic mobilities of many organic ions were often similar and a different pH has to be applied for the measurements to assure separation of the analytes. Thus, the isotachophoretic separation of PEG 250 DA constituents and their biodegradation products was investigated in two different systems of electrolytes. The pK_a values of the investigated analytes are in the range from 1.27 to 4.76 [20] (for dicarboxylated tetraethylene glycol pK_a was estimated experimentally in our laboratory). In the system B at pH 6.0 almost all the analytes were completely ionized while in the system A at pH 3.5 the analytes were only partially ionized which enabled a different selectivity to be obtained.

Two isotachophoretic systems were initially used for the separation of PEG 250 DA constituents without their potential biodegradation products. The separation was achieved in both systems as expected. The isotachopherograms obtained in both conditions show three steps (Fig. 2). The longest step comes from dicarboxylated tetraethylene glycol, which is the main constituent of PEG 250 DA. Two shorter steps come from lower molecular mass homologues. The relative step heights (RSHs), counted as the ratio of the step height of the analyte to the step height of the terminator, were calculated for all the compounds found at both isotachophoretic systems. At pH 3.5 RSHs = 0.293, 0.259 and 0.174 for dicarboxylated tetra-, tri- and diethylene glycol, respectively. At pH 6.0 RSHs = 0.304, 0.234 and 0.149 for the three compounds. The above composition of PEG 250 DA was confirmed by mass spectrometry measurement (Fig. 3). The main peak on the mass spectrum with m/z = 220.9 comes from the dicarboxylated tetraethylene glycol $[M–H]^-$ ion. Two peaks

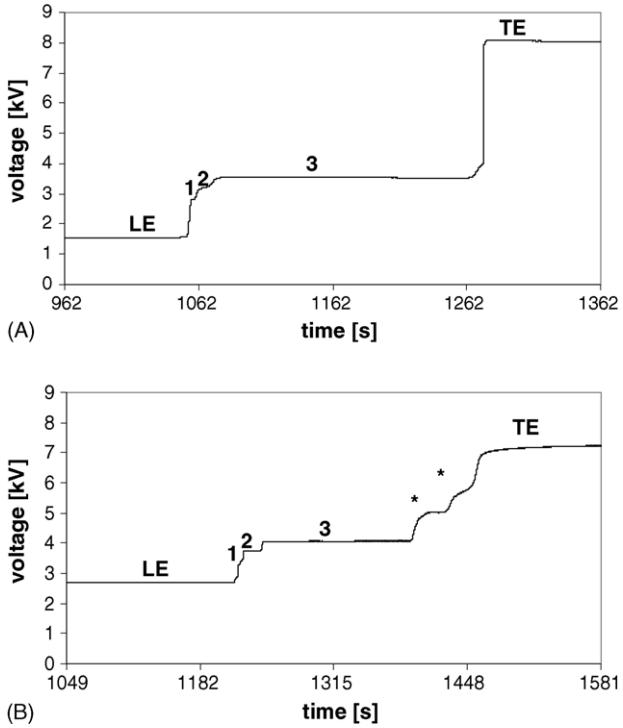

Fig. 2. The isotachophoresis of PEG 250 DA obtained at pH 3.5 (A) and 6.0 (B); (LE—leading electrolyte, 1—dicarboxylated diethylene glycol, 2—dicarboxylated triethylene glycol, 3—dicarboxylated tetraethylene glycol, *—unknown impurities, TE—terminating electrolyte).

with smaller abundances were found for dicarboxylated triethyleneglycol (m/z = 176.9) and dicarboxylated diethylene glycol (m/z = 133.0).

Both the isotachophoretic systems were tested for the possibility of their use in the biodegradation studies of PEG 250 DA. First, the separation of potential biodegradation products was studied. A mixture containing oxalic acid, glycolic

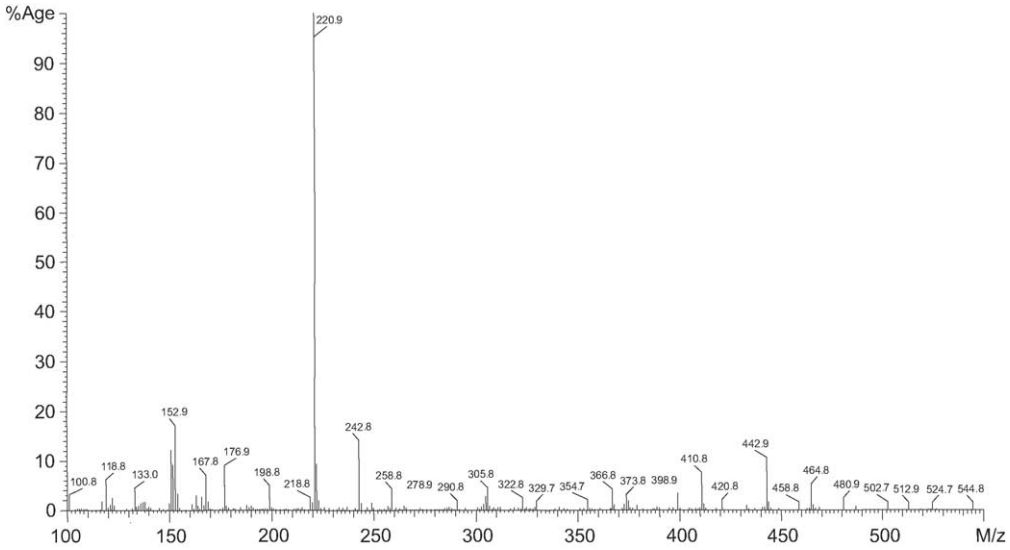

Fig. 3. Mass spectrum of PEG 250 DA.

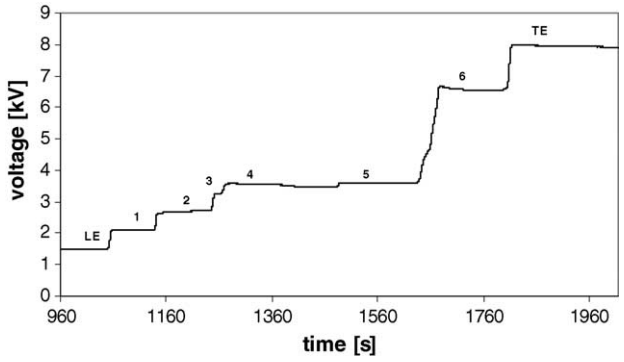

Fig. 4. The isotachopherogram of analysed acids (LE—leading electrolyte, 1—oxalic acid, 2—formic acid, 3—dicarboxylated triethylene glycol, 4—dicarboxylated tetraethylene glycol, 5—glycolic acid, 6—acetic acid, TE—terminating electrolyte).

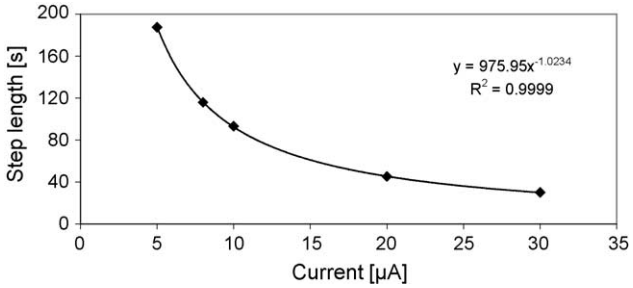

Fig. 5. The dependence of the step length of dicarboxylated tetraethylene glycol at fixed concentration upon the driving current.

acid, acetic acid, formic acid and PEG 250 DA was analysed in the two systems. At pH 3.5, all the analytes were successfully separated (Fig. 4), although RSHs for dicarboxylated tetraethylene glycol and glycolic acid were very similar. At pH 6.0 not all the analytes were separated. Thus, the isotachophoretic system with pH 3.5 was chosen for further experiments. The values of RSHs calculated for all the analytes (Table 1) were used for the identification of ITP zones.

After separation of the analytes was achieved, the method was optimised to obtain the lowest possible limit of detection (LOD) and limit of quantitation (LOQ).

For the limit of detection we used the value $(y + 3S)/b$, where the calculated intercept of the calibration line was used as an estimate of y, S is the deviation in the Y direction of the calibration line and b is the slope of the calibration line [10,21]. For the limit of quantitation, the value $(y + 10S)/b$ was used [21].

To achieve lower LOD and LOQ, the analysis of PEG 250 DA was conducted at different driving current values. Generally, a decrease in the driving current in the analytical capillary allows for longer steps of the analytes to be obtained. Thus, the initial value of 30 μA was reduced to 20, 10, 8 and 5 μA. As a result, a curve showing a dependence of step length from driving current was obtained (Fig. 5). As expected, the longest step of dicarboxylated tetraethylene glycol was obtained when the driving current of 5 μA was used. However, due to limited current stabilization possibilities of the equipment used, the step length in the analysis conducted at 5 μA was unrepeatable. The driving current of 8 μA was used for further experiments, which assured the lowest LOD and LOQ to be obtained. For example, the LOD and LOQ for dicarboxylated tetraethylene glycol analysed at the driving current of 8 μA were 0.002 and 0.004 mM L^{-1}, respectively. At the driving current of 30 μA the LOD and LOQ were 0.006 and 0.008 mM L^{-1}, respectively. Thus, the driving current of 8 μA was used for further experiments which assured the lowest LOD and LOQ with a satisfactory precision of measurement (Table 1).

3.2. Sample preparation

Generally, samples from biodegradation tests of PEG 250 DA do not require difficult preparation before isotachophoretic analysis. The LOQ of dicarboxyleted tetraethylene glycol is low enough to ensure qualitative analysis, even if only a small part of initial PEG 250 DA is present in the biodegradation liquor. Thus, only filtration is required before the sample may be introduced to the isotachopherograph. However, there is the possibility that the concentration of low molecular mass carboxylic acid from the biodegradation

Table 1
Validation parameters of the isotachophoretic method

Analysed compound	Relative step height and its confidence interval	Relative standard deviation of relative step height (%)	Limit of quantitation (mM L^{-1})	Limit of detection (mM L^{-1})	Linearity range (mM L^{-1})	Slope	Intercept	Correlation coefficient (r^2)	Precision (relative standard deviation for five injections) (%)
Oxalic acid	0.093 ± 0.002	0.6	0.005	0.001	0.002–1	730	−0.2	0.9999	1.4
Formic acid	0.189 ± 0.006	1.1	0.006	0.001	0.002–1	506	−0.2	0.9999	0.9
Dicarboxylated diethylene glycol	0.174 ± 0.006	0.4	N/D	N/D	N/D	N/D	N/D	N/D	N/D
Dicarboxylated triethylene glycol	0.259 ± 0.032	1.4	N/D	N/D	N/D	N/D	N/D	N/D	3.1
Dicarboxylated tetraethylene glycol	0.293 ± 0.003	0.8	0.004	0.002	0.001–1	969	1.2	1.0000	0.2
Glycolic acid	0.317 ± 0.006	0.7	0.006	0.003	0.002–1	787	1.2	1.0000	0.1
Acetic acid	0.778 ± 0.018	0.8	0.007	0.003	0.001–1	663	0.8	0.9999	1.4

N/D: not determined.

process is low. Therefore, solid phase extraction was considered to obtain preconcentration of the analytes.

The technique of SPE is well established. However, recovery rates of polar carboxylic acids from the water matrix are generally low due to the high polarity of these substances and the resulting isolation problems. For the isolation of the acids from the water matrix, we tested two sorbents: the anion exchanger and the graphitised carbon black. The anion exchanger was an obvious choice for carboxylic acid adsorption. However, the eluent used in SPE and electrolytes used in isotachophoresis had to be matched to each other. Therefore, the mixture of methanol and 0.2 M hydrochloric acid used for elution was diluted tenfold with water and 5% of methanol was added to the leading and terminating electrolytes. As a result, the ionic strength as well as density of the samples and the electrolytes were equal which prevented problems during ITP analysis. The recovery of dicarboxylated tetraethylene glycol from this sorbent was $97 \pm 2\%$. However, the recovery of oxalic acid was only $35 \pm 8\%$ and glycolic acid was not recovered at all.

The use of GCB in isolation of polar and low molecular mass compounds including carboxylic acids has already been described in the literature [3,7,15,22–24]. Thus, this sorbent was also considered for use in our studies. In this study GCB was eluted in normal and back-flush mode. In the back-flush mode, the SPE sorbent was turned upside down after adsorption of the analytes. Thus, elution was made in a direction opposite to adsorption. This method of elution was recommended for GCB sorbent by some authors [25,26]. However, in our study recovery of dicarboxylated tetraethylene glycol eluted with 4 mL of methanol in normal mode of elution ($32 \pm 1\%$) was better than that obtained in back-flush mode ($9 \pm 3\%$). The use of 10 mL of methanol or 10 mL of the mixture of dichloromethane–methanol (8:2, v/v) only slightly improved the recovery of dicarboxylated tetraethylene glycol ($42 \pm 1\%$ and $43 \pm 2\%$, respectively). Also, the mixture of methanol with dichloromethane used with the addition of acids was recommended in the literature [22–24]. This indeed improved the recovery of dicarboxylated tetraethylene glycol. However, the overall results in recovery studies with GCB sorbent were not satisfactory. The recoveries of dicarboxylated tetraethylene glycol and glycolic acid were $66 \pm 3\%$ and $57 \pm 2\%$, respectively. The recoveries of acetic acid and formic acid were $11 \pm 3\%$ and $6 \pm 4\%$, respectively. The oxalic acid was not recovered at all.

The results obtained for both types of sorbents made it impossible to use them in the biodegradation studies of PEG 250 DA. The results obtained for SPE isolation of polar carboxylic acids are similar to those obtained by other researchers. None of them could obtain high recoveries of the low molecular mass carboxylic acids from the water matrix [3,7,15,22].

Thus, the samples from the biodegradation tests were filtrated without any preconcentration, which was enough for the biodegradation studies. There were no losses of the analytes and sample preparation was faster. However, the method used in the biodegradation studies cannot be used for the analysis of environmental samples, due to low concentration of carboxylated oligo(ethylene glycol)s in these samples.

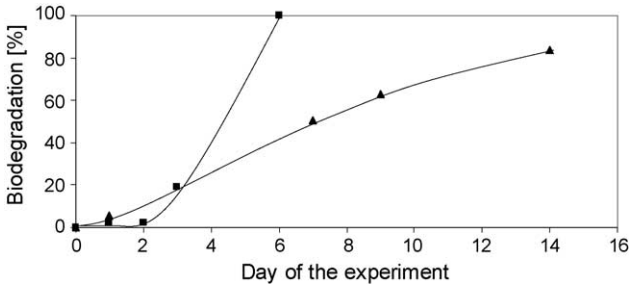

Fig. 6. The biodegradation of PEG 250 DA in the river water die-away test (triangles) and the OECD screening test (squares) calculated as the percentage decline in dicarboxylated tetraethylene glycol concentration.

3.3. Biodegradation tests

The biodegradation tests proved the biodegradation of PEG 250 DA (Fig. 6). However, the biodegradation in the OECD screening test was faster than in the river water die-away test. This can be ascribed to a higher concentration of bacteria in the OECD screening test.

The biodegradation rate of PEG 250 DA was different in the two tests. In the river water die-away test, the process started early but the degree of biodegradation was only about 50% after 7 days of biodegradation. On day 14 of the test about 20% of dicarboxylated tetraethylene glycol was still present in the test liquor. Also, no degradation products were found in this test.

During the first days of the OECD screening test, only the residual low molecular mass carboxylic acids (formic acid and acetic acid) from the activated sludge were being degraded. The presence of the two acids was confirmed by ITP measurements. The biodegradation of PEG 250 DA in the screening test started after 2 days of adaptation. However, no PEG 250 DA was found in the samples on day 6 of the test. On this and the next day high concentrations of glycolic acid were found. Also, the concentration of acetic and formic acids began growing.

The course of the biodegradation process was confirmed by HPLC–MS measurements. The abundance of the ions from dicarboxylated tetraethylene glycol was smaller after 3 days of biodegradation and no PEG 250 DA constituents were found on day 6 of the OECD test. The increase of signal from dicarboxylated tri- and diethylene glycols was not observed. Also, low concentrations of monocarboxylated glycols were found only in the first 3 days of the test and formation of low molecular mass acids in the last days of the biodegradation was confirmed. The course of the biodegradation found in the literature [5] was confirmed.

The literature describes many examples of the biodegradation tests of surfactants and their intermediate biodegradation products. Among them, linear alcohol ethoxylates are good examples of relatively fast biodegradation. Their

biodegradation reaches over 80% within 28 days. In contrast, the branched alcohol ethoxylates reached hardly half that level [27]. In this context, we can say that the rate of the biodegradation process of PEG 250 DA was proved to be relatively fast, especially in the conditions present in sewage treatment plants.

4. Conclusion

The isotachophoresis was proved to be a simple and inexpensive technique in organic acid analysis. The possibility of its use in biodegradation studies was shown. However, the use of isotachophoresis for environmental analysis would require a time-consuming pretreatment, which is also difficult because of the high requirements of isotachophoresis regarding low sample ionic strength and organic solvent content.

The carboxylated metabolites of oligo(ethylene glycol)s were proved to be biodegradable. The biodegradation process was relatively fast, leading to the formation of low molecular mass carboxylic acids.

Acknowledgement

This work was supported by the KBN grant 3 T09A 13626.

References

[1] Information of Association of the Industry Producting Chemicals for Textile, Leather and Surfactant Plant TEGEWA, Germany, 2001.
[2] R.D. Swisher, Surfactant Biodegradation, second ed., Revised and Expanded, Marcel Dekker, Basel, New York, 1987.
[3] A. Marcomini, G. Pojana, Analusia Mag. 25 (1997) 35.
[4] M. Frańska, R. Frański, A. Szymański, Z. Łukaszewski, Water Res. 37 (2003) 1005.
[5] T. Balson, M.S.B. Felix, in: D.R. Karsa, M.R. Porter (Eds.), Biodegradability of Surfactants, Blackie, Glasgow, UK, 1995, p. 204.
[6] A. Szymański, B. Wyrwas, M. Szymanowska, Z. Łukaszewski, Water Res. 35 (2001) 3599.
[7] C. Crescenzi, A. Di Corcia, A. Marcomini, R. Samperi, Environ. Sci. Technol. 31 (1997) 2679.
[8] M. Shirao, R. Furuta, S. Suzuki, H. Nakazawa, S. Fujita, T. Maruyama, J. Chromatogr. A 680 (1994) 247.
[9] W. Buchberger, K. Winna, J. Chromatogr. A 739 (1996) 389.
[10] J. Sádecká, J. Polonský, J. Chromatogr. A 988 (2003) 161.
[11] P. Kosobucki, B. Buszewski, Chem. Anal. 48 (2003) 555.
[12] J. Polonsky, A. Horvathova, M. Panik, J. Chromatogr. 390 (1987) 141.
[13] T. Barth, Anal. Chem. 59 (1987) 2232.
[14] M. Koval, D. Kaniansky, M. Hutta, R. Lacko, J. Chromatogr. 325 (1985) 151.
[15] M. Hutta, E. Simunicova, D. Kaniansky, J. Tkacova, J. Brtko, J. Chromatogr. 470 (1989) 223.
[16] P. Bocek, P. Gebauer, V. Dolnik, F. Foret, J. Chromatogr. 334 (1985) 157.
[17] European Economic Community, Council Directive of 31 March 1982 on the approximation of the laws of the Member States relating to the methods of testing the biodegradability of non-ionic surfactants and amending Directive 73/404/EEC(82/242/EEC), Official Journal of the European Communities.
[18] P. Schöberl, Tenside Surf. Det. 28 (1991) 6.
[19] R.D. Swisher, Surfactant Biodegradation, Marcel Dekker, New York, Basel, 1987.
[20] T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi, J.-I. Akiyama, J. Chromatogr. 271 (1983) D1.
[21] J. Sádecká, J. Polonský, Talanta 59 (2003) 643.
[22] A. Di Corcia, C. Crescenzi, A. Marcomini, R. Samperi, Environ. Sci. Technol. 32 (1998) 711.
[23] N.S. Battersby, A.J. Sherren, R.N. Bumpus, R. Eagle, I.K. Molade, Chemosphere 45 (2001) 109.
[24] F. Houde, Ch. DeBlois, D. Berryman, J. Chromatogr. A 961 (2002) 245.
[25] A. Di Corcia, A. Bellioni, M. Diab Madbouly, S. Marchese, J. Chromatogr. A 733 (1996) 383.
[26] N. Masqué, R.M. Marcé, F. Borrull, J. Chromatogr. A 793 (1998) 257.
[27] L. Kravetz, J.P. Salanitro, P.B. Dorn, K.F. Guin, J. Am. Oil Chem. Soc. 68 (1991) 610.