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Selectivity of solid-phase extraction phases in the determination of biodegradation products ☆

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Abstract

The extraction techniques connected with gas chromatography were used to describe quantitatively and qualitatively the biodegradation process. We investigated the biodegradation of hydrocarbons and non-ionic surfactants. Solid-phase extraction (SPE) and liquid–liquid extraction were used for the isolation of the non-degraded compounds and their degradation products. The selectivity of SPE has a significant influence on the isolation and preconcentration of organic compounds from water. © 2001 Published by Elsevier Science B.V.

Keywords: Solid-phase extraction; Hydrocarbons; Surfactants

1. Introduction

The great development of new technology very often can cause the great environmental pollution. The use of liquid fuels induces large amounts of hydrocarbons and their degradation products in water and soil. These substances can influence the ecological situation in water systems. Biotechnological methods are most often used to solve the problem of the environment pollution. The microbe can transform organic compounds into simple environmentally friendly chemicals. For the description of these processes the appropriate analytical procedures are required. Environmental analysis, especially analysis of water samples, includes an appropriate sample preparation step followed by an instrumental analysis.

Sample preparation procedure is usually the most laborious and time-consuming part of the overall analysis. Therefore, it is very important to choose an appropriate analytical way. In environmental analysis the whole range of compounds of interest should be efficiently recovered from the original water sample.

Solid-phase extraction (SPE) is one of the possible techniques which may be used in the preconcentration step of the analytical procedure. The principles of SPE and the use of this kind of extraction are widely described in the literature. The historical and new developments are also reviewed [1–11].

SPE is a multistage process. There are four steps in this extraction technique:

(1) Sorbent conditioning – the solvent is passed

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through the sorbent to wet the packing material and to solvate the functional groups of the sorbent.

(2) Sample application and retention step – the analyte is concentrated on the sorbent.

(3) Interference elution – this step is to rinse the column of interference and to retain the analyte.

(4) Desorption – analyte elution with an appropriate solvent [12–15].

The interaction of various sorbent materials with compounds having different physico–chemical properties lead inevitably to large differences in preconcentration efficiency. Specific differences in the recovery of the analytes were found for almost each sorbent.

SPE procedures were compared to traditional liquid–liquid extraction (LLE) methods. LLE is still used in routine water analysis, but its selectivity is very low, it needs large amounts of toxic organic solvents. There are some advantages of SPE over LLE: (1) minimized consumption of organic solvents; (2) no emulsion formation; (3) reduced contact of analyst with potentially toxic substances; (4) solvent replacement; (5) cleaning and preconcentration of the sample [15,16].

The sorbents in SPE techniques are placed in cartridges; these columns are typically constructed of polypropylene, polyethylene or glass, and filled with 40-μm packing material with different functional groups. A 20-μm polypropylene frit usually contains from 50 mg to 10 g of packing material. A liquid sample is passed through the column and analytes are concentrated and purified. The sample volume that can be applied ranges from 1 ml to over 1 l.

The sorbents used for SPE are similar to those used in liquid chromatography, including normal-phase, reversed-phase, size-exclusion and ion-exchange sorbents. Normal-phase sorbents consist of a stationary phase that is more polar than the solvent or sample matrix. These sorbents are used in SPE when an analyte of interest is dissolved in an organic solvent. There is a chemically modified silica gel among this kind of sorbents, for normal-phase, cyanopropyl (CN), aminopropyl (NH_2), and diol functional groups are chemically bonded to the silica gel.

Reversed-phase sorbents are packing materials that are more hydrophobic than the sample. These sorbents are commonly used in SPE when aqueous samples are involved. For reversed-phase sorbents, octadecyl (C_{18}), octyl (C_8), ethyl (C_2), cyclohexyl, and phenyl functional groups are bonded to the silica gel.

Ion-exchange sorbents usually contain both weak and strong cation and anion functional groups bonded to the silica gel. Strong cation-exchange sorbents contain ion-exchange sites consisting of sulfonic acid groups, weak cation-exchange sorbents contain sites consisting of sulfonic acid groups, and weak cation-exchange sorbents contain sites consisting of carboxylic acid groups. Anion-exchange sites are amines bonded to the silica gel.

Size-exclusion sorbents utilize a separation mechanism based on the molecular size of the analyte. For this kind of interaction butyl [$-(CH_2)_3CH_3$] and carboxylic acid (–COOH) functional groups are bonded to the silica gel.

Another type of packing material is a mixed-mode resin. This packing material contains both a bonded reversed-phase group (typically a C_8) and a cation-exchange group on a silica gel or a polymeric matrix [12]. Apart from classical, accessible, sorbents – bonded silica (as C_8, C_{18}, COOH, CN), polymers and carbon materials, some modified sorbents were used, for example, modified resins containing surface sulfonic acid groups introduced onto polystyrene–divinylbenzene (PS–DVB). These resins displayed excellent surface hydrophilicity and improved extraction efficiencies [17]. Silicalite was introduced in the extraction techniques as a sorbent complementing polystyrene and bonded-phase silica adsorbents. It is a molecular sieve. It is a polymorph of silica with an unusual crystal structure. Silicalite adsorbs organic analytes through hydrophobic interactions and additionally possesses size-exclusion properties.

Silicalite was used in cartridges and it has been embedded in polydimethylsiloxane, ethylene–propylene, and polychloroprene and nitrilebutadiene rubbers to form composite membranes. It was used in the extraction of the following compounds: aldehydes, ketones, esters, chlorinated alkanes, carboxylic acids, amines and phenols. The samples after extraction were analyzed with high-performance liquid chromatography (HPLC) [14].

Various phases of SPE have been blended or copolymerised in order to use multiple interactions for the isolation and purification of analytes called

mixed-mode resins. These resins have the potential to recover analytes covering a wider range of polarity by utilizing specific, simultaneous interactions. Elution steps, in which different solvents are used, can be selective by sequentially cancelling the specific mechanisms of interaction in the operation on one resin. These resins show the duality of isolation mechanisms, so multiple functionality is bonded onto a copolymerization or different resins are blended into one cartridge [18].

The main aim of this work was to develop an analytical procedure for the description of the biodegradation process using bonded silica and polymer as sorbents for SPE. Some groups of SPE sorbents were tested in the extraction of different compounds from water samples. The SPE technique connected with gas chromatography (GC) was used for the investigation of the biodegradation processes. Hydrocarbons in the presence of emulsifier (non-ionic surfactant) were degraded with bacteria strains: *Pseudomonas putida* and *Pseudomonas aeruginosa*.

Biodegradation processes are very complex, they can proceed in very different ways, and many biodegradation products may be present in the post-biodegradation mixture. The analytical procedure should be very precise and sensitive for the reliable qualitative and quantitative determination of those samples.

2. Experimental

2.1. Chemicals

Aliphatic hydrocarbons (C_{12}, C_{14}, C_{16}) were obtained from Acros Organics (NJ, USA), oxyethylates $C_{12}(EO)_n$ (n=3, 6, 7, 10) were obtained from Sigma (St. Louis, MO, USA), the solvents *n*-hexane, dichloromethane (pure for analysis) were obtained from Merck (Darmstadt, Germany), and methanol, trichloromethane, and ethyl acetate (pure for analysis), were obtained from POCh (Gliwice, Poland). The derivatization reagent *N*,*O*-bis-(trimethylsilyl)acetamide (BSA) and polyglycols with various number of oxyethylene groups (PEG_n, n=2, 3, 4, 9) were obtained from Fluka (Buchs, Switzerland).

The concentrations of analytes in water samples were the following: (i) aliphatic hydrocarbons: 6 mg/l; (ii) oxyethylene ten lauryl ether [polydispersive $C_{12}(EO)_{10}$ will be signified with bold letters]: 4 mg/l; (iii) oxyethylates $C_{12}(EO)_n$: 5 mg/l; (iv) polyethylene glycols (PEGs): 5 mg/l.

2.2. GC analysis

GC analyses were made on a HP 5890II gas chromatograph (supplied by Hewlett-Packard Poland, Warsaw, Poland) on a capillary column of dimethyl–diphenyl polysiloxane with a film thickness of 0.25 μm, 30 m×0.28 mm I.D. MXT-5 (Resteck, supplied by AnaSerwis, Baranowo, Poland). Helium was used as the carrier gas at a flow-rate of 2.5 ml/min and a head pressure of 140 kPa. The column temperature was held at 115°C for 1 min, then ramped at 10°C/min to 125°C, then at 25°C/min to 175°C where it was held for 2 min, then ramped at 30°C/min to 350°C, where it was held for 7 min.

2.3. Solid-phase extraction

We have used various sorbents (in normal-phase and reversed-phase systems) and various solvents to choose the best arrangement for the analysis of the following compounds: aliphatic hydrocarbons, oxyethylene lauryl ether, fatty alcohols and acids, polyglycols.

All sorbents were placed in cartridges produced by J.T. Baker (Deventer, The Netherlands, supplied by Witko, Łódź, Poland).

(i) C_{18} sorbent – silica bonded with octadecyl; cartridge volume: 6 ml; sorbent mass: 500 mg.

(ii) C_8 sorbent – silica bonded with octyl; cartridge volume: 6 ml; sorbent mass: 500 mg.

(iii) COOH sorbent – silica bonded with carboxylic acid; cartridge volume: 8 ml (glass); sorbent mass: 500 mg (hand packed).

(iv) SDB sorbent – copolymer PS–DVB; cartridge volume: 6 ml, 8 ml; sorbent mass: 200 mg, 500 mg.

During optimization of the SPE procedure aliphatic hydrocarbons and emulsifier (oxyethylene lauryl ether) were analyzed separately and in the mixture, with the use of different sorbents. We used the following sorbents: bonded-silica (C_8, C_{18}, COOH), PS–DVB (SDB).

In each extraction methanol and water were used

as the conditioning solvents, different organic solvents were tested as eluents: methanol, *n*-hexane, dichloromethane, trichloromethane and ethyl acetate (5 ml each).

Alcohol $C_{12}H_{25}OH$ and acid $C_{11}H_{23}COOH$ were separated on C_8, C_{18}, COOH bonded silica, using methanol and water as the conditioning solvents and ethyl acetate (5 ml) as eluent.

Polyglycols from the water samples with various number of oxyethylene groups ($n=2$, 3, 4, 9) were separated with LLE and SPE. In LLE ethyl acetate was used as the extracting solvent, while in SPE C_8, C_{18}, COOH and SDB sorbents were used [13].

3. Results and discussion

We have tested sorbents and solvents which may be used in the separation of non-ionic surfactants: oxyethylene lauryl ether $C_{12}(EO)_n$ homologues, where $n=3$, 6, 7, 10. The shorter oxyethylates can be the biodegradation products of $\mathbf{C_{12}(EO)_{10}}$. Moreover, $\mathbf{C_{12}(EO)_{10}}$ is not an individual compound; it is polydispersive and contains oxyethylates having more or less than 10 units. Oxyethylene lauryl ether is the non-volatile compound so the derivatization was necessary to produce the volatile derivatives for GC analysis. BSA was used as the derivatizing agent.

After extraction the solvent was evaporated and the residue was derivatized.

The extraction results of $C_{12}(EO)_n$ ($n=3$, 6, 7, 10), are given in Table 1. The best recovery of $C_{12}(EO)_3$ is on the C_8 sorbent, the best recovery of $C_{12}(EO)_6$ is on the C_{18} sorbent, while for $C_{12}(EO)_7$ the highest recovery is achieved on the COOH sorbent.

Table 1
Recovery of oxyethylene lauryl ether $C_{12}(EO)_n$ ($n=3$, 6, 7, 10) on different sorbents; eluent: ethyl acetate

Oxyethylate, $C_{12}(EO)_n$	Recovery (%)				
	C_8	C_{18}	COOH	CN	SDB
$C_{12}(EO)_3$	93.1	90.6	56.3	–	–
$C_{12}(EO)_6$	63.2	83.2	66.8	–	–
$C_{12}(EO)_7$	82.1	83.0	85.3	–	–
$\mathbf{C_{12}(EO)_{10}}$ (polydispersive)	68.0	55.0	99.1	70.1	71.4

Comparing the recoveries of longer oxyethylates and sorbents' polarity we can see that the shorter oxyethylates [$C_{12}(EO)_3$, $C_{12}(EO)_6$] are better sorbed on non-polar sorbents (C_8, C_{18}) while the longer oxyethylates ($C_{12}(EO)_7$, $\mathbf{C_{12}(EO)_{10}}$) are better sorbed on more polar sorbents (COOH). The recovery of $C_{12}EO_{10}$ is near 100%. In the analyses of oxyethylates mixture we observed very similar dependence. The average recoveries are 70–80%. The analysis of $\mathbf{C_{12}(EO)_{10}}$ shows that ethyl acetate is the best eluent on all examined sorbents, while the weakest one is methanol.

3.1. Biodegradation products

Among oxyethylates' biodegradation products we can find lauryl alcohol, lauric acid and polyglycols. C_8 sorbent and ethyl acetate as eluent give the best recovery for alcohol and acid (Fig. 1).

Polyglycols are not extracted with ethyl acetate in LLE, while only longer polyglycols ($n>3$) are extracted with trichloromethane. Their recovery in LLE is rather low; the emulsions are formed during extraction and salting out may be required.

The SPE recovery of polyglycols is relatively low on C_8 and C_{18} sorbents and on the COOH sorbent polyglycols are not sorbed at all; but the recovery on SDB sorbent is near 100% (Table 2). Both in LLE and in SPE the recovery increases when molecular mass of the polyglycols increases. It is connected with decreasing polyglycols' polarity.

Smaller recovery of hydrocarbons on the C_{18} sorbent can be connected with the stronger sorption of aliphatic hydrocarbons C_{12} and C_{16} on octadecyl bonded silica (Table 3). This means that very often analytes cannot be to eluted, even with solvent of high eluotropic strength. Octyl bonded silica has weaker sorption properties because of its short hydrocarbon chain. This sorbent was found to be somewhat better for the separation of aliphatic hydrocarbons; the best eluent was *n*-hexane. However, *n*-hexane is not an efficient eluent after sorption on SDB (Table 3). Here, trichloromethane was found to be the best eluent. LLE with a relatively large amount of the solvent (2×20 ml; diethyl ether and trichloromethane) gave much lower recoveries than

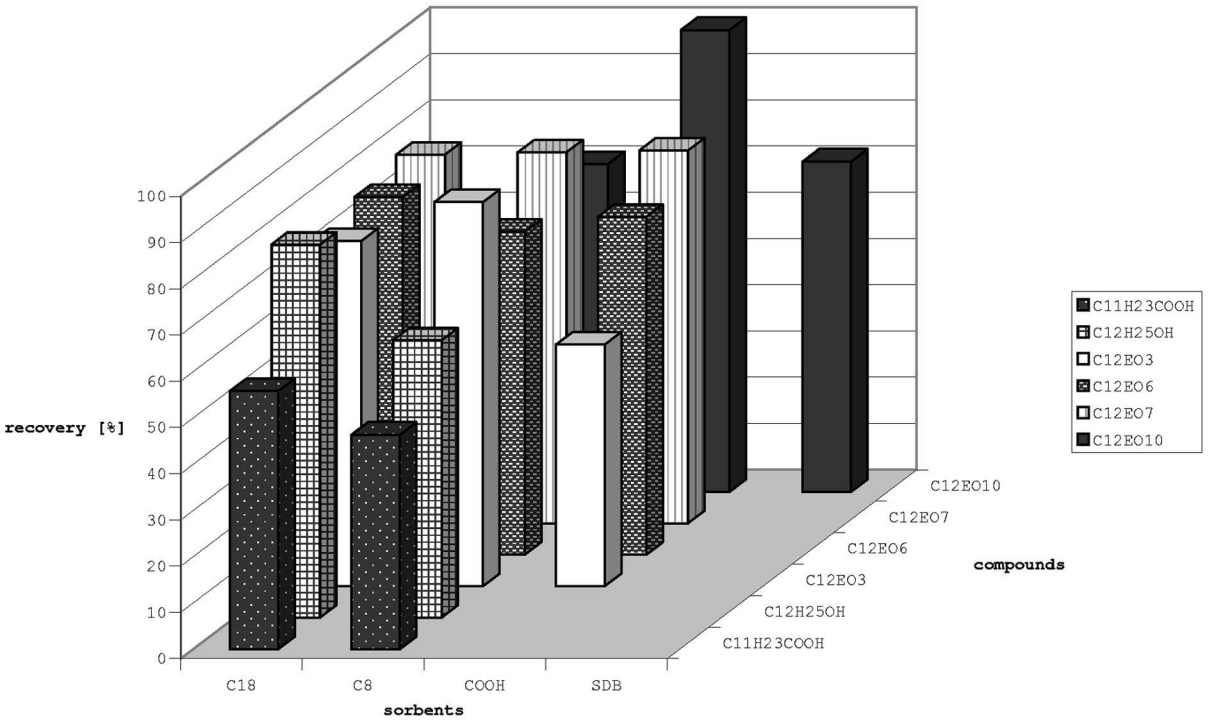

Fig. 1. Recovery of oxyethylates, acid and alcohol on different sorbents; eluent: ethyl acetate.

those obtained in SPE procedures. These were: 44.6% for C_{12} and 46.8% for C_{16} ($CHCl_3$ as eluent).

Aliphatic hydrocarbons (C_{12}, C_{16}) are non-soluble in water. They gather on the surface of water. They are additionally highly hydrophobic compounds, so they deposit on the laboratory vessels. It is a reason for their high loss during the analytical procedure. It does not happen when we have samples with the emulsifier. The presence of surfactant prevents the settling of analytes on the laboratory vessels. However, recoveries of hydrocarbons are rather low: *n*-hexane removes C_{14} better than ethyl acetate, while C_{16} is better eluted with ethyl acetate.

The results of the extraction of hydrocarbons and oxyethylene lauryl ether [$\mathbf{C_{12}(EO)_{10}}$] on C_8 sorbent are given in Table 4. The aim of experiments was to study the selectivity of this sorbent and to compare the elution properties of *n*-hexane and ethyl acetate. Oxyethylene lauryl ether, as surfactant, is very soluble in water, much better than in organic sol-

Table 2
Recovery of polyglycols on different sorbents; eluent: trichloromethane

n-Glycol	Recovery (%)			
	C_8	C_{18}	COOH	SDB
PEG2	0.26	0.57	0.0	0.48
PEG3	0.32	4.14	0.0	20.1
PEG4	2.37	–	–	87.3
PEG9	52.0	25.0	5.7	100.0

Table 3
Recovery of aliphatic hydrocarbons in different sorbent/eluent systems

Sorbent	Eluent (5 ml)	Recovery (%)	
		C_{12}	C_{16}
C_{18}	*n*-Hexane	63.3	74.2
C_8	*n*-Hexane	72.2	77.3
SDB	*n*-Hexane	17.1	22.5
	Ethyl acetate	28.3	18.8
	Trichloromethane	44.6	46.8

Table 4
Recovery of C_{14} and C_{16} hydrocarbons in the presence of $\mathbf{C_{12}(EO)_{10}}$ emulsifier (C_8 sorbent)

Compound	Average recovery (%) (n=5)		Σ
	n-Hexane	Ethyl acetate	
C_{14}	3.04	2.09	5.5
C_{16}	0.78	10.8	11.6
Emulsifier $C_{12}EO_{10}$	30.5	57.3	87.7

vents. This may influence the recovery of $\mathbf{C_{12}(EO)_{10}}$. Ethyl acetate is also a good eluent for oxyethylene lauryl ether.

Experiments with model mixtures of all analytes mentioned above were carried out during the optimization of the SPE procedure, the highest recovery for all compounds and the small volume of eluents were found. The conditions of the chromatographic analysis were also optimized. Such a procedure of biodegradation of sample for GC analyses allowed the quantitative separation of analytes.

4. Conclusions

Application of the SPE–GC procedure allows the analysis of the real samples after the biodegradation process.

The selectivity of the sorbents was used at this step of the analytical procedure. We can separate even complex mixtures with appropriately chosen sorbents and eluents. The mixture of polar and non-polar compounds may be separated in the sequentially used sorbent/eluent steps (Fig. 2). Hydrocarbons (C_{12}, C_{16}) as non-polar compounds are sorbed on the non-polar C_8 phase and they are eluted with the non-polar solvent *n*-hexane, while the polar surfactant [$\mathbf{C_{12}(EO)_{10}}$] is sorbed on the C_8 phase in only small amount. It is eluted quite well with polar solvent (ethyl acetate). $\mathbf{C_{12}(EO)_{10}}$ is effectively separated on SDB and well eluted with ethyl acetate.

The recovery of polar compounds – polyoxyethylene lauryl ethers and polyglycols – depends on the number of ethoxyl groups and the type of polar sorbent.

The selectivity of SPE allows one to separate even complex mixtures after a biodegradation process. It allows the isolation of individual compounds or groups of compounds. In this way we can separate biodegradation products and non-degraded residues.

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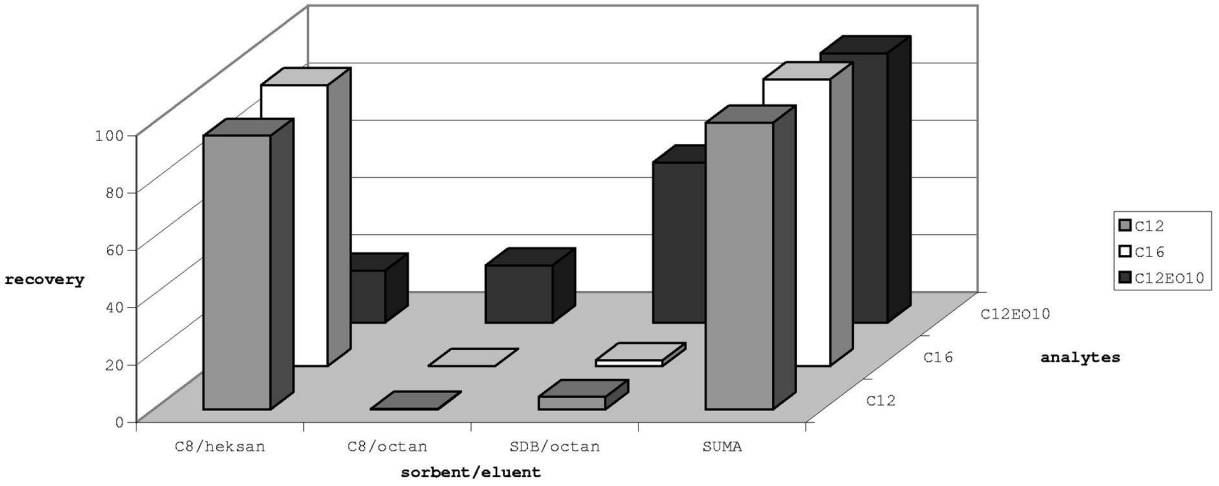

Fig. 2. The influence of the type of sorbent/eluent step on the recovery of non-polar and polar analytes from post-biodegradation mixture (*Pseudomonas aeruginosa*).

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