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DETERMINATION OF ALKYLPHENOLS AND ALKYLPHENOL ETHOXYLATES IN WATER USING MAGNETICALLY MODIFIED CHROMATOGRAPHIC COLUMN PACKING FOR EXTRACTION

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A rapid and simple magnetic solid phase extraction (MSPE) was used to pre-concentrate alkylphenols (APs) and nonylphenol mono- and di-ethoxylates (NP1EO and NP2EO) from water samples before capillary gas chromatographic

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(CGC) determination. AP, NP1EO and NP2EO are biodegradation products ethoxylated nonylphenols (NPnEO) surfactants. MSPE was tested for extraction of these pollutants from water. This method was compared with liquid - liquid extraction (LLE). Different types of sorbents and column packing such as Chezacarb B, S, Tenax GC, TA, GR, Porapak S and Chromosorb 101 - 106 were magnetically modified and used for the preconcentration and isolation of the target compounds. The four extraction parameters were optimized. Water samples from the reservoir Rozkos, river Labe and from water phase after biodegradation tests of oxyethylenated nonylphenols were analysed. Detection limits of the method were between 0.65-1 $\mu g\, l^{-1}$.

Introduction

Alkylphenols (APs) with longer alkyl chain, such as 4-octylphenol (OP) and 4-nonylphenol (NP) are mainly used to produce alkylphenol ethoxylate (APnEO) surfactants. Although APs themselves can be used for example as plasticizers in plastics, most frequently used compounds are NPnEO. They are synthesized from technical mixture of NP. NP is produced through Friedel–Crafts alkylation of phenol with technical nonene. Technical nonene is not a simple linear alpha-alkene but it is a complex mixture. The resultant NP is a very complex mixture of 22 isomers containing also 2-nonylphenol and decylphenol [1]. NPnEO, produced by oxyethylation of technical NP, is a very complex mixture of many oligomers with various numbers of oxyethylene functional groups. NPnEO has still been used in many applications due to its favourable physicochemical characteristics. These mixtures have been used as emulsifiers and solubilizers in pharmaceutical and agrochemical formulations, in cosmetics, as well as in various biotechnological processes. Furthermore, NPnEO is used in the industrial production of cleaning products, textiles, petroleum, pulp and paper and pesticides formulation.

During biological waste water treatment, these mixtures are partially converted to more toxic and persistent metabolites such as NP1EO, NP2EO, NP, 4-nonyl-phenoxyacetic acid (NP1EC) and 4-nonylphenoxy ethoxyacetic acid (NP2EC) [2]. The endocrine-disrupting effect of these metabolites formed from industrial non-ionic surfactants such as NPnEO is well known today [3].

AP, AP1EO and AP2EO were determined not only in samples of surface water, but also in various matrices, such as in sediments [2] and aquatic organisms and animals [2,4].

Concentrations of NP, NP1EO and NP2EO in surface water and in water after biodegradation tests of NPnEO are low therefore it is necessary to perform extraction before chromatographic separation.

Extraction of AP, AP1EO and AP2EO was carried out with organic solvents. This extraction method is the older way. Water samples were acidified

to pH 2 with sulphuric acid or hydrochloric acid and then extracted with an organic solvent. For LLE it is recommended to use toluene [5], dichloromethane [6], hexane [7] or ethyl acetate [8]. Nowadays, solid-phase extraction (SPE) [9-11], solid-phase microextraction (SPME) [12,13], stir-bar-sorptive extraction (SBSE) [14-16], liquid-gas-liquid microextraction (LGLME) [17] and liquid-liquid-liquid microex-traction (LLLME) [18] are preferred extraction methods. Extracted NP, NP1EO and NP2EO are analysed using of CGC either directly as non-derivatized or after their derivatization [19,20].

Magnetic solid extraction (MSPE) [21] has been used also for the extraction of non-ionic surfactants — oxyethylated aliphatic alcohols, methyl esters of rape oil and NP [22-24]. This technique enables adsorption of a target analyte on the magnetic adsorbent with subsequent direct separation of the magnetic complex. Then, adsorbed analytes are eluted with solvents. The obtained extract is analyzed after concentration using of CGC [25,26].

The advantage of this technique is mainly in the possibility to isolate the target analyte from water samples containing suspended solids, microorganisms or salts which can cause difficulties when column methods are used.

The aim of this study was to extract NP, NP1EO and NP2EO in spiked water samples, in water phase after biodegradation tests of NPnEO and in the river water samples using magnetically modified chromatographic sorbents and determine the target compounds by CGC.

Experimental

Materials

Porapak S (80-100 μm) was from Water Assoc., USA, Tenax GC (200-320 μm) was from Serva, Germany, Tenax TA and Tenax GR (140-210 μm) were from Scientific Instrument Services, USA, Chromosorb 101 (100-120 μm), 102 (120-200 μm), 103-105 (100-140 μm) and Chromosorb 106 (150-200 μm) were from Johns-Manville, USA; Al_2O_3 (1-5 μm), Poly(oxy-2,6-dimethyl-1,4-phenylene) (PODMP) (5-50 μm) were from Aldrich, USA, DPA-6S (polyamide derivatives) (30-60 μm) was from Supelco, USA. Sorbents Tonsil and Rudex (1-5 μm) were from the company Farmet a.s., the Czech Republic), activated carbon Chezacarb B and Chezacarb S were produced by Chemopetrol, Czech Republic in the form of small beads (between 0.2-1.5 mm in diameter); before use, everyone was milled in a knife coffee mill to obtain fine particles (1-10 μm).

4-Nonylphenol, technical mixture, 4-*n*-Propylphenol, 2,4-Diisopropylphenol, 2,4-Di-*tert*-butylphenol, 4-*tert*-Octylphenol, 2,4-Di-*tert*-pentylphenol and 4-*n*-Octylphenol were from Aldrich, USA; 4-*n*-Nonylphenol was from Lancaster Synthesis, Germany; 4-Nonylphenol mono- and diethoxylates technical mixtures

were from SLOVECA Sasol, s.r.o., Slovakia. 4-*n*-Nonylphenol mono- and diethylenglycol ethers were from the University of Pardubice, the Czech Republic. Methanol, toluene and hydrochloric acid were from Lachema Brno, the Czech Republic. All the chemicals were of analytical grade. Reax Top mixer (used for MSPE) and Reax 2 (for LLE) were from Heidolph, Germany.

The samples from river Labe in Pardubice and the reservoir Rozkos were taken into 2 l glass sample bottles, transported to laboratory and filtered. One litre of ground water sample was taken for LLE. The samples of water phase after biodegradation tests of NPnEO were taken in 1 l sample bottles to VŠCHT in Prague where these tests were carried out. After cooling to 4 °C, these samples were transported in thermos box to our laboratory.

Magnetic Modification of Sorbents

Porapak S, Chezacarb B and S, Chromosorbs 101 – 106, Al_2O_3, Rudex and Tonsil were incorporated into magnetic iron oxides during precipitation of iron (II) and iron (III) chlorides with alkaline solution [21]. Sorbents of type Tenax TA, GC, GR and DPA-6S were postmagnetized with magnetic fluid stabilized with perchloric acid [27]. Magnetic PODMP was melted with ε-caprolactam and powdered iron (II, III) oxide, milled and washed [28]. The dry weight (mg ml^{-1}) of magnetically modified sorbents in 1 ml settled suspension were: Tonsil: 145; Rudex: 156; DPA-6S: 51; Al_2O_3: 138; Chromosorb 101: 46.9; Chromosorb 102: 79.5; Chromosorb 103: 76.0; Chromosorb 104: 85.2; Chromosorb 105: 116; Chromosorb 106: 50.5; Porapak S: 64.8; Tenax TA: 45.5; Tenax GC: 49; Tenax GR: 80; Chezacarb B: 73.0; Chezacarb S: 27.5.

Extraction of APs and NPnEO

a) Spiked water samples
Model water samples were prepared by adding methanolic stock solution of APs or NP1EO and NP2EO to distilled and spiked water to achieve the concentration of 50 μg ml^{-1}.

b) Extraction of water samples by magnetically modified sorbents
10 ml of model water sample or real water samples were extracted in 15 ml test tubes with screw cap, where 50 μl of settled adsorbent was added. The sample was acidified to pH 2.0 with hydrochloric acid and stirred for selected time using the vortex mixer; mixing frequency 2400 min^{-1}. Then the magnetic particles were separated using NdFeB permanent magnet and water was poured out from the test tube. The adsorbed compounds were eluted from magnetically modified sorbent

with 1 ml methanol added into the test tube. The test tube was closed and then the suspension was mixed on a vortex mixer, the mixing frequency being the same as that for sorption. The sorbent was magnetically separated and the extract was poured out into a vial and after evaporation analysed by CGC. The determination of the target compounds was accomplished by the method of external calibration curve.

c) Liquid-liquid extraction
For LLE of APs, NP1EO, NP2EO from spiked and real water samples, the recommended method was used [5]. 200 ml water sample was extracted by 10 ml toluene. Five extracts were poured together and after evaporation the extract was analysed by CGC.

Capillary Gas Chromatography

The CGC analyses were performed using a gas chromatograph Mega 5160 coupled with a flame ionization detector (Carlo Erba Fisons Instruments, Milano, Italy). Clarity software (DataApex, Czech Republic) was used for data processing. The chromatographic fused silica column DB-5HT, 15 m × 0.25 mm × 0.1 μm (Supelco, USA) was used. The helium carrier gas was maintained at a constant flow rate 1.3 ml min^{-1}. The GC column was programmed from 80 °C (2 min), 10 °C min^{-1} to 180 °C (2 min) for AP analysis and from 120 °C (2 min), 10 °C min^{-1} to 220 °C (1 min) for NP1EO and NP2EO analysis. The extract (0.5-2 μl) was injected manually in the injection port using split mode (split ratio 1:10). The temperature of injector was 280 °C, the temperature of detector was 250 °C.

Optimization of Extraction by Magnetically Modified Sorbents

Four basic variables of extraction were optimized:

a) the time of static sorption (time of vortex mixing of sample with magnetic sorbent),
b) the time of static elution (time of vortex mixing of magnetic sorbent with adsorbed analytes with elution solvent - methanol),
c) the volume of elution solvent (methanol),
d) the number of repeated elutions.

The dependence of these parameters on the recovery was determined. Firstly, the dependence of the time of static sorption on the recovery was measured while the other variables were kept constant. Then the optimum time of static sorption was used when the dependence of time of static elution was measured.

Finally, the volume of elution solvent and the number of repeated elutions were optimized using optimum values of the first, the second and the third variable [29].

Analysis of Real Water Samples

After optimization of extraction procedure using spiked water samples, the same method with the same conditions was used for real water samples.

Water samples from water phase after biodegradation tests of oxyethylenated nonylphenols, from river Labe and the reservoir Rozkos were analysed [30]. Extraction at room temperature was used in all the experiments.

Results and Discussion

The optimized time of static sorption for tested magnetically modified sorbents ranged from 0.5 to 6 min. Typical dependence of recovery on the time of sorption for chosen sorbent is shown in Fig. 1a. The optimized time of static elution for tested magnetically modified sorbents ranged from 0.33 to 2 min. The dependence of recovery on the time of elution for chosen sorbent is shown in Fig. 1b. The dependence curves of recovery on elution solvent amount and on the number of repeated elutions for chosen sorbent and analytes are shown in Fig. 1c and Fig. 1d. In this case, the highest recovery was achieved when three times repeated elution with 1 ml methanol was used.

Extraction recoveries and optimized times of static sorption and elution are shown in Table I. Recoveries of extractions with magnetically modified Tenax GC, TG and TC were between 15 and 65 % and therefore these values are not given the Table. Relative standard deviation (*RSD*; $n = 3$) values in the tables are not higher than 10 %. The data in Table I show that the extraction recovery depends on the type of sorbent and chemical structure of analytes. Recovery of 4-*n*-propylphenol (short alkyl chain) is low, but for alkylphenols with longer alkyl chain and higher number of carbon atoms, such as *n*-octyl- and *n*-nonylphenol, is the highest. Higher recovery was observed for one non-branched chain alkyl APs in comparison with branched chain APs.

For extraction of APs from real water samples, the magnetically modified Chromosorb 103 was used, because of the highest determined recoveries of spiked samples (see Table I) while for extraction of NP1EO and NP2EO, the magnetically modified Chezacarb B was used (see Table I).

The recoveries of both method LLE and MSPE of technical mixtures of NP, NP1EO and NP2EO from distilled and tap water samples were determined and compared, see Table II. This was carried out because of extraction recoveries comparison of extractions from different matrices. Low extraction recovery of NP

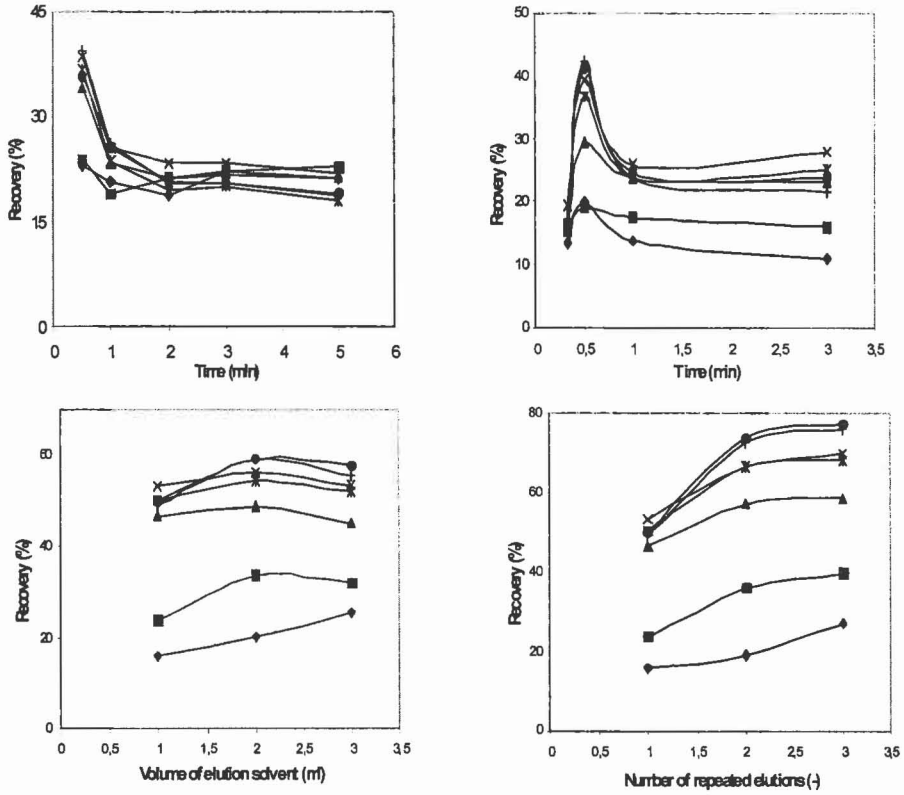

Fig. 1 Dependence of APs recovery for Chromosorb 101 on: a) time of sorption for time of elution 1 min (1 × 1 ml); b) time of elution for time of sorption 0,5 min (1 × 1 ml); c) amount of elution solvent – methanol, time of sorption 0.5 min and time of elution 0.5 min; d) number of repeated elutions with 1 ml of methanol for time of sorption 0.5 min, time of elution 0.5 min (3 × 1ml), —◆— 4-*n*-Propylphenol, —■— 2,4-Diisopropylphenol, —▲— 2,4-Di-*tert*-butylphenol, —○— 4-*tert*-Octylphenol, —✳— 2,4-Di-*tert*-pentylphenol, —●— 4-*n*-Octylphenol, —□— 4-*n*-Nonylphenol

technical mixtures is caused by absence of 4-*n*-nonylphenol in the mixtures, while branched 4-nonylphenol isomers form the majority of the mixtures.

The magnetically modified sorbent was used for extraction of NP, NP1EO and NP2EO from water phase of water samples after biodegradation test of NPnEO with different state of oxyethylation because stable emulsions were formed during solvent extraction. Biodegradation tests were carried out in VŠCHT Praque. The found values are given in Table III.

Finally, the concentrations of NP, NP1EO and NP2EO in water samples from river Labe, and reservoir Rozkos were determined (see Table III). Table III shows that the concentration of nonylphenols is under the detection limit but concentration of NP1EO and NP2EO were detected and quantified. For both extraction method, the *RSD* values for $n = 3$ were between 1-12 %, but mostly they

Table I Optimum times of sorption, elution and recoveries of MSPE of APs, NP1EO and NP2EO after three times elution with 1 ml of methanol using magnetically modified sorbents

Magnetically modified sorbent	Chromosorb						Porapak
	101	102	103	104	105	106	S
Time of sorption, min	0.5	2	2	0.5	6	3	3
Time of desorption, min	0.5	2	1	0.5	0.33	0.33	0.33
Compound	Recovery, %						
4-*n*-propylphenol	27	42	27	32	49	47	51
2,4-Diisopropylphenol	40	57	41	33	64	59	50
2,4-Di-*tert*-butylphenol	59	66	62	46	70	46	44
2,4-Di-*tert*-pentylphenol	68	70	76	55	77	45	53
4-*tert*-Octylphenol	70	77	82	60	82	63	69
4-*n*-Octylphenol	77	71	96	58	92	56	77
4-*n*-Nonylphenol	76	71	99	57	97	77	77
4-*n*-Nonylphenols tec. mix.	-	-	96	-	91	-	-
NP1EO	-	-	-	-	-	-	-
NP2EO	-	-	-	-	-	-	-

Magnetically modified sorbent	Chezacarb		PODPM	Al_2O_3	DPA-6S	Tonsil	Rudex
	B	S					
Time of sorption, min	2	1.5	6	1	1	1	2
Time of desorption, min	1	0.5	3	1	0.5	0.33	2
Compound	Recovery, %						
4-*n*-propylphenol	57	48	49	19	19	25	25
2,4-Diisopropylphenol	51	50	67	52	47	25	40
2,4-Di-*tert*-butylphenol	49	47	82	69	58	32	62

Table I – Continued

2,4-Di-*tert*-pentylphenol	63	71	93	75	59	41	78
4-*tert*-Octylphenol	47	72	89	76	64	43	68
4-*n*-Octylphenol	78	91	94	86	73	49	77
4-*n*-Nonylphenol	87	93	91	84	73	49	76
4-*n*-Nonylphenols tech. mix.	50	70	-	-	-	-	-
NP1EO	98	-	-	-	-	-	-
NP2EO	99	-	-	-	-	-	-

Table II Comparison of recoveries and *RSD* (n = 3) of NP, NP1EO, NP2EO after liquid extraction and extraction with magnetically modified sobent in model water samples

Extraction method		LLE				MSPE			
Water sample		Distilled water		Tap water		Distilled water		Tap water	
Compound	Conc. $\mu g\ l^{-1}$	Recovery, %	*RSD* %	Recovery, %	*RSD* %	Recovery, %	*RSD* %	Recovery, %	*RSD* %
NP1	1.1	87.8	3.7	87.9	7.8	96.0	6.6	93.2	9.5
NP1EO	1.0	94.8	4.3	95.7	6.9	98.3	4.7	94.5	4.9
NP2EO	1.0	93.2	7.3	94.2	5.6	98.8	1.5	98.1	1.8

Table III Comparison of determined contents of NP, NP1EO, NP2EO after liquid extraction and extraction with magnetically modified sorbent in real water samples

Extraction method	LLE			MSPE		
Compound / water sample	NP $\mu g\ l^{-1}$	NP1EO $\mu g\ l^{-1}$	NP2EO $\mu g\ l^{-1}$	Tech. NP, $\mu g\ l^{-1}$	NP1EO $\mu g\ l^{-1}$	NP2EO $\mu g\ l^{-1}$
NP1EO	6.3	1.1	LOD	6.2	1.0	LOD
NP15EO+4-*n*-NP3EO	7.0	1.7	LOD	6.4	1.3	LOD
NP15EO+4-*n*-NP3EC	6.7	1.6	LOD	6.2	1.2	LOD
Rozkos	LOD	1.5	1.8	LOD	1.5	1.9
Labe	LOD	2.1	LOD	LOD	2.2	LOD

LOD – below detection limit

were lower than 8 %. It is also obvious, that LLE can be superseded by MSPE (with magnetically modified sorbents prepared in laboratory), because the results of both methods are comparable. Besides, MSPE is economical and less demanding than LLE and it is especially suitable for emulsion forming samples.

Conclusion

The described extraction method showed to be a suitable procedure for fast extraction of APs with middle length alkyls, NP1EO and NP2EO from water samples using laboratory prepared magnetically modified sorbents, which are commonly used as chromatographic column packing or as industrial sorbents in industry for various technological processes. Especially, magnetically modified Chromosorbs 103 and 105 and Chezacarbs B and S show good sorption qualities. Magnetically modified active carbon Chezacarb B is appropriate for extraction of NP1EO and NP2EO to achieve the recovery of 98 %. All the extraction conditions, such as time of static sorption and time of static elution, depend on the used adsorbent and analytes. From the optimized conditions it is obvious that the extraction with magnetically modified sorbents is a very fast extraction method which uses a very small amount of solvent and adsorbent. It is accomplished in 10-15 minutes. Therefore, the extraction method was used for extraction of metabolites from samples of water phase after biodegradation tests of NPnEO instead of LLE with toluene.

Residues of NPnEO and also their biodegradation products can occur everywhere in the environment, which was demonstrated by our analysis of water samples from river Labe and the reservoir Rozkos. Therefore, it is very important to monitor their concentrations in water samples.

MSPE is an equivalent alternative to SPE and SPME, and it has a few advantages in comparison with LLE.

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