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Journal of Chromatography A 819 (1998) 297–302

JOURNAL OF CHROMATOGRAPHY A

Separation and identification of nonylphenylethylene oxide oligomers by high-performance liquid chromatography with UV and mass spectrometric detection

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Abstract

Nonylphenylethylene oxide surfactants were separated on an alumina column using an ethylene oxide–*n*-hexane mixture as the mobile phase, and UV and MS detection. Several well separated, large peaks and some small, partly separated peaks were detected. It was found that the main fractions elute log-equidistantly and they correspond to the nonylphenylethylene oxide oligomers with a given number of ethylene oxide units, and the small peaks contain isomers, probably surfactants with different positions of the nonylgroup at the phenyl ring. The method validation showed no significant differences between the intra-day and inter-day values of the area percentages and their standard deviations, showing the good reproducibility of the determination of the area percentages during at least three consecutive days. Significant differences were found between the intra-day and inter-day values of the standard deviation of the retention times, which indicates that the determination of the retention time is less reproducible than that of the area percentages.

Keywords: Nonylphenylethylene oxide oligomers; Surfactants, non-ionic

1. Introduction

Nonionic surfactants, like nonylphenylethylene oxide oligomers, are amphipatic molecules consisting of a hydrophobic and a hydrophilic part. Due to their favourable physicochemical characteristics the nonionic surfactants are extensively used in pharmaceutical [1] and agrochemical formulations [2], in cosmetics [3], and in various biotechnological processes [4]. Nonionic surfactants can show biological activities, like enhancing the decomposition rate of polychlorinated biphenyls [5] and polycyclic aromatic hydrocarbons [6]. They also can be toxic, and can cause ocular [7,8] and skin irritancy [9,10] and skin dehydration [11].

It has been proved many times that the character of both the hydrophobic and hydrophilic parts influences the biological efficacy of nonionic surfactants [12,13]. Therefore, many efforts have been devoted to the development of high-performance liquid chromatography (HPLC) methods to separate those surfactants according to the differences in their hydrophobic and hydrophilic moiety. Reversed-phase supports such as C_{18} [14], C_8 and C_6 [15] and polyethylene-coated silica [16] have been successfully used for the separation of various surfactants according to the character of their hydrophobic moiety. On the other hand silica [17], and C_1 silica supports separated well various surfactants according to the length of their ethylene oxide chain (hydrophilic moiety) [18]. It is generally accepted that for the separation of surfactants due to both characteristics two different supports are needed.

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0021-9673/98/$19.00
PII: S0021-9673(98)00567-6

In recent years aluminium oxide was developed as a suitable stationary phase in HPLC. The retention characteristic of alumina columns has been recently reviewed [21]. Good separations of heroin derivates [22], proteins [23] and drugs [24] have been achieved on alumina columns. It was recently reported that an aluminium oxide column is suitable for the separation of nonionic surfactants according to both the length of the ethylene oxide chain and the character of hydrophobic moiety in one run [19,20].

Separation of a commercial nonylphenylethylene oxide oligomer surfactants containing, on average, four ethyleneoxide groups per molecules on an alumina column [20] resulted in six main peaks containing some unresolved fractions. Due to the fact that pure standards were not available to identify the peaks, it was assumed that the main peaks correspond to surfactants with different numbers of ethyeleneoxide units in the molecule. The presence of the unresolved fractions suggested, that the sample might contain some other surfactants perhaps differing in the hydrophobic moiety.

The objective of this work was to separate a commercial nonylphenylethylene oxide oligomer surfactant mixture on an alumina column, to identify the fractions by HPLC–MS, and to determine the validation parameters of the method.

2. Experimental

Alumina support of 5 μm particle size was produced by the research group of Dr. L. Zsembery at the Research Institute of the Hungarian Alumina Trust (Budapest, Hungary). A 250×4 mm I.D. column was filled in our laboratory with a Shandon analytical HPLC packing pump (Pittsburgh, PA, USA). A sample of a commercial nonylphenylethylene oxide surfactant containing on average four ethyleneoxide groups (n_e) per molecule (Hoechst, Frankfurt, Germany) was dissolved in the eluent at a concentration of 0.5 mg ml^{-1}. The experiments were carried out by HPLC–UV and HPLC–MS. The following experimental parameters were the same in both cases: the flow-rate was 1 ml min^{-1}, ethyl acetate–*n*-hexane mixture (70:30, v/v) was used as mobile phase. The solvents were purchased from Chemolab (Budapest, Hungary). The measurements were carried out at room temperature (22±2°C).

The chromatographic setup for UV detection consisted of a Merck–Hitachi L-6000A pump (Tokyo, Japan), a Rheodyne injector 7125 (20 μl) (Cotati, CA, USA), a Merck–Hitachi L-4000A UV detector and a Merck–Hitachi D-2500A Chromato-Integrator. The detection wavelength was set to 254 nm.

The HPLC setup of the HPLC–MS system consisted of a Perkin-Elmer 200 lc micro pump (Toronto, Canada) and a Rheodyne injector 7125 (20 μl).

All the mass spectra were acquired using a Perkin-Elmer SCIEX API 165 mass spectrometer equipped with atmospheric pressure chemical ionization (APCI) heated nebulizer ion source interface operated in the positive mode. The corona discharge needle (NC) was set to 5 μA and the orifice potential (OR) was 20 V. The quartz tube temperature was 200°C. Dry nitrogen was used as the nebulizing and curtain gas. Full scan (100–650 u) acquisitions were performed, cycle time was 2 s.

Both the total ion chromatogram (TIC) and selected ion chromatograms (SICs) corresponding to fractions with different number of ethyeleneoxide groups per molecules were determined.

Linear correlation was calculated between the log k' and the n_e values for the main peaks, where k' is the capacity factor of the solutes, and n_e is the number of the ethyleneoxide groups per molecule determined by HPLC–MS.

The intra-day and the inter-day reproducibility of the HPLC–UV and HPLC–MS methods were determined by ten independent measurements carried out on three consecutive days. The relative standard deviation of the retention times and that of the area percentages of the peaks was calculated.

The comparison of the means of area percentages of inter-day and intra-day reproducibility measurements was carried out with the method of paired means [25] both for UV and MS detection. The comparison of the standard deviation of the retention times and the area percentages of the same measurements were carried out by the Bartlett-test [25].

3. Results and discussion

The separation of nonylphenylethylene oxide oligomers by HPLC–UV is shown in Fig. 1. In the majority of the cases baseline separation was achieved proving the good separation capacity of the HPLC system. It was assumed, that each main peak corresponds to a surfactant containing a given number of ethyleneoxide groups and the fractions eluted later contain the highest number of ethyleneoxide units. However, besides of the main peaks some additional not well resolved peaks were observed on the chromatogram.

The selected ion chromatograms of the protonated molecular ions corresponding to nonylphenylethylene oxide oligomers with given numbers of the ethyleneoxide units are shown in Fig. 2A and B (note that the SICs are always scaled to the maximum intensity of the peaks). The six main peaks can clearly be identified as compounds with 3, 4, 5, 6, 7 and 8 ethyleneoxide groups per molecule.

The minor peaks on the selected ion chromatograms for $n_e=3$, 4, 5 and 6 indicate that more then one fraction with the same ion mass occurs in the sample. This result can be explained by the supposition that the sample contains isomers, probably surfactants with different positions of the nonylgroup at the phenyl ring. The presence of isomers accounts for the unresolved peaks on the chromatograms.

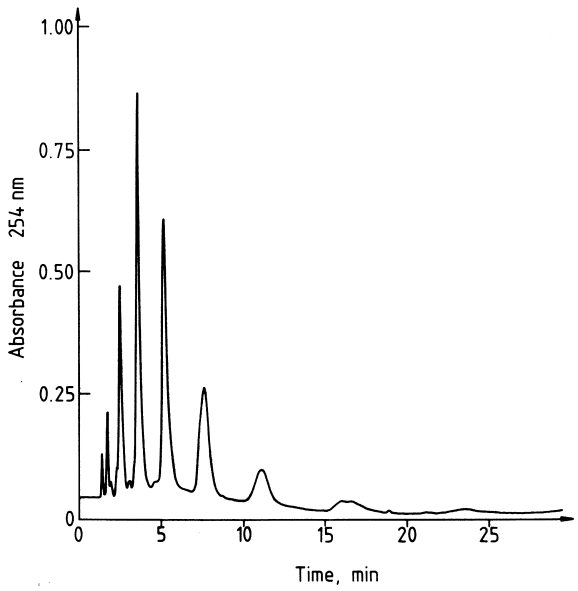

Fig. 1. UV chromatogram of nonylphenylethylene oxide oligomer surfactants separated on an alumina column. Mobile phase: ethyl acetate–*n*-hexane (70:30, v/v), flow-rate: 1 ml min^{-1}, detection wavelength 254 nm.

The sample specified by the producer to contain on the average four ethyleneoxide groups per molecule was found to consist mainly of compounds with four hydrophilic groups, but compounds with $n_e=5$ and $n_e=6$ are abundant as well.

Significant linear correlations were found between the log k' and the n_e values, the correlation coefficients being $r_{UV}=0.9958$ and $r_{MS}=0.9987$ for the UV and the MS measurements, respectively. This finding indicates, that the main fractions of the surfactants elute log-equidistantly and they correspond to a homologue series of ethylene oxide oligomer surfactants with different numbers of ethyleneoxide groups.

The intra-day and the inter-day reproducibility values of the retention time and of the area percentage of the fractions are collected in Tables 1 and 2, for UV and MS detection, respectively. No significant differences were found between the intra-day and the inter-day values of the means of the area percentages using either UV or MS detection, the values of the *t*-probe being $t_{UV}=0.18$ and $t_{MS}=0.01$. This means, that the determination of the area percentages does not change significantly due to the longer analysis time, the determination of the area percentages is reproducible during at least three consecutive days.

No significant differences were found neither between the intra-day and the inter-day values of the standard deviations of the area percentages using either UV or MS detection ($\chi^2_{UV}=3.10$ and $\chi^2_{MS}=5.71$). This shows, that the standard deviation of the area percentages does not increase within a three days long period of time.

On the other hand significant differences were found between the intra-day and inter-day values of the standard deviation of the retention times for both detection systems ($\chi^2_{UV}=22.05$ and $\chi^2_{MS}=10.70$). This indicates that the determination of the retention time is less reliable than that of the area percentages.

It can be concluded from the data that the

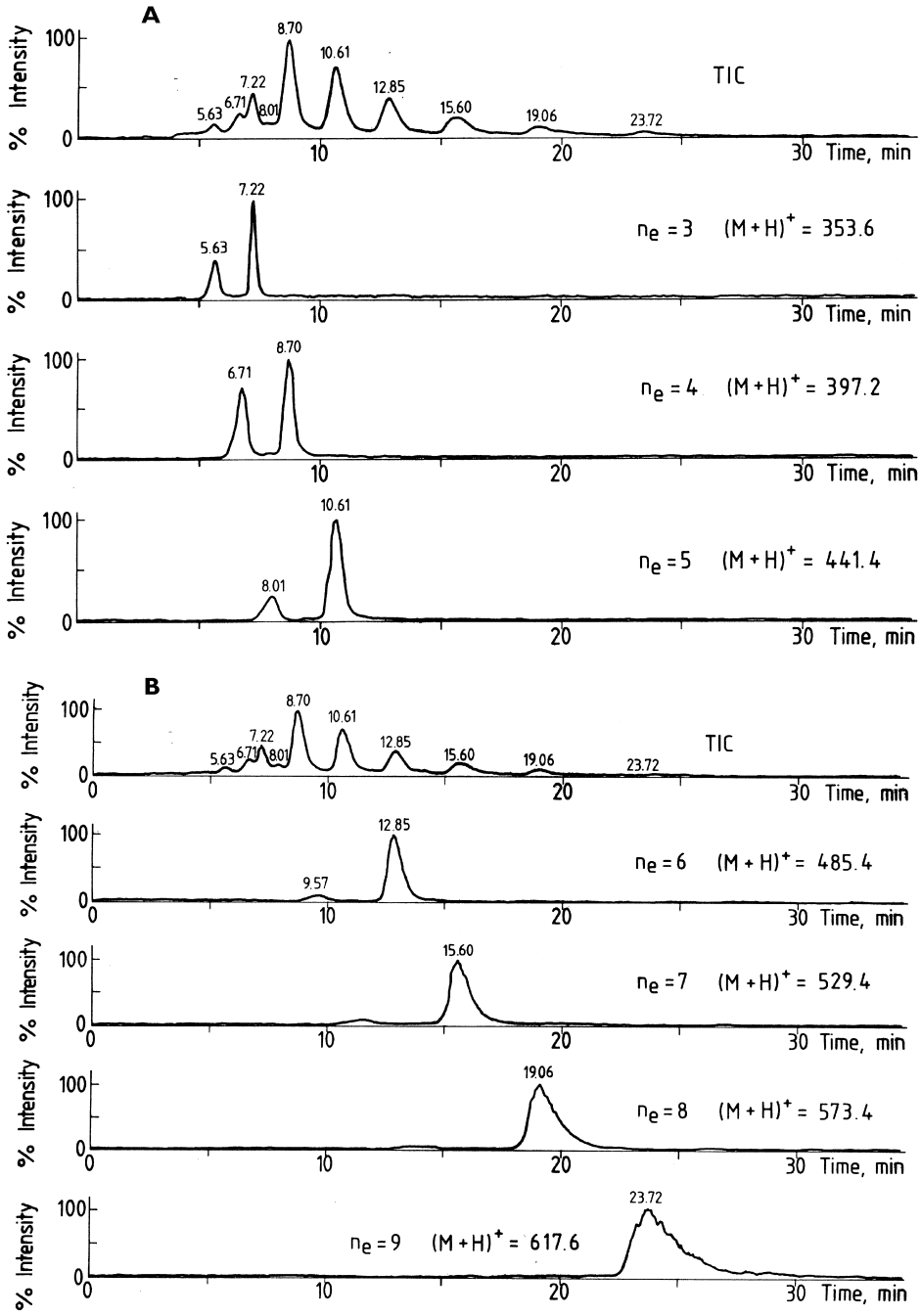

Fig. 2. (A) Total ion and selected ion chromatograms of the nonylphenylethylene oxide oligomer surfactants separated on an alumina column, detected by HPLC–MS. For experimental conditions see Section 2. The three selected ion-chromatograms correspond to different numbers of the ethylene oxide groups, (n_e=3, 4 and 5), $(M+H)^+$ is the corresponding mass of the protonated molecular ions. (B) Total ion and selected ion chromatograms of the nonylphenylethylene oxide oligomer surfactants separated on an alumina column, detected by HPLC–MS. For experimental conditions see Section 2. The four selected ion chromatograms correspond to different numbers of the ethyleneoxide groups, (n_e=6, 7, 8 and 9), $(M+H)^+$ is the corresponding mass of the protonated molecular ions.

Table 1
Intra-day and inter-day reproducibility of HPLC–UV system

Intra-day reproducibility				Inter-day reproducibility			
t_R		Area		t_R		Area	
(min)	R.S.D. (%)	%	R.S.D. (%)	(min)	R.S.D. (%)	%	R.S.D. (%)
2.13	0.01	2.79	0.44	2.35	0.05	2.91	0.56
2.87	0.06	0.86	0.00	2.98	0.09	0.60	0.01
3.21	0.07	9.18	0.15	3.65	0.18	9.03	1.35
3.72	0.17	2.72	0.23	4.14	0.32	3.58	0.39
4.51	0.10	33.06	0.19	4.46	0.38	32.55	0.48
5.20	0.16	23.42	0.47	5.29	0.74	23.68	0.59
7.11	0.22	15.69	0.50	7.98	1.43	15.69	0.72
10.98	0.46	8.51	0.22	11.65	2.71	8.30	0.53
18.20	0.54	3.78	0.51	19.45	4.42	3.66	0.49

The table shows the retention time (t_R), its relative standard deviation (R.S.D.) percentage, the area percentages and its R.S.D. percentage of the fractions.

Table 2
Intra-day and inter-day reproducibility of HPLC–MS system

Intra-day reproducibility				Inter-day reproducibility			
t_R		Area		t_R		Area	
(min)	R.S.D. (%)	%	R.S.D. (%)	(min)	R.S.D. (%)	%	R.S.D. (%)
5.63	0.99	2.01	0.10	5.25	1.09	1.71	0.13
6.71	0.77	4.90	0.11	6.22	1.29	4.07	0.35
7.22	0.78	9.61	0.12	6.70	0.82	9.45	1.44
8.01	0.29	2.27	0.07	7.81	4.67	2.15	0.15
8.70	0.27	31.53	0.03	8.14	0.95	31.94	0.62
10.61	0.00	23.09	0.03	9.94	1.07	23.51	0.22
12.82	0.13	14.29	0.03	12.02	1.32	14.97	0.42
15.60	0.11	7.65	0.13	14.63	1.78	7.42	0.41
19.06	0.33	3.34	0.20	17.81	2.01	3.22	0.46
23.72	0.94	1.31	0.49	23.68	3.45	1.54	0.59

The table shows retention time (t_R), its relative standard deviation (R.S.D.) percentage, the area percentages and its R.S.D. percentage of the fractions.

nonylphenylethylene oxide surfactants can be successfully separated, and quantitatively determined by HPLC–UV, and the fractions can be identified by HPLC–MS.

Acknowledgements

The present work was supported by the grant OTKA No. T 023422.

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