Mass Spectrometry in the Analysis of Non-ionic Detergents

I. Molecular mass distribution of polyethyleneglycol by derivatization and separation by HPLC coupled with ultraviolet detection in parallel with mass spectrometry

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The molecular mass distribution of polyethyleneglycols was thoroughly determined after derivatization with phenylisocyanide. The resulted carbamic diesters were analyzed by HPLC coupled with mass spectrometry and ultraviolet detection. The mass spectrometry was used for structure confirmation and the ultraviolet spectra for establishing the molecular mass distribution of the carbamic diesters.

Keywords: non-ionic detergents, liquid chromatography coupled with mass spectrometry, molecular mass distribution

Detregents represent one of the most important classes of synthetic materials largely used in modern society. In this class, non-ionic detergents play an important role, due to some valuable specific properties. Generally, a non-ionic detergent is a macromolecule with a nonpolar hydrophobic chain and a polar hydrophilic chain, consisting of variable length chains of polyethoxylate or polypropoxylate.

Critical micellar concentration of non-ionic detergents is not dependent on the ionic strength of the solutions they are dissolved in, so it can be easily modulated by varying the lengths of the hydrophobic and hydrophilic chains.

Synthesis of non-ionic detergents is performed by polycondensation of ethyleneoxide or propyleneoxide with a convenient substrate possessing a hydrophobic chain of convenient length, such as fatty alcohols, fatty acids, fatty amines or alkylphenols. The non-ionic detergents are not unitary compounds. Besides the dispersion due to different degrees of polycondensation, these detergents contain variable amounts of polyethyleneglycol (PEG) or polypropilenglycol.

The analysis of polyethoxylated non-ionic detergents can not be made by classical chemical methods due to potential high errors. Gas chromatography is also not an option due to the lack of volatility and thermal instability of the compounds [1]. Under these circumstances, high performance liquid chromatography (HPLC), where the macromolecules are preserved intact, represents the appropriate separation technique, and was already successfully applied for the analysis of various non-ionic detergents [2]. Still, the most common detector for HPLC, based on UV-Vis absorbance, can only be used for the analysis of polyethoxylated or polypropoxylated alkylphenols and not for other macromolecules, which do not contain chromophores. This shortcoming can be solved by introducing in the molecule a chromophore moiety, by example through derivatization of terminal -OH groups with phenylisocyanide [3]. Even so, this method can not establish if the derivatization is complete and in addition it is not applicable to non-ionic detergents with both terminal -OH groups blocked.

The HPLC separation technique coupled with mass spectrometry detection has been successfully applied for all types of non-ionic detergents [4, 5], and modern interfaces allow qualitative as well as quantitative analyses. Although it is the most utilized method for the analysis of these compounds, until now the quantitative determination of PEG in various commercial products was not successful. Therefore the intention of this work is to make a step towards solving this problem by establishing the molecular mass distribution in liquid PEG making use of HPLC with ultraviolet and mass spectrometry detection.

Experimental part

Reagents: methanol and acetonitrile of chromatographic purity (Fluka); methanol, chloroform and isopropyl alcohol of analytical purity (Fluka); phenylisocyanide 99% purity (Aldrich); ultrapure water (18.2 MΩ) supplied by a Millipore purification system; PEG 99% (PEG) 400 (Fluka); argon for the fragmentation by collision in the second quadrupole (Linde).

The High Performance Liquid Chromatography system: Prostar 240 SDM pump, Prostar 410 automatic injector, Prostar 330 diode array UV-Vis detector and a 1200 L/MS/MS mass spectrometer with triple quadrupol, all from Varian. The system was controlled by MS Workstation software, version 6.4.1.

PEG derivatization was carried out as follows: 10 mg PEG 400 were mixed in a vial with 10 mg phenylisocynide; then the sealed vial was maintained at 60^{0}C for half an hour. After cooling, 1 mL of (10/1 vol) mixture of chloroform and isopropyl alcohol was added. 5 μL of the resulting solution were injected in the chromatographic system. The separation was performed under isocratic conditions, using as mobile phase a mixture of water/methanol/acetonitrile: 50/10/40 %, at a flow rate of 0.8 mL/min. The column used was a 150 mm length and 4.6 mm diameter Waters Nova Pak column, with 4 μm particle size. Ultraviolet detection was performed at 236 nm, with an acquisition frequency of 2.5 Hz. An atmospheric pressure chemical ionization (APCI) was used as interface with air as drying gas, at

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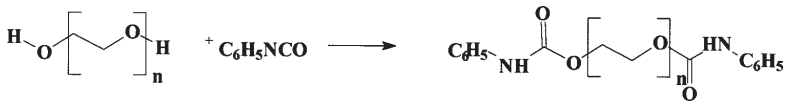
200^{0}C and 20 psi, and nitrogen as nebulisation gas, at a pressure of 40 psi. Corona needle was loaded at a current of 10 μA and the source was heated to 50 ^{0}C.

Results and discussion

PEG derivatization with phenylisocyanide takes place at both free terminal -OH groups, as shown in scheme 1. The resulted product was analysed by HPLC, with 90% of the effluent passing through the UV-Vis diode array detector cell and the rest sent into the APCI interface to generate protonated molecular ions of the analyte.

By reversed phase separation of the PEG-bis phenylcarbamates and UV detection it was possible to ascertain each individual chromatographic peak; the obtained chromatogram is shown in figure 1.

The mass spectrometry coupled to the HPLC allowed the detection of the PEG-bis phenylcarbamates with a degree of polycondensation **n** between 3 and 14. As expected, the difference between molecular masses of subsequent oligomers is of 44 daltons due to the ethylenoxide moiety $(CH2)_2O$. Individual assignments were done by scanning with the first quadrupole for protonated molecular ion of every PEG-bis phenylcarbamate starting from m/z = 389, corresponding to **n** = 3. The chromatogram from figure 2 was obtained by selecting the ions with differences of 44 daltons until m/z = 873. Comparing the chromatogram generated by the mass spectrometer with the one obtained using UV detection, a delay of 2.5 min was observed for the first peak, corresponding to the oligomer with **n** = 3.



Scheme 1

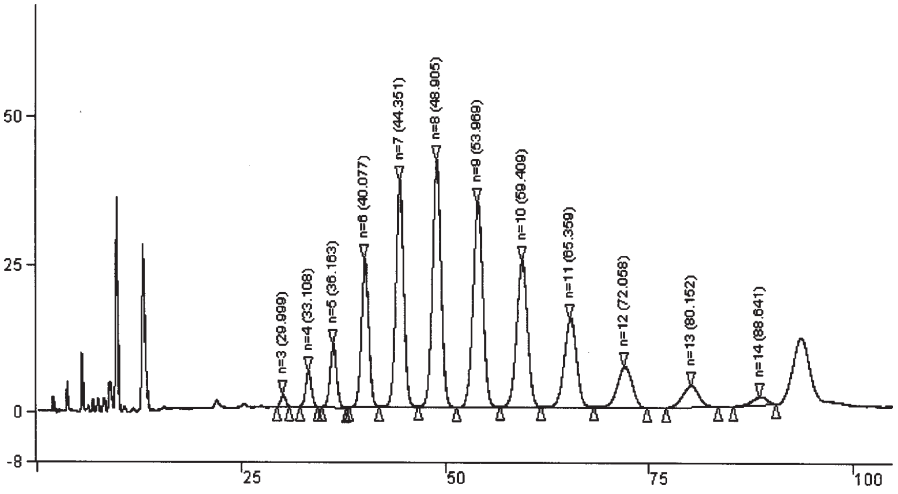


Fig. 1. Individual distribution of bis carbamates determined by detection in UV

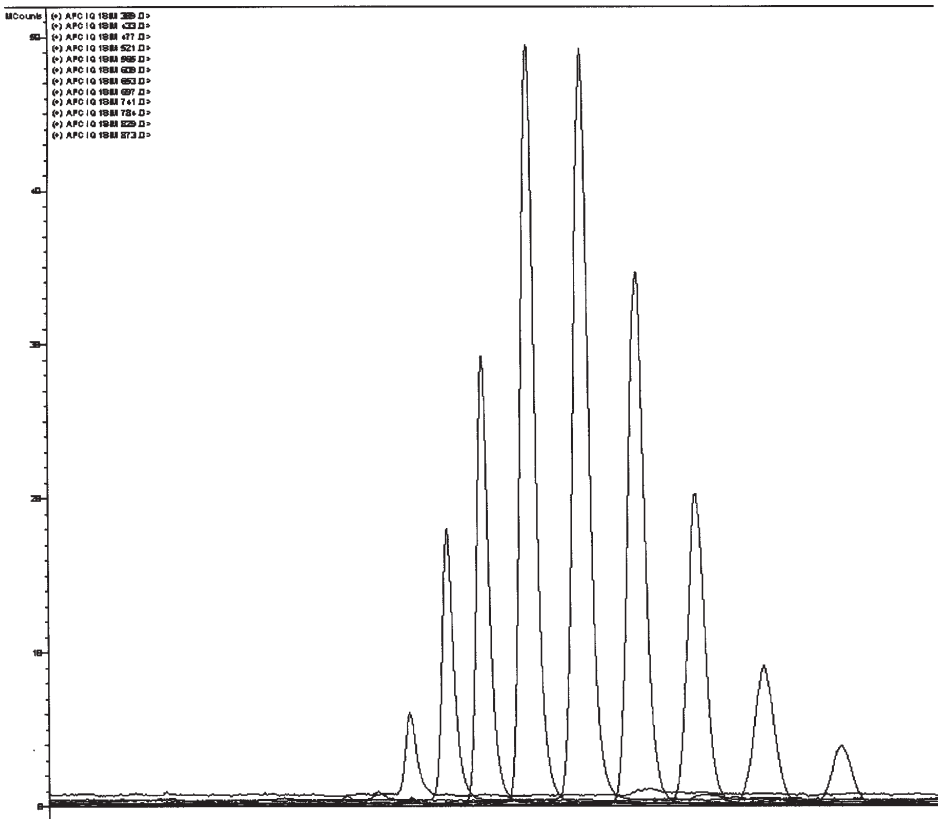


Fig. 2. Individual distribution of bis carbamates determined by mass spectrometry

Table 1
MOLECULAR MASS DISTRIBUTION BY INTEGRATION PEAKS FROM UV CHROMATOGRAM

Peak No.	Peak Name	Result ()	Ret. Time (min)	Time Offset (min)	Area (counts)	Sep. Code	Width 1/2 (sec)
1	n=3	0.4748	29.999	0.190	410380	BB	39.2
2	n=4	1.7393	33.108	-0.001	1503319	BB	44.9
3	n=5	3.4727	36.163	-0.000	3001607	BB	49.6
4	n=6	9.2664	40.077	-0.000	8009348	BV	56.0
5	n=7	15.6191	44.351	-0.000	13500250	VV	62.0
6	n=8	18.5832	48.905	0.000	16062254	VV	68.4
7	n=9	17.6117	53.969	0.000	15222576	VV	78.4
8	n=10	13.9812	59.409	-0.000	12084548	VV	86.2
9	n=11	9.7329	65.359	-0.000	8412524	VV	97.5
10	n=12	5.5106	72.058	0.000	4763049	VB	122.4
11	n=13	2.9874	80.152	0.000	2582124	BB	126.5
12	n=14	1.0208	88.641	0.084	882297	BB	133.0
	Totals:	100.0001		0.273	86434276		

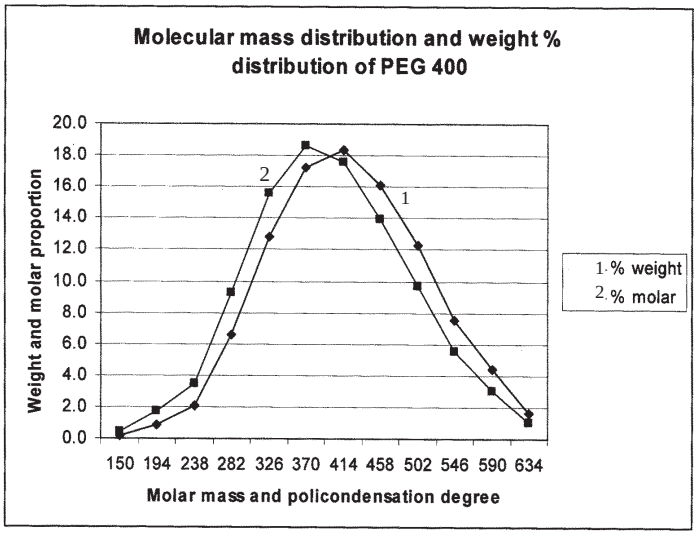
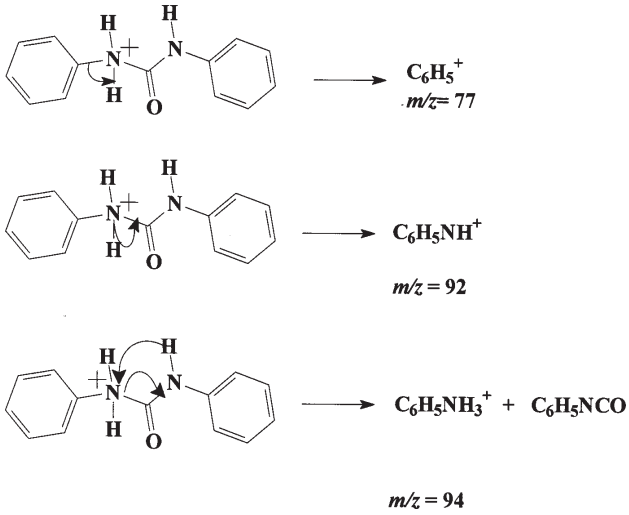


Fig. 3. Molecular mass distribution of PEG 400 after derivatization with phenylisocyanide

Purity assessments for each peak of the phenylcarbamates mixture showed a constant value, indicating that co-elution was avoided. Consequently, the molar composition of each oligomer can be accurately determined by integrating the peak areas, and the computed molar composition is given in table 1. By calculating the mass composition, the mass distribution depicted in figure 3 was obtained. It is worth mentioning that when put side by side the mass distribution and the molar one appear shifted. The average molar mass of the commercial analysed PEG thus calculated, namely 398 g/mol, has a value very close to the one declared by the manufacturer.

The compounds corresponding to chromatographic peaks with retention times lower than 30 min, present in figure 1, correspond to PEG monocarbamates and other by-products such as diphenylurea and tri-phenyl-isocyanurate, generated from phenylisocyanide in the derivatization step. Thus, by reaction with traces of water, aniline is formed, which immediately reacts with phenylisocyanide forming diphenylurea (A). The trimerization of the phenylisocyanide leads to isocyanuric acid triphenylester (B). For a structure confirmation for these last two products, (A) and (B), a new analysis was performed, with the mass spectrometer set to record only their corresponding protonated molecular ions. The obtained peaks with m/z = 213 and m/z = 358 confirmed the presence of the respective ions, along with fragments obtained by their collision with argon. For diphenylurea fragments the recorded m/z and abundance were (%): 77 (49), 92 (15), 94 (100, base peak), and for isocyanuric acid

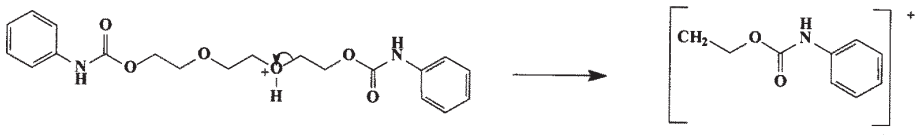


Scheme 2

triphenylester fragments (%): 92 (12), 120 (100, base peak). The possible fragmentation mechanisms start with the protonation of one of the nitrogen atoms, as shown in scheme 2. The base peak for isocyanuric acid triphenylester, with m/z = 120, is obtained by the protonation of phenylisocyanide. Another experiment was performed by infusing a solution obtained from PEG 400 with derivatization directly into the APCI interface without chromatographic separation.

As previously shown, 10 % of the chromatographic effluent was transfered in the APCI interface to generate protonated molecular ions of the analyte. This allowed us to bring together a large amount of protonated molecular ions of derivatizated PEG 400 in order to sufficiently increase the abundance of the fragments. An experiment with multiple monitorization of the fragmentation reactions

was performed. The protonated molecular ions of derivatizated PEG with m/z = 389, 433, 477, 521, 565, 609, 653, 697, 743 were fragmented into the second quadrupole and the obtained fragments were scanned with the third quadrupole. The fragment with m/z = 164 can be considered the most characteristic one for all parents ions. The available mass spectrometer provides collision energies up to 50 eV, i.e. low energy fragmentation by collision with argon. As a consequence, the most likely mechanism for the fragmentation of PEG-bis phenylcarbamates is charge induced: the proton is attached to one of the oxygen atoms of the polyethoxylated chain and the fragment with m/z = 164 is obtained, as shown in scheme 3.



m/z = 389 *m/z* = 164

Scheme 3

The experiment monitoring the fragmentation reactions of parent molecular ions with m/z = 389, 433, 477, 521, 565, 609, 653, 697, 743 to daughter ion with m/z = 164, evidenced a relative distribution virtually identical to that obtained from the experiment with selected ion monitorization presented in figure 2. This suggests that the presence of the fragment with m/z = 164 is due to phenylisocyanide and that the fragmentation path shown in scheme 2 is not affected by the polyethoxylated chain, being practically the same for any degree **n** of polycondensation.

Conclusions

This study substantiates the possibility to establish the exact molecular masses distribution for polyethyleneglycol 400 through derivatization with phenylisocyanide, HPLC separation and ultraviolet spectrometry. The analysis performed by HPLC coupled with mass spectrometry has shown that similar distributions are obtained both by selected ion monitoring and by multiple ion monitoring of the fragmentation reactions. The presence of the fragment with m/z = 164 is characteristic for the splitting of the terminal carbamate ester groups and as such this fragment can be used to establish the distribution of oligomers in the mixture. Compared to the distribution obtained by liquid chromatography with UV detection at 236 nm, the distribution given by mass spectrometry is shifted. The last is influenced by the set up parameters of the ionization source, so that the maximum corresponds to a degree of polietoxylation of **n** = 7 compared to UV where the maximum corespondents to **n** = 8. Previous studies using mass spectrometry for the determination of polietoxylated detergents composition show that the real distribution of oligomers can not be obtained directly by mass spectrometry. Even by the derivatization procedure described in the present paper the actual molar mass distribution of PEG can not be unambiguously established by mass spectrometry. Therefore, the derivatization and ultraviolet detection remains in this case essential for the accurate determination of the molar mass distribution of PEG.

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