Environmental behaviour of onylphenol ethoxylates in coastal waters

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Adduct formation of nonylphenol ethoxylates in LC-ESI-MS: mass spectrometrical, theoretical and quantitative analytical aspects

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Abstract

The analysis of alkylphenol ethoxylate (A\textsubscript{9}PEO\textsubscript{n}) surfactants with LC-ESI-MS was investigated in a detailed study of the formation of different types of adducts. Part of the observations were explained by calculating their relative stabilities using molecular dynamics techniques. Strong differences in adduct formation behaviour were found for different oligomers. Beside the common sodium adducts, surfactant dimer adducts [2×A\textsubscript{9}PEO\textsubscript{1,2}+Na]\textsuperscript{+}, adducts including a solvent molecule [A\textsubscript{9}PEO\textsubscript{1,2}+MeOH+Na]\textsuperscript{+} and doubly charged adducts [A\textsubscript{9}PEO\textsubscript{>11}+2×Na]\textsuperscript{2+} were found. Molecular dynamics calculations showed that the A\textsubscript{9}PEO\textsubscript{n} molecule wraps itself around the complexing sodium ion in a way that negative electronic charges on oxygen have optimum electrostatic interaction with this ion. Van der Waals interactions between alkyl chains are of less importance for the stability of these adducts. Both [2×A\textsubscript{9}PEO\textsubscript{2,5}+Na]\textsuperscript{+} dimer and [A\textsubscript{9}PEO\textsubscript{2,5}+Na]\textsuperscript{+} monomer adducts turned out to be stable from an energetic point of view with adducts of A\textsubscript{9}PEO\textsubscript{3} being more stable than adducts of A\textsubscript{9}PEO\textsubscript{2}. Only for the monomer adduct the latter is in accordance with experimental observations. Consequences of the formation of several adducts per A\textsubscript{9}PEO\textsubscript{n} oligomer for the quantitative analysis of environmental samples were evaluated. In clean samples, it was found that the presence of short chain A\textsubscript{9}PEO\textsubscript{1,2} can cause an overestimation of long-chain A\textsubscript{9}PEO\textsubscript{2}. In real environmental extracts, other processes like matrix effects have a stronger influence on the quantitative result, and therefore no significant influence of adduct formation processes could be observed. However, inclusion of [A\textsubscript{9}PEO\textsubscript{1,2}+MeOH+Na]\textsuperscript{+} adduct signals does improve the detection limits of these two oligomers. Correct quantitative results are obtained when A\textsubscript{9}PEO\textsubscript{1} and A\textsubscript{9}PEO\textsubscript{2} are quantified separately, and longer oligomers with a molar calibration followed by correction of the average molar weight of the A\textsubscript{9}PEO\textsubscript{2} in the sample.

2.1. Introduction

Liquid-chromatography – mass spectrometry (LC-MS) has in recent years become the preferred analytical instrument for analyzing relatively polar organic molecules. The reasons for this development are the high amount of information and low detection limits which can be obtained with this technique, as well as decreasing prices of the instrumentation. An important additional advantage of LC-MS compared to GC-MS is that there is no need to derivatize the often non-volatile analytes in LC-MS applications.
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For nonionic surfactants, the trend is no different, and it is becoming increasingly difficult to find any recent publication in which LC-MS has not been applied. Alkylphenol ethoxylates (APEO_n) are one of the principal groups of nonionic surfactants, and their environmental relevance lies in the aquatic toxicity, in the estrogeneric activity caused by some of their metabolites, and in the large amounts in which APEO_n are still being produced. APEO_n consist of many alkyl (mainly nonyl, A_9PEO_n) isomers and ethoxylate oligomers, which makes the analysis of even standards rather complicated. Several aspects of LC-MS analysis of A_9PEO_n have been described in detail in recent studies [1-4]. When different studies using LC-MS analysis of A_9PEO_n are compared, the variety in methods applied is enormous. The variety of LC-MS methods makes the comparison of analytical results difficult and may even lead to systematic errors [5]. This diversity could be considered as the main drawback of LC-MS compared to GC-MS, as methods for the latter technique are much more comparable.

Reversed phase C_{18} is most often used, as the solvents used with this stationary phase are polar and have a high dielectric constant, which facilitates electrospray ionization. The fact that surfactants with an equal alkyl chain length elute simultaneously in reversed phase LC is considered an advantage by most scientists, as it facilitates peak integration. Other scientists prefer to use normal phase columns (in which all ethoxylate oligomers are separated), to have a more reliable identification of every separate ethoxymer [2].

The LC-MS interface applied can be either the electrospray ionization (ESI) interface or the Atmospheric Pressure Chemical Ionization (APCI) interface, although the use of the latter is only reported sporadically in literature. For nonionic surfactants, the ionization mode is always positive. Adduct ions detected depend on the mobile phase buffers used, and involve usually sodium [2,3] or ammonium [6,7].

Responses in LC-ESI-MS analysis vary between individual A_9PEO_n oligomers, with the short chain A_9PEO_{1,2} adducts having a relatively low response. For this reason, there is an ongoing discussion on the validity of quantitative analysis of environmental samples in which the standard composition is not equal to the A_9PEO_n mixture in the sample (which is usually the case). The analysis is further complicated by the formation of various types of adducts in the LC-MS interface. In most studies, only single metal adducts are selected in the selected ion monitoring (SIM) analysis, whereas other adducts are formed as well.

Several aspects of the adduct formation processes occurring in the LC-MS analysis of A_9PEO have been discussed in literature, such as the formation of the doubly charged adducts [A_9PEO_n+2×Na]^2+ [2,3,7,8], or dimer adducts [2×A_9PEO_n+Na]^+ [9]. However, no systematic study providing an overview of these processes and determining the influence of LC-MS conditions has been reported so far.

In low resolution mass spectrometry, no distinction can be made between dimer adducts and some longer A_9PEO_n oligomer monomer adducts, as their m/z value is equal, e.g. both
[2×A\textsubscript{9}PEO\textsubscript{2}+Na]\textsuperscript{+} and [A\textsubscript{9}PEO\textsubscript{2}+Na]\textsuperscript{+} have m/z=639. A similar observation is made for [A\textsubscript{9}PEO\textsubscript{n}+2×Na]\textsuperscript{2+} adducts, which have an m/z equal to either short-chain [A\textsubscript{9}PEO\textsubscript{n}+Na]\textsuperscript{+} or [A\textsubscript{9}PEO\textsubscript{n}+H]\textsuperscript{+}, e.g. both [A\textsubscript{9}PEO\textsubscript{15}+2×Na]\textsuperscript{2+} and [A\textsubscript{9}PEO\textsubscript{5}+Na]\textsuperscript{+} have m/z=463. Evidently, this complicates their identification in RP-LC-MS, when all ethoxylates elute simultaneously.

Molecular modeling calculations may provide a theoretical tool to explain the observed adduct formation behaviour of compounds in LC-MS. A number of studies in this field have been successfully performed in recent years, showing a good complementarity of LC-ESI-MS analysis and molecular modeling in the study of the stability of organic molecule – alkali metal complexes [10-12]. Hofmeister et al. provided experimental and theoretical proof (using molecular mechanics calculations) that during mass spectrometry ionization, alkali metals tend to coordinate with several oxygen atoms in one molecule [13]. Studies on crown ethers gave similar results [10, 14, 15].

Several molecular modeling studies on surfactants can be found in literature. All of these studies focus on the application of surfactants as emulsifier/dispersant, and therefore study their behaviour at interfaces, expressed by parameters like the molecular conformation at the interface [16], interfacial energy [17] alkane-water partitioning coefficients [18], molecular interfacial area [19] and micelle formation mechanisms [20]. Only one study is known in which molecular modeling is applied to A\textsubscript{9}PEO\textsubscript{n}. In that study, physical parameters such as dipole moment and molecular area at interfaces were determined by molecular mechanics and molecular dynamics [19].

**Objectives**

This research aims to describe and explain the main adduct formation processes of A\textsubscript{9}PEO\textsubscript{n} in LC-ESI-MS analysis. The study will mainly focus on the electrospray interface and reversed phase LC column, but a number of deviations from this base method is investigated, including different buffers, solvents and column types.

An attempt is made to explain some of the adduct formation processes by molecular modeling calculations. The main theoretical, molecular dynamics, objective is a preliminary study of the relative stabilities of [2×A\textsubscript{9}EO\textsubscript{2,5}+Na]\textsuperscript{+} dimer and [A\textsubscript{9}EO\textsubscript{2,5}+Na]\textsuperscript{+} monomer adducts from an energetic point of view. In addition we would like to obtain insight in the three-dimensional structure of the adducts.

The consequences of the observed adduct signals for the detection and quantification of A\textsubscript{9}PEO\textsubscript{n} in environmental samples are evaluated by analyzing and quantifying samples using several combinations of adduct signals. Finally, the influence of the use of different A\textsubscript{9}PEO\textsubscript{n} calibration standard mixtures on the quantitative result is determined.
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2.2. Experimental section

The LC-MS instrument used was a Thermoquest Navigator aQa single quadrupole system with an electrospray (ESI) interface. Two LC-columns were used: a reversed phase Lichrospher RP-C18 column (dimensions 125x2 mm, 3μm) and a normal phase Hypersil NH₂ column (dimensions 100x2 mm, 3μm), both from Phenomenex, Torrance, USA.

Chemicals

HPLC-grade methanol and acetonitrile were used (Rathburn, Walkerburn, Scotland). Nanopure water was produced with a Barnstead D4742 ultrapure water system. Sodium acetate and potassium acetate (p.a.) were purchased from Baker (Deventer, NL) and ammonium acetate from Merck (Amsterdam, NL). Pure A₉PEO₁ and A₉PEO₂ standards were synthesized by dr. F. Ventura of AGBAR, Barcelona. Technical mixtures of on average A₉PEO₁₀ (with an ethoxylate range of 3 to 16 units, mixture II), A₉PEO₄ (ethoxylate range of 2 to 9, mixture IV), A₉PEO₂ (ethoxylate range of 1 to 5, mixture V) and A₉PEO₈ (ethoxylate range of 3 to 17) were provided by Shell Amsterdam, The Netherlands, and ICI, Wilton, UK. The exact oligomer distributions of the A₉PEOₙ mixtures are shown in table 2.1. Pure A₉PEO₂ and NP standards containing ¹³C labeled aromatic rings were purchased from Cambridge Isotope Laboratories, Andover, USA.

LC-MS Approach

As our interest lies mainly in the processes occurring during the LC-MS analysis of environmental samples, our approach deviated somewhat from earlier studies on the formation and stability of adducts in LC-ESI-MS. Our approach focused on the phenomena occurring at low analyte concentrations of around 10⁻⁶ M with a high excess of metal ions (10⁻⁴ M). Most attention was paid to the formation of adducts containing sodium in the electrospray interface using a reversed phase LC-column. The ionization voltage was set to +4.0 kV, and a cone voltage of 20 V was used.

In this “base method”, the mobile phases consisted of pure methanol (A) and water-methanol 3:1 (v:v) containing 0.1 mM sodium acetate (B). This method was previously described by Jonkers et al. [21]. A fast LC gradient elution was used for the adduct formation studies: starting at 60% A for two minutes, the mobile phase was brought to 100% A in 9 minutes, where it was kept for 10 minutes, before returning to the starting composition in one minute and equilibrating for 8 minutes.

A programmable valve was inserted between the column and the interface, diverting the mobile phase away from the interface during the first 2 and the last 9 minutes of each run.
In this way, precipitation of the buffer in the interface was strongly reduced. Diverting the mobile phase away from the interface did not compromise the stability of the signals. Full scan analyses (m/z range 100-1100) were performed on pure standards of \( \text{A}_9\text{PEO}_1 \) and \( \text{A}_9\text{PEO}_2 \), and the technical \( \text{A}_9\text{PEO}_n \) mixtures mentioned above. In addition, for several adducts the stability was studied by determining the effect of increased cone voltages on the signal intensity.

First, the adduct formation using the base method was studied in detail. Then, several parameters in this method were varied systematically (keeping all other parameters unchanged) to investigate the effect on the type of adducts detected. The following parameters were changed:
- the sodium acetate (NaAc) buffer was changed to ammonium acetate (NH\(_4\)Ac) (2 mM), potassium acetate (0.1 mM) or an equimolar mixture of ammonium, potassium and sodium acetate (all 0.1 mM)
- the mobile phase solvent was changed from methanol to acetonitrile
- the column was changed to a normal phase NH\(_2\)-column, using a mobile phase of hexane/isopropanol 98/2 (A) and isopropanol/water 97/3 with NaAc (0.1 mM) (B). A gradient was applied which started at 99% A for 8 minutes, then decreased to 55% A in 18 minutes and further to 1% A in 2 minutes.
- the electrospray ionization mode was changed from positive to negative (-4.0 kV)
- the LC-MS interface was changed from ESI to APCI (positive ionization, +4.0 kV corona voltage)

**Molecular modeling approach**

A preliminary theoretical study was performed in which molecular dynamics was applied in order to explain some of the phenomena occurring in the ESI ionization of \( \text{A}_9\text{PEO}_n \). First, relative stabilities of the observed adducts were calculated and used to explain the formation of dimer adducts \([2\times\text{A}_9\text{PEO}_n+\text{Na}]^+\). Next, the calculated relative stabilities of these dimer adducts were compared for the short chain and longer chain oligomers \( \text{A}_9\text{PEO}_2 \) and \( \text{A}_9\text{PEO}_5 \).

To investigate the influence of the type of alkyl isomer on adduct stability, all calculations were done for two types of alkyl chains of the \( \text{A}_9\text{PEO}_n \) molecules. Two structurally extreme isomers were chosen: linear nonyl-1 (denoted by N later on) and the maximally branched 2,2,4,4-tetramethylpentyl-3 (denoted by T). In reality, \( \text{A}_9\text{PEO}_n \) mixtures exist of at least 23 alkyl isomers, of which the degrees of branching are somewhere in between the two structures chosen [22, 23].

The calculated stabilities of the lowest energy conformers should reflect the observed signals in LC-MS, while the three-dimensional conformation can give insight into the types
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of interaction which are responsible for the observed phenomena, i.e. van der Waals interaction between alkyl chains or Coulomb interactions in the polar part of the molecule. Relative stabilities of adducts were calculated by subtracting the energy (E in kcal/mol) of the reagents from the energy of the adduct product according to the following equations:

$$E_{\text{dimer adduct}} = E([2 \times A_9\text{PEO}_n+\text{Na}]^+) - 2 \times E(A_9\text{PEO}_n) - E(\text{Na}^+) \quad (1a)$$

$$E_{\text{monomer adduct}} = E([A_9\text{PEO}_n+\text{Na}]^+) - E(A_9\text{PEO}_n) - E(\text{Na}^+) \quad (1b)$$

where $n=2$ or $5$.

The molecular dynamics method for the calculation of energy was a slightly modified version of a procedure previously described by our group [24]. The details of this modification will be described elsewhere. Essential characteristics included the use of the Amber96 force field for the calculation of bond stretching, angle bending and torsional interactions and nonbonded interactions within and between molecules. Electrostatic interaction was calculated from PM3 generated quantum mechanical atomic charges. The charge on the sodium ion was +0.862 as determined by fit to the experimental value of the enthalpy of hydration of the sodium ion, and neutral organic (parts of the) molecules were assumed. Hydrogen bonding was neglected. $E(\text{Na}^+) = 0$ in this approach. Molecules were placed in cubic boxes with an edge of 23 Å. Switched inner/outer summation cut off distances of 5/9 Å were applied in combination with scaling factors of 1.58 as determined elsewhere [25].

Prior to full molecular dynamics runs cyclic annealing took place (three cycles with 8, 4 and 8 ps as heating, run and cooling periods, respectively). Starting structures were obtained by sketching in 2D followed by 3D model building, geometrical optimization in PM3 (see above) and Amber96. Full molecular dynamics runs at 300 K and 495 K were carried out with heating from 0 K to these temperatures during 16 ps followed by 144 ps equilibration plus run times and cooling down to 0 K in 8 ps with time steps of 0.0008 ps. 0 K energy values were considered to be equal to the lowest geometrically optimized values obtained. Representative structures of reagents and adducts were saved.

Prior to its application the method was validated by calculation of the energy of vaporization of methanol, n-nonane, 2,2,4,4,-tetramethylpentane, phenetole and ethyleneglycol monoethylether. These compounds contain all types of atoms also present in $A_9\text{PEO}_n$. Experimental values were reproduced within 0.3 kcal/mol.

**Comparison of quantification methods**

In order to further evaluate the results obtained regarding the formation of different $A_9\text{PEO}_n$ adducts, the consequences for the quantitative determination of $A_9\text{PEO}_n$ in environmental
samples were investigated. To that end, both distilled water (blanks) and extracts from field samples were spiked with known amounts of several types of \( A_9 \text{PEO}_n \) mixtures. These amounts were then quantitatively determined selecting and comparing different (combinations of) adducts for the calibration.

Blanks were spiked with one of four different mixtures:
- a mixture of pure \( A_9 \text{PEO}_1 \) and \( A_9 \text{PEO}_2 \) (mixture I)
- an industrial standard containing a mixture of nonyl isomers with an average ethoxylate chain length of 10 units (range \( A_9 \text{PEO}_{3-16} \), mixture II)
- a mixture of pure \( A_9 \text{PEO}_1 \), \( A_9 \text{PEO}_2 \) and \( A_9 \text{PEO}_3-16 \) (mixture III)
- an industrial standard containing a mixture of nonyl isomers with an average ethoxylate chain length of 4 units (range \( A_9 \text{PEO}_{2-9} \), mixture IV)

All mixtures were spiked in amounts of 200 ng to approximately 1 mL. Four replicates were prepared for each \( A_9 \text{PEO}_n \) mixture.

The sediment and water samples used for spiking had been collected in the Dutch coastal area and the Rhine and Scheldt estuaries (The Netherlands). For the 6 water samples, dissolved organic carbon varied from 1.5 to 2.9 mg L\(^{-1}\). In the 6 sediment samples, the organic carbon content ranged from 0.09 to 1.5%, with fractions of sediment particles smaller than 63\( \mu \)m (clay/silt fraction) between 0.01 and 0.35. The samples had been pre-treated using Soxhlet and SPE according to methods described earlier [21].

The extracts were split, and to one part the spike was added. Three different \( A_9 \text{PEO}_n \) mixtures were tested: mixture I, II and III (see above). Each \( A_9 \text{PEO}_n \) mixture was spiked to two water and two sediment extracts, in amounts of 200 ng.

To all calibration standards, spiked blanks and extracts used in the quantification tests, \( ^{13} \text{C}-A_9 \text{PEO}_2 \) was added as internal standard, to correct for matrix effects or fluctuations in LC-MS sensitivity.

The concentrations of the \( A_9 \text{PEO}_n \) in all spiked blanks, spiked and non-spiked extracts were determined using our base method. However, the LC gradient was slower than in the adduct formation studies described above to improve separation of analytes and interferences, with runs of 45 minutes, as reported in Jonkers et al. [21]. The selected ion monitoring mode was used, selecting m/z values of all possible monomer, dimer, disodium and solvent adducts (described in the LC-MS results section) for detection.

8-point calibration curves were used for the standards \( A_9 \text{PEO}_1 \), \( A_9 \text{PEO}_2 \), \( A_9 \text{PEO}_{3-16} \) (mixture II), \( A_9 \text{PEO}_{2-9} \) (mixture IV) and \( A_9 \text{PEO}_{1-5} \) (mixture V). For \( A_9 \text{PEO}_1 \), \( A_9 \text{PEO}_2 \) and mixture II, several calibration curves were constructed, using either the mono sodium adduct signals, or the sum of monomer, dimer and solvent adducts.
In addition, a comparison was made of the quantification of samples using calibration curves of the different $A_9$PEO$_n$ mixtures II, IV and V, both on a molar and on mass basis. The summation of the mono and disodium adduct signals was used for this quantification. Each test sample was analyzed in duplicate.

Table 2.1: Oligomer distributions in $A_9$PEO$_n$ standard mixtures used for quantifications, expressed as molar fractions.

<table>
<thead>
<tr>
<th>oligomer</th>
<th>standard mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$A_9$PEO$_3$-16 (II)</td>
</tr>
<tr>
<td>$A_9$PEO$_1$</td>
<td>0.001</td>
</tr>
<tr>
<td>$A_9$PEO$_2$</td>
<td>0.005</td>
</tr>
<tr>
<td>$A_9$PEO$_3$</td>
<td>0.02</td>
</tr>
<tr>
<td>$A_9$PEO$_4$</td>
<td>0.07</td>
</tr>
<tr>
<td>$A_9$PEO$_5$</td>
<td>0.08</td>
</tr>
<tr>
<td>$A_9$PEO$_6$</td>
<td>0.08</td>
</tr>
<tr>
<td>$A_9$PEO$_7$</td>
<td>0.11</td>
</tr>
<tr>
<td>$A_9$PEO$_8$</td>
<td>0.12</td>
</tr>
<tr>
<td>$A_9$PEO$_9$</td>
<td>0.12</td>
</tr>
<tr>
<td>$A_9$PEO$_{10}$</td>
<td>0.11</td>
</tr>
<tr>
<td>$A_9$PEO$_{11}$</td>
<td>0.09</td>
</tr>
<tr>
<td>$A_9$PEO$_{12}$</td>
<td>0.08</td>
</tr>
<tr>
<td>$A_9$PEO$_{13}$</td>
<td>0.06</td>
</tr>
<tr>
<td>$A_9$PEO$_{14}$</td>
<td>0.03</td>
</tr>
<tr>
<td>$A_9$PEO$_{15}$</td>
<td>0.02</td>
</tr>
<tr>
<td>$A_9$PEO$_{16}$</td>
<td>0.01</td>
</tr>
</tbody>
</table>
2.3. Results

2.3.1. LC-MS results

Adduct formation in the base LC-ESI-MS method.

In our base LC-ESI-MS method, several pronounced series of adducts were observed. Examples of mass spectra of mixture I and II are shown in figure 2.1. For most \( A_9 \text{PEO}_n \) oligomers, additional signals were observed besides the sodium adduct. The signals with normalized abundances are given in table 2.2, showing the remarkable differences in ionization behaviour for this so-called "homologous series" of oligomers. In table 2.3, an overview is given of all m/z values for \( A_9 \text{PEO}_n \) detected in all methods tested in this study.

For short chain \( A_9 \text{PEO}_n \) only, signals corresponding to methanol-sodium \([A_9 \text{PEO}_n+\text{MeOH}+\text{Na}]^+\) and dimer adducts \([2\times A_9 \text{PEO}_n+\text{Na}]^+\) were observed. Especially the \([A_9 \text{PEO}_1+\text{MeOH}+\text{Na}]^+\) signal is relevant, as it has a higher abundance than \([A_9 \text{PEO}_1+\text{Na}]^+\).

Overlooking this adduct may be an explanation why many studies from literature reported a very low response for this oligomer [7, 26-28]. \([A_9 \text{PEO}_{1,2}+\text{MeOH}+\text{Na}]^+ / [A_9 \text{PEO}_{1,2}+\text{Na}]^+\) signal intensity ratios were on average 6.0 (\( A_9 \text{PEO}_1 \)) and 0.19 (\( A_9 \text{PEO}_2 \)) and remain constant with increasing \( A_9 \text{PEO}_n \) concentration.

Figure 2.2 shows that the relative abundance of the \([2\times A_9 \text{PEO}_n+\text{Na}]^+\) adduct is concentration dependent. The \([A_9 \text{PEO}_n+A_9 \text{PEO}_{n+1}+\text{Na}]^+\) adduct signal (n= 1 or 2) of two different short chain oligomers was also detected (m/z 595 and 683), albeit at lower intensity than the homogeneous dimer adducts. For longer oligomers, neither \([2\times A_9 \text{PEO}_{>3}+\text{Na}]^+\) nor \([A_9 \text{PEO}_{>2}+\text{MeOH}+\text{Na}]^+\) signals were detected.

Table 2.2: Normalized signal intensities of the adducts of several \( A_9 \text{PEO}_n \) for the base method, expressed as percentages of the most abundant signal. Average signals of concentrations from 0.5 to 10 \( \mu \text{g mL}^{-1} \) are shown.
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Figure 2.1: Mass spectra of two A₉PEOₙ mixtures using full scan positive ES ionization (reversed phase column, with MeOH and NaAc in the mobile phase): a) spectrum of a A₉PEO₃-16 mixture (mixture II). Only for even-numbered A₉PEOₙ, the [A₉PEOₙ+2xNa]²⁺ signals can be observed separately. b) spectrum of a A₉PEO₁₋₂ mixture (mixture I). Besides sodium adducts, dimer adducts and adducts containing a solvent molecule are visible.
For A₉PEO₁₂ and longer, disodium adducts [A₉PEO₁₁₋₁₂×Na⁺]²⁺ were observed besides the common [A₉PEOₙ+Na⁺]⁺ signals. The ratios of [A₉PEOₙ+2×Na⁺]/[A₉PEOₙ+Na⁺]⁺ are difficult to determine, as the m/z values of short chain [A₉PEOₙ+Na⁺]⁺ and [A₉PEOₙ+H⁺]⁺ happen to be equal to those of long chain [A₉PEOₙ+2×Na⁺]²⁺. For example, the [A₉PEO₁₃₋₁₄×Na⁺]²⁺ adduct has an m/z equal to that of [A₉PEO₄+Na⁺]⁺ (m/z 419). However, in analyses using a normal phase column, it was found that the proton adduct of A₉PEO is not formed (see section Variation of stationary phase, p. 44), and therefore the [A₉PEOₙ+2×Na⁺]/[A₉PEOₙ+Na⁺]⁺ signal intensity ratios of even-numbered long chain A₉PEOₙ can be obtained. The [A₉PEOₙ+2×Na⁺]²⁺ adduct increases in importance with increasing ethoxylate chain length, while with increasing A₉PEOₙ concentration, the ratio tends to decrease (see figure 2.3).

Table 2.3: Overview of A₉PEOₙ signals detected and their identities for different LC-MS methods. MeOH and ACN signify: only occurring with methanol or acetonitrile in the mobile phase, respectively.

<table>
<thead>
<tr>
<th>m/z value</th>
<th>Identity</th>
<th>LC-MS conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>287, 331, 375, + n×44</td>
<td>[A₉PEO₁₂,₃ etc+Na]⁺</td>
<td>ESI+</td>
</tr>
<tr>
<td>282, 326, 370, + n×44</td>
<td>[A₉PEO₁₂,₃ etc+NH₄]⁺</td>
<td>ESI+</td>
</tr>
<tr>
<td>551, 639, 727</td>
<td>[2×A₉PEO₁₂,₃+Na]⁺</td>
<td>ESI+</td>
</tr>
<tr>
<td>595</td>
<td>[A₉PEO₁+₂×[PEO₂+Na]⁺</td>
<td>ESI+</td>
</tr>
<tr>
<td>397, 419, 441 + n×22</td>
<td>[A₉PEO₁₂,₁₃,₁₄etc+2×Na⁺]²⁺</td>
<td>ESI+</td>
</tr>
<tr>
<td>413</td>
<td>[A₉PEO₂+NaAc+Na⁺</td>
<td>ESI+</td>
</tr>
<tr>
<td>319, 363</td>
<td>[A₉PEO₁₂+MeOH+Na⁺]⁺</td>
<td>ESI+ (MeOH)</td>
</tr>
<tr>
<td>328, 372, 416</td>
<td>[A₉PEO₁₂,₃+ACN+Na⁺]⁺</td>
<td>ESI+ (ACN)</td>
</tr>
<tr>
<td>495, 577</td>
<td>[A₉PEO₂+2,3×NaAc+Na⁺]⁺</td>
<td>ESI+ (ACN)</td>
</tr>
<tr>
<td>323, 367, 411, + n×44</td>
<td>[A₉PEO₁₂,₃ etc+Ac⁻]⁻</td>
<td>ESI-</td>
</tr>
<tr>
<td>263, 307, 351, + n×44</td>
<td>[A₉PEO₁₂,₃ etc−H]⁻</td>
<td>ESI-</td>
</tr>
<tr>
<td>265, 309, 353, + n×44</td>
<td>[A₉PEO₁₂,₃ etc+H]⁺</td>
<td>APCI+</td>
</tr>
<tr>
<td>297, 341</td>
<td>[A₉PEO₁₂+MeOH+H⁺]⁺</td>
<td>APCI+</td>
</tr>
</tbody>
</table>
Adduct formation of nonylphenol ethoxylates in LC-ESI-MS

Figure 2.2: Ratios of $[2 \times A_9 PEO_n + Na]^+ /[A_9 PEO_n + Na]^+$ adduct signal intensities for short chain $A_9 PEO_n$ at five surfactant concentrations, using the base LC-ESI-MS method. The relative abundance of dimer adducts increases with increasing surfactant concentration.

Figure 2.3: Ratios of $[APEO_{n+2}Na]^+/ [APEO_n + Na]^+$ adduct signal intensities for long chain $A_9 PEO_n$ and $A_9 PEO_n$ at three surfactant concentrations, using the base method. * indicates that the ratio could not be determined, due to overlapping signals of $[A_9 PEO_{13,15,17} + 2Na]^+$ and short chain $[A_9 PEO_{4,5,6} + Na]^+$. 
For the octylphenol ethoxylate surfactants ($A_8\text{PEO}_n$), the problem of overlapping m/z values does not occur. Analysis of a standard mixture of $A_8\text{PEO}_{3.17}$ resulted in ratios of $[A_8\text{PEO}_n+2\times\text{Na}]^{2+}/[A_8\text{PEO}_n+\text{Na}]^+$ shown in figure 2.3. Analogous to $A_9\text{PEO}_n$, for $A_8\text{PEO}_n$ an increase of this ratio with increasing ethoxylate chain length is observed.

At higher cone voltages, the signal intensities of $[2\times A_9\text{PEO}_n+\text{Na}]^+$ and $[A_9\text{PEO}_n+2\times\text{Na}]^{2+}$ drop more sharply than $[A_9\text{PEO}_n+\text{Na}]^+$, indicating that dimer and disodium complexes are significantly less stable than the mono sodium adducts (see figure 2.4). The signal intensities of $[A_9\text{PEO}_n+\text{MeOH}+\text{Na}]^+$ and $[A_9\text{PEO}_n+\text{Na}]^+$ decrease to the same extent at higher cone voltages (their ratio remains constant in figure 2.4), indicating these adducts are of comparable stability.

A closer inspection of the background signal revealed that much of this signal can be attributed to constantly eluting clusters of buffer ions $[n\times\text{NaAc}+\text{Na}]^+$ (m/z 269, 351, 597, 679 for $n=3,4,7,8$).

Figure 2.4: Signal ratios of disodium, dimer or solvent adducts and their corresponding mono sodium adducts at different cone (fragmentation) voltages. ■: $[A_9\text{PEO}_{16}+2\times\text{Na}]^{2+}/[A_9\text{PEO}_{16}+\text{Na}]^+$; ○: $[A_9\text{PEO}_{14}+2\times\text{Na}]^{2+}/[A_9\text{PEO}_{14}+\text{Na}]^+$; +: $[2\times A_9\text{PEO}_2+\text{Na}]^+/[A_9\text{PEO}_2+\text{Na}]^+$; x: $[A_9\text{PEO}_2+\text{MeOH}+\text{Na}]^+/[A_9\text{PEO}_2+\text{Na}]^+$ (ESI+ mode, with MeOH and NaAc in mobile phase). All disodium and dimer adducts are less stable than the sodium adduct at increasing voltages, while the solvent adduct remains equally stable.
Adduct formation of nonylphenol ethoxylates in LC-ESI-MS

Influence of the mobile phase buffer composition on adduct formation

In general, when the NaAc buffer in the mobile phase was replaced by a NH4Ac or KAc buffer, the adduct types observed were similar to those of the base method (with Na replaced by NH4 or K). It has been mentioned (although not demonstrated) before in literature that the cation in [A9PEOₙ⁺NH₄⁺] adducts is less tightly bound than in [A9PEOₙ⁺Na⁺] adducts [7]. The relatively low stability of ammonium adducts is confirmed in the present study by the relatively high abundance of sodium adducts despite an ammonium acetate buffer concentration of 2 mM. For both the ammonium acetate and potassium acetate buffers, [A9PEOₙ⁺Na]⁺/[A9PEOₙ⁺NH₄ or K]⁺ ratios were highest for the A9PEO₃ oligomer (0.39 and 0.14, respectively).

Similar to the analyses using the base method, for short A9PEO₁₋₄ oligomers the dimer adducts [2×A₉PEOₙ⁺NH₄⁺] and [2×A₉PEOₙ⁺K⁺] and for long A₉PEO₁₋₁₃ oligomers the doubly charged [A₉PEOₙ⁺2×NH₄⁺⁺] and [A₉PEOₙ⁺2×K⁺⁺] signals were observed when using the ammonium or potassium buffer, respectively. Relative abundances of dimer and doubly charged adducts were somewhat lower than in the base method, but showed the same dependence on concentration and ethoxylate chain length. However, no adducts including a methanol molecule were observed for either buffer.

To compare the metal preference of A₉PEOₙ during adduct formation, equimolar amounts of the three buffers NaAc, NH₄Ac and KAc were added to the mobile phase (0.1 mM of each). The results showed that for all oligomers, the potassium adduct [A₉PEOₙ⁺K⁺] gave a more abundant signal than [A₉PEOₙ⁺Na⁺] and [A₉PEOₙ⁺NH₄⁺⁺]. The ratio of the sodium or ammonium and the potassium adduct signals [A₉PEOₙ⁺Na or NH₄⁺⁺]/[A₉PEOₙ⁺K⁺⁺] was highest for the A₉PEO₄ oligomer (0.28 for sodium and 0.19 for ammonium).

Influence of the organic modifier on adduct formation

When the organic modifier in the mobile phase was substituted by acetonitrile (ACN) using a NaAc buffer, disodium and dimer adducts were formed similar to the base method. Adducts including a solvent molecule were formed as well, with the [A₉PEO₁₋₂⁺ACN⁺Na⁺]⁺ adduct abundances being higher than those of [A₉PEO₁₋₂⁺Na⁺]⁺.

In addition, a significant series of signals with m/z = 413, 495, 577 and 659 (m/z = 413+n×82) were observed for short A₉PEOₙ oligomers. Two types of adducts could be responsible for this: [A₉PEO₂⁺2×ACN⁺Na⁺]⁺ or [A₉PEO₂⁺NaAc⁺Na⁺]⁺ (for m/z 413). Signals with m/z values corresponding to A₉PEOₙ adducts containing 3 or 5 acetonitrile molecules were not observed, which would mean that acetonitrile would only cluster to an A₉PEOₙ adduct in pairs of two, which seems unlikely. Additional analyses were performed with ammonium acetate and acetonitrile in the mobile phase, but neither the signals of [A₉PEO₂⁺2×ACN⁺NH₄⁺]⁺ (m/z = 408) nor [A₉PEO₂⁺NH₄Ac⁺NH₄⁺]⁺ (m/z = 403) were observed, and therefore no additional clues were obtained for the identity of the previously
mentioned sodium adducts of m/z 413+n×82. However, after a closer inspection of mass spectra of A₉PEO₂ obtained with the base method, a small trace of the signal with m/z 413 was found, confirming its identity as [A₉PEO₂+NaAc+Na]⁺. Therefore, we conclude that the series of signals with m/z = 413+n×82 belong to [A₉PEO₂+n×NaAc+Na]⁺ adducts with n=1-4. The [A₉PEO₂+n×NaAc+Na]⁺ adduct ions were relatively unstable, as their signal decreased steeply with increasing cone voltage.

A peculiar detail was found in the elution order of the A₉PEOₙ oligomers from the C₁₈-column. All A₉PEOₙ eluted almost simultaneously as one broad peak of about 2.5 minutes, but with methanol as modifier, short oligomers eluted slightly earlier than long oligomers (with 10-15 seconds between the apexes of consecutive oligomers), while with ACN as mobile phase, this was reversed. Probably, with ACN in the mobile phase, C₁₈-chains of the stationary phase are oriented more linearly and orderly, optimizing the hydrophobic interactions with the analytes, and causing the most apolar oligomer (A₉PEO₁) to elute last. With methanol, hydrophilic interaction between the analytes and the stationary phase has slightly more influence. In both situations, all A₉PEO could be integrated as one broad peak.

Variation of the stationary phase
When using a normal phase NH₂-column, with an appropriate normal phase mobile phase (hexane/isopropanol/water), the observed adducts are similar to those of the base method. Both dimer and disodium adducts are formed. However, no adducts containing a solvent molecule are observed.

As the different A₉PEOₙ oligomers are chromatographically separated in normal phase analysis, it was possible to distinguish between e.g. [A₉PEO₁₃+2×Na]²⁺ and [A₉PEO₄+Na]⁺ (m/z = 419), and therefore ratios of [A₉PEOₙ+2×Na]²⁺/[A₉PEOₙ+Na]⁺ signal intensity could be determined reliably. These ratios, measured in a sample with a concentration of 15 µg mL⁻¹ increased from 0.01 (A₉PEO₁₁) to 0.87 (A₉PEO₁₆), which is similar to those observed in reversed phase analysis at high concentrations (for A₉PEO₁₂, A₉PEO₁₄ and A₉PEO₁₆).

ESI in negative mode and APCI in positive mode
Two additional analytical methods were tested, which will be discussed only briefly. In one method negative ionization was used with the ESI interface, and in the other method APCI was applied in the positive mode.

The main signals for A₉PEOₙ in negative ionization were found to correspond to the acetate adducts [A₉PEOₙ+CH₃COO]⁻, and to a minor extent deprotonated [A₉PEOₙ-H]⁻ ions were also formed. The acetate adducts were relatively unstable, as their signals dropped sharply at higher cone voltages. The detection limits were 4 to 50 times higher for [A₉PEOₙ+Ac]⁻ than for the A₉PEOₙ adducts of the base method, and therefore the practical utility of this method is limited.
Adduct formation of nonylphenol ethoxylates in LC-ESI-MS

The main difference between the APCI+ method and the base method was that with APCI mainly proton adducts were formed. Sodium adducts were of less importance (despite the presence of a sodium acetate buffer). For A₉PEO₂, the most abundant signal was the methanol-proton adduct \([A₉PEO₂+MeOH+H]^+\), while the abundance of the \([A₉PEO₁+MeOH+H]^+\) adduct was relatively low.

2.3.2 Molecular Dynamics results

Calculated formation energies of monomer and dimer adducts of the two alkyl isomers of A₉PEO₂ and A₉PEO₅ using equations (1a) and (1b) are presented in table 2.4. Uncertainties in the calculated energies amount to about 3 (at 0 K), 1 (at 300 K) or 2 (at 495 K) kcal mol⁻¹ at maximum. In all cases adduct formation energies are negative and the adducts are stable with respect to the reagents. In view of the uncertainties in the calculations no difference was found for the adduct formation energies of nonyl-1 and 2,2,4,4-tetramethylpentyl-3 isomers. Taking into account the higher uncertainties in the 0 K results, no significant differences in adduct formation energies are found between 0 and 300 K for all but one calculations. A comparison between the results for 300 and 495 K shows that adduct formation energies become less negative at higher temperature. Finally, the 0 K results show that the major contribution to the total energy of adduct formation originates from the electrostatic interaction, whereas the van der Waals contribution \((E_{\text{total}} - E_{\text{elec}})\) is much less negative (all dimer adducts) or even positive (all monomer adducts).

Table 2.4: Adduct formation energies \((E_{\text{total}})\) of sodiated nonyl-1- (N) and 2,2,4,4-tetramethylpentyl (T) phenol ethoxylates at various temperatures calculated by molecular dynamics. For 0 K also the electrostatic contribution is given \((E_{\text{elec}})\). Energies are in kcal/mol.

<table>
<thead>
<tr>
<th>Adduct</th>
<th>0 K (E_{\text{total}})</th>
<th>0 K (E_{\text{elec}})</th>
<th>300 K (E_{\text{total}})</th>
<th>300 K (E_{\text{elec}})</th>
<th>495 K (E_{\text{total}})</th>
<th>495 K (E_{\text{elec}})</th>
</tr>
</thead>
<tbody>
<tr>
<td>([2\times \text{NPEO}_2+\text{Na}]^+)</td>
<td>-39.1</td>
<td>-31.9</td>
<td>-41.9</td>
<td>-26.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([2\times \text{TPEO}_2+\text{Na}]^+)</td>
<td>-37.7</td>
<td>-32.3</td>
<td>-31.2</td>
<td>-26.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([2\times \text{NPEO}_5+\text{Na}]^+)</td>
<td>-53.9</td>
<td>-43.9</td>
<td>-56.2</td>
<td>-49.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([2\times \text{TPEO}_5+\text{Na}]^+)</td>
<td>-53.3</td>
<td>-43.4</td>
<td>-54.4</td>
<td>-45.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{NPEO}_2+\text{Na}]^+)</td>
<td>-12.5</td>
<td>-17.5</td>
<td>-14.5</td>
<td>-9.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{TPEO}_2+\text{Na}]^+)</td>
<td>-13.4</td>
<td>-16.2</td>
<td>-12.7</td>
<td>-10.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{NPEO}_5+\text{Na}]^+)</td>
<td>-31.3</td>
<td>-33.5</td>
<td>-28.1</td>
<td>-27.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>([\text{TPEO}_5+\text{Na}]^+)</td>
<td>-28.7</td>
<td>-32.5</td>
<td>-30.6</td>
<td>-23.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
A9PEO3 adducts invariably are more stable than the corresponding A9PEO2 adducts. For the monomer adducts, this is in accordance with LC-MS results, which show that short chain A9PEO1,2 have a considerably higher detection limit than longer A9PEO2, which apparently is caused by the relative instability of the adducts. However, for the dimer adducts the calculations are not in accordance with the observed adducts. First, the calculations suggest that the [2×A9PEO2+Na]+ dimer adduct is more stable than the [A9PEO2+Na]+ monomer, while the adduct intensities observed in LC-ESI-MS are higher for the monomer than for the dimer adduct (see table 2.2). Next, the [2×A9PEO5+Na]+ dimer is calculated to be more stable than the [2×A9PEO2+Na]+ dimer, while in LC-ESI-MS the latter dimer adduct is observed and the former not at all. It is clear that a direct correlation between calculated stability and observed signal intensity cannot be made for these compounds.

Figure 2.5: Lowest energy conformers obtained after molecular dynamics of the dimer adducts [2×A9PEO2+Na]+ and [2×A9PEO5+Na]+. Oxygen atoms (small dark) are coordinated around the sodium atom (large dark). Alkyl chains are relatively far apart and have little interaction with each other.
According to the Molecular Dynamics calculations, the A₉PEOₙ molecule wraps itself around the complexing sodium ion in a way that negative electronic charges on oxygen have optimum electrostatic interaction with this ion. This is the case for both the monomer and dimer adducts. Figure 2.5 shows the 3D orientation of the lowest energy conformations for [2×A₉PEO₂+Na]⁺ and [2×A₉PEO₃+Na]⁺. The two alkyl moieties are far apart in these conformations, indicating that van der Waals interactions between alkyl moieties are not the main driving force for the formation of these dimer adducts.

Table 2.5: Influence of the selection of different combinations of adduct signals on the quantification. Results are shown for three A₉PEOₙ mixtures which had been spiked to blanks and to environmental extracts. Quantifications of A₉PEO₁, A₉PEO₂ and A₉PEO₃-16 are expressed as average percentages of the nominal spiked amount quantified in the samples, with standard deviations (n=4 for each spike mixture). (- = not quantified in this mixture).

<table>
<thead>
<tr>
<th>quantification method</th>
<th>adduct signal(s) used for quantification</th>
<th>mixture spiked to blank</th>
<th>mixture spiked to environmental extract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mixture I</td>
<td>mixture II</td>
</tr>
<tr>
<td>A</td>
<td>[A₉PEO₁+Na]⁺</td>
<td>97 (7)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>[A₉PEO₁+MeOH+Na]⁺</td>
<td>101 (2)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>[A₉PEO₁+Na]⁺ + [A₉PEO₁+MeOH+Na]⁺</td>
<td>102 (1)</td>
<td>-</td>
</tr>
<tr>
<td>A</td>
<td>[A₉PEO₂+Na]⁺</td>
<td>99 (3)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>[A₉PEO₂+MeOH+Na]⁺</td>
<td>101 (3)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>[A₉PEO₂+Na]⁺ + [A₉PEO₂+MeOH+Na]⁺</td>
<td>101 (2)</td>
<td>-</td>
</tr>
<tr>
<td>B</td>
<td>[A₉PEO₃-16+Na]⁺</td>
<td>-</td>
<td>102 (4)</td>
</tr>
<tr>
<td>C</td>
<td>[A₉PEO₃-16+Na]⁺ + [A₉PEO₃-16+2×Na]⁺</td>
<td>-</td>
<td>102 (4)</td>
</tr>
</tbody>
</table>

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2.3.3. Influence of adduct and standard selection on quantitative analysis

Quantification of spiked blanks

Table 2.5 presents the results of the quantitative analysis of the spiking experiments. The results are expressed as percentages of the spiked amounts found in the samples. Columns 3, 4 and 5 of table 2.5 show that in most cases the amounts of \( A_9\text{PEO}_n \) mixtures I, II and III spiked to the blanks was recovered quantitatively, independent of the adduct signals selected for quantification.

The exceptions are the quantification of \( A_9\text{PEO}_1 \) and \( A_9\text{PEO}_2 \) in mixture III when using quantification method C, and \( A_9\text{PEO}_{3-16} \) in mixture III when using either method D or E. In these cases, a considerable overestimation resulted. This can be explained by the overlapping signals of \([2\times A_9\text{PEO}_1+\text{Na}]^+\) and \([A_9\text{PEO}_7+\text{Na}]^+\) (m/z = 551) and similarly, \([2\times A_9\text{PEO}_2+\text{Na}]^+\) and \([A_9\text{PEO}_9+\text{Na}]^+\) (m/z = 639).

Limits of detection

When a comparison is made of detection limits using different adduct signals, the most significant difference is observed for \( A_9\text{PEO}_1 \). The detection limit improved considerably with the inclusion of the \([A_9\text{PEO}_1+\text{MeOH}+\text{Na}]^-\) signal, with a detection limit of 2.2 ng injected for quantification method A and 0.3 ng injected for method B. A smaller increase in sensitivity was observed for \( A_9\text{PEO}_2 \) with detection limits of 0.5 ng injected for method A and 0.4 ng injected for method B. For neither \( A_9\text{PEO}_1 \) nor \( A_9\text{PEO}_2 \), additional selection of the dimer adducts (method C) further improved the detection limits. For \( A_9\text{PEO}_{9-11} \), no difference in detection limit (1.1 ng injected) was observed between quantification method D and E.

Table 2.6: Comparison of results of quantitative analysis of different \( A_9\text{PEO}_n \) spikes added to blanks, based on different calibration standards. Quantifications of \( A_9\text{PEO}_{1-15} \) are expressed as average percentages of the nominal spiked amount quantified in the samples, with standard deviations (n=4 for each spike mixture).

<table>
<thead>
<tr>
<th>calibration curve used</th>
<th>standard mixture spiked to blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( A_9\text{PEO}_{3-16} ) (II)</td>
</tr>
<tr>
<td>( A_9\text{PEO}_{3-16} ) (II) mass</td>
<td>100 (4)</td>
</tr>
<tr>
<td>( A_9\text{PEO}_{3-16} ) (II) molar</td>
<td>100 (4)</td>
</tr>
<tr>
<td>( A_9\text{PEO}_{2-9} ) (IV) mass</td>
<td>81 (3)</td>
</tr>
<tr>
<td>( A_9\text{PEO}_{2-9} ) (IV) molar</td>
<td>113 (4)</td>
</tr>
<tr>
<td>( A_9\text{PEO}_{1-5} ) (V) mass</td>
<td>109 (4)</td>
</tr>
<tr>
<td>( A_9\text{PEO}_{1-5} ) (V) molar</td>
<td>195 (7)</td>
</tr>
</tbody>
</table>
Adduct formation of nonylphenol ethoxylates in LC-ESI-MS

Selection of the calibration standard
In table 2.6, a comparison is made of quantifications of \( \text{A}_9\text{PEO}_{1-15} \) in spiked blanks using different standard mixtures, and calibrating on a molar or mass basis. For the blanks spiked with the \( \text{A}_9\text{PEO}_{2-9} \) mix (mixture IV), an overestimation occurred when quantifying with the calibration of \( \text{A}_9\text{PEO}_{3-16} \) (mixture II) based on mass. This is at least partly caused by the difference in average molecular weight between the two standard mixtures. The error introduced is around 35%. This error can be considered as a maximum error which may occur when using this calibration based on mass, as the difference between standard and sample oligomer composition is at its maximum here: normally the \( \text{A}_9\text{PEO}_1 \) and \( \text{A}_9\text{PEO}_2 \) oligomers are quantified with 2 separate standards, while \( \text{A}_9\text{PEO}_n \) mixtures with average ethoxylate chain lengths higher than 15 are hardly ever present in environmental samples.

Alternatively, when the samples spiked with the \( \text{A}_9\text{PEO}_{2-9} \) mix are quantified using the \( \text{A}_9\text{PEO}_{3-16} \) mixture in a calibration on a molar basis, and afterwards the result is multiplied by the correct average molar weight of the \( \text{A}_9\text{PEO}_{2-9} \) mix, a correct quantification is obtained. From this exercise, it can be concluded that the \( \text{A}_9\text{PEO}_{3-16} \) mix can be used for quantification of all \( \text{A}_9\text{PEO}_{n} \), as long as molar calibrations are used.

Results of the quantification with a \( \text{A}_9\text{PEO}_{1-5} \) mixture (V) on a molar basis show a considerable overestimation for both the spiked mixtures II and IV. This indicates that a high percentage of \( \text{A}_9\text{PEO}_1 \) and \( \text{A}_9\text{PEO}_2 \) in the calibration standard mixture (like mixture V) leads to increasing deviations in the quantification of \( \text{A}_9\text{PEO}_{n} \).

As the right column of table 2.6 shows, none of the standard mixtures is able to quantify correctly the spike of \( \text{A}_9\text{PEO}_{1-2} \) (mixture I). Considerable underestimations are found in all cases, underlining the need for separate quantification of these two oligomers.

Quantification of spiked environmental extracts
For the spiked environmental extracts, the \( \text{A}_9\text{PEO}_n \) mixtures used for spiking were equal to the calibration mixtures, and therefore no comparison between calibrations on molar and mass basis is necessary. For all samples, the percentage of the spiked amount recovered in the sample was determined ((\( \text{A}_9\text{PEO}_n \) in spiked sample - \( \text{A}_9\text{PEO}_n \) in non-spiked sample) / \( \text{A}_9\text{PEO}_n \) spiked). As shown in table 2.5 (right side), most of the results are below 100%, indicating that in most cases, an underprediction of the spiked amount was found. This underprediction can probably be attributed to matrix effects for which apparently the internal standard does not correct completely. The large variations in composition of the environmental samples (e.g. organic carbon content) explains the relatively high standard deviations in the quantifications (10-20%) compared to those of the spiked blanks. Standard deviations are higher for the samples spiked with mixture III, indicating that more complicated oligomer patterns reduce the reliability of the quantification.

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Table 2.5 shows that for the spiked extracts, neither between methods A, B and C for the quantification of A₉PEO₁ and A₀PEO₂ nor between method D and E for the quantification of A₀PEO₃₁₆ any significant differences were found. For quantification method C, the average A₀PEO₁ and A₀PEO₂ spikes recovered in the samples spiked with mixture III are not significantly different from those in the samples spiked with mixture I. This indicates that the overlap of the [2×A₀PEO₁₂⁺Na]⁺ and [A₀PEO₇₉⁺Na]⁺ signals has no significant consequences for the quantification in these cases. Possibly, in the environmental samples A₀PEO₂ (in combination with the matrix) suppresses the A₀PEO₁₂ signals, which is not the case in the spiked blanks.
On average, the A₀PEO₃₁₆ spike recovered in the samples spiked with mixture III was higher than the spike recovered in the samples spiked with mixture II. However, due to the relatively large variations between samples, the difference is not significant. This signifies that in environmental samples, the differences in matrix effects between samples cause larger variations in the quantification of A₀PEOₙ than possible interferences due to the presence of dimer [2×A₀PEO₁₂⁺Na]⁺ adduct signals.

2.4. Discussion

Formation of adducts in LC-ESI-MS
It must be noted that the formation of adducts in LC-MS depends not only on their stability, but is also determined by ‘macroscopic’ factors, such as the design of the interface: e.g. the electrospray construction type (orthogonal or off-axis) or material of the ESI probe (conducting or non-conducting material) [29,30]. However, several of the adducts investigated in this work ([A₀PEOₙ⁺2×Na]²⁺ and [2×A₀PEOₙ⁺Na]⁺) have been reported by other scientists. Their formation can therefore be considered as generally occurring processes in LC-ESI-MS analysis. All investigated LC-MS parameters had some influence on the A₀PEOₙ adduct formation. However, none were able to prevent the formation of additional adducts besides the single cation adducts. An advantage of using NH₄Ac or KAc as buffer is that solvent adducts [A₀PEOₙ⁺MeOH+NH₄/K]⁺ are eliminated. However, the relatively high Na/NH₄ or Na/K ratios still complicate the spectra. Using acetonitrile instead of methanol in the mobile phase leads to even more adduct types than those formed with methanol. Analyzing in the negative ionization mode using acetate adducts for detection results in poorer detection limits.
Therefore, we conclude that in spite of the formation of several types of adducts, the most appropriate LC-ESI-MS conditions for the analysis of A₉PEOₙ are a mobile phase composition of methanol and water using NaAc as buffer, in positive ionization mode. A possible solution for the formation of disodium adducts [A₉PEOₙ+2×Na]⁺ was proposed by Shao et al., who showed that the formation of disodium adducts can be significantly reduced by ramping the cone voltage from 25 to 70 V, instead of using a constant low cone voltage [8].

Explanation of adduct formation using molecular dynamics calculations
The predicted higher stability of A₉PEO₅ dimer adducts compared to the A₉PEO₂ dimer adduct is not in accordance with the observed LC-MS signal intensities. The higher predicted stability of both A₉PEO₂,₅ dimer adducts compared to their corresponding A₉PEO₂,₅ monomer adducts is not in agreement with the observed signal intensities either. Looking for potential causes on the side of the calculations it must be emphasized that entropy contributions were neglected - as often done in this type of molecular modeling -, though these could contribute substantially to stability in the case of the pertinent flexible molecules at 495 K. In addition, major uncertainties remain as a consequence of the neglect of the influence of solvents (water, methanol) on the values of the adduct formation energy. This refers both to uncertainties in what actually happens during the formation of the gas-phase adduct (the two common theories being the ion evaporation model and the charge residuum model) [31] and to uncertainties caused by the neglect of the very high electrostatic interactions to be expected between polar adducts and solvents. Calculations on adduct formation in a solvent surrounding will be included in future molecular dynamics studies. In addition, the partitioning of analytes between the interior and the surface of an electrospray droplet may be of influence on the observed signal intensities, as pointed out previously by Sherman et al. [32]. In general, apolar analytes tend to have a greater affinity for the surface of the polar solvent droplet, which facilitates their evaporation from the droplet. Possibly, this explains why [2×A₉PEOₙ+Na]⁺ dimer adduct signals are observed for the relatively apolar A₉PEO₁,₂, and not for longer oligomers. Moriwaki et al. found an influence of the mobile phase composition on the formation of A₉PEOₙ dimer adducts in LC-ESI-MS, using methanol and water with sodium perchlorate. The ratio of [2×A₉PEOₙ+Na]⁺/[A₉PEOₙ+Na]⁺ adduct signal intensities was higher at higher water percentages. The author suggested from this observation that the formation of the [2×A₉PEOₙ+Na]⁺ complex is driven by van der Waals interactions which are enhanced at higher water percentages. However, from our calculations it is concluded that van der Waals interactions are not very important in these adducts. An alternative explanation for the dependence of adduct formation ratios on water percentage could be that desolvation energies for the sodium atom increase with increasing water percentage. This effect is more
pronounced for the monomer adduct than for the dimer adduct, as the sodium in the dimer adduct is more shielded from solvent molecules than in the monomer adduct. Hence, this would lead to an increase in the ratio of \( [2 \times A_9 \text{PEO}_n + \text{Na}]^+/[A_9 \text{PEO}_n + \text{Na}]^- \) adduct signal intensities. These differences in desolvation energies were also mentioned as an explanation for the preferential formation of dimer over monomer adducts of several crown ethers in LC-ESI-MS [33].

**Quantification of \( A_9 \text{PEO}_n \) in environmental samples**

All presented results show that \( A_9 \text{PEO}_1 \) and \( A_9 \text{PEO}_2 \) are the most ‘deviating’ oligomers in terms of the types and responses of adducts formed. It is therefore necessary to quantify these two oligomers separately with pure standards. Another reason to treat these oligomers with special care is from a toxicological point of view, as these two are the only \( A_9 \text{PEO}_n \) oligomers which are proven to be weak endocrine disruptors [34].

For the higher \( A_9 \text{PEO}_{n>2} \) oligomers, it has been shown that a reliable quantification can be performed with calibration curves based on molar amounts using an \( A_9 \text{PEO}_n \) standard containing as little of the \( A_9 \text{PEO}_{1,2} \) oligomers as possible, even if the average ethoxylate chain length deviates between samples and the standard mixture. The selection of adduct signals for quantification is based on detection limits and the possible interference by other \( A_9 \text{PEO}_n \) oligomers. For \( A_9 \text{PEO}_1 \) and \( A_9 \text{PEO}_2 \), the detection limits improved significantly by including the solvent adduct, while inclusion of the dimer adduct is not advised, due to the overlap with the signals of \( [A_9 \text{PEO}_7 + \text{Na}]^+ \) and \( [A_9 \text{PEO}_9 + \text{Na}]^- \). For \( A_9 \text{PEO}_{n>2} \), the inclusion of signals other than the sodium adduct does not lead to improved detection limits or accuracy, and is therefore not recommended.

**2.5. Conclusions**

In this paper, the occurrence and consequences of the formation of different types of adducts of \( A_9 \text{PEO}_n \) in LC-ESI-MS analysis were investigated. Under all LC-MS conditions investigated several adduct types were present. In clean samples, these additional adducts can lead to overestimations of other \( A_9 \text{PEO}_n \) oligomer concentrations. However, in actual environmental extracts the influence of the formation of additional adducts is minor compared to other influences on the quantitative result such as matrix effects. Judging from these results, the formation of disodium, dimer and solvent adducts is a complication, but it does not compromise reliable quantification in the environmental analysis of \( A_9 \text{PEO}_n \).
In the choice of calibration standards, it is important to quantify the $A_9$PEO$_{1,2}$ oligomers separately, and use a $A_9$PEO$_n$ standard containing as little $A_9$PEO$_{1,2}$ as possible for longer oligomers, while using molar calibrations.

Explanation of the formation of the observed adducts by molecular dynamics calculations was partially possible. However, additional factors (both at the molecular and macroscopic level) will have to be taken into account in the calculations before a complete theoretical explanation for all observed types of $A_9$PEO$_n$ adducts and their relative intensities can be provided.

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References


Adduct formation of nonylphenol ethoxylates in LC-ESI-MS


"I scrambled up to the crest of Primrose Hill, the Martians' camp was below me. A mighty space it was, and scattered about it, in their overturned machines, were the Martians – dead! – slain after all man's devices had failed, by the humblest things that God, in his wisdom, had put upon this earth: bacteria. Minute, invisible, bacteria. Directly the invaders arrived, and drank and fed, our microscopic allies attacked them. From that moment they were doomed."

H.G. Wells – The War of the Worlds (1898)