# Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry of Synthetic Polymers. 7. Analysis of Fatty Alcohol Ethoxylates by Coupled Liquid Chromatography at the Critical Point of Adsorption and MALDI-TOF Mass Spectrometry

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SUMMARY: Fatty alcohol ethoxylates can be analyzed efficiently with respect to functionality and molar mass by coupled liquid chromatography and MALDI-TOF mass spectrometry. Both techniques are coupled via a robotic interface, where the matrix is coaxially added to the eluate and spotted dropwise onto the MALDI target. It is shown that liquid chromatography at critical conditions of adsorption coupled to MALDI-TOF yield useful structural information on oligomer masses and chemical composition. In particular, the analysis of technical fatty alcohol ethoxylates by LC-CC/MALDI-TOF reveals the presence of different functionality fractions in one sample. The oligomer distributions of all functionality fractions are determined.

## Introduction

Fatty alcohol ethoxylates (FAE) are in widespread use in technical applications. The polymerization of ethylene oxide with fatty alcohols as starters yields polymers with amphiphilic properties which are used as surfactants. Depending on the chemical composition of the starter molecules, FAE or formed that have endgroups of different chain lengths from $C_{10}$ up to about $C_{18}$.

In addition to the molar mass distribution (MMD) FAEs are distributed with respect to functional endgroups. This functionality type distribution (FTD) results from the type of starter fatty alcohol that is used. Very frequently, technical fatty alcohols are mixtures of molecules with different carbon numbers, e.g. $C_{13}/C_{15}$ or $C_{16}/C_{18}$. For a proper evaluation of the product properties of FAEs it is, therefore, important to determine FTD as a function of molar mass.

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It has been established theoretically and verified experimentally that liquid chromatography at the critical point of adsorption (LC-CC) provides selective information on FTD of functional homopolymers [1-5]. Different from any other liquid chromatographic technique, LC-CC operates at conditions where the polymer chain does not contribute to retention and the elution behaviour of the sample is solely directed by the type and number of the functional endgroups.

Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry is a particularly effective tool for the molar mass determination of natural and synthetic polymers. The ability to ionize a broad range of materials, its high sensitivity, large mass range, fast sample preparation, and the absence of fragmentation are characteristics of this powerful technique. In a MALDI-TOF instrument, short-duration laser pulses are directed at a sample dispersed in a specific matrix. The laser energy causes a portion of the sample/matrix mixture to be desorbed from the surface and ionized. The produced ions are analyzed in a TOF mass analyzer. Initially developed for use with large biomolecules in 1988 by Karas and Hillenkamp [6,7], MALDI-TOF has advanced very rapidly into a powerful technique for synthetic polymer analysis [8-15].

One major concern is the ability of MALDI-TOF to provide accurate molar mass measurements. It has been shown that for polydispersities $M_w/M_n>1.1$ there is a significant discrepancy between molar masses calculated from MALDI-TOF vs. that calculated from size exclusion chromatography (SEC). Polymers with higher polydispersities may be completely inaccessible to analysis by MALDI-TOF [16-19]. A solution to this problem has been proposed recently by combining a SEC prefractionation with a subsequent MALDI-TOF analysis of the resulting fractions [16,18,20-23]. The feasibility of direct deposition has been shown in [22], where the SEC effluent was sprayed onto a moving matrix-coated substrate using a modified LC-Transform Series 100 IR interface of Lab Connections. Alternatively, Nielen described the coupling of SEC to a robotic interface Probot of Bioanalytical Instruments, where the matrix is coaxially added to the SEC effluent and spotted dropwise onto the MALDI target [23]. It has been shown by us that functional polypropylene oxides can be analyzed in detail by coupled LC-CC and MALDI-TOF [24].

In this paper, the analysis of fatty alcohol ethoxylates by LC-CC shall be discussed. For the analysis of FTD as a function of molar mass, liquid chromatography shall be interfaced with MALDI-TOF MS. As the liquid chromatographic separation technique LC-CC shall be used.

## Experimental

***SEC/LC-CC***: The measurements were conducted using a modular system, comprising a Waters HPLC pump Model 510, a Rheodyne six-port injection valve, a Waters refractive index detector Model 410 or an Altech Model 500 ELSD detector. The column for the LC-CC was Macherey&Nagel $100C_{18}$, 250x4.6 mm i.d. For the experiments a mobile phase of methanol-water 79:21 v/v was used.

***MALDI-TOF-MS***: The spectra were recorded on a KRATOS Kompact MALDI 4 instrument. The irradiation source was a pulsed nitrogen laser with a wavelength of 337 nm. The length of one laser pulse was 3 ns. The measurements were carried out using the following conditions: polarity-positive, flight path-linear, mass-high (20 kV acceleration voltage), 100-150 pulses per spectrum. The delayed extraction technique was used applying delay times of 200-800 ns.

***MALDI-TOF Sample Preparation***: For interfacing liquid chromatography and MALDI-TOF mass spectrometry a software operated robotic interface Probot of Bioanalytical Instruments (Bensheim, Germany) was used. The eluate stream coming from LC-CC was deposited dropwise on MALDI-TOF targets. The matrix was added coaxially to the eluate stream. As the matrix dithranol-LiTFA was used.

***Samples***: All samples were technical products of BASF AG, Ludwigshafen, Germany.

## Results and Discussion

The experimental setup for the coupled LC/MALDI-TOF experiments is schematically presented in Fig. 1. The chromatographic separations are carried out using standard equipment, a refractive index detector is applied for monitoring the separation [24]. The flow rate is 0.5 mL/min. After the chromatography the eluate stream is split with 40 parts of the eluate going to the detector and 1 part going to the Probot interface. With an eluate flow of 12.5 µL/min reaching the interface, the dual collection mode is used to deposit 2 µL/min of the eluate on the MALDI-TOF target. The matrix is coaxially added to the eluate stream before deposition. After the evaporation of the solvent, the MALDI-TOF target is subjected to the spectrometer and spectra are taken from all positions using an automatic scan mode.

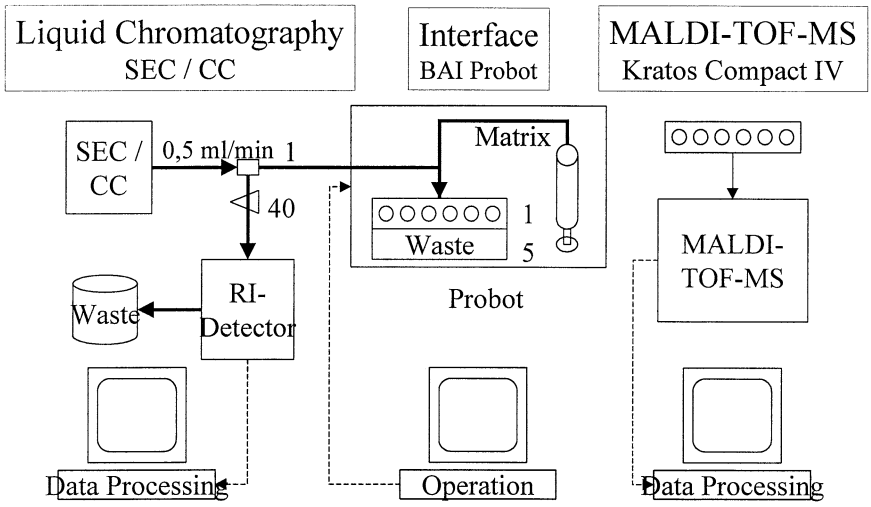



**Fig. 1** Schematic representation of the coupling of LC-CC and MALDI-TOF-MS

In a first experiment, a technical FAE with $C_{13}/C_{15}$-fatty alcohol endgroups is separated by LC-CC. The chromatogram in Fig. 2 clearly indicates the presence of different functionality fractions. Tentatively, the first peak at 2.5 mL can be assigned to polyethylene glycol while the second and third peaks at 16 and 30 mL, respectively, are assumed to correspond to the $C_{13}$- and the $C_{15}$-FAE. The minor peaks that are identified in the chromatogram are assumed to be due to different isomeric structures of the endgroups.

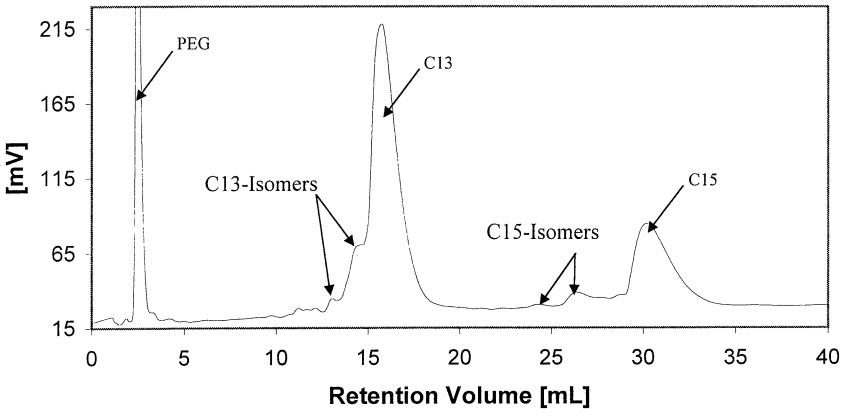
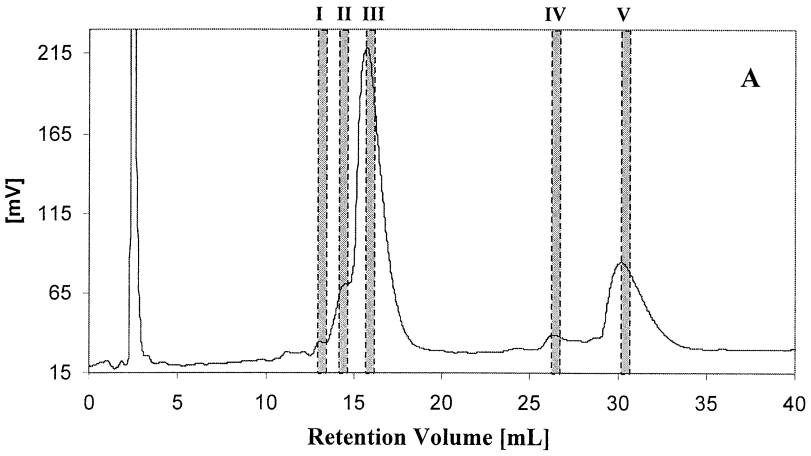
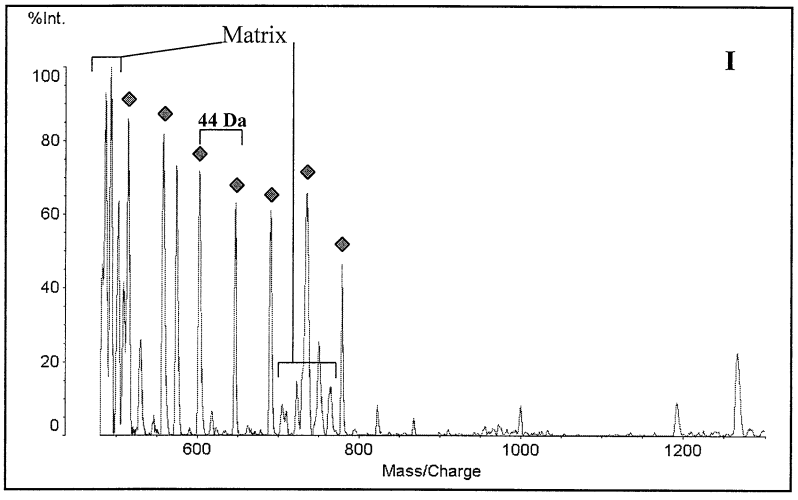
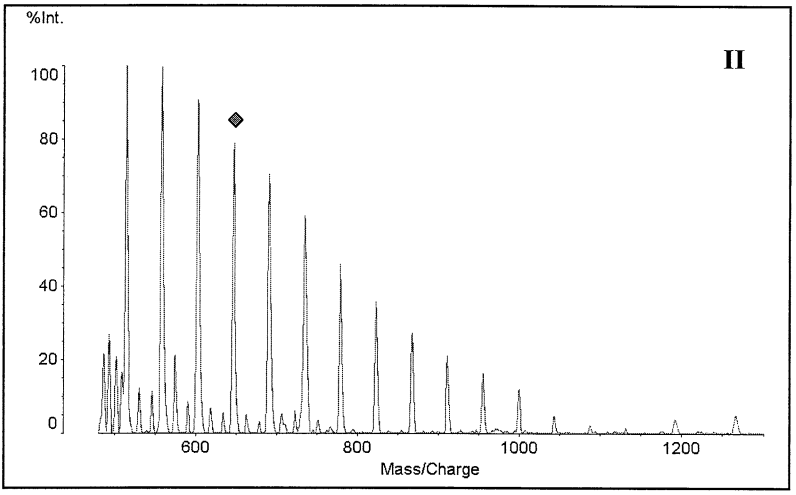


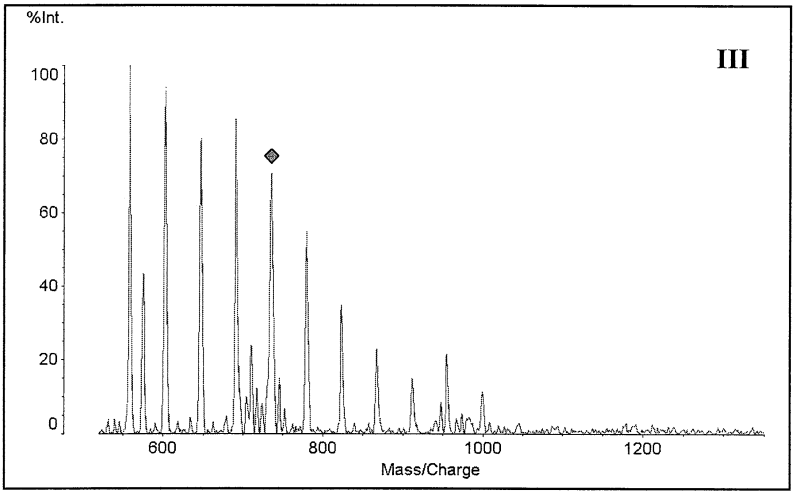

**Fig. 2** LC-CC chromatogram of a $C_{13}/C_{15}$-fatty alcohol ethoxylate, stationary phase: M&N $100C_{18}$, mobile phase: methanol-water 79:21 v/v, RI detector

The identification of the different elution peaks is done by the combination of LC-CC with MALDI-TOF mass spectrometry. The sample is separated with respect to functionality by LC-CC and different fractions are subjected to MALDI-TOF mass spectrometry via automated fraction collection and deposition on the MALDI-TOF target. The LC-CC chromatogram indicating the different fractions and the corresponding mass spectra are presented in Fig. 3.




%Int.
II
100
80
60
40
20
0
600
800
1000
1200
Mass/Charge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Mass/Charge

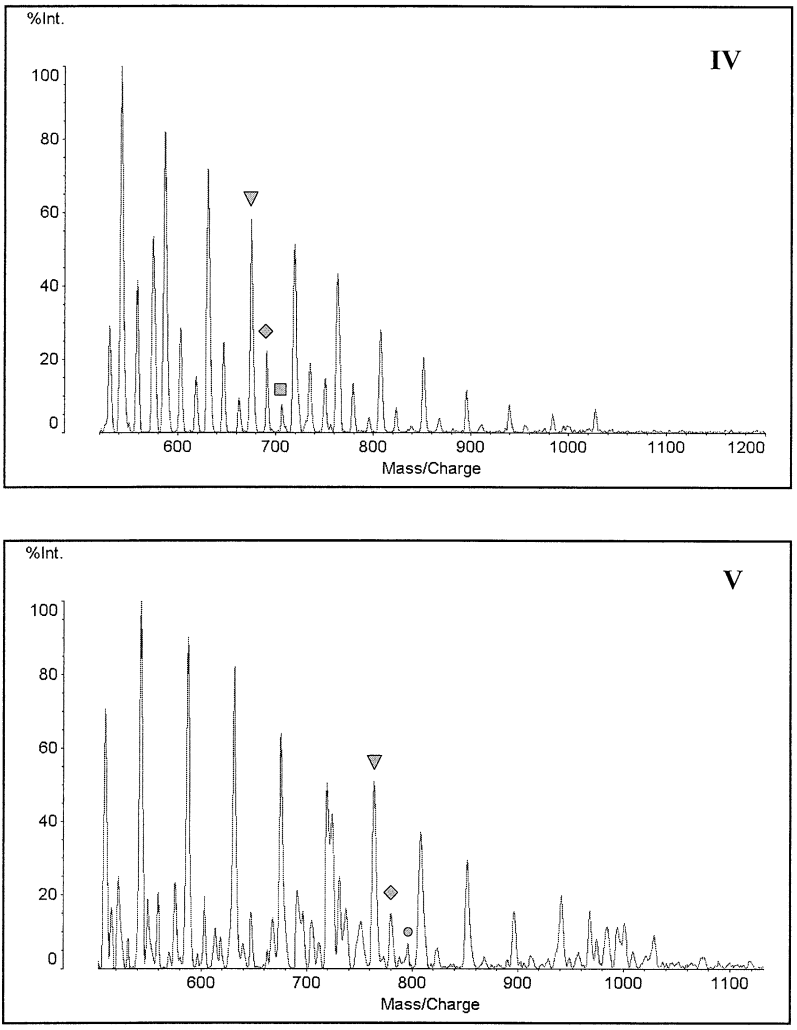



**Fig. 3** LC-CC (A) and MALDI-TOF analysis of a $C_{13}/C_{15}$-fatty alcohol ethoxylate, numbers I-V indicate the different fractions

As can be seen, all MALDI-TOF spectra exhibit well resolved mass peaks that can be assigned to the different oligomer series. The peak-to-peak mass increment in all cases is 44 Da and indicating that all oligomer series have a polyethylene oxide polymer backbone.

Fractions I-III show oligomer peaks that can be assigned to the $C_{13}$-PEO fraction. Due to the addition of lithium trifluoroacetate (LiTFA) as cationization agent, all mass peaks correspond to the $[M+Li]^+$-molecular ions. The peak masses of this series correspond to $[M+Li]^+ = 207 + 44n$, where n is the degree of polymerization. The fact that the $C_{13}$-oligomer series is found in fractions I, II, and III indicates that the $C_{13}$-endgroup has three different oligomers. LC-CC separates with regard to the chemical composition of endgroups and even different isomers can be separated. Unfortunately, MALDI-TOF does not differentiate between isomers since separation occurs with regard to mass/charge ratio.

While in fractions I-III one oligomer series each have been found in the MALDI-TOF spectra, fractions IV and V exhibit three oligomer series. These also have peak-to-peak mass increments of 44 Da and correspond to ethylene oxide based oligomer series. Mass difference between the oligomer series is 16 Da and equals the mass difference between $Li^+$, $Na^+$, and $K^+$. Accordingly, the oligomer series can be assigned to the $[M+Li]^+$-, $[M+Na]^+$-, and $[M+K]^+$-molecular ions. Similar to fractions I-III it can be assumed that fractions IV and V are due to different isomeric endgroups.

The analysis of a technical FAE with $C_{16}/C_{18}$-fatty alcohol endgroups is described in the next application. The off-line MALDI-TOF spectrum of this sample is shown in Fig. 4 and exhibits two oligomer series that can be assigned to the $[M+Li]^+$-molecular ions of the $C_{16}$-series (▽) and the $C_{18}$-series (◇), respectively.

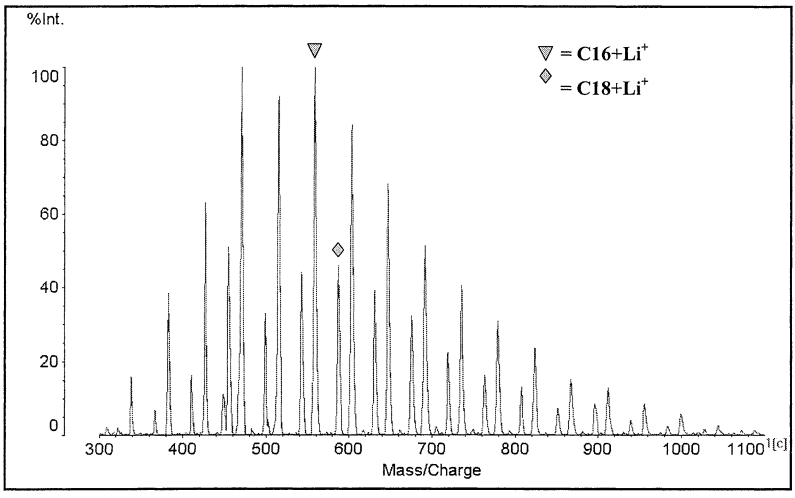



**Fig. 4** MALDI-TOF spectrum of a $C_{16}/C_{18}$-fatty alcohol ethoxylate

The LC-CC separation of this sample is presented in Fig. 5.

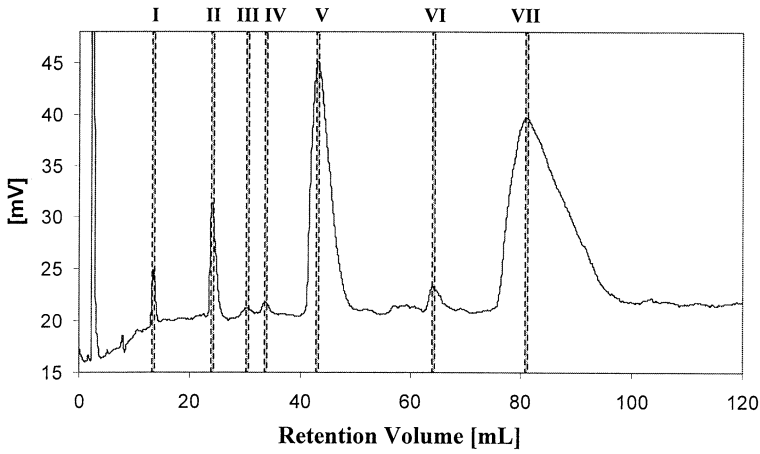



**Fig. 5** LC-CC separation of a $C_{16}/C_{18}$-fatty alcohol ethoxylate, numbers I-VII indicate the different fractions taken for MALDI-TOF analysis, stationary phase: M&N $100C_{18}$, mobile phase: methanol-water 79:21 v/v, RI detector

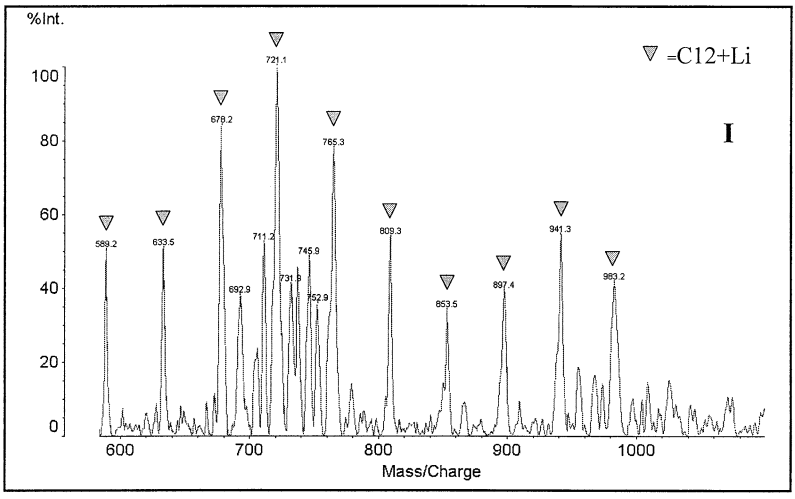
While in the off-line MALDI-TOF spectrum only two functionality fractions were identified, the LC-CC separation reveals more than five elution peaks corresponding to different functionalities. As in the previous case, the first elution peak is due to polyethylene glycol. Assuming that the detector response factors of the different fractions are rather similar, a first information on the relative concentrations of the fractions can be obtained, see Table 1.

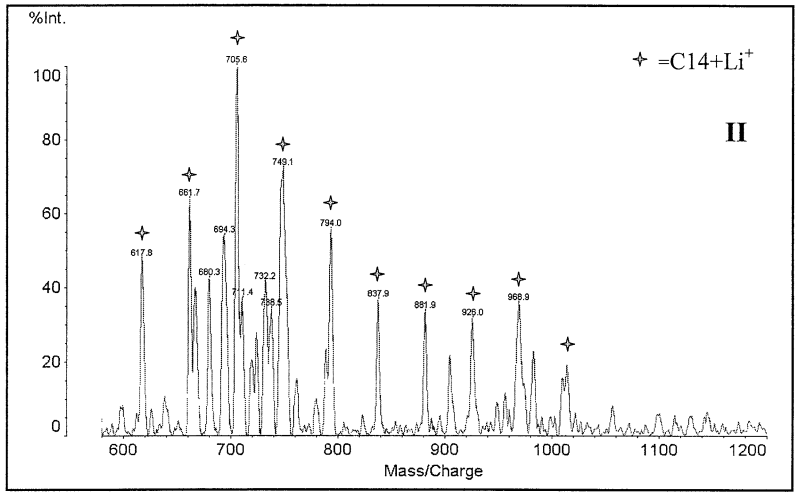
**Table 1** Relative concentrations of the chromatographic fractions determined through the peak areas

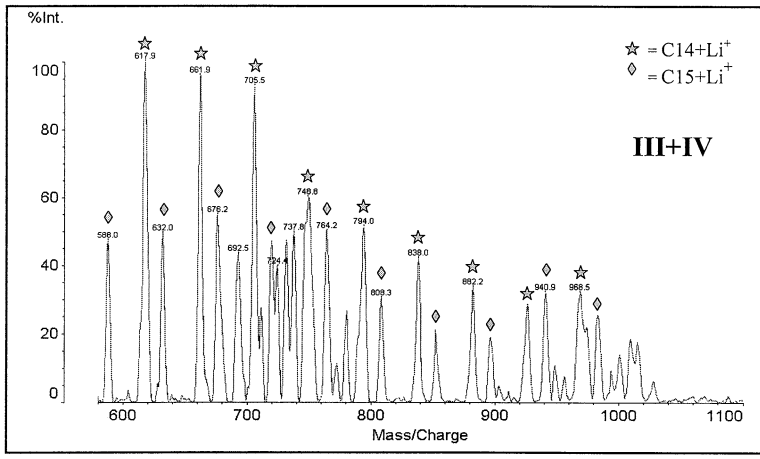
| Peak | relative concentration [%] |
|---|---|
| I | 1.0 |
| II | 4.6 |
| III | 4,7 |
| IV | 5.3 |
| **V** | **28,6** |
| VI | 2,7 |
| **VII** | **60,2** |

The fractions of highest concentration obviously are fractions V and VII. Considering the off-line MALDI-TOF analysis, these fractions can tentatively be assigned to the $C_{16}$- and the $C_{18}$-oligomer series, respectively.

The unambigous analysis of the chromatographic peaks is done by coupled LC-CC and MALDI-TOF. The MALDI-TOF spectra of the fractions are presented in Fig. 6.




%Int.
☆ = C14+Li+
◊ = C15+Li+
III+IV
617.9
661.9
705.5
586.0
632.0
676.2
692.5
724.4
737.8
749.8
764.2
794.0
809.3
838.0
882.2
940.9
968.5
100
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40
20
0
600
700
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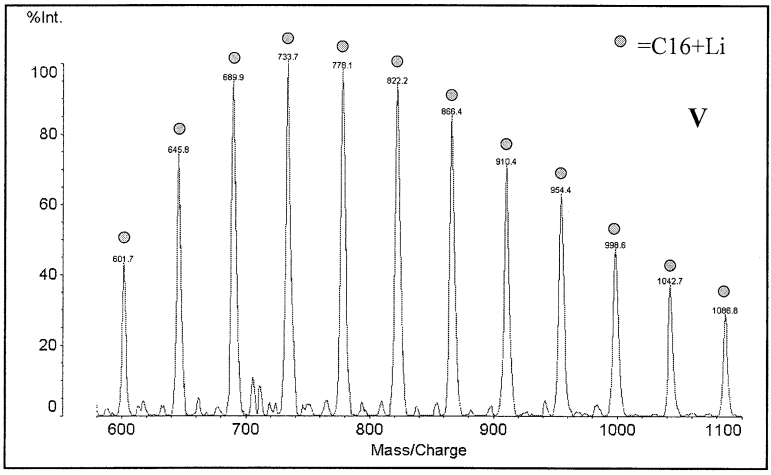
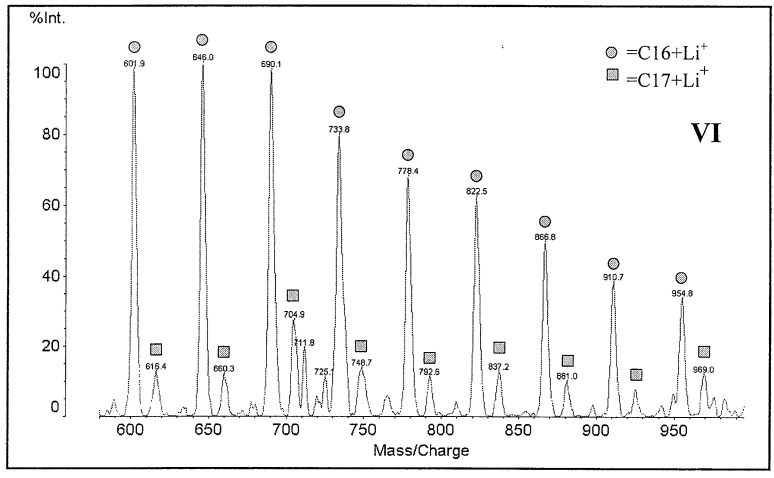
1000
1100
Mass/Charge


%Int.
=C16+Li
V
601.7
645.8
689.9
733.7
778.1
822.2
866.4
910.4
954.4
998.6
1042.7
1086.8
100
80
60
40
20
0
600
700
800
900
1000
1100
Mass/Charge


%Int.
=C16+Li+
=C17+Li+
VI
601.9
646.0
690.1
733.8
778.4
822.5
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954.8
616.4
660.3
704.9
711.8
725.1
748.7
792.6
837.2
881.0
969.0
100
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Mass/Charge

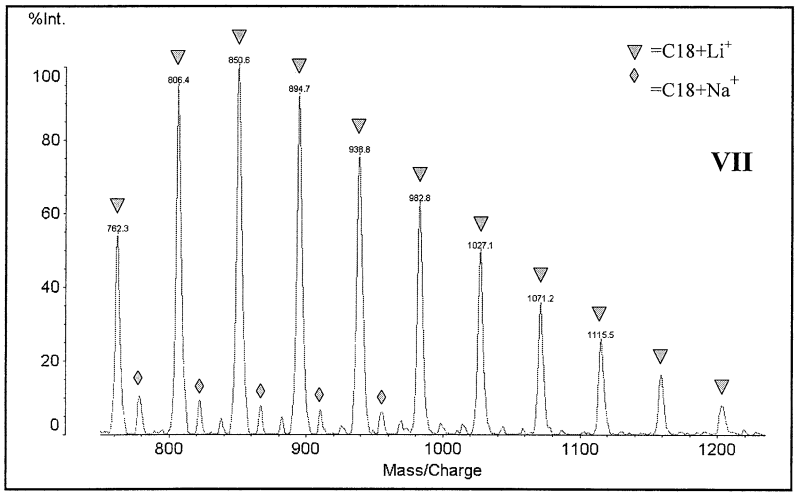


**Fig. 6** MALDI-TOF spectra of LC-CC fractions from the separation of a $C_{16}/C_{18}$-fatty alcohol ethoxylate, numbers I-VII indicate the different fractions taken for MALDI-TOF analysis

The MALDI-TOF analyses of the fractions give a clear picture of the molecular heterogeneity of the present sample. All fractions can be assigned to fatty alcohol ethoxylate oligomer series. The only difference between the fractions is the type of endgroup. In most cases, the oligomer peaks are due to $[M+Li]^+$-molecular ions.

The MALDI-TOF spectrum of fraction I in Fig. 6 shows an oligomer series with a calculated endgroup mass of 169.3 Da. This mass corresponds to a $C_{12}$-fatty alcohol endgroup (▼). The mass range is 500-1,000 Da. Accordingly, the elution peak I in Fig. 5 can be assigned to a $C_{12}$-PEO functionality fraction. The oligomer distribution in fraction II also exhibits a mass increment of 44 Da (★). The mass of the endgroup is 197.4 Da, corresponding to a $C_{14}$-fatty alcohol endgroup. The mass range in this case is 500-1050 Da.

Following the analysis of fractions I and II, the other fractions can be identified as follows: the oligomer distribution (◆) in fractions III+IV has an endgroup mass of 211.4 Da and corresponds to a $C_{15}$-PEO functionality fraction. The mass peaks in fraction V are due to a $C_{16}$-PEO functionality fraction (●), while the oligomer series in fractions VI and VII can be assigned to $C_{17}$-PEO (■) and $C_{18}$-PEO functionality fractions (▼). All oligomer series are in the mass range of 500-1,500 Da.

In conclusion, the combination of liquid chromatography and MALDI-TOF mass spectrometry is a powerful tool for the detailed analysis of complex polymer systems. Fatty alcohol ethoxylates can be analyzed with regard to functionality and molar mass which is not possible by either LC-CC or MALDI-TOF alone.

## Acknowledgement

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