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Towards a molecular characterization of pharmaceutical excipients: Mass spectrometric studies of ethoxylated surfactants

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Abstract

The in-depth characterization of excipients is a prerequisite for their safe application in pharmaceutical products. In case of surfactants, this task can be a challenge, since many industrial products are mixtures of variable composition. In this work, mass spectrometric methods are applied to characterize some ethoxylated surfactants that are widely used by the pharmaceutical industry. Among them are ethoxylated fatty alcohols with ether structure (e.g., Brij®), ethoxylated fatty acids with ester structure (e.g., Myrj®), ethoxylated sorbitane fatty acid esters (e.g., Tween®), ethoxylated glycerides (e.g., Tagat®), and Triton® X-100. MALDI-TOF mass spectrometry is best suitable to obtain molecular mass distributions of polymeric products, namely those with higher molecular mass. Electrospray and nanoelectrospray molecular mass shows a greater tendency for multiple charges. However, it is best suitable for small MM products, and multiple charges have been de-convoluted successfully using the MaxEnt™ 3 algorithm. Tandem mass spectrometry helps to identify the chemical composition, e.g. for identification of acyl chains. The work is intended to serve as a reference for mass spectrometric characterization of surfactants in the course of R&D, validation or change control.

Keywords: Surfactants; Mass spectrometry; MALDI; ESI; Brij; Myrj; Tagat; Tween

1. Introduction

Excipients are important constituents of pharmaceutical preparations, and the choice of excipients can determine the quality of a product. Frequently bioavailability, therapeutic activity and stability of preparations are influenced remarkably by excipients. Surfactants are extremely important, versatile excipients. They can be divided into anionic, cationic, and nonionic surfactants. The latter can be further divided into more lipophilic and more hydrophilic compounds. Ethoxylation is a common way to increase the hydrophilicity of nonionic surfactants. Since polyethylene glycol (PEG, also known as Macrogol) is strongly hydrophilic, the degree of ethoxylation, i.e. the number and/or length of PEG side chains determines the hydrophilicity of a surfactant. The amphiphilic nature can be described by the HLB value (hydrophilic lipophilic balance) according to Griffin (1949). When used as an emulsifier, surfactants with HLB < 10 result in water-in-oil emulsions (W/O), whereas surfactants with HLB > 10 give oil-in-water emulsions (O/W). Frequently, two or more emulsifiers are blended to obtain a product with tailored characteristics, and the HLB of the mixture can be theoretically calculated from the HLBs of the single components.

Hydrophilic nonionic surfactants are widely used as detergents or solubilizers. For technical purposes such ethoxylated surfactants are characterized by HLB value, average molecular mass, and viscosity. However, practical experience tells that these parameters alone are not sufficient to foresee the characteristics, especially when surfactant mixtures are applied. This can make the setup of new recipes, the change of parameters or even the use of a new batch a trial-and-error approach. Responsible for these effects is the variability in the molecular structure, namely the degree of ethoxylation and the chain length of fatty acids or alcohols. Therefore, an in-depth characterization on the molecular level is required. This might also help to understand the multidrug resistance modulating effect of such surfactants (Lo, 2003).

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Mass spectrometry is a powerful tool for the investigation of polymers (Montaudo and Lattimer, 2001), in particular MALDI-TOF mass spectrometry (Karas and Hillenkamp, 1988; Pasch and Schrepp, 2003). Recently, some commercial polysorbates have been studied with the help of MALDI-TOF MS (Ayorinde et al., 2000; Frison-Norrie and Sporns, 2001). The surfactant Cremophor® EL, which is widely used in pharmaceutical preparations for parenteral application, was investigated, too (Meyer et al., 2001, 2002a,b). In that case a structure elucidation was urged by reports on serious side effects such as anaphylactic reactions caused by byproducts (Meyer et al., 2002a and literature given there). MALDI-TOF was compared to conventional chromatographic methods in detergent analysis (Cumme et al., 1997). The coupling of liquid chromatography and MALDI-TOF MS via a robotic interface has recently been applied to analyze some fatty alcohol ethoxylates (Spriestersbach et al., 2003). Electrospray MS (Yamashita and Fenn, 1984; Whitehouse et al., 1985) is a viable alternative for the analysis of ethoxylated compounds, especially for low MM compounds, when matrix peaks disturb the evaluation of MALDI spectra. However, suppression effects are more prominent than with MALDI, but they are reduced in nanoelectrospray due to the improved ionization efficiency. The interpretation of ESI mass spectra is frequently complicated by the presence of multiply charged species. The software MaxEnt™ is designed for de-convolution of multiply charged raw data into monoisotopic singly charged spectra (Ferrige et al., 1992). It is applied widely to proteins and peptides but so far not to polymers, which was subject to this study. The big advantage of ESI however is its applicability as an online interface in LC/MS. It has been used for quantification of ethoxylated surfactants in pharmaceutical and environmental studies (Sparreboom et al., 2002; Barco et al., 2003; Baker et al., 2004; Cantero et al., 2005).

The investigated compounds include polyoxyethylene fatty ethers such as the Brij® series, polyoxyethylene fatty esters such as the Myrj® series, ethoxylated sorbitane fatty acid esters (e.g., Tween®) ethoxylated glycerides (e.g., Tagat®), and the alkylphenylethoxylate Triton® X-100. The Brij® series is stable to acids and alkalies and serves as O/W or W/O emulsifier, solubilizer for oils and wetting agents that are widely used in pharmaceutical products, personal care, fiber finish, household and industrial cleaning, crop protection, adhesives, paints and coatings. Myrj® is used as O/W emulsifiers, frequently blended with sorbitane fatty acid esters (Span®), and applied in the same fields as Brij®.

Tween® products are obtained from Span® by ethoxylation and serve as O/W emulsifiers with applications as mentioned before. The Tagat® series consists of ethoxylated glyceryl or castor oil esters. Triton® X-100 is a widely used biochemical reagent.

The purpose of this study was to show the possibilities of mass spectrometry to characterize ethoxylated nonionic surfactants. Characterization was mainly carried out using MALDI-TOF MS. Some typical and instructive nano-ESI mass spectra are shown to demonstrate the differences of this technique compared to MALDI.

2. Experimental

2.1. Materials

The investigated surfactants were from different suppliers as indicated: Uniqema (Everberg, Belgium), Goldschmidt (Essen, Germany), Serva (Heidelberg, Germany), Merck (Darmstadt, Germany), Ferak (Berlin, Germany), COM Pharma (Hamburg, Germany), Caelo (Hilden, Germany) and Sigma (Deisenhofen, Germany).

2.2. MALDI-TOF

MALDI-TOF experiments were carried out using a delayed extraction time-of-flight (TOF) mass spectrometer Voyager-DE™ PRO (Applied Biosystems, Framingham, MA, USA) equipped with a pulsed nitrogen laser ($\lambda = 337$ nm, 3 ns pulse width, 20 Hz repetition rate). The instrument was calibrated using calibration mixtures 1 and 2 of the Sequazyme™ Peptide Standards Kit (Applied Biosystems). As matrix a solution of 10 mg/mL of CHCA in acetonitrile/0.1% TFA (1:1, v/v) was used. The samples (typical concentration, 100 μg/mL) were mixed with the matrix solution 1:9 (v/v). 0.7 μL of the resulting mixture were applied to each spot of the sample plate using the dried droplet method. Then, the samples were dried in a gentle stream of air at ambient temperature (24 °C). Measurements were performed operating in the positive-ion reflector mode at a total acceleration voltage of 20 kV, grid voltage set to 75%, 0.005% guide wire voltage and an extraction delay of 100 ns. Survey spectra for the *m/z* range of between 200 and 3000 were obtained by accumulating data from 500 laser shots. If required, a low mass gate set to 400 u was used to prevent detector saturation from matrix peaks. The mass spectra were evaluated with Data Explorer™ (version 4, Applied Biosystems).

2.3. Nanoelectrospray

Nanoelectrospray measurements were carried out using a quadrupole time-of-flight mass spectrometer Q-TOF-2™ (Waters Micromass, Manchester, UK) equipped with a nanoelectrospray ZSpray™ source. The nano-ESI glass capillaries were obtained precoated from DNU (Berlin, Germany). The instrument was calibrated using a mixture of sodium iodide and caesium iodide. Two microliters of the sample solution were loaded into the capillary using Microloader pipette tips (Eppendorf, Hamburg, Germany).

The typical operating conditions for the qTOF mass spectrometer were as follows: capillary voltage, 900 V; sample cone voltage, 35 V; source temperature, 80 °C. The instrument was operated in the positive ion mode (unless otherwise indicated). Full scans were performed between *m/z* 50 and 3000. All mass spectra were recorded in the profile and processed using MassLynx™ (version 3.4, Micromass). When indicated, mass spectra were processed by the MassLynx™ add-on Maximum Entropy™ 3 (MaxEnt™ 3). The algorithm of MaxEnt™ 3 deconvolutes charge state and isotopic information in a

continuum spectrum to generate a centroid spectrum containing only monoisotopic, singly charged peaks.

3. Results and discussion

3.1. MALDI-TOF MS

MALDI-TOF mass spectrometry is a suitable method for structure elucidation of polyethoxylated multicomponent mixtures, since the softness of ionization conditions prevents fragmentation, which would be detrimental for evaluation. In positive mode, instead of protonation, polyethoxylates are ionized by adduct formation with alkali metal ions, mostly sodium or potassium, which can lead to different homologue series referring to $[M+Na]^+$, $[M+K]^+$, and more rarely $[M+Li]^+$. Furthermore, the single charge state is found almost exclusively, which facilitates molecular mass calculations. However, the problem of varying end groups remains, e.g. due to the presence of different fatty acid residues.

The assignment of a homologue peak series to the corresponding polyethoxylate and thus the identification of the components is achieved by calculating the residue masses:

$$m_{\mathrm{res,calc}} = m_{\mathrm{signal}} - n m_{\mathrm{mon}}$$

where m_{signal} is the observed mass and m_{mon} the mass of the repeat unit, in case of polyethoxylates $m_{\mathrm{mon}} = 44$ u. The calculated residue mass consists of the mass of the residue $m_{\mathrm{res,\,theo}}$ and the mass of the alkali metal ion that forms the particular adduct. In other words, the subtraction of adduct mass and residue mass from the observed mass must give a multiple of $m_{\mathrm{mon}} = 44$ u.

3.1.1. Polyoxyethylene fatty ethers (see Table 1)

Molecular masses can be calculated from the end group, the fatty alcohol ($m = 186$ u in case of lauryl alcohol), plus $n \times 44$ u for the POE repeat unit, plus the mass of the alkali ion (sodium, $m = 23$ u, and potassium, $m = 39$ u, respectively).

Brij® 30 is declared to be POE (4) lauryl ether. In case of Brij® 30 (Uniqema), the potassium adduct series showed the highest intensity, with a distribution corresponding to $n = 4$–15 (maximum at $n = 6$). Besides, a series of the corresponding sodium adducts coexisted. Brij® 30 (Serva) yields the sodium adducts from $n = 4$–14 (maximum at $n = 6$). Another series with $\Delta m = 16$ u results presumably from $[M+Li]^+$.

Brij® 35 is declared to be POE (20) lauryl ether. In case of Brij® 35 (Uniqema), the sodium adducts prevail, with a distribution corresponding to $n = 8$–38 (maximum at $n = 23$). The potassium adducts and another series with $\Delta m = 28$ u follow, the latter presumably corresponding to the myristyl ethers. Brij® 35 (Merck) yields distributions corresponding to $n = 8$–37 (maximum at $n = 22$).

Brij® 35 (Ferak), a relatively old product, shows remarkable differences. Only sodium adducts were observed corresponding to $n = 10$–38 (maximum at $n = 25$). In addition, series with $\Delta m = 28$ u and $\Delta m = 56$ u follow, representing the myristyl and cetyl ethers, respectively.

Table 1
Investigation of polyethoxylated fatty alcohols with MALDI-TOF MS

Product	Structure	Distribution	Max.	Int.
Brij® 30 (Uniqema)				
$[M+Na]^+$	POE (n) lauryl ether	385.3–869.5	561.4	80
$[M+K]^+$	POE (n) lauryl ether	401.2–885.5	489.3	100
Brij® 30 (Serva)				
$[M+Na]^+$	POE (n) lauryl ether	385.2–825.4	473.3	100
Brij® 35 (Uniqema)				
$[M+Na]^+$	POE (n) lauryl ether	561–1881	1221.8	100
$[M+K]^+$	POE (n) lauryl ether	577–1897	1237.8	30
$[M+Na]^+$	POE (n) myristyl ether	589–1911	1249.8	25
Brij® 35 (Merck)				
$[M+Na]^+$	POE (n) lauryl ether	561.4–1838.2	1177.8	100
$[M+K]^+$	POE (n) lauryl ether	577.4–1810.1	1193.8	83
$[M+Na]^+$	POE (n) myristyl ether	589.4–1822.2	1205.8	33
Brij® 35 (Ferak)				
$[M+Na]^+$	POE (n) lauryl ether	649.5–1882.3	1309.9	100
$[M+28+Na]^+$	POE (n) myristyl ether	677.5–1910.4	1425.9	70
$[M+56+Na]^+$	POE (n) cetyl ether	705.5–1938.4	1365.9	33
Brij® 52 (Uniqema)				
$[M+Na]^+$	POE (n) cetyl ether	397.3–1101.8	529.4	100
$[M+K]^+$	POE (n) cetyl ether	413.3–941.7	545.4	28
Brij® 52 (Serva)				
$[M+Na]^+$	POE (n) cetyl ether	397.3–1233.7	441.3	100
$[M+K]^+$	POE (n) cetyl ether	413.2–941.5	501.3	14
Brij® 58 (Serva)				
$[M+Na]^+$	POE (n) cetyl ether	573.4–1808.2	1189.8	100
$[M+K]^+$	POE (n) cetyl ether	765.5–1734.1	1205.8	19
Brij® 96 V (Uniq.)				
$[M+Na]^+$	POE (n) oleyl ether	467.3–1391.8	775.5	95
$[M+K]^+$	POE (n) oleyl ether	483.3–1363.8	791.5	88
$[M+Na]^+$	POE (n) cetyl ether	441.3–1365.9	749.5	100
$[M+K]^+$	POE (n) cetyl ether	457.3–1337.8	765.5	80

Intensities are related to the maximum and are given in % of base peak.

Brij® 52 is theoretically POE (2) cetyl ether with an HLB of 5.3, what makes it one of the most lipophilic compounds of the series. However, the investigated products show a different picture. Brij® 52 (Uniqema) shows a $[M+Na]^+$ series corresponding to $n = 3$–19 (max. $n = 6$) and a $[M+K]^+$ series with lesser intensity. Brij® 52 (Serva) is very similar.

Brij® 58 is described as POE (20) cetyl ether. Brij® 58 (Serva) shows an almost ideal bell-shape distribution representing $n = 7$–35 with a maximum at $n = 21$. The potassium series follows with weaker intensity (Fig. 1).

Brij® 96 V (Uniqema) should be POE (10) oleyl ether. Surprisingly, the MALDI-TOF mass spectra reveal four series of high intensity referring to the sodium and potassium adducts of the oleyl and cetyl ethers, respectively. The degree n ranges from approx. 5–25 (max. $n = 11$).

3.1.2. Polyoxyethylene fatty esters (see Table 2)

Molecular masses can be calculated from the end group, the fatty acid ($m = 284$ u in case of stearic acid), plus $n \times 44$ for the POE repeat unit, plus the mass of the alkali ion (sodium, $m = 23$ u, and potassium, $m = 39$ u, respectively).

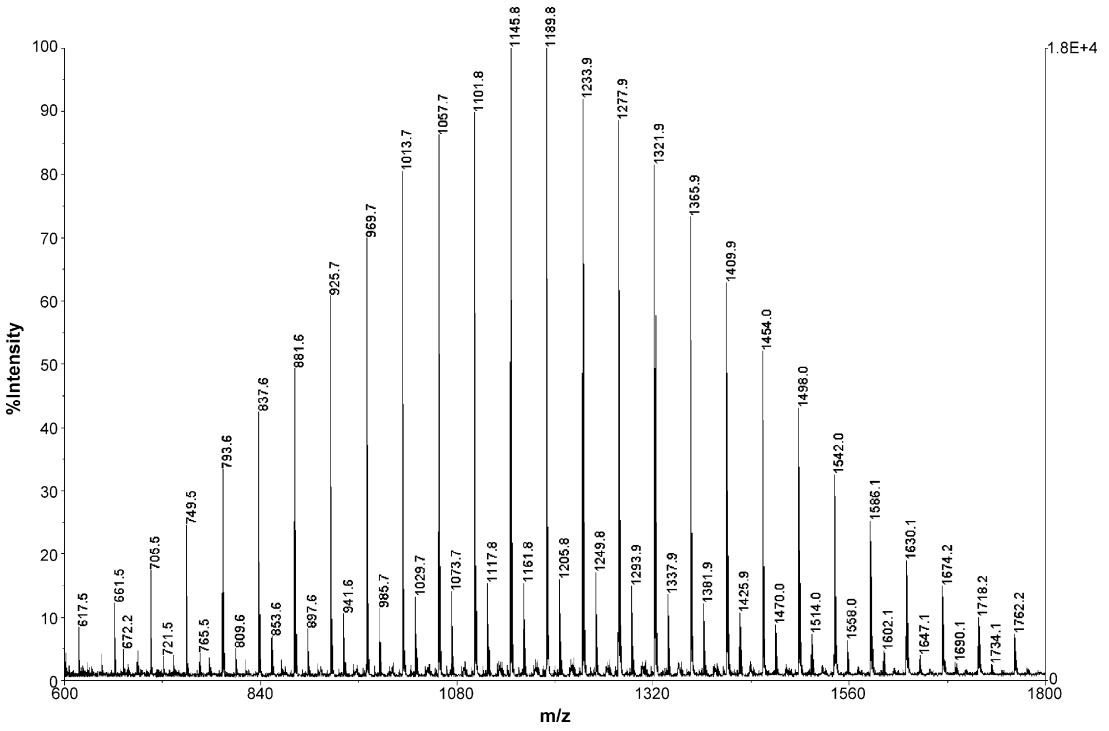

Fig. 1. MALDI-TOF mass spectrum of Brij® 58 (Serva).

Table 2
Investigation of polyethoxylated fatty acids with MALDI-TOF MS

Product	Structure	Distribution	Max.	Int.
Myrj® 45 (Uniqema)				
$[M+Na]^+$	POE (*n*) stearate	483.4–923.7	659.5	75
$[M+K]^+$	POE (*n*) stearate	499.4–939.7	675.5	100
$[M+K]^+$	POE (*n*) palmitate	471.3–911.6	647.5	63
Myrj® 52 (Serva)				
$[M+Na]^+$	POE (*n*) palmitate	543.4–763.6	631.5	100
$[M+Na]^+$	POE (*n*) stearate	571.4–791.6	659.5	92
$[M+Na]^+$	POE (*n*) oleate	965.6–1582.1	1317.9	42
$[M+Na]^+$	POE (*n*) stearate	1363.9–2024.4	1628.1	70
$[M+K]^+$	POE (*n*) stearate	1380.0–2040.4	1644.2	45
Myrj® 52S (Uniq.)				
$[M+K]^+$	POE (*n*) stearate	1424.0–2172.5	1776.2	100
$[M+Na]^+$	POE (*n*) stearate	1408.0–2156.5	1760.2	67
$[M+K]^+$	POE (*n*) palmitate	1528.0–2100.4	1748.2	31
Myrj® 53P (Uniq.)				
$[M+Na]^+$	POE (*n*) palmitate	1379.6–2039.9	1687.8	42
$[M+Na]^+$	POE (*n*) oleate	1405.6–2065.9	1713.7	59
$[M+Na]^+$	POE (*n*) stearate	1627.7–2728.4	2112.0	71
$[M+K]^+$	POE (*n*) stearate	1643.7–2744.4	2217.0	100
$[M+K]^+$	POE (*n*) palmitate	1880.9–2676.3	2322.1	43
Cremophor® S9 (Caelo)				
$[M+Na]^+$	POE (*n*) stearate	483.4–923.7	659.5	50
$[M+K]^+$	POE (*n*) stearate	499.4–939.6	675.5	100
$[M+K]^+$	POE (*n*) palmitate	471.3–911.6	647.4	59
Cremophor® S9 (COM-Pharma)				
$[M+Na]^+$	POE (*n*) stearate	483.4–923.7	659.5	38
$[M+K]^+$	POE (*n*) stearate	499.4–939.6	675.5	100
$[M+K]^+$	POE (*n*) palmitate	471.3–911.6	647.4	80

Myrj® 45 is an ester product, described as POE (8) stearate. Myrj® 45 (Uniqema) shows a distribution corresponding to $n=4$–14 (max. $n=8$). The $[M+K]^+$ series shows the highest intensity, accompanied by $[M+Na]^+$. Another series can be observed that can be deduced to be $[M+K]^+$ of the palmitate esters. Unfortunately, the possibly also existing $[M+Na]^+$ is hidden under the isobaric $[M+K]^+$ of the stearates (Fig. 2). The spectrum of Myrj® 52 (Serva) appears to be an overlay of several distributions. In general, sodium adducts dominate, but potassium adducts can also be found, as long as the intensity is high enough and they are not hidden under overlapping distributions. First, there are distributions with $n=6$–11 (max. $n=8$) with an palmitoyl and stearoyl residue, respectively. $\Delta m=28$ u between both series indicates this relation. Second, there are the $[M+Na]^+$ and $[M+K]^+$ series of the stearates with $n=22$–42 (max. $n=30$). In between lies another $[M+Na]^+$ distribution, this time with an oleoyl residue ($n=15$–29; max. $n=23$). This astonishingly complex mixture might be the result of blending.

Myrj® 52S (Uniqema) looks similar. At least three distributions can be distinguished: $[M+K]^+$ and $[M+Na]^+$ referring to the stearate esters ($n=25$–42; max. $n=33$), and the $[M+K]^+$ series referring to the palmitate esters.

Myrj® 53P (Uniqema) shows several distributions. The main distribution represents the $[M+K]^+$ ions of the stearate esters, although it seems to be bimodal ($n=31$–55; max. $n=42$). The preceding $[M+Na]^+$ series repeats this bimodal appearance. A series of $[M+K]^+$ of the palmitate esters is shifted towards higher masses ($n=36$–54; max. $n=46$). Two series of sodium adducts referring to palmitate and oleate esters, respectively, coexist in the mass range approx. 1300–2100 u ($n=25$–40; max. $n=32$). The reason, why within the same preparation the higher

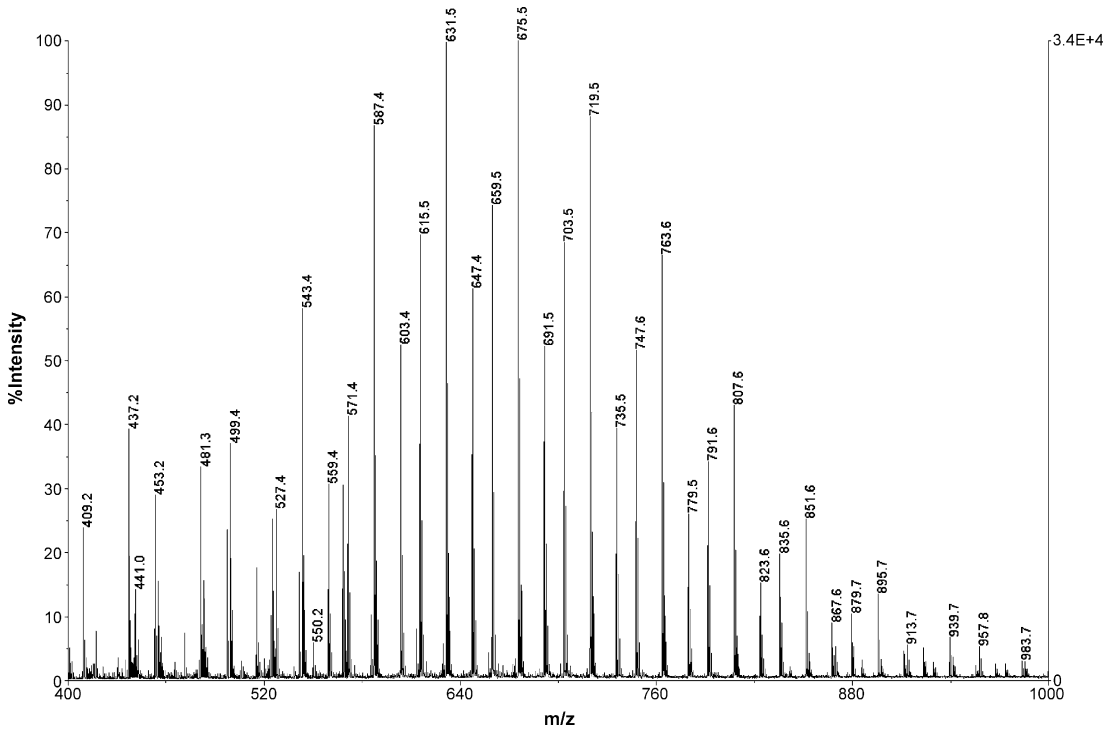

Fig. 2. MALDI-TOF mass spectrum of Myrj® 45 (Uniqema).

mass distributions show preferably potassium adducts whereas the lower mass distributions show sodium adducts, remains unclear.

Cremophor® S9 (Caelo) shows a striking similarity to Myrj® 45 (Uniqema). The same holds for Cremophor® S9 (COM-Pharma).

3.1.3. Polysorbates

Some commercial polysorbates (Tween® 20, 40, 60, and 80) have been analyzed by MALDI-TOF MS before (Ayorinde et al., 2000). We analyzed Tween® 20 (Ferak and Serva, resp.), Tween® 40 (Uniqema), Tween® 60 (Caelo), and Tween® 80 (Synopharm and Uniqema, respectively). We could confirm these results in all major points (data not shown). In strict contrast to textbook knowledge and manufacturers' declarations, which claim that polysorbates are polyethoxylated sorbitan fatty acid esters, these products are rather crude mixtures of sorbitan polyethoxylates (SPE), polysorbate diesters (PDE), polysorbate monoesters (PME), polyethylene glycols (PEG), and isosorbide polyethoxylates (IPE). $[M+Na]^+$ predominates throughout. At the first view, the spectra of Tween® 20, 40, 60, and 80 look essentially very similar. The low mass region is characterized by the IPE series, whereas the high mass region shows SPEs. The PMEs seem to be a minor component, even though quantitative calculations should be made with caution. Masses of SPEs are calculated from sorbitan ($m = 164$ u), plus $n \times 44$ for the POE repeat unit, plus the mass of the alkali ion (sodium, $m = 23$ u, and potassium, $m = 39$ u, respectively). IPEs are calculated from isosorbide ($m = 146$ u), plus $n \times 44$ for the POE repeat unit, plus the mass of the alkali ion (sodium, $m = 23$ u, and potassium, $m = 39$ u, respectively). PEGs are calculated from the end group water ($m = 18$), plus $n \times 44$ for the POE repeat unit, plus the mass of the alkali ion (sodium, $m = 23$ u, and potassium, $m = 39$ u, respectively). PMEs are calculated from the mass of sorbitan ($m = 164$ u), plus the fatty acid ($m = 284$ u in case of stearic acid), minus one water ($m = 18$ u, lost in the course of esterification), plus the mass of the alkali ion (sodium, $m = 23$ u, and potassium, $m = 39$ u, respectively). Masses for diester and triester are calculated in an analogous way.

In this work, we focus on results for Tween® 61 and Tween® 85 that were not reported before (see Table 3, Figs. 3 and 4).

Tween® 85 (Uniqema) is described as polysorbate (20) trioleate. It was not investigated before and it turned out that the MALDI-TOF mass spectra look quite different from the aforementioned Tween® 20, 40, 60, and 80. In principle, again the low mass region is dominated by the IPE and PEG series, but this time the PEGs prevail ($n = 11$–18; max. $n = 14$). In the high mass

Table 3
Investigation of polyethoxylated sorbitan fatty acid esters with MALDI-TOF MS

Product	Structure	Distribution	Max.	Int.
Tween® 61 (Serva)				
$[M+Na]^+$	Polysorbate (n) monopalmitate	601.4–821.6	689.5	100
$[M+Na]^+$	Polysorbate (n) monostearate	629.5–849.6	717.5	56
$[M+Na]^+$	SPE	363.2–583.4	451.2	31
Tween® 85 (Uniq.)				
$[M+Na]^+$	PEG	525.3–833.6	657.5	100
$[M+Na]^+$	IPE	521.2–961.6	785.5	20
$[M+Na]^+$	SPE	979.6–1860.2	1243.8	20

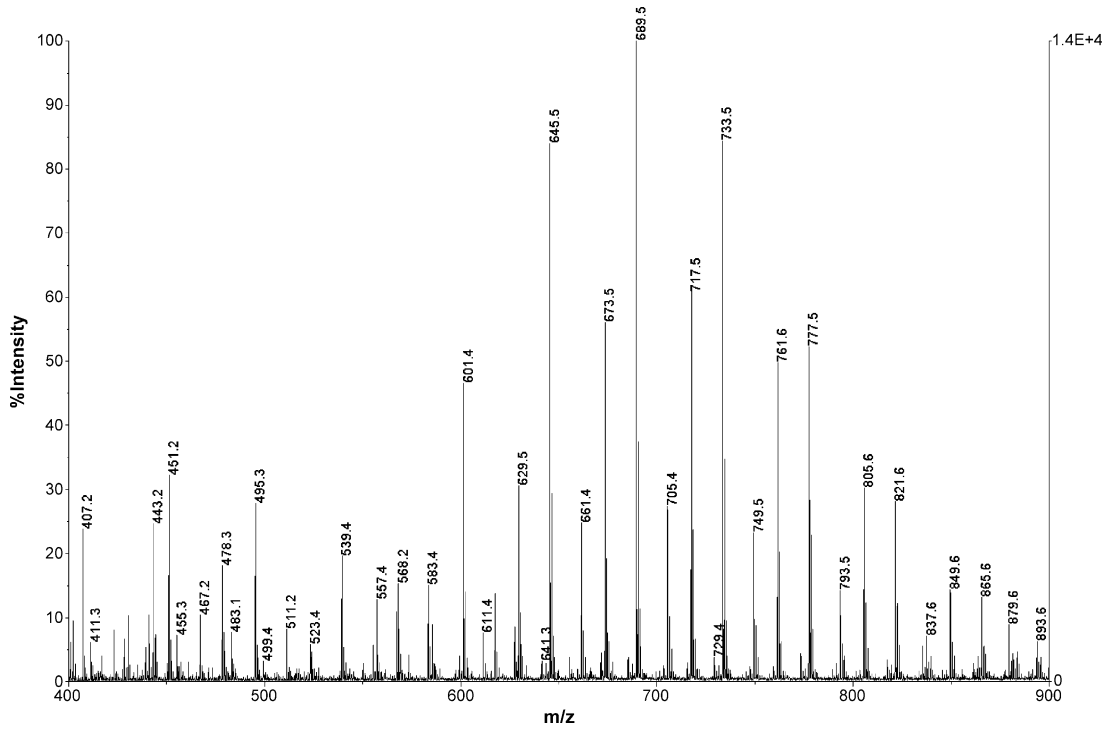

Fig. 3. MALDI-TOF mass spectrum of Tween® 61 (Serva).

region again the SPE species dominate ($n = 18$–38; max. $n = 24$), but the intensity is just 20% of the PEGs. In between the SPE series appear minor series that might result from polysorbate mono-, di-, and triesters.

Tween® 61 (Serva) is described as polysorbate (4) monostearate. This is also the first MALDI-TOF report on this. As stated elsewhere, the spectra for each Tween® species are "unique and instructive" (Ayorinde et al., 2000), and Tween® 61 looks surprisingly different. Three distinct bell-shaped distributions appear. The most intense refers to the polysorbate monopalmitate series ($n = 4$–9; max. $n = 6$). With a mass difference of $\Delta m = 28$ u appears the polysorbate monostearate series

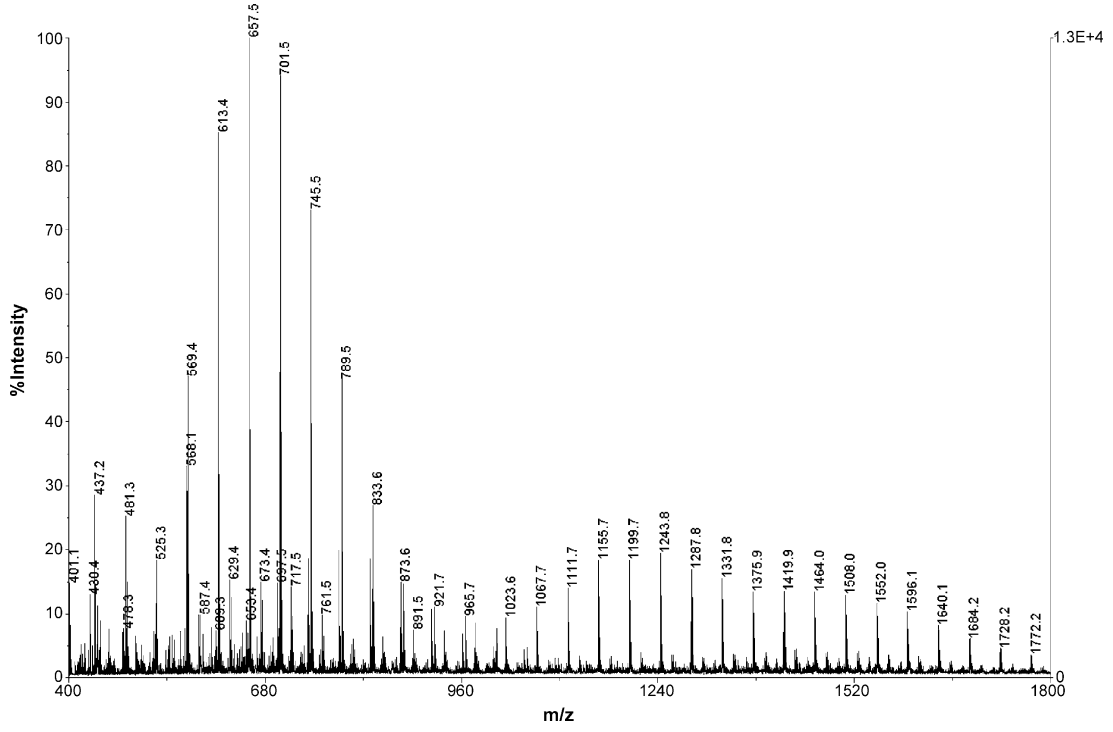

Fig. 4. MALDI-TOF mass spectrum of Tween® 85 (Uniqema).

Table 4
Investigation of polyethoxylated glycerides with MALDI-TOF MS

Product	Structure	Distribution	Max.	Int.
Tagat® L2 (Goldsch.)				
$[M+Na]^+$	POE (*n*) glycerol-monolaurate	957.6–1486.0	1177.8	100
$[M+K]^+$	POE (*n*) glycerol-monolaurate	973.6–1502.0	1237.8	75
$[M+Na]^+$	POE (*n*) glycerol	775.5–1391.9	1083.6	40
$[M+Na]^+$	POE (*n*) laurate	575.4–1015.7	795.5	48
$[M+Na]^+$	PEG	613.4–877.5	701.4	23
Tagat® S2 (Caelo)				
$[M+K]^+$	POE (*n*) glycerol	703.4–1407.9	1055.7	100
$[M+Na]^+$	POE (*n*) glycerol	731.5–1303.9	1039.7	72
$[M+K]^+$	POE (*n*) glycerol-monostearate	1013.8–1674.2	1277.9	90
$[M+Na]^+$	POE (*n*) glycerol-monostearate	1129.9–1570.1	1261.9	51
$[M+K]^+$	POE (*n*) glycerol-distearate	1147.9–1940.4	1544.2	52
$[M+Na]^+$	POE (*n*) glycerol-distearate	1308.0–1836.4	1528.2	28
$[M+K]^+$	PEG	453.3–893.6	673.4	28
$[M+Na]^+$	PEG	437.3–877.6	701.5	20
Tagat® R7 (Goldsch.)				
$[M+Na]^+$				
$[M+K]^+$	POE (*n*) glycerol-monoricinolate	529.4–1013.6	705.4	100
$[M+Na]^+$	POE (*n*) glycerol-monoricinolate	589.4–1029.6	765.4	85
$[M+Na]^+$	POE (*n*) glycerol	423.2–555.3	467.2	20
$[M+Na]^+$	POE (*n*) ricinolate	499.4–631.4	543.4	15
Tagat® CH 40 (Goldschmidt)				
$[M+K]^+$	PEG	717.4–1421.8	1069.6	100
$[M+K]^+$	POE (*n*) glycerol	1319.8–2068.2	1672.0	100
$[M+K]^+$	POE (*n*) glycerol-monoricinolate	1690.1–2218.3	1954.2	50
$[M+K]^+$	POE (*n*) ricinolate	1087.7–1572.0	1395.9	30

($n=4$–9; max. $n=6$). The SPE series that dominates the high mass region of other Tween® products is shifted to lower masses because of the relatively short PEG chains. The series appears in the region between *m/z* 350 and 600 ($n=4$–9; max. $n=6$). It is noteworthy that the polysorbate fatty acid esters dominate the spectrum of Tween® 61. This proves that they do have sufficiently good desorption/ionization properties. In reverse, i.e. that the minor intensity of the PME in other Tween® spectra indicates minor concentrations.

3.1.4. Ethoxylated glycerides (see Table 4)

Molecular masses are calculated from glycerol ($m=92$ u), plus fatty acid ($m=200$ u in case of lauric acid), minus one water ($m=18$ u, lost in the course of esterification), plus the mass of the alkali ion (sodium, $m=23$ u, and potassium, $m=39$ u, respectively). Masses for diester and triester are calculated in an analogous way.

Tagat® L2 (Goldschmidt) is described as POE (20) glycerol-monolaurate. The obtained MALDI-TOF mass spectrum (see Fig. 5) meets the expectations in the way that indeed the polyethoxylated glycerol monolaurate ester series shows the highest intensity and $n=20$ is the maximum of a bell-shape distribution. The $[M+Na]^+$ series is accompanied by a $[M+K]^+$ series, both with $n=15$–27. At least three more series can be distinguished: the polyethoxylated glycerol ($n=15$–29; max. $n=22$), the polyethoxylated laurate ($n=8$–18; max. $n=13$), and the free PEG ($n=13$–19; max. $n=15$). Tagat® S2 (Caelo) is described as POE (20) glycerol-monostearate. Therefore, one would expect a spectrum similar to Tagat® L2. However, the result was different. This time the $[M+K]^+$ is generally more intense than the corresponding $[M+Na]^+$ series, and the polyethoxylated glycerol shows the highest intensity ($[M+K]^+$: $n=13$–29; max. $n=21$). The polyethoxylated glycerol-monostearate shows a distribution from $n=14$–29 with a maximum at $n=20$. The low mass range is dominated by PEG ($n=9$–19, max. $n=14$). In the high mass range, a $[M+K]^+$ series of polyethoxylated glycerol-distearate could be clearly identified, accompanied by a $[M+Na]^+$ series and a series that can be deduced to relate to the polyethoxylated glycerol-monostearate-monopalmitates. Furthermore, a series related to the tristearates can be assumed.

Tagat® R7 (Goldschmidt) is described as POE (7) glycerol-monoricinolate. Again, the obtained spectrum shows as expected a $[M+Na]^+$ series of polyethoxylated glycerolmonoricinolate with $n=7$ as maximum of the distribution ($n=3$–14). The following $[M+K]^+$ series shows a similar distribution, but the maximum is shifted to $n=8$. This seems to be in coincidence with the observation that within the investigated substance classes higher MM compounds tend to form a higher share of $[M+K]^+$ versus $[M+Na]^+$. The polyethoxylated glycerols and polyethoxylated ricinolates also occur in the range between *m/z* 400 and 650, but the intensity is relatively weak and their assignment is hampered by disturbing peaks from MALDI matrix.

Tagat® CH 40 (Goldschmidt) should be POE (40) hydrogenated castor oil, which is known to contain triglycerides with mostly ricinolic acid, and to a lesser degree, stearic acid. The

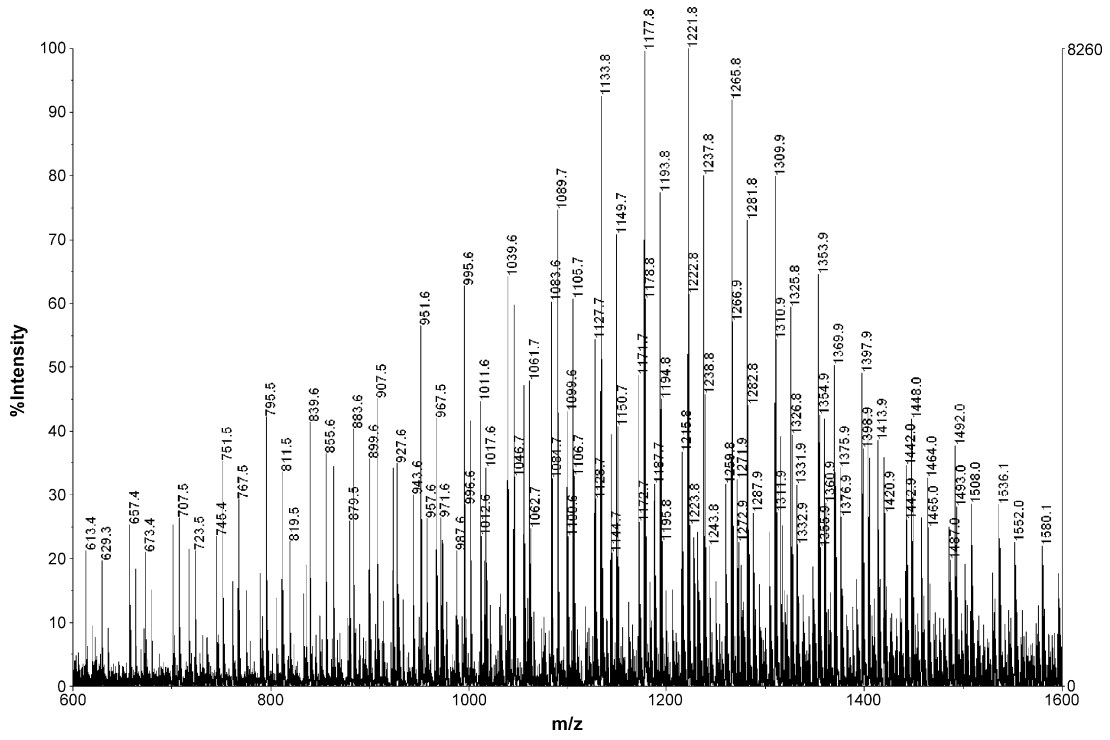

Fig. 5. MALDI-TOF mass spectrum of Tagat® L2 (Goldschmidt).

MALDI-TOF mass spectrum, however, shows none of these triesters. $[M+K]^+$ dominates throughout in case of these high MM compounds. Two series show equal intensity: in the low mass region, the PEG series ($n=15$–31; max. $n=23$). In the high mass region dominates the polyethoxylated glycerol series ($n=27$–44; max. $n=35$). Two more series have been identified: the POE (n) glycerol-monoricinolates ($n=29$–41; max. $n=35$) and the polyethoxylated ricinolates ($n=17$–28; max. $n=24$).

The MALDI-TOF mass spectrum of Triton® X-100 (see Fig. 6), a widely used biochemical reagent with a polyethoxylated tert-octylphenyl ether structure, suffers from disturbing CHCA matrix peaks in the low mass region. Apart from that, interpretation is very simple because it shows only the expected $[M+Na]^+$ series ($n=5$–20; max. $n=10$) followed by the corresponding $[M+Na]^+$ series with lesser intensity (Table 5).

Pluronic® L101 (Serva), a copolymer of ethylene glycol and propylene glycol, was also analyzed (data not shown). Multiple different combinations of the units complicate the interpretation and make high resolution a necessity. Literature (Schriemer et al., 1997) deals with this topic.

Table 5
Investigation of Triton® X-100 with MALDI-TOF MS

Product	Structure	Distribution	Max.	Int.
Triton® X-100 (Sigma)				
$[M+Na]^+$	POE (n) tert-octylphenyl ether	449.3–1109.8	669.5	100
$[M+K]^+$	POE (n) tert-octylphenyl ether	465.2–905.6	685.5	22

3.2. Nanoelectrospray MS

Nano-ESI is also a soft ionization technique that can be applied successfully to polyethoxylated surfactants. A sufficient resolution is mandatory to resolve isotope patterns, particularly in case of multiply charged species. In this study a quadrupole/time-of-flight hybrid instrument was used which combines high resolution with MS/MS capabilities. ESI spectra are complicated by the formation of multiply charged species. To overcome this drawback, we have processed the raw mass data using the software MaxEnt™ 3 to de-convolute the multiply charged raw data into singly charged, monoisotopic spectra. MaxEnt™ 3 finds the simplest molecular mass spectrum (spectrum of maximum entropy) that could account for the observed m/z data. It works iteratively starting from an initial approximation. Whereas this algorithm is widely used for peptides and other biomolecules, to our knowledge this is the first report on its application to surfactants with polymeric chains. In general, polyethoxylated compounds are preferably ionized in the positive mode. Negative mode, however, also works and is particularly useful for MS/MS studies in order to find out the fatty acid residues.

Fig. 7(A) and (B) shows nanoelectrospray mass spectra before and after MaxEnt™ 3 processing. The spectrum of Brij® 58 (Serva) confirms the structural data obtained by MALDI-TOF-MS (maximum and width of distribution), although it looks different. The adduct pattern depends on a number of factors including interface design and operational parameters. With the equipment and conditions described, $[M+Na]^+$ dominates the nanoelectrospray mass spectra. No potassium adducts are found.

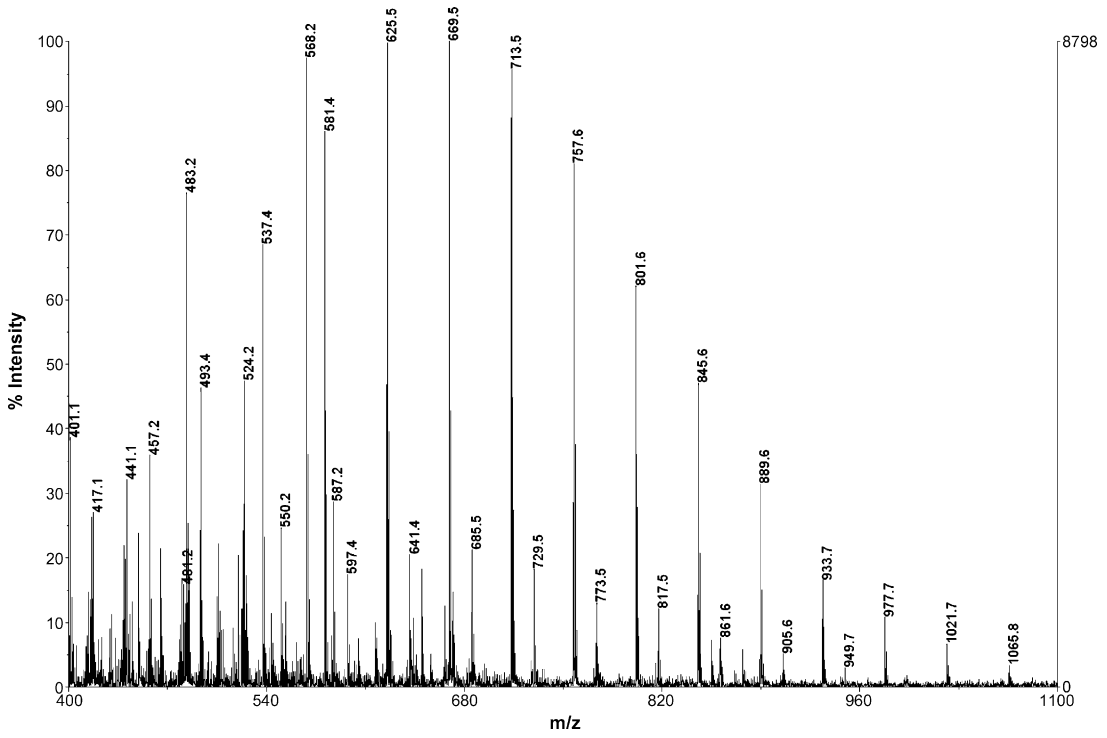

Fig. 6. MALDI-TOF mass spectrum of Triton® X-100.

As a typical feature for ESI, multiple charges are important. For Brij® 58, $[M+2\mathrm{Na}]^{2+}$ is prominent. In the raw spectrum this series appears to be shifted to smaller masses because m/z is displayed with $z=2$. In spectra processed with MaxEnt™ 3, however, the series follows the $[M+\mathrm{Na}]^{+}$ with $\Delta m=22$. The positive nano-ESI mass spectrum of Myrj® 45 (Uniqema) shows a rather confusing picture (see Fig. 8). As with MALDI, $[M+\mathrm{K}]^{+}$ predominates, but for a reason that is unknown the

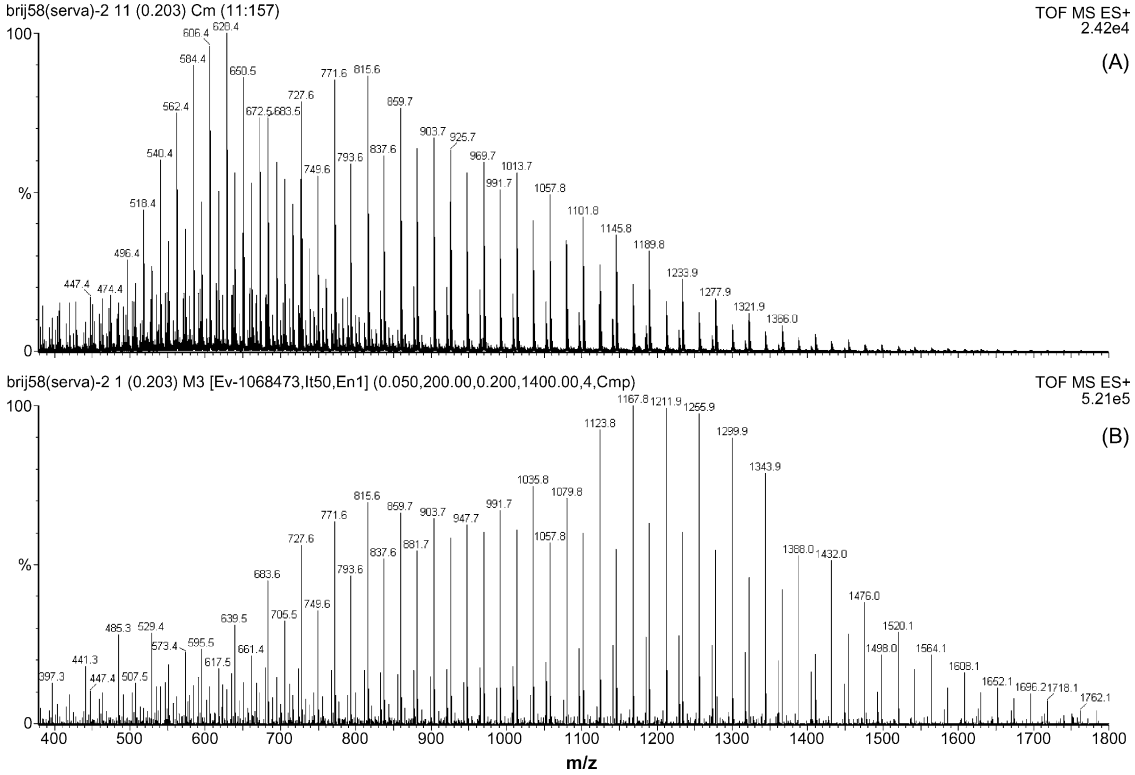

Fig. 7. Positive nanoelectrospray mass spectra of Brij® 58 (Serva). (A) Unprocessed, combined spectrum; (B) spectrum processed with MaxEnt™ 3 (see text for further explanation).

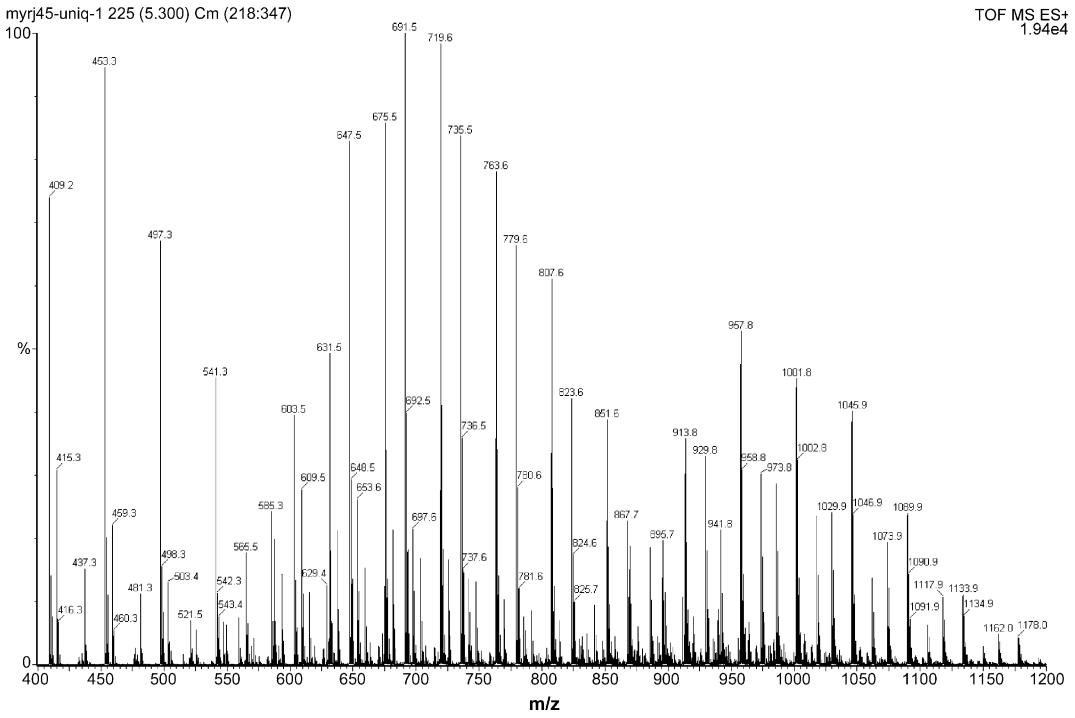

Fig. 8. Positive nanoelectrospray mass spectrum of Myrj® 45 (Uniqema).

series assigned to the palmitates shows higher intensity than the one assigned to the stearates. Furthermore other series in the higher mass range appear (e.g., $m/z = 869$, 913, 957, 1001, 1045, 1089; maximum at $m/z = 957$). Re-inspecting the MALDI-TOF spectrum, the series can be found there, too, but with a weak intensity so that it will usually be overlooked. These can be assigned to the $[M + K]^+$ of diacylated PEG chains. For example, $m/z = 957$ refers to stearyl nonaethylene glycol palmitate ester. Series with $\pm\Delta m = 28$ u are additionally present (two stearoyl and palmitoyl residues, respectively). Since no multiple charges occur, the spectrum processed using MaxEnt™ 3 is practically identical (data not shown). Interestingly enough, the

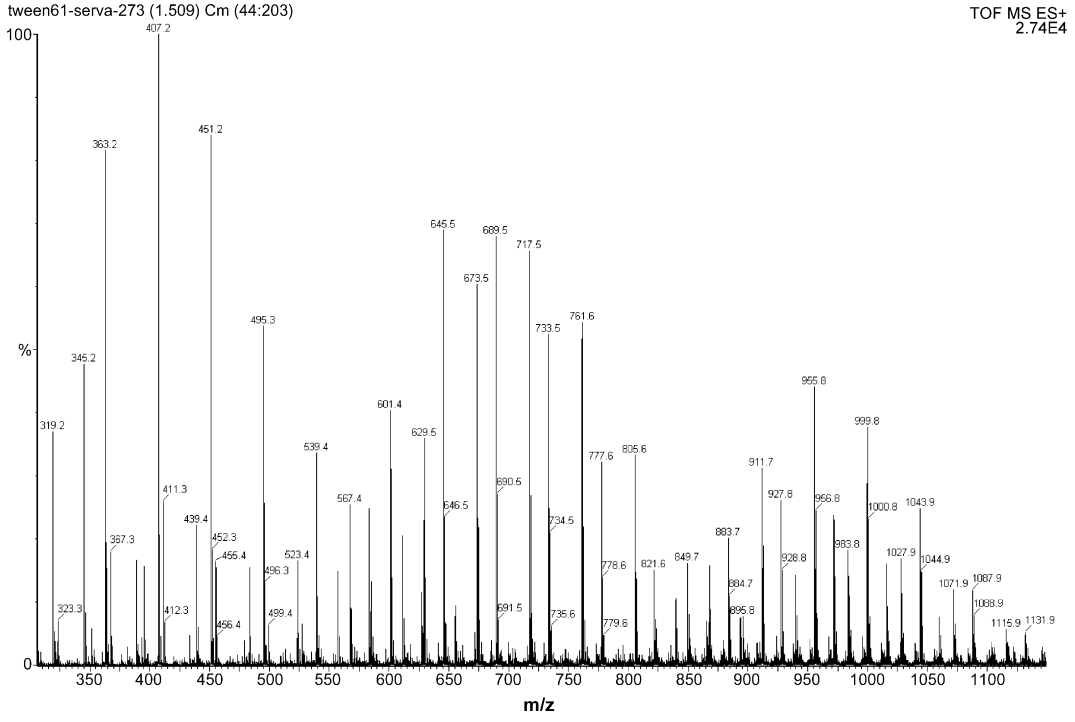

Fig. 9. Positive nanoelectrospray mass spectrum of Tween® 61 (Serva).

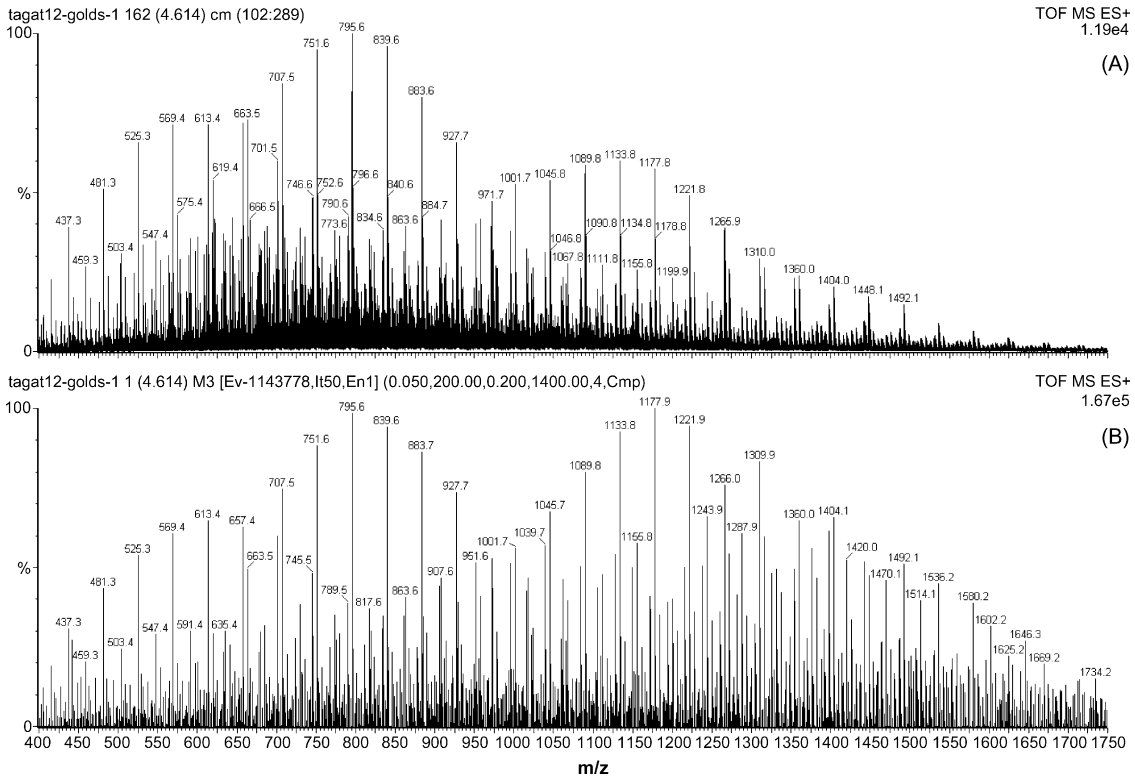

Fig. 10. Positive nanoelectrospray mass spectra of Tagat® L2 (Goldschmidt). (A) Unprocessed, combined spectrum; (B) spectrum processed with MaxEnt™ 3 (see text for further explanation).

negative nano-ESI spectrum (data not shown) shows only the distributions described before under MALDI-TOF. Tween® 61 (Serva) gives in positive nanoelectrospray MS the same series for $[M+Na]^+$ of the PMEs and SPEs as compared to MALDI (see Fig. 9). However, again another series rises ($m/z = 867, 911, 955, 999, 1043, 1087$; maximum at $m/z = 955$). It is not encountered in the negative spectrum.

Tagat® L2 (Goldschmidt) gives a positive nano-ESI spectrum that largely confirms the MALDI results (see Fig. 10(A) and (B)). $[M+Na]^+$ dominates, no $[M+K]^+$ is found. The most obvious difference is that the polyethoxylated glycerol series shows the highest intensity, which might be due to the better ionization ability compared to the more lipophilic acylated species. The series referring to the polyethoxylated glycerol monolaurates is also prominent. Processing with MaxEnt™ 3 helps to simplify the spectrum. The signal-to-noise is clearly improved for species that form multiple charges, since the intensities of the different charge states are summed up.

3.3. Conclusion

The overall purpose of this study was to show how mass spectrometric techniques, namely MALDI-TOF and nanoelectrospray MS, can be used to characterize polyethoxylated surfactants. Therefore, instructive spectra of typical compounds representing different compound classes are shown. It was not intended to perform a detailed compositional analysis of one particular product. For products containing different fatty acid moieties ($\Delta m = 28$ u) results an overlay of different series in the presence of sodium and potassium adducts ($\Delta m = 16$ u). Interpretation of this type of spectra requires knowledge about the occurrence of fatty acids and/or knowledge which alkali ion prevails. For reliable quantification, much more effort has to be undertaken, including chromatographic or electrophoretic separation, isotopically labeled standards a.s.o. Furthermore, it is recommendable to influence adduct formation in a way that only one type of adducts is formed, preferably by addition of appropriate amounts of the cation of choice (sodium or potassium).

The results show, that products with the same name from different suppliers may differ from each other (e.g., polyethoxylated fatty acids), that related fatty acids may occur, and that mixtures are more complicated than expected (e.g., polysorbates).

A comparison of the spectra obtained with different mass spectrometric methods (MALDI versus nano-ESI, positive versus negative ionization) shows that complementary information is needed to complete the structural information. Each ionization technique discriminates ions in a different way. Even though absolute quantification cannot be derived from such data, it serves well as a fingerprint for reference, e.g. to measure batch-to-batch reproducibility, compare products from different suppliers, and obtain at least semiquantitative data about molecular mass distribution, degree of ethoxylation, and residual groups. Once established in the R&D or QC lab, these methods can help greatly to reduce the trial-and-error in the change or setup of surfactant formulations.

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