Identification of Surfactants by Electrospray Liquid Chromatography-Mass Spectrometry

Introduction

Surfactants are used in a variety of products and also found in many environmental samples. Their identification can be a challenge due to the number of commercial products available and the fact that they are often complex mixtures. They are broken down into four classes: cationic, nonionic, cationic, and amphoteric. There is a wide diversity in types of structures including simple monomers and fairly complex mixtures of polymeric type species. Furthermore, they are often compounded as mixtures of individual surfactants to enhance performance. Surfactants are prepared by functionalizing a wide variety of lipophilic end-groups with a variety of hydrophilic end-groups such as ethylene oxides, propylene oxides, sulfates, phosphates, amines, etc.

We have developed an electrospray LC-MS method that is very successful in identifying surfactants from a variety of sources. This paper includes a description of the process plus literature references to other approaches for characterizing surfactants by mass spectrometry.

Experimental

HPLC Separations: Schmidt’s surfactant series book lists a wide variety of information on HPLC methods. There are also many literature references listed for reversed phase, size exclusion, and ion chromatography separations. An especially novel separation method was noted for differentiating the classes of surfactants present in samples using dyes as ion pair reagents.

It is difficult to develop a standard HPLC method that will work for such a diverse collection of chemicals. However, we have found a method that works reasonably well for all the surfactants that we have analyzed. We tried a variety of reversed phase columns including C-8, C-18, cyano, and phenyl phases. The C-18 column seemed to be a good compromise for separation efficiency and reasonable peak shape. We also tried a variety of additives including acetic acid, formic acid, ammonium acetate, etc. The ammonium acetate seemed to yield reasonable peak shape and retention time reproducibility and did not suppress mass spec ionization efficiency.

We also tried a variety of solvents and mixtures of solvents such as acetonitrile (ACN), methanol, tetrahydrofuran (THF), and isopropanol. The acetonitrile seemed to elute the majority of the compounds in a reasonable amount of time. For certain specific applications, we used a mixture of 50/50 vol/vol ACN/isopropanol because there were some indications that the isopropanol yielded less carry-over when analyzing fluorinated surfactants at lower levels. A mixture of 50/50 ACN/THF would be useful for eluting components that are highly retained using acetonitrile alone.
We initially employed 100% water containing 2.5 mmolar ammonium acetate, but switched to 3% ACN/97% water containing 2.5 mmolar ammonium acetate because bacterial growth in the 100% ammonium acetate buffer solution rapidly plugged our HPLC system filters. We normally prepare the fresh solutions of the aqueous/ACN phase each week and store the solutions in an amber bottle, which is routinely washed with methanol, for storage to further minimize bacterial growth.

A solution of 25 mmolar ammonium acetate in methanol is added at 0.1 ml/min post-column to enhance ionization at high organic concentrations. We split the flow after the diode-array detector and post column addition so that about 90 ul/min of the total flow is introduced into the electrospray probe.

An Agilent 1100 HPLC and Diode Array Detector (190-900 nm) was used for all separations employing a Varian Polaris C-18 column (3 micron PN 2001-050x046 50 x 4.6 mm).

A= 97:3 water:ACN containing 2.5 mmolar ammonium acetate  
B=ACN

<table>
<thead>
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<th>Time (mins)</th>
<th>A%</th>
<th>B%</th>
<th>Flow (ml/min)</th>
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<td>100.0</td>
<td>1.500</td>
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<tr>
<td>40.00</td>
<td>100.0</td>
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Sample Preparation: There are a wide variety of sample preparation methods for surfactant samples. If the surfactant is present in an aqueous matrix, many different concentration methods have been developed. The main approach is concentration using solid phase extraction (SPE) with either C-18 cartridges, polymeric tubes, ion exchange tubes, XAD resin, or granulated activated carbon. The tubes are then either selectively desorbed, totally desorbed, or Soxhlet extracted to recover the surfactants. Another interesting approach employed sublation.

Most of our samples were either commercially purchased materials in their concentrated form or part of a product in which the surfactant was used to disperse various solids. The neat samples (5-10 mg) were dissolved in 1 ml of 50/50 acetonitrile/4 mmolar ammonium acetate in water and 5-25 ul’s injected onto the HPLC column. The dispersions were diluted 1:1 with water and centrifuged for 2 hours at 15,000 rpm at 10 °C. The supernatant was diluted 1:1 in 1 ml of 50/50 acetonitrile/4 mmolar ammonium acetate in water and 10-25 ul’s injected onto the HPLC column.
Mass Spectrometry: The samples were analyzed on a Waters/Micromass LCT-TOF LC-MS. Normally a 4-function experiment was performed with two functions in the positive ion mode (25 and 75 sample cone volts) and two functions in the negative ion mode (25 and 75 sample cone volts). However, the sample cone voltage was increased or decreased as needed to obtain additional in-source collision induced dissociation (CID) data. More detailed instrument parameters are shown in Table 1.

The instrument is calibrated with polypropylene glycol 1000 in both positive and negative ion modes. The calibration tables are shown in Tables 2 and 3. The positive ion table is normally included with the MassLynx software and is labeled Ppgnh4mo.ref. PEG 1000 can also be used for calibration, but we found it more difficult to “flush” from the system after using when compared to polypropylene glycol.

15-25 ul’s of a stock solution of 33 mg/ml of polypropylene glycol in acetonitrile is added to 1 ml of a 50/50 mixture of acetonitrile/4 mmolar ammonium acetate. This solution is infused into the instrument at a flow rate of 5-10 ul/min. In the positive ion mode, the cone voltage is adjusted to get ions from m/z 50 to 1500 m/z. A typical cone voltage of between 50-75 volts gives a good ion distribution. This minimizes the M+2NH₄⁺ doubly charged ions, yields M+NH₄⁺ ions for the PPG 1000 components at higher MW, some M+H⁺ ions at lower MW, and a series of low MW fragment ions at 59, 117, 175, etc. The instrument is calibrated in the negative mode with a sample cone voltage of 17 volts which yields M+OAC⁻ (M+59) ions for the PPG oligomers and ions at m/z 59 for AcO⁻ (acetate anion) and the deprotonated acetic acid dimer (AcOH.ACO)⁻ at m/z 119.

Results and Discussion

General Overview: Computer searching the in-source collision induced dissociation (CID) spectrum of the unknown against on in-house database is the easiest means to identify surfactants. There are currently about 1800 spectra in our MS/MS database. About 200 of these spectra are positive and negative ion in-source CID spectra of surfactants.

The other approach is to search the molecular weights of monomeric surfactants or residual molecular weights (RMW) of polyethoxylated surfactants in our various Excel-based databases. Either nominal or accurate mass molecular weights are searched for monomers, but only nominal values of the RMW are searched for polymeric surfactants. The in-source CID spectra of the unknown is used to further eliminate possible structures from the candidates list plus additional information such as presence of UV chromophores and the types of molecular ion adducts noted in both the positive and negative ion modes.

The Excel-based databases were compiled from a variety of literature and internet resources and from the TSCA database. Fragmentation patterns were determined from a
wide variety of literature references and by analyzing many commercially available surfactants with known structures.

Sometimes in-source CID spectra do not clearly indicate the identity of the end-groups in polyethoxylated nonionic or anionic surfactants. These surfactants are can be cleaved with a mixed anhydride reagent and the end-groups identified by GC-MS.

**Molecular Weight Information:** Ammonium acetate is employed as a buffer in the HPLC separation and added post-column to enhance ionization efficiency. This simplifies the types of ions noted and minimizes fragmentation at lower sample cone voltages. The ions discussed in the following paragraphs are those used in determining the molecular weights of the unknowns. A separate section discusses the types of substructural information available from in-source CID spectra.

The nonionic surfactants containing polyethylene glycol groups give their best response in the positive ion mode as M+NH₄⁺ ions with smaller intensities of M+H⁺ and M+Na⁺ adducts observed. In addition doubly, (M+2NH₄)⁺² and triply, (M+ 3NH₄)⁺³ charged ions are observed as the number of polyethylene glycol repeat groups increase or the sample cone voltage of the instrument is decreased. Alkyl dimethylamine N-oxides showed primarily (M+H)⁺ ions at higher cone voltages and (2M+H)⁺ cluster ions at lower cone voltages. The positive ion mode is usually the best indication of the average molecular weight for the polyethylene glycol containing surfactants (see Figure 5). A lower response and somewhat skewed MW distribution can be noted in negative ion mode for some components from M+OAc⁻ (acetate, M+59) adducts.

The anionic surfactants yield their best response and MW distribution information in the negative ion mode showing primarily M-H⁻ ions (see Figure 3). If two acid groups are present in the molecule, the M-2H⁻² doubly charged ions will also be observed. Anionic surfactants containing polyethylene glycol repeat groups can also show positive ions such as M+NH₄⁺⁺ (M+2NH₄)⁺², (M+NH₄+H)⁺² and (M+ 3NH₄)⁺³ ion adducts. Often in the positive ion mode the loss of the phosphate and sulfate are also observed. All information about the associated cation for the anionic surfactant is lost in the HPLC MS analysis since it is exchanged with ammonium ion during the separation. Sometimes the salts eluting in the unretained solvent peak can lend insight on the identity of the cation. Otherwise other methods need to be employed.¹²

The cationic surfactants are essentially pre-ionized and do not form ammonium, sodium, or proton adducts. Sometimes it is difficult to determine if a cationic surfactant is present in a sample instead of a nonionic or anionic surfactant. As the sample cone voltage is increased for anionic and nonionic surfactants, the ammonium adds fragment back to the protonated adducts and the sodium adducts intensity actually increases. The cationic surfactants, on the other hand, only fragment by dealkylation. It would be nice to have a routine and reliable SPE method to determine if the type of surfactant present in a mixture (see Experimental Sections on HPLC separations and Sample Preparation for leading references), but we have not taken time to develop it for our applications. The cationic surfactants did show relatively intense ions for the cation clustered with two
acetate anions (M+118). This is due to the fact that we are using ammonium acetate in the HPLC MS separation. The chloride or bromide anion actually attached to the cationic surfactant is exchanged-out by the acetate from ammonium acetate. Another acetate clusters with this ion pair to yield the (M+118) singly charged negative ion observed.

We have very little personal experience with the ionization of amphoteric species, but information is available in the literature. The only one we have analyzed is cocamidopropyl betaine (CAPB) which is found in most commercially available shampoos. They are used as secondary surfactants in shampoos to enhance the performance of the primary surfactant’s performance and to lessen the irritancy of anionics. The major component in CAPB is the C12 fatty acid-amide shown below:

![Chemical Structure of Cocamidopropyl Betaine (CAPB)](attachment:image)

The component as drawn above has a MW of 342. However, we detect the component in positive ion electrospray at m/z 343, (M+H),+ since the acid is protonated by the ammonium acetate buffer solution used in the chromatography. Also a small (2M+H)+ gas-phase dimer ion is noted at m/z 685 at low cone voltages. In the negative ion mode, the component shows primarily a (M+acetate)⁻ adduct at m/z 401 and a gas phase dimer at (2M+acetate)⁻ at m/z 743.

**Substructural Information from In-Source CID:** Ammonium acetate is employed as the eluent and also added post-column. This eluent is ideal for the analysis of most surfactants since it yields good molecular information (see previous section), but still offers excellent lipophilic end-group substructural information in both positive and negative modes. Proton and alkali adducts can also be employed, but they yield more hydrophilic end-group substructural information.

The identity of end-groups can often be determined by in-source CID or by CID spectra obtained with an inert gas in multistage mass spectrometers (Q-TOF, triple quads, ion traps, etc.). In many cases the in-source CID and CID obtained in the multistage instruments compare reasonably well. However, differences are observed and arise from the fact that the solvent is the collision gas for in-source CID instead of inert gases such as helium or Argon. Nevertheless, the in-source CID can be obtained on any electrospray instrument with good sensitivity and the majority of our data was obtained in this manner.

We normally acquire our in-source CID spectra at 75 volts, but often more information is acquired by varying the voltage between 40 to even 150 volts for certain components.
The sample cone voltage and the instrument name are usually entered into the comment field of our mass spectral database.

A “beta version” of MS Interpreter from NIST (Version 0.9f, 5/21/2003) is very useful for automatically correlating observed fragment ions with substructural information (see Figure 1). The ion of interest is selected and the possible substructures are highlighted in color and its relative rate of formation calculated. All the ions noted in black in the spectrum are correlated to one or more possible structures. We normally use the “show unspecified cleavage” to give better coverage of possible substructures that would not be displayed when fragments are considered by rate alone.

A few examples are shown for several end-groups to illustrate the general types of fragments observed. There are also many excellent literature references for MS/MS and in-source CID fragmentation3,4,5,7,8,9,10,11,14,15,18,23 employing either FAB, electrospray, or thermospray ionization. We have stored our fragmentation information using the NIST Version 2 mass spectral search software which also allows users to search by spectrum, structure/substructure, and other parameters.

m/z 89, 133, 177, etc. for:

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{OR} & \quad \text{CH}_2^+ \\
\text{HO} & \quad \text{O} \\
\text{OR} & \quad \text{CH}_2^+ \\
n & \quad n
\end{align*}
\]

m/z 59, 117, 175, etc for:

\[
\begin{align*}
\text{OH} & \quad \text{O} \\
\text{CH}_3 & \quad \text{CH}_3 \\
\text{OR} & \quad \text{CH}_2^+ \\
\text{CH}_3 & \quad \text{CH}_3 \\
n & \quad n
\end{align*}
\]

m/z 311, 355, etc. plus glycol-end series at 89,133, 177:
m/z 57, 71, 85, etc for alkyl, m/z 183 for alkyl carbocation, 227, 271, 305 for ether with alkyl end group:

mainly\textsuperscript{14} m/z 254, very little of the glycol-end series at 89, 133, 177

m/z 268 for dealkylation\textsuperscript{14} of the amine plus some of the 89, 133, 177 for glycol-end group:
m/z 212 and 91 for dealkylation and tropylium ion respectively, also note m/z 132 for in-source CID, not shifted when long-chain alkyl group varied; m/z 132 not noted in triple quad MS/MS spectrum. Accurate mass data for the m/z 132 is consistent with the aziridinium cation which could be formed by some type of rearrangement of the molecule parent compound or clustering of the tropylium ion with the solvent, acetonitrile. However, shifting of the m/z ion from 132 to 135 when deuterated acetonitrile was substituted for acetonitrile showed it is an adduct ion with acetonitrile.

dealkylation of amphoteric surfactant followed by loss of water and sulfinic acid:

another amphoteric surfactant, CAPB, commonly found in commercial shampoos yielded the following predominant fragment ions in positive and negative ion modes:
m/z 80 and 97 for sulfate anionic surfactants:

\[
\text{H}_3\text{C} - \text{CH} - \text{CH} - \text{CH} - \text{C}_8\text{H}_{17} - \text{SO}_4^2- \\
\rightarrow \text{SO}_4^2- + \text{SO}_4^2- \\
\]

m/z 79 and 97 for phosphate surfactants:

\[
\text{H}_3\text{C} - \text{CH} - \text{CH} - \text{CH} - \text{C}_8\text{H}_{17} - \text{PO}_4^2- \\
\rightarrow \text{PO}_4^2- + \text{PO}_4^2- \\
\]

m/z 81 for some, not all, sulfonate groups attached to carbon instead of oxygen:

\[
\text{H}_3\text{C} - \text{CH} - \text{CH} - \text{CH} - \text{C}_8\text{H}_{17} - \text{SO}_3^- \\
\rightarrow \text{SO}_3^- \\
\]

Phenol containing species form their stable phenolic anion, e.g. m/z 219:

\[
\text{H}_3\text{C} - \text{CH} - \text{CH} - \text{CH} - \text{C}_8\text{H}_{17} - \text{OH} \\
\rightarrow \text{SO}_3^- \\
\]

m/z 79, 97, 159, 177 for minor “anhydride-type” components found in phosphate anionic surfactants:
NIST Mass Spectral Databases: The reference spectra of surfactants in both positive and negative ion mode are stored in Version 2.0 of the NIST MS Search Program. This allows us to search unknowns against the database to identify unknowns. Even if the exact species is not in the database, similar structures often lead to the identification of the unknown from similar spectra. Also, MS Interpreter (see Substructural Information from In-Source CID) allows the fragmentation pattern of components in the database to be easily displayed and correlated to substructure.

The creation and use of the surfactant in-source CID database is somewhat different than that of the typical electron impact mass spectral database. The following processes need to be understood and followed to obtain useful results:

1. For surfactants containing polyethylene glycol repeat units, the in-source CID spectra for the whole “envelope” of components differing by the number of polyethylene glycol units is entered as one library spectrum if the components are not chromatographically resolved.

All ions above m/z 350 in the spectra of polyethoxylated surfactants (ones for loss of small molecules, protonated adducts, sodium adducts) are deleted employing the Librarian editing function within the NIST Search Program. Likewise, the same editing process needs to be performed to the unknown spectrum before it is searched against the database to be searched against the database.

2. The whole spectrum including the ion adducts (ammonia, sodium, proton, etc.) for monomers are entered into the library for storage.

3. Structures are added with Chemsketch (available free on internet from ACD). It is best to enter 3-4 polyethylene groups on surfactants to best predict fragment ions when using MS Interpreter.
4. As the number of polyethylene glycol end-groups increases in a nonionic and anionic surfactant, the ions noted for the lipophilic end group will decrease and the ions for the hydrophilic end-group oligomeric ion series (e.g. 89, 133, 177) will increase.

The amount of fragmentation information in the in-source CID spectrum is dependent on the sample cone voltage on our instrument. This voltage is not standard between instruments and the fragmentation noted at 75 cone volts on one particular type of instrument might require 100 volts on another type of instrument. Normally 75 volts is the standard sample cone voltage used to obtain our database spectra. However, we often vary this voltage between 50-150 volts for some species. The actual voltage and instrument utilized is included in the comment field for the spectrum in our NIST MS/MS database.

We often note additional ions not found for the same compound analyzed on a triple quadrupole mass spec with Argon as the collision gas. The differences are probably due to the fact that the solvent and air are the collision gases for the in-source CID. Sometimes the ions cannot be understood, but often the in-source CID offers more abundant ions that can be correlated with substructural information that are not found in the triple quadrupole CID spectra. Nevertheless, the NIST library algorithm normally yields reasonable results when searching in-source CID spectra against triple quadrupole CID spectra and vice versa.

The negative and positive spectra are stored in separate libraries. However, we usually search both the positive and negative libraries together. Searching the libraries together does not cause any problems because there are major differences in the types of observed fragments note in negative versus positive in-source CID spectra. There are currently around 150 positive ion and 50 negative ion surfactant spectra in our in-source CID database of around 1800 spectra.

The NIST library offers several ways to query databases. We always include the common name or names of the surfactant, MW, CAS number if available, a label of “Surfactant” in the synonym field, the RMW of the observed component in the synonym field, and the MW and MF. If the component is a polyethoxylated surfactant, a typical structure should be drawn containing 3-4 polyethoxylated repeat units and its MF and MW entered into the appropriate field.

The inclusion of additional data, e.g. RMW and Surfactant, in addition to alternate names in the Synonym field is very useful. The information can be used with the “Names Function” to display the components sorted by their RMW and to display all the surfactants present in our database. This latter capability is useful since many other types of compounds are also stored in the same databases.

The RMW values are stored as nominal values as two digits to make the Names Display ordered them sequentially. Thus a compound with RMW of 6 has the value entered into the Synonym field as RMW 06.
Residual Molecular Weight Calculations (RMW): A single molecular weight cannot be readily calculated for a surfactant which is a mixture of components due to the presence of various numbers of polyethylene glycol repeat units. There are many such surfactants in this category including nonionic, cationic, anionic, and amphoteric surfactants. An idea was conceived to calculate the Residual Molecular Weight from earlier work done by MALDI on various anionic surfactants. The current approach is very similar to the original approach, but simplified significantly for anionic surfactants since the exchange of sodium for acidic protons does not occur under our MS electrospray conditions employing ammonium acetate.

The RMW for a surfactant with the same end-groups but differing numbers of polyethylene glycol repeat units can be calculated from any one of the observed ions in the series. If it is a nonionic surfactant, the MW of the species is calculated by subtracting the MW of the ion adduct or ion adducts (doubly charged ions) from the observed ion. The ion representing the mass in atomic mass units of a molecule composed of the most abundant isotope of each atom. Note, this is not always the largest ion in the isotopic cluster as the m/z of the ion approaches 1500 or isotopes such as chlorine or bromine are present! Our LCT-TOF easily resolves the isotopes of doubly charged ions and singly charged ions with m/z’s of 2000 and the mass accuracy without internal calibration is usually within 150 ppm if the instrument is calibrated daily.

The RMW of the anionic surfactants is calculated for the species in its acid form. In other words, if an M-H ion is noted, a proton is added back to the observed ion for the RMW calculation. For nonionic surfactants, the ammonium adducts are subtracted from the observed ions. For cationic and amphoteric surfactants, the RMW is calculated from the observed ion, e.g. N(R)4⁺.

The RMW molecular weight is calculated as follows:

\[ \text{RMW} = ((x/44.026)-y)44 \]

\( x = \) the observed accurate molecular weight of the surfactant species
\( y = \) integer value for \( x/44.026 \), i.e. the whole number to the left of the decimal

The RMW will only equal the actual end-group’s actual MW if its MW is less than or equal to 44. Thus, the concept is really just a way to assign each component a number between 0-44 for indexing and searching since most surfactants contain end-groups with MW’s greater than 44.

The “Typical Example” section demonstrates the use of RMW in the identification of unknowns. Components that are suspected to be anionic sulfate or phosphate surfactants can have 80 subtracted from their MW before their RMW is calculated. Then the resulting RMW would be of the corresponding nonionic surfactant. That would be useful if only the nonionic surfactant is listed in the Excel databases. However, we have
attempted to enter all reasonable anionic surfactants of known nonionic surfactants in the Excel databases.

The same value can be obtained and used with the Excel spreadsheet when polypropylene glycol is the repeat unit by using the following formula:

\[
RMW = ((x/58.042) - y)58
\]

\(x\) = the accurate mass molecular weight of the surfactant species
\(y\) = integer value for \(x/58.042\), i.e. the number to the left of the decimal

**Excel Databases of RMW and MW:** The molecular weights (MW’s) and Residual Molecular Weights (RMW’s) obtained by electrospray LC/MS are used to identify the surfactants. The information is separated into several Excel spreadsheets. The RMW is calculated as described in a separate section of this report and is demonstrated in the “Typical Example Section” of this report.

The molecular weights of the monomers are based on the mass in of a molecule composed of the most abundant isotope of each atom.\(^2\) The molecular weight of the anionic surfactants are calculate in their acid form. For example, if a \((M-H)\) ion is noted, the mass of hydrogen is added to the observed ions for reference to the Excel spreadsheet.

For cationic surfactants, the \(N(R)4\) ion without its counter-anion is used.

The most useful Excel spreadsheet (see Figure 7) is the one labeled “Literature_Real_Samples.” It is composed of entries that were added from various literature articles and from surfactant samples analyzed in our laboratory. The structures are drawn with Chemskech (ACD labs, freeware on internet) and can be opened by “double-left clicking” on them. Then partial parts of the structure can be “lassoed” from within Chemskech to get the MW of substructures which are displayed on the bottom of the Chemskech program window (monoisotopic mass must be selected in bottom right corner of Chemskech window). This is very useful for determining likely fragments in the in-source CID spectrum.

The “Literature_Real_Samples” spreadsheet is composed of several columns (~400 components) besides the structure. The “MW Monomer” column is the nominal MW using the most abundant isotope. Often an end group is actually a surfactant itself. The next column is the RMW of the component. All components have RMW values, but some species do not exist commercially as polyethylene glycol containing surfactants. Other information is included in the spreadsheet such as common names, type of surfactant (anionic, cationic, etc.), comments about its use or references to the literature, repeat unit (non PEG, e.g. 28 units for fatty acids from natural sources), and types of fragments observed.

The RMW values are easily calculate with the MOD function in Excel. It is very important that the structures are sized so that they are smaller than the Excel block in which they are stored. In addition, all the Excel structure blocks need to be the same size.
If these guidelines are not followed, the structures will not be sorted properly when the spreadsheet is sorted by RMW, Monomer MW, etc. Also it is very important to select the radio button “move but don’t size with cells” is selected under the properties tab. This field information is accessed by “right-clicking” on the structure and selected the Format Object option.

The “TSCA_PEG” spreadsheet is a list of PEG containing surfactants obtained by parsing the TSCA CD ROM. The TSCA Chemical Inventory list contains public information for 64,000+ chemicals or chemical substances manufactured or imported into the U.S. for commercial purposes as defined under the Toxic Substances Control Act (TSCA). There are chemicals substances with confidential identities that are not included on the distributed TSCA list.

Our “TSCA_PEG” spreadsheet includes the RMW’s for all the PEG containing components (~700 components) present in the list. Unfortunately there are no structures and the surfactants are not defined by class of surfactant (e.g. anionic, nonionic, etc.). Furthermore, it takes a little practice to learn their nomenclature. However, the list can be very useful if one cannot identify the surfactant with the “Literature_Real_Samples” spreadsheet. A “TSCA_PEG_Questionable” spreadsheet contains some possible polyethylene glycol type surfactant candidates that could not be easily parsed.

The last spreadsheet is the “TSCA_Monomers.” It contains all the well-defined chemicals (nonpolymeric, non PEG-containing) in the TSCA list with both nominal and accurate mass values calculated. The monomeric surfactants would be a subset of this total list. The ability of the LCT-TOF to obtain accurate mass data makes this list very valuable for identifying commercial surfactants and a variety of other components found in commercial products.

**Cleavage of Ether Bonds with Mixed Anhydride Reagent:** Sometimes it is difficult to identify the types of end-groups in surfactants employing only RMW calculations and in-source CID. Especially if the number of the repeating polyethylene glycol groups is large or if some polypropylene glycol repeat units are mixed with the polyethylene glycol repeat unites. The ether groups can be cleaved with the mixed anhydride of acetic acid and p-toluenesulfonic acid prepared in-situ. The resulting alcohols are converted during the process into their corresponding acetate esters which can be identified by GC-MS analyses. If the end group was a long chain ester group, the end-group will be detected by GC-MS as its free acid. Sulfate and phosphate anionic surfactant end-groups will also be cleaved, but will not be detected since the reaction mixture is extracted with diethyl ether/water mixture.
Example One--Identification by RMW: Figure 2 shows the electrospray MS and UV Diode array total ion chromatograms for an anionic surfactant. The summed mass spectra in positive and negative ion mode at a sampling cone voltage of 25 volts are shown in Figure 3.

The presence of M-H⁻ ions and M+NH₄⁺ and M+H⁺ ions indicate that the first component is an anionic surfactant. The RMW can be calculated from any of the ions in noted in the negative ion spectrum. For example if 783.41 is used, one must add back 1.01 to convert the oligomer to its acid form.

\[
RMW = \left[\frac{(783.41+1.01)/44.026}{44}\right] - y
\]

RMW = (17.82-17)/44 = 35.95 rounded to integer value of 36

The RMW of 36 is looked up in the “Literature_Real_Samples” Excel spreadsheet of our Excel database, which is sorted first by RMW and second by surfactant type (see Figure 7). There are 5 possible candidates which have a RMW of 36 and are anionic surfactants. Only 2 of the 5 candidates would have UV absorptions, so that narrows the possibilities to the two following surfactants:
The two candidate structures can easily be differentiated by their negative ion in-source CID spectra at 75 volts (see Figure 4) as the phosphate anionic surfactant. Phosphate anionic surfactants yield m/z 79 for the PO\(^{3-}\) ion. Sulfate anionic surfactants yield m/z 80 for the SO\(^{3-}\) ion (also a m/z 82 for presence of S\(^{34}\) isotope). Interpretation of the positive ion in-source CID spectrum further confirms the proposed identification.

The positive mass spectrum at 25 volts for unknown is very interesting. As the number of polyethylene glycol groups increases, the (M+NH\(_4\))^+ predominates. At lower numbers of polyethylene glycol repeat units, the (M+H)^+ ion predominates. The doubly charged ion series at lower mass is quite confusing. It is the (M+NH\(_4\)+H)^+2 series of ions!

The RMW for unknown 2 is calculated as follows from the (M+NH\(_4\))^+ at m/z 722.50:

\[
RMW = \frac{[(722.50-18.03)/44.026]-y}{44}
\]
\[
RMW = (16-16)44 = 0
\]

From the RMW=0, in-source CID, and types of ion adducts noted at low sample cone voltage (see Figure 5), unknown 2 was identified as the following nonionic surfactant:

It is not unusual for anionic surfactants to contain the corresponding nonionic surfactant and even polyethylene glycol which are present as by-products in their syntheses.

The RMW for unknown 3 is calculated as follows from the (M-H)^- at m/z 1205.72:

\[
RMW = \frac{[((1205.72+1.01)/44.026)-y]44}{44}
\]
\[
RMW = (27.41-27)44 = 18.01 \text{ rounded to } 18
\]

From the RMW=18, in-source CID, and types of ion adducts noted at low sample cone voltage (see Figure 6), unknown 2 was identified as the following anionic diphosphate surfactant:
Phosphate anionic surfactants normally have varying ratios of the mono- to diphosphate depending on the method of preparation.

**Example Two--Identification by Accurate Mass and Excel “TSCA_Monomers”**

**Excel Spreadsheet:** The electrospray LC-MS analysis of a mixture of cationic surfactants is shown in Figure 8. The accurate mass of 304.3039 was searched against the “TSCA_Monomers” Excel spreadsheet sorted first by accurate monoisotopic MW and second by MF. The results are shown in Figure 9. There are four structures consistent with the accurate mass data, but only two are consistent with the in-source CID spectrum. The counter anion is not detected since it would be exchanged-out during the HPLC separation with acetate. Thus, we could not determine if the count ion was bromide or chloride with this data.

The observed accurate mass value was within 14 ppm of the actual accurate mass. This is quite surprising since no lockmass was added to correct for errors due to temperature gradients in the time of flight tube and other instrument variables. However, the instrument was calibrated an hour before the sample was analyzed. In general, accurate mass data is best done with a lockmass.

The fragment ion at m/z 132 was initially proposed to be the aziridinium ion from the fragmentation of the cationic surfactants:
This ion was not seen in the MS/MS spectrum of the cationic surfactant obtained on a triple quadrupole MS/MS. Additionally, the ion was not noted in the Platform single quadrupole MS in-source CID spectrum (“Pepper-pot type source”), but was noted on the LCT-TOF MS (Z-spray source) in-source CID spectrum.

Shifting of the m/z ion from 132 to 135 when deuterated acetonitrile was substituted for acetonitrile showed it is an adduct ion with acetonitrile. Thus it is more likely an ion adduct of acetonitrile with the tropylium ion:

$$\text{CH}_3\text{CN}^+$$

Others have noted similar adduct ions with acetonitrile, water, and aliphatic alcohols. The other two compounds in the sample at 15.32 and 18.01 minutes were the C\(_{14}\)H\(_{29}\) (m/z 332) and C\(_{16}\)H\(_{33}\) (m/z 360) homologues of the compound with m/z 304.

**Example Three--Identification by NIST Search of Eastman MS/MS Database:** The LC-MS data from a solution obtained from an inkjet printer is shown in Figure 10. It was obviously a polyethylene glycol nonionic surfactant from its MS data and has a RMW of 6. No response was noted in the negative ion mode and a very intense ammonium adduct was noted for all the components. Furthermore, the (M+Na)** ion was noted at higher cone voltages as the (M+NH\(_4\))** ion decreased.

Searching the NIST library gave a very good fit for the ethoxylated Surfynol nonionic Surfactant (see Figure 11). Note that the RMW of the reference spectrum is included in the comment field for the library spectrum plus information on the origin of the reference spectrum and the average MW of the surfactant standard.
As the number of spectra in our MS/MS database increases, the ability to identify unknown surfactants employing this database will become more and more important in our surfactant characterization studies.

Conclusions

Surfactants are easily analyzed by positive and negative ion electrospray LC-MS. At low sample cone voltages, primarily MW information and molecular weight distributions are obtained. At higher sample cone voltages, abundant fragments are noted which correlate with the substructure of the surfactants.

We have been very successful in identifying nonionic, cationic, and anionic surfactants employing MW (nominal and accurate), RMW, and fragmentation data. Candidates are proposed from Excel databases from MW or RMW data. The candidates are then eliminated by in-source CID spectra and other analytical information.

In-source CID spectra and structures of standards and identified components are added to a NIST in-house MS/MS database. NIST search software, normally used for EI searches, does an excellent job of searching the MS/MS spectra and identifying surfactant unknowns. Furthermore, the NIST MS Interpreter software is an excellent, cost effective tool for understanding MS/MS fragmentation.

References with Comments

1. “Determination of Anionic and nonionic Surfactants, Their Degradation Products, and Endocrine-Disrupting Compounds in Sewage Sludge by Liquid Chromatography/Mass Spectrometry,” M. Petrovic and D. Barcelo, Anal. Chem., 72 4560-4567 [Describes use of solid phase extraction to concentrate and separate surfactants, used triethylamine/acetic acid to ion pair in negative mode with methanol/acetonitrile with C-18 reverse phase separation, and acetic acid in positive mode]

2. “Analysis of Surfactant in Formulations by Mass Spectrometry,” R. Spilker, B. Menzebach, K Kube, La Rivista Italiana Delle Sostanze Grasse, Vol LXXVIII, Aprile 2001, p 241-246 [Used a C18 Reverse phase separation using methanol and water, addition of 0.01 mol/l ammonium acetate in both solvents]
3. “Forensic Toxicological Determination of Surfactant by Liquid Chromatography/Electrospray Ionization Mass Spectrometry Ionization Tandem Mass Spectrometry,” M. Nishikawa et al, Journal of Health Science, 49(2) 138-148 (2003) [Article discusses both reverse phase C-18 using 10 mM ammonium acetate and methanol and with a Asahipak GC-310HQ size exclusion column with methanol containing 10 mM ammonium acetate, the reverse phase retained some cationic surfactants too strongly, the size exclusion worked for the ones retained strongly and the nonionic surfactants, but eluted most cationic and anionic surfactants with little retention; weak cation and anion exchange SPE cartridges used to concentrate anionic and cationic surfactants, surprisingly the recovery for nonionic surfactants on both cartridges poor, also many references in article on C-18 SPE cartridge concentrations]

4. “Characterization of Poly(Ethylene Glycol) Esters Using Low Energy Collision Induces Dissociation in Electrospray ionization Mass Spectrometry,” R. Chen, X. Yu, and L. Li, J. Am. Soc Mass Spectrom, 2002, 888-897 [Very interesting article, talks about the loss of end-groups when using cationization with Li and Na via 4- and 6-member transition states, while with ammonium adducts and some other transition metals you get the end-group ionized with one CH2CH2+ group attached which is typical of our in-source CID spectra, also talks about loss of water and formic acid to give ionized alkyl chain which rearranges]

5. “Lithium and transition Metal Ions Enable Low Energy Collision-Induced Dissociation of Polyglycols in Electrospray Ionization Mass Spectrometry,” R. Chen, L. Li, J. Am. Soc Mass Spectrom, 2001, 12, 832-839. [Good article on fragmentation mechanisms from R end group of substituted versus the free OH group, Also discusses why sodium adducts don’t give fragmentation and lithium adducts do, also show M+H fragmentation information and M+NH4+).

6. “Determination of Block Length Distributions of Poly(oxypropylene) and Poly(Oxyethylene) Block Copolymers by MALDI-FTICR Mass Spectrometry,” G.van Rooij et al, Anal chem., 1998, 70, p 843-850. [MALDI used to determine block length distributions, also nice background introduction on this type of surfactant, commonly called Pluronics.]

7. “Identification of Alkyl Dimethylbenzylammonium Surfactants in water Samples by Solid-Phase Extraction Followed by Ion trap LC/MS and LC/MS/MS,” T. Furlong et al, environ. Sci. Technol 2001, 35, 2583-2588 [Nice article, tells about in-source CID spectra, chromatography, and concentration on polymeric SPE cartridges (important to use Acetonitrile in SPE method to get good recovery); use acetonitrile and waters with 10 mmolar ammonium formate at pH 3.5 to protonate free Si-O groups on C18 column; interesting that we see m/z 132, shown to be the ion adduct of tropylium ion at m/z 91 with acetonitrile by employing deuterated acetonitrile.]

separation by ion exchange chromatography to separate, but no details, a wealth of information on fragmentation by MS/MS]

9. “Identification and Quantitation of Surfactants in Consumer Products by Ion-Spray Mass Spectrometry,” JAOCs, Vol 73, no 1, 1996 [They did no chromatography, diluted formulation with ethanol and removed insoluble inorganics with centrifuge, if anionic surfactants interfered with nonionic, removed by passing supernatant through an anion-exchange SPE cartridge, talks about problems of trying to quantitate by infusion due to variation in response as a function of chainlength; used TFA to protonate or ammonium acetate to form adducts in methanol/water solutions]

10. Applications of LC-MS in Environmental Chemistry, Chapter 6: Separation, Identification, and Quantification of Surfactants and their Metabolites in Water, Surface Water, and Drinking Water by LC-TSP-MS, FIA-TSP-MS and MS-MS, Horst Friedrich Schroder Chapter Author, book Editor D. Barcelo pp 263-324 [Somewhat dated, but contains a lot of references to SPE using C-18 cartridges for concentration then a variety of solvents used to selectively elute surfactant of interest, says Cationic difficult to do since strongly adsorbs to particles and surfaces, also lists Sublation, a process used to remove surfactants from water, producing a dry form of the material for analysis. It involves bubbling N₂ through surfactant solution over which an organic phase of ethyl acetate is laid. The resultant foam dissolves into the organic layer. Evaporation of the organic layer leaves the surfactant as a dried residue. Specialty glassware to do sublation available]

11. “New Methods for the Identification of Surfactants and Their Acidic Metabolites in Raw and Drinking Water: FAB MS and MS-MS,” F. Ventura et al., Wat. Sci. Tech, Vol 25, No 11, pp 257-264, 1992 [Used GAC (granulated activated carbon) and XAD-2 adsorbents, but few details, also fractionated by HPLC and split into acidic and neutral components, but again very little detail]

12. Analysis of Surfactants, Second edition, Thomas M. Schmitt; Surfactant Science Series Volume 96; [Great overall view of surfactants and analytical methods by a wide variety of techniques including mass spectrometry]


14. “Electrospray Ionization Tandem Quadrupole Mass Spectrometry for the Characterization and Identification of Ethoxylated Surfactants,” G. Socher, Tenside Surf. Det 38 (2001) 2 pp 80-85 [Great article, shows that the fatty acid ethoxylates fragment to the RCOCH₂CH₂ group plus back to the protonated acid and the protonated ketene equivalent plus the PEG series at 89, 133, 177, etc, while the RCONHC₃H₂CH₂OH fragment mainly to RCONHC₃H₂CH₂ with only minor other fragments and little of the 89, 133 series, the latter matches our in-source CID well, we don’t have any for the fatty acid ethoxylates; the fatty alcohol ethoxylates fragment back to the carbocation, e.g.
ROCH2CH2OH fragments to the R+ group, but in addition see the R(OCH2CH2)n+ series with n = 1, 2, 3 at varying amounts, sometimes the n=1 can be fairly small in our in-source CID spectra; our in-source CID compares reasonably well to their MS-MS, but intensities are much different; the alkylamine ethoxylates mainly loose EO from the molecular ion and stop at the RNH3+ ion with very 89, 133, 177 series ions.


17. “Surfactants: Non-Biodegradable, Significant Pollutants in Sewage Treatment Plant Effluents; Separation, Identification, and Quantification by Liquid Chromatography, Flow-Injection Analysis-Mass Spectrometry and Tandem Mass Spectrometry,” Journal of chromatography, 647 (1993) 219-234; H. Fr. Schroder [Effluents from water were concentrated by liquid-liquid extraction and C18 SPE cartridges; the SPE cartridges were selectively desorbed and samples analyzed by LC-MS via Thermospray, the partially biodegraded samples gave ions in their CID MS-MS at 147 and 103 for the oxidized end, HO2CCH2OCH2CH2OCH2CH2 type series ions from ammonium adduct; problems with irreproducible retention times required that the reverse phase column be flushed between runs with 3:3:3:3 Acetonitrile, Chloroform, methanol, dimethylsulfoxide (vol:vol)]

18. “Identification of Surfactants in Water by FAB Mass Spectrometry,” Wa. Res Vol 23 No. 9, pp 1191-1203, F. Ventura et al. [Used Granulated activated carbon, similar to that used in wastewater treatment, desorbed with dichloromethane by Soxhlet extraction, ether soluble fraction separated into acidic, basic, neutral fractions by a classic fractionation scheme. Also fractionated by preparative reverse phase HPLC; extensive table of M+Na ions of a wide variety of surfactants included]

19. “Surfactant Analysis by Ion Chromatography/Particle Beam Mass Spectrometry” Chad J. Quinn, Rohm and Haas Company, Research Laboratories, p 1322-1323, The 39th Conference on Mass Spectrometry and Allied Topics, Nashville, TN, 1991. [Separated and identified many different surfactants using ammonium hydroxide as ion pair reagent and a Polymer Laboratories PLRP-S column. Used EI and negative and positive on methane CI as reagents.]

method was studied, which responded to particular ionic surfactants using a system of connecting the post-column reaction detector with a high-performance liquid chromatograph (HPLC). Orange II was used to form the hydrophobic ion-pair complexes for cationic and amphoteric surfactants, and Methylene blue for anionic surfactants. By this method, cationic, amphoteric, and anionic surfactants were easily identified in the chromatograms.


22. “Gas Chromatographic Analysis of Ester-type Surfactants by Using Mixed Anhydride Reagent,” Kazuro Tsjui et al., Journal of the American Oil Chemists’ Society, Vol 52, pp 106-109. [p-Toluenesulfonic acid (120 g) was placed in a round-bottom flask and 80 g of acetic anhydride added dropwise with stirring. The mixture was heated under reflux at 120°C for 30 minutes and cooled to room temperature. The acetic acid was not removed from the reagent. The reagent was stored in a brown bottle and will keep about a month. 100 mg of sample and 2 g of reagent were heated under reflux at 120°C for 2 hours. The reaction mixture was cooled to room temperature and extracted 3-4 times with deionized water until the washings were no longer acidic as measured by methyl orange. The organic solution was dried over anhydrous sodium sulfate and part of the organic solvent removed. The concentrate was analyzed by GC].


25. Conversations and Reports from work performed by Joost W. Gouw and Peter C. Burgers, August 27, 2001 [Development of initial idea to characterize compounds by their Residual MW]


27. “Chemical Listings Annual Subscription Includes TSCA & SARA Title III,” from Solutions Software Corp, www.env-sol.com, Annual Subscription $159. [P. Wehner at Eastman Chemical wrote Delphi Program to parse the TSCA Excel Database on the CD.
into Tab-Delimited Files for Import into an Excel or Access Database, the RMW and MW values were not in the original CD file from Solutions software, but instead, were calculated in the Delphi program, two entries for the phosphate anionic surfactants are found in the TSCA_PEG spreadsheet, this is because a parsed MF and associated RMW was calculated for both the monophosphate and diphosphate esters that would be found in a sample, Feb 2003]

28. “Comparison of Dissociation of Ions in an Electrospray Source, or a Collision Cell in Tandem Mass Spectrometry,” Corinne Bure, Catherine Lange, Current Organic Chemistry, 2003, 7, 1613-1624. [Excellent article, Concludes that in-source CID is a low energy process which yield ions very similar to those noted in low-energy condition with triple-quads, iontraps, QTOF’s, and ICRMS instruments. The fragment ion intensities appear to be higher when the fragmentation is achieved in the source instead of the collision gas cell.]


30. “Solvation of acylium fragment ions in electrospray ionization quadrupole ion trap and Fourier transform ion cyclotron resonance mass spectrometry.” Guan, Ziqiang; Liesch, Jerrold M. Merck Research Laboratories, Rahway, NJ, USA. Journal of Mass Spectrometry (2001), 36(3), 264-276. [They noted in electrospray ionization on quadrupole ion trap and Fourier transform ion cyclotron resonance mass spectrometry that certain fragment ions such as acylium ions can undergo ion-molecule reactions to yield adduct ions. These ions can complicate the interpretation of in-source CID spectra. Ion adducts noted with water, acetonitrile and aliphatic alcohols. High-resolution mass measurements were used to establish the structures.]
Table 1: LC-TOF Experimental Parameters

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  - Mass range: 90 to 3000

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  - Scan duration (secs): 0.50
  - Interscan delay (secs): 0.50
  - Ionization mode: ES+
  - Mass range: 50 to 3000

- **Function 3**
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  - Scan duration (secs): 0.50
  - Interscan delay (secs): 0.50
  - Ionization mode: ES-
  - Data type: Enhanced Mass
  - Mass range: 90 to 3000

- **Function 4**
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Figure 1: NIST Mass Spec Interpreter Program for Correlating Substructural Fragments with Observed Ions
Figure 2: Multi-Function *Single Analysis*, MS and Diode-Array Chromatograms of Anionic Surfactant Shown

Diode Array 190-900 nm

75 sample cone Neg Ion

75 sample cone Neg Ion

75 sample cone Pos Ion

25 sample cone Pos Ion
Figure 3: Positive and Negative Ion Spectra with Sample Cone at 25 Volts

- $(M-H)^-$ (n=11)
- $(M+H)^+$ (n=11)
- $(M+NH_4)^+$ (n=11)
- $(M+H+NH_4)^{+2}$ (n=15)
Figure 4: Negative and Positive ion In-Source CID Spectra for Unknown 1 at Sample Cone of 75 Volts
Figure 5: Positive and Negative Mass Spec at Sample Cone of 25 Volts for Unknown 2

\[
\text{H}_9\text{C}_9\text{O}\left[\text{H}_{n}\right]_{\text{O}}\text{H}
\]

\[
\text{(M+OAc)}^\cdot \text{[n=11]}
\]

\[
\text{(M+NH}_4^\text{)}^\cdot \text{[n=11]}
\]
Figure 6: Positive and Negative Mass Spec at Sample Cone of 25 Volts for Unknown 3

- $(M-H)^-$, $[m+n=16]$
- $(M+NH_4)^+$, $[m+n=16]$
- $(M+2NH_4)^{+2}$, $[m+n=21]$

Chemical structure with labels:
- $(M+NH_4)^+$, $[m+n=16]$
- $(M+2NH_4)^{+2}$, $[m+n=21]$
- $(M-H)^-$, $[m+n=16]$
Figure 7: "Literature-Real Samples" Excel Worksheet Sorted by RMW then Type

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<td>344</td>
<td>36</td>
<td>Called &quot;SDS&quot; for C12 Homolog when no PEG groups added</td>
<td>anionic</td>
<td>mainly C12-C1- unsaturated, but C8-C24 and u-series</td>
</tr>
<tr>
<td>253</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>344</td>
<td>36</td>
<td>Hiterol BC Series, found in residue from Hiterol BC Series polymerizable surfactant</td>
<td>anionic</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>344</td>
<td>36</td>
<td>mixture of mono and diester phosphates</td>
<td>anionic</td>
<td>exists as monodiester, reduces respectively</td>
</tr>
<tr>
<td>140</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>256</td>
<td>36</td>
<td>Salts of Fatty Acids</td>
<td>anionic</td>
<td>simple soap for hydrolysis of the</td>
</tr>
</tbody>
</table>
Figure 8: Analysis of Cationic Surfactant by Accurate Mass and "TSCA Monomers" Excel Worksheet

- +ion 25 v
- +ion 75 v

Unknown

- Tropylium ion
- Tropylium ion + ACN adduct
- M+ - CH2-phenyl
- M+
Figure 9: Search of "TSCA Monomers Worksheet" with m/z 304.3039, Two Possible Structures Noted Consistent with In-Source CID Spectrum.
Figure 10: LC-MS Data for an Unknown Surfactant Noted in a Commercial Inkjet Cartridge.
Figure 11: LC-MS Data for an Unknown Surfactant Noted in a Commercial Inkjet Cartridge.