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Structural elucidation of co-polymer-like surfactants using MALDI High-Energy Collision Induced Dissociation

ASMS 2015 ThP 068

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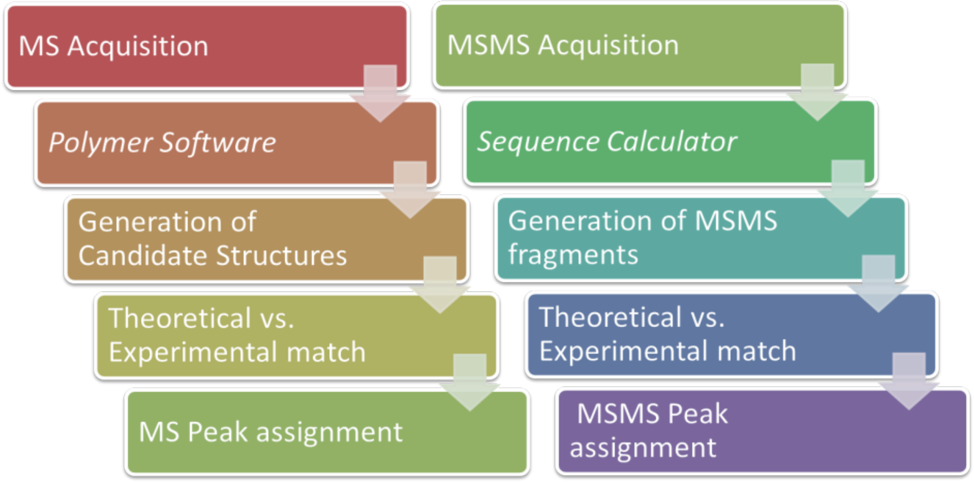
Introduction

- Despite the extensive use of surfactants in household and industry, there is a lack of accessible data in the literature describing their analysis.
- Most of this information is generally proprietary and not available in public domain. The rising interest in surfactant analysis relies in the heavy pollutant power of untreated wastewaters that diffuse in environments and represent a high risk of contamination for ecosystems and population.
- In this regards, MALDI analysis appears particularly suited for polymer-like structures such as surfactants, offering an easy and versatile technique to investigate with high accuracy structural details such as chain derivatisations and terminal groups.

Aim

- Describe the complete fragmentation pathway of co-polymer-like neutral surfactants using High-Energy Collision Induced Dissociation (HE-CID), followed by a software-guided peak assignment. Polyethylene oxide (PEO) structures, linked with saturated fatty alcohol chains of different length, i.e. C12-C18, were subjected to HE-CID, revealing chain linkages, length of fatty acid chains and end-capping group.
- Software guided peak identification was applied in both MS and MSMS, allowing quick and accurate assignments for most of the detected signals, including those not directly assignable and otherwise likely to be discarded.

Workflow in the software guided structure elucidation



MS Acquisition

Polymer Software

Generation of Candidate Structures

Theoretical vs. Experimental match

MS Peak assignment

MSMS Acquisition

Sequence Calculator

Generation of MSMS fragments

Theoretical vs. Experimental match

MSMS Peak assignment

Results

MS

- The MS analysis in reflectron positive mode, showed multiple gaussian distributions belonging to different fatty acid chain lengths and PEO units. The most intense signals were later confirmed to be part of the lauryl fraction (C12:0).

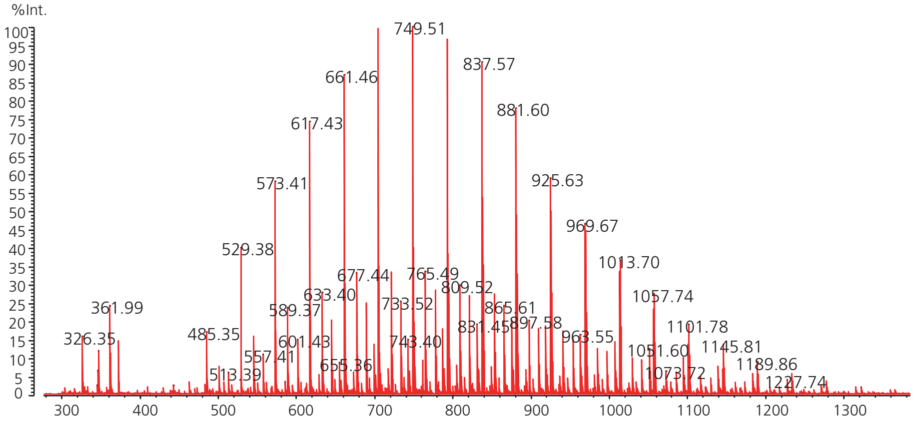
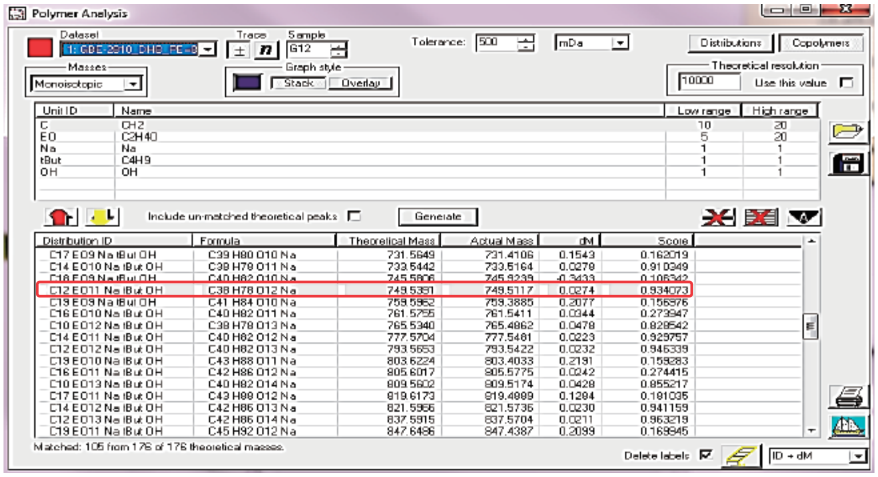


Figure 1: Reflectron positive MS analysis of poly-ethoxylated fatty alcohol surfactant. Different distribution refer to combinations of saturated alkyl chains (C12-C18) with variable EO units.

- Distributions from MS analysis, were subjected to combinatorial search matching theoretical values over experimental m/z signals. A software guided procedure using *Polymer Analysis* (a Shimadzu proprietary tool), allowed to match over 60% of the total chains within a tolerance of 500 mDa.
- The remaining portion was associated to unsaturated structure and deliberately not investigated in this instance.
- Figure 2 shows the list of theoretical candidates per each peak. Additional information such as error and raw formula are accessible along with a score that shows the likelihood of that species over its isotopic distribution match, i.e. exact match, score=1.



Dataset: 1: GDE-2910_DHB_FE-0
Sample: G12
Tolerance: 500 mDa
Masses: Monoisotopic
Theoretical resolution: 10000
Use this value ☐

Unit ID	Name	Low range	High range
C	CH2	10	20
EO	C2H4O	5	20
Na	Na	1	1
tBut	C4H9	1	1
OH	OH	1	1

Include un-matched theoretical peaks ☐

Distribution ID	Formula	Theoretical Mass	Actual Mass	dM	Score
C17 EO9 Na tBut OH	C39 H80 O10 Na	731.5649	731.4106	0.1543	0.162019
C14 EO10 Na tBut OH	C38 H78 O11 Na	733.5442	733.5164	0.0278	0.910349
C18 EO9 Na tBut OH	C40 H82 O10 Na	745.5806	745.9239	-0.3433	0.106342
C12 EO11 Na tBut OH	C38 H78 O12 Na	749.5391	749.5117	0.0274	0.934073
C19 EO9 Na tBut OH	C41 H84 O10 Na	759.5962	759.3885	0.2077	0.156976
C16 EO10 Na tBut OH	C40 H82 O11 Na	761.5755	761.5411	0.0344	0.273947
C10 EO12 Na tBut OH	C38 H78 O13 Na	765.5340	765.4862	0.0478	0.828542
C14 EO11 Na tBut OH	C40 H82 O12 Na	777.5704	777.5481	0.0223	0.929757
C12 EO12 Na tBut OH	C40 H82 O13 Na	793.5653	793.5422	0.0232	0.946339
C19 EO10 Na tBut OH	C43 H88 O11 Na	803.6224	803.4033	0.2191	0.159283
C16 EO11 Na tBut OH	C42 H86 O12 Na	805.6017	805.5775	0.0242	0.274415
C10 EO13 Na tBut OH	C40 H82 O14 Na	809.5602	809.5174	0.0428	0.855217
C17 EO11 Na tBut OH	C43 H88 O12 Na	819.6173	819.4889	0.1284	0.181035
C14 EO12 Na tBut OH	C42 H86 O13 Na	821.5966	821.5736	0.0230	0.941159
C12 EO13 Na tBut OH	C42 H86 O14 Na	837.5915	837.5704	0.0211	0.963219
C19 EO11 Na tBut OH	C45 H92 O12 Na	847.6486	847.4387	0.2099	0.169945

Matched: 105 from 176 of 176 theoretical masses.

Delete labels ☑ ID + dM

Figure 2: *Polymer Analysis* software. The monomer units composing the molecule are combinatorially generated over the experimental mass list and a table of potential candidates is generated with an error and score per each detected signal.

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- The combinatorial approach is used for a first approach towards the peak identification, although MSMS was required to confirm the structure of each species.
- Figure 3 shows the peak assignments as per MS analysis in the range of 749 m/z and 793 m/z.

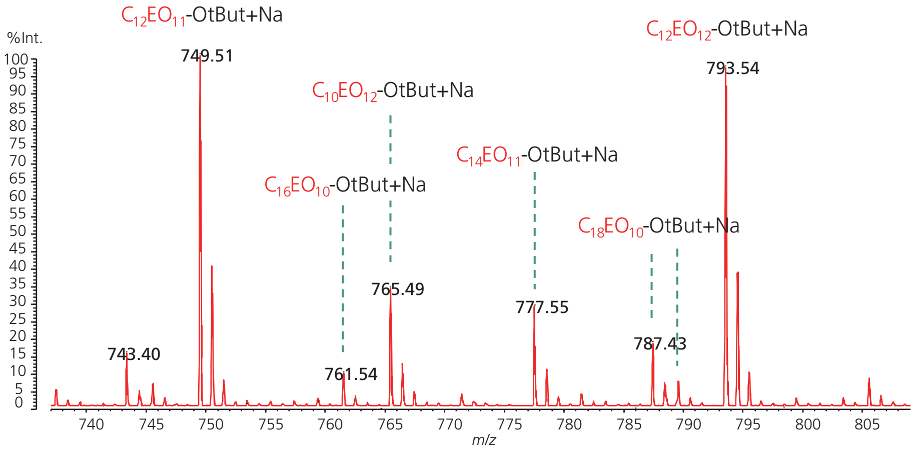
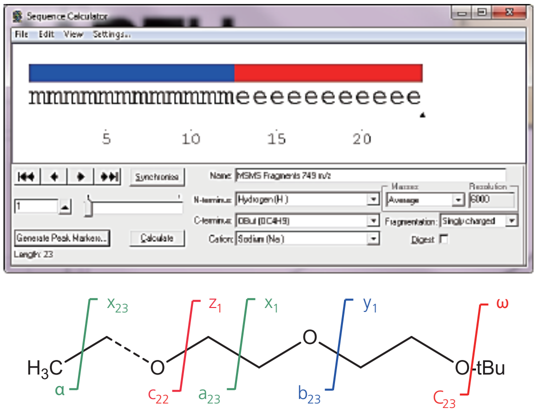


Figure 3: Following to software based assignments, peak are labelled according to the highest probability

MS²

- Being one of the most intense in the gaussian distribution, the peak at 749 m/z, was taken as a precursor model for MSMS investigations.
- Spectra were typically dominated by fragments from the PEO backbone, likely due to the higher stability of oxide ions in the sodiated form.
- The aim was to confirm: 1) the relative length for both PEO and alkyl chains and 2), the species was end-capped with a t-Bu (tert-Butyl) moiety in the PEO portion.
- By using the Shimadzu proprietary software, *Sequence Calculator*, MSMS fragments were generated *in-silico* from the candidate structure (C12EO11OtBut+Na) previously obtained in MS (Figure 4).
- The resulting ions, *x,y,z* from ethylene oxide side, and *a,b,c*, from alkyl chain side, were subsequently matched over the experimental data with a tolerance of 400 mDa.


mmmmmmmmmmmmeeeeeeeeeee

Name: MSMS Fragments 749 m/z
N-terminus: Hydrogen (H)
C-terminus: OBut (OC4H9)
Cation: Sodium (Na)
Masses: Average
Resolution: 5000
Fragmentation: Singly charged
Digest ☐
Length: 23

Figure 4: *Sequence calculator* software. Using custom moieties such as methyl (m) and ethylene oxides (e), it is possible to calculate expected fragments from a candidate structure. The nomenclature scheme is reported in the bottom part of the figure.

- The HE-CID at 20keV, led to complete fragmentation of the chain backbone, providing the full sequence of the molecule including the portion end cap, lost as C2H4Ot-Bu (ion *23b*).
- Typical losses of 2 Da were also observed as a result of PEO rearrangements as reported in other literature data.
- The full list of matched ions, was reported in the software-generate table that includes mass and error per each corresponding peak Figure 5.

MSMS Fragments 749 m/z [L:23]

N-terminus : Hydrogen (H)
C-terminus : OBut (OC4H9)
Sequence composition : e11 m12
Elemental composition (M+Na)+ : C38H78O12Na
Average mass (M+Na)+ : 750.02
Most abundant mass (M+Na)+ : 749.54
Monoisotopic mass (M+Na)+ : 749.54

Fragment	Predicted	Actual	Difference
13a	207.3566	207.5507	-0.1942
14a	251.4099	251.6029	-0.1930
15a	295.4631	295.5657	-0.1026
16a	339.5164	339.5876	-0.0712
17a	383.5697	383.6319	-0.0622
18a	427.6230	427.6670	-0.0440
19a	471.6763	471.6697	0.0066
20a	515.7296	515.6540	0.0756
21a	559.7828	559.6735	0.1093
22a	603.8361	603.7024	0.1337
23a	647.8894	647.6643	0.2251
12b	191.3136	191.2411	0.0725
13b	235.3669	235.6534	-0.2865
14b	279.4202	279.7026	-0.2824
15b	323.4735	323.6012	-0.1277
16b	367.5268	367.6702	-0.1434
17b	411.5800	411.6876	-0.1075
18b	455.6333	455.6566	-0.0233
19b	499.6866	499.6182	0.0684
20b	543.7399	543.6280	0.1119
21b	587.7932	587.7684	0.0248
22b	631.8465	631.6871	0.1594
23b	675.8997	675.7109	0.1889
4c	96.1284	96.5005	-0.3721
5c	110.1554	110.5307	-0.3753
6c	124.1824	124.5190	-0.3366
15c	340.5041	340.7758	-0.2717
16c	384.5574	384.7761	-0.2187
17c	428.6107	428.9973	-0.3866
18c	472.6640	473.0060	-0.3420
19c	516.7173	516.9481	-0.2309
2x	211.2139	211.5771	-0.3631
3x	255.2672	255.5631	-0.2959
4x	299.3205	299.5513	-0.2308
5x	343.3738	343.5470	-0.1732
6x	387.4271	387.5994	-0.1724
7x	431.4803	431.6023	-0.1219
8x	475.5336	475.6191	-0.0854
9x	519.5869	519.5416	0.0453
10x	563.6402	563.6348	0.0054
11x	607.6935	607.4765	0.2169
12x	621.7205	621.6009	0.1196
13x	635.7475	635.6616	0.0858

Fragment	Predicted	Actual	Difference
14x	649.7744	649.5806	0.1938
15x	663.8014	663.6632	0.1382
16x	677.8284	677.5923	0.2361
17x	691.8554	691.7051	0.1504
18x	705.8824	705.7129	0.1695
22x	761.9904	761.6305	0.3598
1y-2	139.1503	139.5364	-0.3861
2y-2	183.2036	183.5142	-0.3106
3y-2	227.2569	227.5504	-0.2935
4y-2	271.3102	271.5425	-0.2324
5y-2	315.3634	315.5375	-0.1741
6y-2	359.4167	359.5576	-0.1409
7y-2	403.4700	403.6015	-0.1315
8y-2	447.5233	447.5697	-0.0464
9y-2	491.5766	491.5781	-0.0015
10y-2	535.6299	535.5649	0.0650
11y-2	579.6831	579.6215	0.0616
13y-2	607.7371	607.4765	0.2606
14y-2	621.7641	621.6009	0.1632
15y-2	635.7911	635.6616	0.1295
16y-2	649.8181	649.5806	0.2375
17y-2	663.8451	663.6632	0.1819
18y-2	677.8721	677.5923	0.2798
19y-2	691.8990	691.7051	0.1940
20y-2	705.9260	705.7129	0.2131
1y-1	140.1583	140.5567	-0.3984
2y-1	184.2116	184.5947	-0.3831
3y-1	228.2649	228.5562	-0.2913
5y-1	316.3714	316.7100	-0.3386
8y-1	448.5313	448.8698	-0.3385
10y-1	536.6378	536.9909	-0.3531
1y	141.1663	141.5054	-0.3391
4y	273.3261	273.4786	-0.1525
5y	317.3794	317.4306	-0.0512
7y	405.4860	405.7663	-0.2803
12y	595.7261	595.6737	0.0523
23y	750.0230	749.7119	0.3111
1z	124.1356	124.5190	-0.3834
2z	168.1889	168.5534	-0.3645
3z	212.2422	212.5503	-0.3081
4z	256.2955	256.6722	-0.3768
6z	344.4020	344.7142	-0.3122
10z	520.6152	520.8068	-0.1916

Figure 5: Table of MSMS ions generated with *Sequence Calculator* for the precursor at 749 m/z. Matches of theoretical values are compared towards experimental peak signals using an error tolerance of 400 mDa.

- The linkage between the PEO chain and the alkyl chain was characterised by an intense peak at 579 m/z (ion 11y-2), confirming the 11EO units estimated in MS analysis by the software.
- Confirmation of the C12 chain, was given by the presence of the ion [C12H24O+Na]$^+$ at 207 m/z, representing the carbonyl from of 13a.
- The peak list was finally exported in the spectrum and assigned to each signal. Some of the assignments are listed in Figure 6.

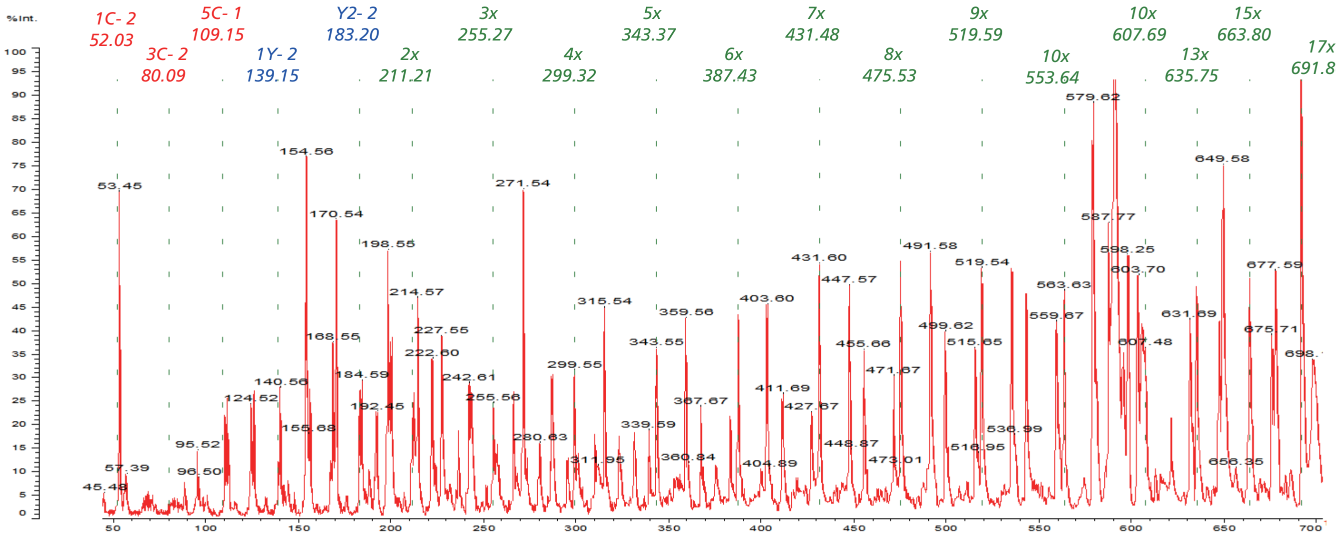


Figure 6: Resulting MSMS ions obtained in the HE-CID of the precursor at 749 m/z.

Materials & Methods

- Saturated fatty alcohol ethoxylated with variable EO units, were kindly provided by Clariant (Germany).
- All solvents and chemicals were purchased from Sigma-Aldrich (UK). Surfactants were dissolved in 1:1 CH3CN/H2O acidified with 0.1% v/v trifluoroacetic acid (TFA). 1%v/v of 0.1 M NaCl was added as the doping agent, whereas 10 mg/mL DHB in 1:1 CH3CN/H2O 0.1% v/v TFA, was used as the matrix.
- An *Axima Performance*, MALDI TOF-TOF mass spectrometer (Shimadzu, UK) was used in MS and MSMS analyses in reflectron positive mode. MALDI ions were generated under high vacuum conditions and fragmented by collisional induced dissociation using helium (20keV (lab frame of reference). All acquisition parameters used for fragmentation experiments were set by software control.

Conclusions

- We used proprietary software developed for the interpretation of polymers and co-polymers in order to confirm our finding.
- Peak assignment was successfully achieved for fragments belonging to different ion series resulting from both, PEO and Alkyl portions of the surfactant.
- The complementary use of software guided approach crucially helped in the accuracy of the assignment speeding up data interpretation.
- Additional structures are currently under investigation.

Acknowledgements

- Clariant for generously donating the surfactants.

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First Edition: June, 2015

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