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## DEGREES OF FREEDOM EFFECT ON FRAGMENTATION IN TANDEM MASS SPECTROMETRY OF SINGLY CHARGED SUPRAMOLECULAR AGGREGATES OF SODIUM SULFONATES

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### Abstract

The characteristic collision energy (CCE) to obtain 50% fragmentation of positively and negatively single charged non-covalent clusters has been measured. CCE was found to increase linearly with the degrees of freedom (DoF) of the precursor ion, analogously to that observed for synthetic polymers. This suggests that fragmentation behavior (e.g. energy randomization) in covalent molecules and clusters are similar. Analysis of the slope of CCE with molecular size (DoF) indicates that activation energy of fragmentation of these clusters (loss of a monomer unit) is similar to that of the lowest energy fragmentation of protonated leucine-enkephalin. Positively and negatively charged aggregates behave similarly, but the slope of the CCE vs DoF plot is steeper for positive ions, suggesting that these are more stable than their negative counterparts.

## Introduction

The effect of molecular size on fragmentation of ions formed by electron ionization (EI) has been largely investigated at the beginning of organic mass spectrometry<sup>1-3</sup>. On the ground of quasi-equilibrium theory (QET), this effect is attributed to the rapid spreading of the energy conferred by electron ionization<sup>4-6</sup> or chemical ionization<sup>7</sup> to overall the vibrational Degrees of Freedom (DoF). Thus, if the same amount of energy is spread over a larger number of DoF, less energy is conferred to each DoF. This slows down fragmentation rates with the size of the ions.

The advent of fast atom bombardment (FAB)<sup>8</sup> followed by other soft ionization techniques, as the most widely used electrospray ionization (ESI)<sup>9</sup> and matrix assisted laser desorption ionization (MALDI)<sup>10,11</sup>, makes Collision Induced Dissociation (CID) and tandem mass spectrometry<sup>12-15</sup> the most widespread method to fragment ions either for analytical purposes or to obtain structural information. The low fragmentation efficiency of singly charged ion with  $m/z \geq 2,000$  shows that this size effect occurs also in CID experiments<sup>16</sup>. This implies that collision energy conferred by the impact of target gas is likely to spread among all DoF in a short time (much less than the residence time in the collision cell, which is typically in the range of  $\mu\text{s}$ - $\text{ms}$ ). In order to observe fragmentation therefore the parent ion needs more internal energy than that of the critical energy. The amount of excess energy required to observe fragments in the timescale of the mass spectrometric experiment has been called the "kinetic shift"<sup>14</sup>. The importance of this effect is generally recognized and has often been discussed. This makes of particular importance, both for practical and for fundamental applications, to study collision energy effects as a function of precursor ion size. However, only few systematic studies concerning the quantitative relationship between collision energy and DoF are reported in literature<sup>17-22</sup>. The relation between fragmentation energetics and molecular size for different type of polymers: poly-ethylene glycols (PEG), poly-tetrahydrofurane (PTHF) and peptides has been reported by Memboeuf *et al.*<sup>21,23</sup>. A good linear correlation between the Characteristic Collision Energy (CCE: the collision energy corresponding to a Survival Yield of 50 %<sup>24-26</sup>) and the mass of the ion has been observed, independently of the type of instrument used. In particular, when the activation energy and entropy do not significantly depend on molecular mass (which is well satisfied for a given oligomer series) the linear correlation observed is excellent ( $R^2 > 0.996$ ). On the other hand for peptides, which have a range of activation energies (and entropies), the correlation is less accurate ( $R^2 = 0.917$ )<sup>21</sup>. A good linear dependence of CCE with ion mass has been also found for singly and doubly lithiated polytetrahydrofuran

(PTHF)<sup>23</sup>. A recent systematic study of various homopolymers confirmed the linear correlation between the characteristic collision energy (CCE) and the precursor ion mass for the investigated polymers. The slopes of the linear trendlines were, however significantly different for various homopolymers. The slope was found to decrease with the activation energy of dissociation, decreasing in the order of polyethers>polymethacrylates> polyesters > polysaccharides. This trend suggested that the slope of the CCE trendline is linearly proportional to the energy required for fragmentation. This also explains that cyclic structures have higher CCE values, due to the additional energy needed for the ring-opening<sup>27</sup>.

All these investigations concerning the DoF effect on the fragmentation relate to compounds characterized by covalently bound repeating units. To the best of our knowledge, the DoF effect has not been studied for non-covalent aggregates. This may be of interest for various organic or inorganic clusters, and also for biomolecular complexes. To shed some light on this subject, we decided to investigate the DoF dependence of the CCE of singly charged aggregates of amphiphilic substances as sodium methanesulfonate (MetSO<sub>3</sub>Na), sodium *n*-butanesulfonate (ButSO<sub>3</sub>Na) and sodium *n*-octanesulfonate(OctSO<sub>3</sub>Na) (Figure 1).

These species have been selected as typical representatives of non-covalent aggregate forming substances<sup>28</sup>, more simple, but structurally similar to the surfactant sodium bis(ethylhexyl)sulfosuccinate (AOT). AOT aggregates have been observed under ESI<sup>29-34</sup> FAB<sup>35</sup>, MALDI<sup>36</sup>, and ToF-SIMS<sup>37</sup> conditions both as positive and negative ions. Self-assembling sodiated AOT aggregates *in vacuo* has also been investigated by molecular dynamics simulation<sup>38,39</sup>. The structurally similar, but more simple amphiphilic MetSO<sub>3</sub>Na, ButSO<sub>3</sub>Na and OctSO<sub>3</sub>Na compounds form analogous positively and negatively charged aggregates under ESI conditions<sup>31,33</sup>. This observation, together with theoretical studies<sup>40</sup> suggests that the aggregation process is determined mainly by electrostatic interactions among head groups while the chain has little influence.

**Please insert here Figure 1**

### **Experimental section**

Methanesulfonic acid Sodium salt, MetSO<sub>3</sub>Na, (≥ 98%), Butanesulfonic acid Sodium salt, ButSO<sub>3</sub>Na (≥ 95%), 1-Octanesulfonic acid sodium salt OctSO<sub>3</sub>Na (approx 98%) were purchased from Sigma- Aldrich (Germany). Solvents used were LC-MS grade from Sigma- Aldrich (Germany).

Q-ToF experiments were carried out using a quadrupole-time of flight high resolution LC/MS system (Waters q-ToF Premier, Manchester, UK), which was equipped with an electrospray source (ESI).

In all the ESI MS and ESI MS/MS experiments the following common operating conditions were chosen: capillary voltage 3.5 kV in ESI (+) and 3 KV in ESI (-), sampling cone voltage: 60 V, extraction cone voltage: 3.2 V, ion guide: 1.2 V, source temperature 90 °C, desolvation temperature 250 °C, cone gas flow 50 l hr<sup>-1</sup>, desolvation gas flow 300 l hr<sup>-1</sup> mass range 50-1000 m/z and collision gas: Argon.

The ESI-MS experiments were performed in positive and negative ion mode using 0.5 mM solutions of each compound in water/methanol (1:1) that were infused at 10 µl min<sup>-1</sup> flow rate. ESI MS/MS measurements were performed using the same sample solutions which were introduced directly into ESI source at 15 µl min<sup>-1</sup> flow rate.

The mix solutions of hetero-oligomers (MetSO<sub>3</sub>Na, ButSO<sub>3</sub>Na, OctSO<sub>3</sub>Na) were prepared using equimolecular starting solutions of each compound at a concentration 0.5mM in water/methanol (1:1).

Leucine-Enkephalin solution has been prepared using a water/acetonitrile (0,1% formic acid) solvent mixture at a concentration of 1mg ml<sup>-1</sup>. Poly(ethylene glycol) samples with average molecular weights varying from 400 up to 1000 Da, were used, at a 50 µM concentration in methanol (MeOH) saturated with lithium bromide.

The Survival Yield curves for each analyte were obtained using the same experimental procedure used for ESI MS/MS experiments, except the collision energies that were changed by discrete values ranging from 5 V to 115 V during the flow injection experiments. For each collision energy an acquisition time of 1 minute was set. Each ESI MS/MS spectrum was obtained by the average of 11 consecutive scans. The percent of each fragment was calculated dividing the counts of the correspondent peak to the sum of counts of all peaks including the precursor ion.

Experimental conditions were maintained constant through the complete set of measurements and each SY curve of positively and negatively singly charged aggregates was repeated 3 times.

## **Results and discussion**

Within the investigated collision energy window, fragmentation of the selected non-covalent aggregates proceeds through consecutive and competitive losses of monomers and/or n-mers. This was established through a careful analysis of the breakdown curves of parent daughter ions.<sup>31</sup> There are practically no fragmentations involving cleavage of covalent bonds<sup>31,33</sup>. Fragmentation efficiency can be described by the SY function defined according to the equation 1:

$$SY = \frac{I_M}{I_M + \sum I_F} \quad \text{eq.1}$$

where  $I_M$  is the intensity of the ionized molecule and  $\sum I_F$  is the sum of all fragment ions intensities. The SY values vs. the collision energy (CE) have been plotted for the singly charged non covalently bounded homo-oligomers of MetSO<sub>3</sub>Na, ButSO<sub>3</sub>Na, OctSO<sub>3</sub>Na and of the hetero-oligomers of MetSO<sub>3</sub>Na-ButSO<sub>3</sub>Na, ButSO<sub>3</sub>Na-OctSO<sub>3</sub>Na, MetSO<sub>3</sub>Na-OctSO<sub>3</sub>Na. Graphs have been plotted for both positively and negatively charged supramolecular aggregates. Collision energy dependent fragmentation of altogether 55 clusters have been studied, including the following species: [(MetSO<sub>3</sub>)<sub>n</sub>Na<sub>n+1</sub>]<sup>+</sup>, [(ButSO<sub>3</sub>)<sub>n</sub>Na<sub>n+1</sub>]<sup>+</sup>, [(OctSO<sub>3</sub>)<sub>n</sub>Na<sub>n+1</sub>]<sup>+</sup>, [(MetSO<sub>3</sub>)<sub>n</sub> (ButSO<sub>3</sub>)<sub>m</sub> Na<sub>(n+m)+1</sub>]<sup>+</sup>, [(MetSO<sub>3</sub>)<sub>n</sub> (OctSO<sub>3</sub>)<sub>m</sub> Na<sub>(n+m)+1</sub>]<sup>+</sup>, [(ButSO<sub>3</sub>)<sub>n</sub> (OctSO<sub>3</sub>)<sub>m</sub> Na<sub>(n+m)+1</sub>]<sup>+</sup> in positive ion mode and [(MetSO<sub>3</sub>)<sub>n</sub>Na<sub>n-1</sub>]<sup>-</sup>, [(ButSO<sub>3</sub>)<sub>n</sub>Na<sub>n-1</sub>]<sup>-</sup>, [(OctSO<sub>3</sub>)<sub>n</sub>Na<sub>n-1</sub>]<sup>-</sup>, [(MetSO<sub>3</sub>)<sub>n</sub> (ButSO<sub>3</sub>)<sub>m</sub> Na<sub>(n+m)-1</sub>]<sup>-</sup>, [(MetSO<sub>3</sub>)<sub>n</sub> (OctSO<sub>3</sub>)<sub>m</sub> Na<sub>(n+m)-1</sub>]<sup>-</sup>, [(ButSO<sub>3</sub>)<sub>n</sub> (OctSO<sub>3</sub>)<sub>m</sub> Na<sub>(n+m)-1</sub>]<sup>-</sup> in negative ion mode (with n+m =6). For positive ions the results cover homo-oligomers with aggregation number (n) ranging from 2 to 10 for MetSO<sub>3</sub>Na, ButSO<sub>3</sub>Na, OctSO<sub>3</sub>Na, while in negative ion mode homo-aggregates of MetSO<sub>3</sub>Na and OctSO<sub>3</sub>Na with n = 3-10, and ButSO<sub>3</sub>Na with n = 3 - 7 were observed. For hetero-oligomers the n+m value ranges from 2 to 6 both in positive and in negative ion mode.

The intensity of the precursor ion, and consequently the SY value, decreases with the collision energy applied. Results for butanesulfonate clusters (n= 3-5) in positive and negative ion mode are shown in Figure 2. The shapes of SY curves as a function of collision energy can be well described by a sigmoid function. Figure 2 shows that the SY curves are shifted to higher collision voltage when the number of monomeric units is increased. This trend is closely analogous to that observed for covalently bound oligomers<sup>21,23,27</sup>.

### **Please insert here Figure 2**

To compare the behavior of different species (by type and number of monomers) we evaluated their Characteristic Collision Energy (CCE) values. First of all reproducibility of Q-ToF measurements was assessed. In order to determine repeatability, the SY curves of the OctSO<sub>3</sub>Na trimer were measured in succession 10 times, both in positive and in negative ion mode. To verify interday reproducibility the octanesulfonate trimer was measured at six different dates; in each case measuring the full SY curve and determining the CCE value. The results show that repeatability (Relative Standard Deviation, RSD) of the CCE value was 4.2% in intraday experiments and 7.8%

in interday ones, both in positive and negative ion mode. To avoid systematic errors, SY curves were acquired for each compound alternatively at low mass and high mass (e.g. SY of OctSO<sub>3</sub>Na with n =2 followed by OctSO<sub>3</sub>Na with n=6, then OctSO<sub>3</sub>Na with n=3 etc.).

The CCE values of all studied homo- and hetero-aggregates, both in positive and negative ion mode are shown in Figure 3 as a function of the degrees-of-freedom (DoF) covering a wide mass range from ca. 100 to 2100 Da. The number of DoF has been calculated by equation 2:

$$DoF = (3 \times n) - 6 \quad \text{eq. 2}$$

where n is the number of atoms constituting the whole aggregate.

This mass range corresponds to 50-900 DoF. The CCE values as a function of DoF for all positively charged aggregates can be well described by a single linear trendline, characterized by a good linear fit ( $R^2 > 0.95$ ). It is worthwhile that the CCE values depends only on size (DoF). This implies that clusters with same DoF but with different composition and aggregation number share the same CCE. A similar trend with a somewhat lower slope is observed for negatively charged aggregates (Figure 3).

### **Please insert here Figure 3**

The present results on clusters may be compared to the behaviour of poly ethylene glycols (PEGs), shown in Figure 4. This shows data on PEGs both measured in this study (triangles) and taken from the literature (filled circles<sup>21</sup>). Both data sets show a similar trendline and are numerically nearly superimposable. Note, the instrument in the present work was tuned according to standard procedures, and not to scale the present results to those in the literature. This may suggest that similar type instruments with roughly similar experimental conditions, yield a similar degree of excitation (tandem mass spectra) under conventional tuning in different laboratories. As a further comparison, CCE of leucine-enkephalin has also been measured, and is shown in Figure 4.

### **Please insert here Figure 4**

It has been reported before<sup>21,23,27</sup> that the linear trendline suggests that the studied series of compounds are all characterized by the same activation energy; and that the slope of the CCE vs. DoF (or molecular mass) trendline closely relates to the activation energy of a series of oligomers. This may be expressed in a different way; that for two compounds having the same mass (or DoF)

and the same charge state, a difference in CCE values represents a difference in activation energies. In the present case the CCE value of protonated leucine-enkephalin practically overlaps with those of the studied clusters having the same DoF, suggesting that the respective activation energies are similar. The activation energy of the lowest energy fragmentation channel of leucine-enkephalin is 1.2 eV (ranging from 1.14<sup>41</sup> to 1.66<sup>42</sup> with average value of 1.2 eV<sup>43</sup>); this suggests that the activation energy of fragmentation for the studied clusters is similar, in the order of 1.2 eV (positively charged clusters) and 1.0 eV (negatively charged clusters).

Comparing the slopes of the trendlines in Figure 4 indicates that the slope for the studied clusters is much less than that of PEGs (by approximately a factor of 3 times); and it is also known that fragmentation of PEGs requires approximately 2.2-2.8 eV<sup>21</sup> activation energy. In qualitative terms this well corresponds to the result based on comparison with leucine-enkephalin: the clusters have a much lower activation energy than those of PEGs. The slope for positive clusters is about 20% higher than that of negative clusters, indicating that positive clusters have higher stability. This well corresponds to relative ion stabilities of these aggregates indicated by both experimental<sup>33</sup> and theoretical (molecular dynamics simulations<sup>38</sup>) data. Note that the intercept of the lines are not exactly zero, usually a small positive value. Its explanation and physical relevance is unknown.

## Conclusions

The experimental data show a linear correlation between the Characteristic Collision Energy (CCE) and the DoF of various non-covalent clusters of surfactants. This behavior is analogous to that observed for various synthetic oligomers, suggesting that the average activation energy (more accurately activation Gibbs energy) of fragmentation does not change with cluster size (i.e. is independent of  $n$ ). The analogy also implies that fragmentation of covalent molecules and non-covalent clusters follows the same scale law, even though in one case bond cleavage, in the other separation of non-covalent clusters take place. It is most tempting to rationalize it by fast randomization of internal energy both in covalent and non-covalent species. These observations are in-line with previous findings based on Infrared Multi-Photon Dissociation (IRMPD) experiments<sup>39</sup>, which also suggest fast spread of energy among vibrational degrees of freedom in non covalent surfactant aggregates.

The slopes of the trendlines characterizing cluster fragmentation are much smaller than those of PEGs – this suggests that the corresponding activation energies are also much smaller than those characterizing PEGs (ca. 2.5 eV<sup>21</sup>). In agreement with this observation, comparison with a well-studied peptide, leucine-enkephalin suggests that its activation energy (1.2 eV<sup>43</sup>) is very close to

that of surfactant clusters. Positive clusters show a slightly higher slope than negative clusters, suggesting a 10-20% higher activation energy. These results are in good agreement with expectations of theoretical studies<sup>44</sup>.

## References

1. F. W. McLafferty, W. S. Pike. Metastable Ion Characteristics. II. Variation of Metastable Ion Abundances in Mass Spectra With Vibrational Degrees of Freedom. *J. Am. Chem. Soc.* **1967**, *89*, 5951.
2. F. W. McLafferty, W. S. Pike. Metastable Ion Characteristics. III. Structures of C<sub>3</sub>H<sub>6</sub>O<sup>+</sup> Ions in the Mass Spectra of Aliphatic Ketones. *J. Am. Chem. Soc.* **1967**, *89*, 5953
3. D. J. McAdoo, P.F. Bente. M.L. Gross, - F. W. McLafferty. Product Ions Formed in Mass Spectral Reactions. *Org. Mass Spectrom.* **1974**, *9*, 525
4. F. Bente, F. W. McLafferty, D. J. McAdoo, C. Lifshitz, Internal Energy of Product Ions Formed in Mass Spectral Reactions. The Degrees of Freedom Effect. *J. Phys. Chem.*, **1975**, *79*, 713
5. F. W. McLafferty, F. Turecek, *Interpretation of Mass Spectra*. University Science Books: Mill Valley, CA 1993.
6. M.C. Natoli, L. Ceraulo, M. Ferrugia, L. Lamartina. Studies in Organic Mass Spectrometry. Part 26. Unimolecular decomposition of ortho-methoxy-substituted diphenylmethyl cations". *Eur. Mass Spectrom.* **1999**, *5*, 363.
7. F. J. Winkler, F. O. Golacar, F. Mermoud, D. Stahl, T. Gäumann, A. Buchs. Degree-of-freedom activation effects in proton-transfer chemical ionization mass spectrometry of open-chain and cyclic alcohols and diols. *Int J Mass Spectrom Ion Phys.* **1983**, *46*, 321.
8. M. Barber, R. S. Bordoli, R. D. Sedgwick, A. N. Tyler. Fast atom bombardment of solids as an ion source in mass spectrometry. *Nature.* **1981**, 293, 270.
9. C.M. Whitehouse, N. Robert M. Yamashita, J.B. Fenn, . Electrospray Interface for Liquid Chromatographs and Mass Spectrometers. *Anal. Chem.*, **1985**, *57*, 675.
10. M. Karas, F. Hillenkamp. , Laser Desorption Ionization of proteins with molecular masses exceeding 10 000 daltons. *Anal Chem.* **1988**, *60*, 2299.
11. K. Tanaka, H. Waki, Y. Ido, S. Akita, Y. Yoshida, T. Yoshida. "Protein and Polymer Analyses up to m/z 100 000 by Laser Ionization Time-of flight Mass Spectrometry. *Rapid Commun Mass Spectrom.* **1988**, *2* (20): 151–3. doi:10.1002/rcm.1290020802.
12. F. W. McLafferty., Introduction and History. In "Tandem Mass Spectrometry". McLafferty, F. W., Ed.; John Wiley: New York, **1983**.
13. R. G. Cooks. , Ed. *Collision Spectroscopy*. Plenum Press: New York, **1978**.



14. K. Levsen, *Fundamental Aspects of Organic Mass Spectrometry*; Verlag Chemie. New York, **1978**.
15. A. K. Shukla, J. H. Futrell. Tandem Mass Spectrometry: Dissociation of Ions by Collisional Activation. *J. Mass Spectrom.* **2000**, 35, 1069.
16. A. L. Burlingame, A. Dell, D.H. Russell. Mass Spectrometry. *Anal. Chem.* **1982**, 54, R363.
17. A. R. Dongre, J. Jones, L. A. Somogyi, V. Wysocki, . Influence of peptide composition, gas-phase basicity, and chemical modification on fragmentation efficiency: Evidence for the mobile proton model; *J. Am. Chem. Soc.* **1996**, 118, 8365.
18. V. Bernshtein, I. Oref, Unimolecular Dissociation of Very Large Polyatomic Molecules; *J. Phys. Chem.* **1994**, 98, 136.
19. J. Laskin, C. Lifshitz. *Principles of Mass Spectrometry Applied to Biomolecules*. Ed., **2006**, pp i-xviii.
20. J. L. Jones A. R. Dongre, A. Somogyi, V. H. Wysocki, Sequence Dependence of Peptide Fragmentation Efficiency Curves Determined by Electrospray Ionization/Surface-Induced Dissociation Mass Spectrometry. *J. Am. Chem. Soc.* **1994**, 116, 8368.
21. A. Memboeuf, A. Nasioudis, S. Indelicato,; F. Pollreis,; A. Kuki, S. Kéki, O. F. Van den Brink,; K. Vékey and L. Drahos Size Effect on Fragmentation in Tandem Mass Spectrometry. *Anal. Chem.* **2010**, 82, 2294
22. V. Gabelica,; E. De Pauw. Internal energy and fragmentation of ions produced in electrospray sources. *Mass Spectrom. Rev.*, **2005**, 24, 566.
23. Á Kuki, L. Nagy, A. Memboeuf, D. László, K. Vékey, M. Zsuga, S. Kéki. Energy-Dependent Collision-Induced Dissociation of Lithiated Polytetrahydrofuran: Effect of the Size on the Fragmentation Properties. *J. Am. Soc. Mass Spectrom.*, **2010**, 21, 10, 1753.
24. C. Collette, L. Drahos, E. D. Pauw, K. Vékey. Comparison of the internal energy distributions of ions produced by different electrospray sources. *Rapid Commun. Mass Spectrom.*, **1998**, 12, 1673.
25. F. Derwa, E. D. Pauw, P. Natalis. New basis for a method for the estimation of secondary ion internal energy distribution in 'soft' ionization techniques. *Org. Mass Spectrom* **1991**, 26, 117.
26. X. H. Guo, M. C. Duursma, P. G. Kistemaker, N. M. M. Nibbering, K. Vekey, L. Drahos, R. M. A. Heeren, Manipulating internal energy of protonated biomolecules in electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry. *J. Mass Spectrom*, **2003**, 38, 597.

27. A. Nasioudis, A. Memboeuf, R. M. A. Heeren, D. F. Smith, K. Vékey, L. Drahos, and O. F. Van den Brink. Discrimination of Polymers by Using Their Characteristic Collision Energy in Tandem Mass Spectrometry. *Anal. Chem.*, **2010**, 82, 22,9350.
28. L. Ceraulo, G. Giorgi, V. Turco Liveri, D. Bongiorno, S. Indelicato, F. Di Gaudio, S. Indelicato. Mass spectrometry of surfactant aggregate. *Eur. J. Mass Spectrom.* **2011**, 17, 525.doi: 10.1255/ejms.1
29. G. Giorgi, L. Ceraulo, V.Turco Liveri, Surfactant Self-assembly in the Gas Phase: Bis(2-ethylhexyl)sulfosuccinate-Alkaline Metal Ion Aggregates; *J. Phys. Chem. B* , **2008**, 112, 1376-1382.
30. G. Giorgi, E. Giocaliere, L. Ceraulo, A. Ruggirello, V.Turco Liveri., Spatially ordered surfactant assemblies in the gas phase: negatively charged bis(2-ethylhexyl)sulfosuccinate-alkaline metal ion aggregates; *Rapid Commun. Mass Spectrom.* **2009**, 23, 2206.
31. D. Bongiorno, L. Ceraulo, G. Giorgi, S.Indelicato, A. Ruggirello, V. Turco Liveri, Supramolecular aggregates in vacuum: positively monocharged sodium alkanesulfonate clusters; *Eur. J. Mass Spectrom.***2010**, 16, 151..
32. Y. Fang, A. Bennett, J. Liu. Multiply charged gas-phase NaAOT reverse micelles: Formation, encapsulation of glycine, and collision-induced dissociation; *Int. J. Mass Spectrom.*, **2010**, 293, 12.
33. D. Bongiorno, L. Ceraulo, G. Giorgi, S. Indelicato, M. Ferrugia, A. Ruggirello and V. TurcoLiveri. Effects of the net charge on abundance and stability of supramolecular surfactant aggregates in gas phase. *J. Mass. Spectrom.* **2011**, 46, 195.
34. Y. Fang, A. Bennett, J. Liu. Selective transport of amino acids into the gas phase: driving forces for amino acid solubilization in gas-phase reverse micelles, *Phys. Chem. Chem. Phys.* **2011**, 13, 1466.
35. P.A. Lyon, W.L. Stebbings, F.W. Crow, K.B. Tomer, D.L. Lippstreu, M.L. Gross. Analysis of anionic surfactants by mass spectrometry/mass spectrometry with fast atom bombardment; *Anal. Chem.* **1984**, 56, 8.
36. D. Bongiorno, L. Ceraulo,, A. Ruggirello, V. Turco Liveri, E. Basso, R. Seraglia, P. Traldi, Surfactant self-assembling in gas phase: electrospray ionization- and matrix-assisted laser desorption/ionization-mass spectrometry of singly charged AOT clusters; *J. Mass Spectrom.* **2005**, 40, 1618.
37. S.A. Burns , PL jr Gardella. Determination of critical micelle concentration of aerosol-OT using time-of-flight secondary ion mass spectrometry fragmentation ion patterns. , *Langmuir*, **2009**, 25, 19, 11244-9

38. G. Longhi, S. Abbate, L. Ceraulo, A. Ceselli, S. L. Fornili and V. Turco Liveri. A molecular dynamics study of structure, stability and fragmentation patterns of sodium bis(2-ethylhexyl)sulfosuccinate positively charged aggregates in vacuo. *Phys. Chem. Chem. Phys.* **2011**, 13, 48, 21423.
39. G. Giorgi, L. Ceraulo, G. Berden, J. Oomens, and V. Turco Liveri. Gas Phase Infrared Multiple Photon Dissociation Spectra of Positively Charged Sodium Bis(2-ethylhexyl)sulfosuccinate Reverse Micelle-like Aggregates, *J. Phys. Chem B*, **2011**, 115, 2282.
40. D. Bongiorno, L. Ceraulo, G. Giorgi, S. Indelicato and V. Turco Liveri, Do electrospray mass spectra of surfactants mirror their aggregation state in solution? *J. Mass Spectrom.*, **2011**, 46, 1262 .
41. K.G. Asano, D.J. Butcher, D.E. Goeringer, S.A. McLuckey. Effective ion internal temperatures achieved via boundary activation in the quadrupole ion trap: Protonated leucine enkephalin. *J. Mass Spectrom.* **1999**, 34, 691.
42. J. Laskin. Energetics and dynamics of fragmentation of protonated leucine enkephalin from time- and energy-resolved surface-induced dissociation studies. *J. Phys. Chem. A* **2006**, 10, 8554.
43. J. Sztaray, A. Memboeuf, L. Drahos, and K. Vékey. Leucine enkephalin—a mass spectrometry standard. *Mass Spectrometry Reviews*, **2011**, 30, 29.
44. G. Longhi, S. L. Fornili, V. Turco Liveri, S. Abbate, D. Rebecani, L. Ceraulo, F. Gangemi. Sodium bis(2-ethylhexyl)sulfosuccinate self-aggregation in vacuo: molecular dynamics simulation. *Physic Chem Chem Physics*, **2010**, 12, 4694

**Figure captions:**

**Figure 1:** Structures of sodium methanesulfonate (MetSO<sub>3</sub>Na), sodium butanesulfonate (ButSO<sub>3</sub>Na), sodium octanesulfonate (OctSO<sub>3</sub>Na).

**Figure 2:** SY curves of ButSO<sub>3</sub>Na singly charged 3-mer, 4-mer, 5-mer A) in positive ion mode and B) in negative ion mode.

**Figure 3:** CCE as function of the DoF for positive (▲) and negative (■) alkanesulfonate aggregates. All compounds are singly charged. Trendlines (with slope and correlation coefficient) are also shown.

**Figure 4:** CCE values of lithiated PEGs taken from ref 21 (●), of lithiated PEGs obtained in the present work (▲), of Leucine-enkephalin measured in the present work (X), and of positive (○) and negative (□) alkanesulfonate clusters.