

Rapid Characterization of Complex Viscous Liquids at the Molecular Level**

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Mass spectrometry (MS) is the method of choice for molecular characterization of complex samples,^[1–3] due to its unparalleled ability to acquire detailed qualitative and quantitative information as well as its exceptional sensitivity and specificity. Recent progress in the rapid characterization of complex samples by MS has mostly been due to the rapid development of ambient ionization techniques,^[4–18] which require no or only minimal sample pretreatment. For instance, analytes present on solid surfaces^[5–9] or in liquid solutions,^[10–14] aerosols,^[15,16] and living objects^[17,18] have been successfully detected at low concentration levels. However, studies on viscous complex mixtures remain sparse.

Herein, we report a simple yet universal method based on extractive electrospray ionization mass spectrometry (EESI-MS) to directly analyze complex liquid samples of high viscosity.^[19,20] As shown in Figure 1, a stream of nitrogen gas is directed into the liquid and causes bubble formation under the liquid surface. Driven by the gas pressure, the emerging bubbles carry molecules of the bulk sample up to the liquid surface.

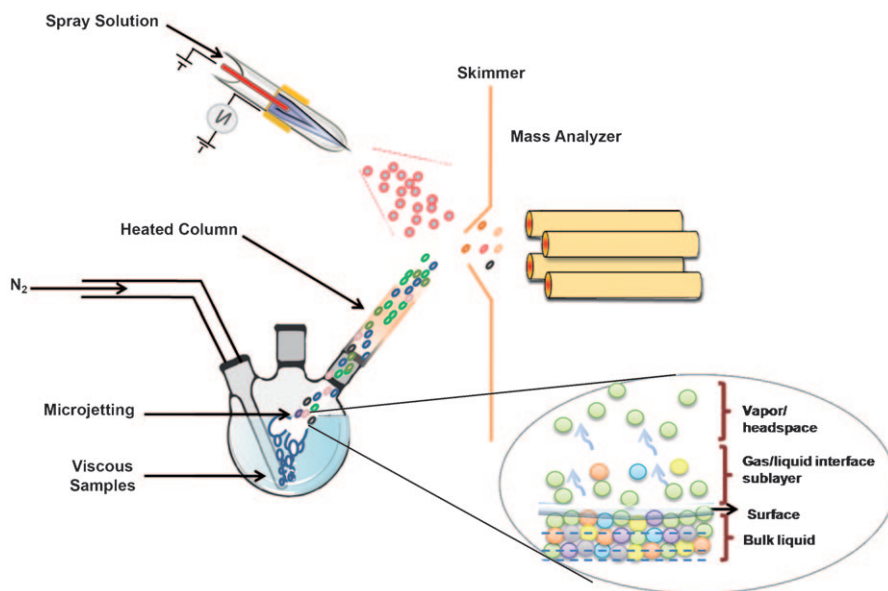


Figure 1. Schematic illustration of the concept and setup of EESI-MS. Inset: compositions and environment of molecules present in bulk liquid, liquid–gas interface, and headspace. The temperature of the heated column is 80 °C.

Eventually, at the gas–liquid interface (inset of Figure 1 and Supporting Information, Figure S1), the bubbles burst, creating microdroplets through a mechanism known as microjetting.^[19,20] Several studies have been conducted to investigate similar processes occurring in highly viscous liquids. These studies, however, only focused on the bursting kinetics of microbubbles rather than on the molecular composition of the microbubbles owing to technological limitations.^[21–23] Unlike previous techniques,^[17] EESI-MS is able to analyze aerosol formed from the bulk liquid, which is representative of the molecular composition of the bulk liquid. Thus, microjetting with subsequent EESI-MS analysis provides a unique opportunity to chemically characterize complex liquid samples, including highly viscous liquids.

Our first example is the online monitoring of the conversion of fructose to 5-hydroxymethylfurfural (HMF, a promising surrogate for petroleum-based chemicals, see the Supporting Information) at 80 °C in an ionic liquid, 1-ethyl-3-methylimidazolium chloride (EMIMCl); chromium chloride (CrCl₃) dissolved in EMIMCl forms the catalyst for this reaction (see the Supporting Information for reaction conditions).^[24,25] At the beginning of the reaction, the mass spectrum was dominated by peaks at *m/z* 83 and 129, which correspond to 1-methylimidazolium (MIM; [C₄H₇N₂]⁺) and hydrated 1-ethyl-3-methylimidazolium (EMIM;

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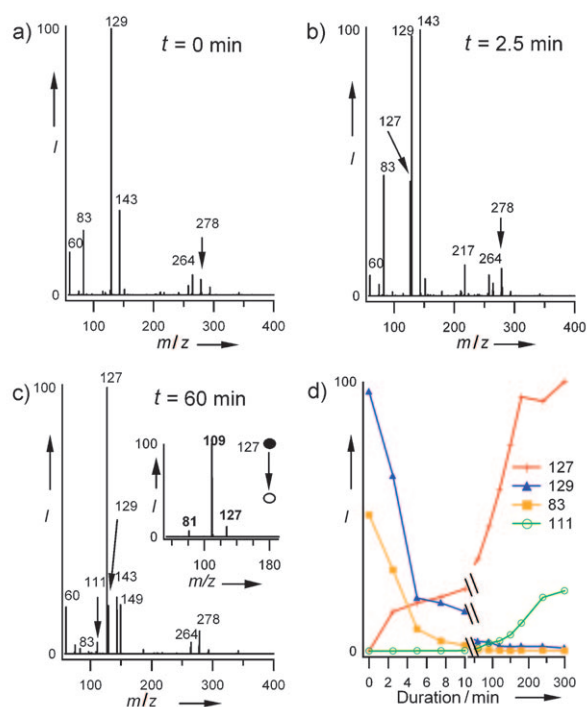


Figure 2. Mass spectra of the fructose dehydration reaction recorded at a) 0, b) 2.5, and c) 60 min. d) Intensities of protonated HMF (m/z 127), 1-methylimidazolium (m/z 83), 1-ethyl-3-methylimidazolium (m/z 111), and hydrated 1-ethyl-3-methylimidazolium (m/z 129). Inset in (c) is the MS/MS (CID) spectrum of HMF.

$[\text{C}_6\text{H}_{11}\text{N}_2\cdot\text{H}_2\text{O}]^+$, respectively (Figure 2a). The signals of EMIM (m/z 111) and protonated HMF (m/z 127) were only observed after 2.5 min (Figure 2b), and their intensities increased over the duration of the reaction (Figure 2c). Probably owing to the decrease of the water content in the reaction mixture as the reaction proceeds, the signal intensity of EMIM increases, resulting in dominant unhydrated EMIM (m/z 111; Figure 2c) instead of hydrated EMIM (m/z 129). This result agrees with previous reports^[24,26] that the HMF yield increases in systems that partition HMF from water. After a reaction time of 60 min, the intensity of the HMF signal increased further, while the intensities of the MIM and hydrated EMIM signals leveled off. The MS/MS spectrum of HMF is shown in the inset in Figure 2c. A reaction intermediate with a peak at m/z 149 was also detected and tentatively interpreted as a lactone/furan-based compound on the basis of the MS/MS spectrum (data not shown). For further information on peak assignments, see the Supporting Information. In the plots of the signal responses versus time (Figure 2d), the simultaneous reduction in the signals of MIM and hydrated EMIM with the increase in intensity of the HMF and EMIM signals as the reaction progressed clearly show that the reaction kinetics can be monitored in real time.

These preliminary results suggest that the intermediates formed between CrCl_2 and EMIM play key roles in fructose dehydration and produce a high yield of HMF without promoting side reactions that form undesired by-products. More mechanistic details are given in the Supporting Information. Ionic liquids (viscosity ca. 100 cP), known as

“solvents of the future”,^[27] are increasingly used as a media in research labs and chemical industry, owing to their negligible vapor pressure, good thermal stability, recyclability, and the availability of a wide varying of binary and approximately 10^{18} ternary ionic liquids.^[28] However, fundamental aspects of chemical reactions in ionic liquids are not well understood, owing to the lack of suitable analytical tools. As demonstrated herein, the EESI-MS is useful for real-time, online monitoring of chemical processes occurring in viscous ionic liquids, providing rich information at the molecular level.

Food quality monitoring is of paramount importance to human health and to the global economy and is another area where viscous liquids occur frequently. Hence, experiments were conducted to perform online monitoring of the quality of extra virgin olive oil (EVOO, viscosity ca. 80 cP) and honey (ca. 8000 cP; see the Supporting Information for details) to explore the scope of EESI-MS for molecular profiling of viscous mixtures. Owing to the intricate matrix of EVOO and its viscosity, solvent extraction is usually necessary prior to MS analysis. Consequently, mass spectral profiling of only the extracted part of the sample is obtained.^[29] As shown in Figure 3, higher peak intensities and a higher peak density

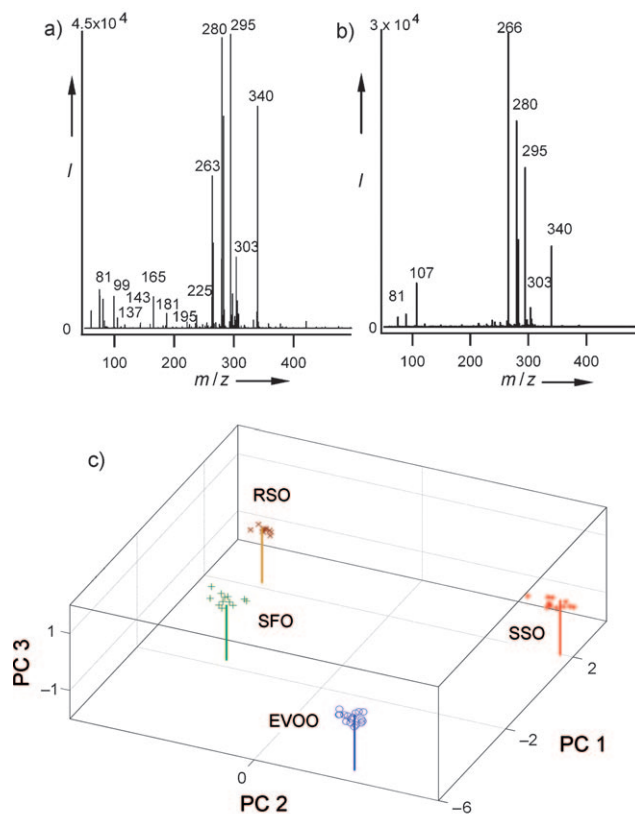


Figure 3. Mass spectra fingerprints of a) extra virgin olive oil b) extra virgin olive oil extract. c) Score plot of principle component analysis of the mass spectra obtained from four edible oils. EVOO: extra virgin olive oil, SSO: sesame oil, RSO: rapeseed oil, and SFO: sunflower oil. The space described by principal components (PC) 1, 2, and 3 is shown.

are observed in the mass spectrum of EVOO compared to an EVOO extract (a MeOH/water mixture was used for extraction, see the Supporting Information). Owing to the intrinsic selectivity of the extraction method, compounds with m/z 57, 99, 121, 137, and 181 were not detected in the olive oil extract. Moreover, signals arising from volatile compounds at m/z 81 and 99 were significantly reduced in intensity in the mass spectrum of the EVOO extract. The latter compound is identified as (*E*)-2-hexenal (Supporting Information, Figure S5), which contributes to the characteristic fruity/sweet taste of EVOO.^[30] The experimental data clearly confirms that important molecular information is lost when extraction of EVOO samples is performed. More interestingly, the major peaks at m/z 340, 303, 295, 280, and 266 observed in the EESI mass spectrum of the EVOO extract were also detected in the pristine EVOO, with similar intensities and relative signal ratios. This result illustrates that the sensitivity of EESI-MS is not compromised when samples with intricate matrices are analyzed; in fact, EESI-MS provides more comprehensive information on molecular composition than is obtained from analysis of an olive oil extract.

Using the EESI mass spectral fingerprints, four EVOO samples (one pure, three adulterated with 5% edible oils), which are difficult to differentiate by smell, were successfully separated with high confidence by principal component analysis (PCA) score plots (Figure 3c and Supporting Information, Figure S5). EVOO adulterated with sun flower oil (SFO) and rapeseed oil (RSO) are grouped near to each other, whereas EVOO with 5% sesame oil (SSO), which also has a distinct smell, is located far from EVOO and SFO/RSO.

Ion–molecule reactions can also be implemented in an EESI source for highly specific detection.^[15] This method is particularly useful in cases where analytes cannot be protonated because of low proton affinity. In this case, the quantitative detection of diethylene glycol (DEG) in toothpaste (viscosity ca. 300 000 cP) is shown as an example of specific detection by EESI. Abundant signals of DEG cationized with ammonium $[M+NH_4]^+$ (m/z 124), sodium

$[M+Na]^+$ (m/z 129), and potassium $[M+K]^+$ (m/z 145) ions were observed (Supporting Information, Figure S6) when ammonium acetate in MeOH/H₂O (1:1) solution was used as the primary spray. In the collision-induced dissociation (CID) spectrum, the ionic ammonium adduct of DEG (m/z 124) generated a distinct fragment at m/z 107 (i.e., protonated DEG), which was used for quantification purposes (Figure 4). These results show that reactive EESI-MS enables rapid and specific detection and quantification of analytes in highly viscous samples.

In conclusion, a simple yet powerful method based on EESI-MS was developed for rapid characterization of complex liquids with viscosities ranging from a few cP to 300 000 cP, without the need for any sample pretreatment. The results show that EESI-MS is potentially an attractive tool to study the mechanisms of chemical reactions and to monitor, characterize, and quantify extremely highly viscous and complex liquid samples. The present experiments can be modified to investigate other viscoelastic systems, such as polymers, gels, and biomaterials.

Experimental Section

Method summary: EESI-MS fingerprints of the samples were obtained in positive-ion mode on a quadrupole time-of-flight mass spectrometer (Q-TOF UltimaTM, Micromass/Waters, Manchester, UK) with very minor modifications of the source. Typical EESI-MS conditions were as follows: source temperature 25 °C, desolvation temperature 50 °C, ESI and cone voltages set at +3.8 kV and +40 V, respectively. An electrospray solvent mixture (MeOH/H₂O/acetic acid in a 2:2:1 ratio) was infused at 2 $\mu\text{L min}^{-1}$. As for detection of DEG in toothpaste, 10 $\mu\text{mol L}^{-1}$ ammonium acetate dissolved in H₂O/MeOH (1:1) was electrosprayed at 5 $\mu\text{L min}^{-1}$. Mass spectra were acquired over the m/z 50–500 range. MS/MS using collision-induced dissociation of selected ions was performed with 10–35 units of collision energy.

The fructose dehydration reaction was conducted according to reported literature with minor modifications^[24] (see the Supporting Information). Honey, oil, and toothpaste samples were obtained from local grocery stores. PCA was performed using the EESI-MS data in TXT. PCA score plots were generated using Matlab (MathWorks, Inc., Natick, MA, United States).

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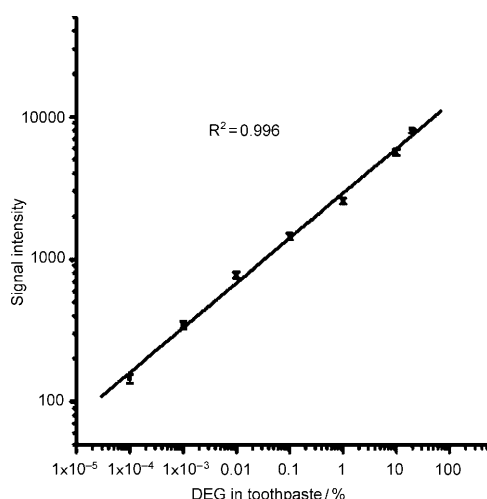


Figure 4. Calibration curve of MS/MS signal intensity at m/z 107 in % of DEG in toothpaste. Each point is the average of six consecutive measurements.

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