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Application of non-polar solvents to extractive electrospray ionization of 1-hydroxypyrene†

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Extractive electrospray ionization of 1-hydroxypyrene (1-OHP) is achieved by using non-polar solvents (*e.g.*, benzene, toluene, xylene, pentane and hexane) as the primary ESI solvent. The extraction of 1-OHP from various polar sample solutions by a non-polar primary ESI solvent is identified and 1-OHP ionization efficiency strongly depends on the sample solution.

Ambient ionization mass spectrometry is an emerging attractive analytical strategy, which enables the high-throughput analysis of various types of samples (*e.g.*, solids, liquids and creams).[1,2] To date, a number of ambient ionization techniques have been developed, including desorption electrospray ionization (DESI),[3] direct analysis in real time (DART),[4] desorption atmospheric pressure chemical ionization (DAPCI),[5] dielectric barrier discharge ionization (DBDI),[6] low-temperature plasma probe (LTP),[7] atmospheric solids analysis probe (ASAP),[8] laser ablation with electrospray ionization (LAESI),[9] electrospray-assisted laser desorption–ionization (ELDI),[10] atmospheric pressure glow discharge ionization (APGDI),[11] *etc.* Among these ionization techniques, extractive electrospray ionization (EESI) facilitates the rapid analysis of complex liquid samples.[2,12]

In EESI, liquid samples are nebulized to intersect a primary ESI plume, leading to the extractive ionization of analytes in liquid samples.[13–15] Selective extraction is the dominant mechanism involved in EESI and analyte signals significantly depend on analyte solubility in the solvents involved (the primary ESI solvent and sample solvent).[13] For example, in studies on the EESI mechanism, Law *et al.* found that the solubility of fatty acids in the primary ESI solvent is crucial for determining the signal intensity of fatty acids. It has also been reported that when extra virgin olive oil samples were analyzed by EESI-MS, compounds with different polarity values can be differentially extracted by adjusting the composition of the primary ESI solvent.[16] By offering an advantage over online extraction of liquid samples, EESI has also been reported as being coupled to conventional liquid chromatography mass spectrometry (LC-MS) to achieve online derivatization.[17]

1-Hydroxypyrene (1-OHP) is the most widely used biomarker of carcinogenic polycyclic aromatic hydrocarbons (PAHs). 1-OHP is adopted to estimate the internal dose of PAHs and multiple routes of PAH exposure, which is important for risk assessment of human health.[18] In the case of risk assessment, high-throughput analysis of 1-OHP is urgently required. EESI-MS is a promising strategy for the fast detection of 1-OHP regarding its advantage of improvement in analysis speed. To apply EESI-MS to 1-OHP analysis, investigation on the extraction and ionization efficiencies is essential. This is because 1-OHP has low polarity (a four-fused-ring structure) and low water solubility (theoretical water solubility 620 μg L^{-1} (ref. 19)), which pose a great challenge for the extractive ionization of 1-OHP in EESI.

Herein we report the successful application of non-polar organic solvents (*i.e.*, arenes and alkanes) as the primary ESI solvent in EESI to extract 1-OHP from polar sample solutions; furthermore, the sample solution is found to be a critical factor for the effective ionization of 1-OHP.

A homemade EESI source (Fig. S1†) coupled to an ion trap mass spectrometer was used for ionizing and detecting 1-OHP, respectively (see ESI† for materials and methods). For the homemade EESI source, two independent sprayers were used to produce charged microdroplets of the primary ESI solvent by electrospray and neutral

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† Electronic supplementary information (ESI) available: (i) Materials and methods; (ii) schematic diagram of the homemade EESI source (Fig. S1); (iii) EESI-MS spectra of 1-OHP water solution (50 μg L^{-1}) when six non-polar organic solvents were used as the primary ESI solvent, respectively (Fig. S2); (iv) MS spectrum of 1-OHP water solution (50 μg L^{-1}) when only the water solution was introduced into the EESI source (Fig. S3); (v) EESI-MS spectra of 1-OHP water solution (50 μg L^{-1}) when six polar solvents were used as the primary ESI solvent, respectively (Fig. S4); (vi) EESI/ESI-MS spectra of different sample solutions (50 μg L^{-1} of 1-OHP) (Figs. S5–S11); (vii) some chemical and physical properties of the solvents used in this study (Table S1). See DOI: 10.1039/c2ay25178g

microdroplets of the sample solution by pneumatic nebulization. With the collision between charged and neutral microdroplets, the target compound in the sample solution would be extracted and ionized. Non-polar organic solvents benzene (BeNE), toluene (ToNE), *o*-xylene (*o*-XyNE), *m*-xylene (*m*-XyNE), *p*-xylene (*p*-XyNE), *n*-pentane (PeNE) and *n*-hexane (HeNE) were used as the primary ESI solvent. Some chemical and physical properties of these solvents are provided in Table S1†.

1-OHP in water (50 μg L^{-1}) was analyzed by EESI-MS by applying different non-polar organic solvents as the primary ESI solvent. In the EESI-MS spectrum (Fig. 1a), the peak at *m*/*z* 217 appeared as the base peak and was assigned as the deprotonated molecule ion of 1-OHP. The MS/MS spectrum of the ion at *m*/*z* 217 showed that the parent ion (*m*/*z* 217) decomposed into the fragment ion at *m*/*z* 189 through the loss of CO (28 Da) (inset in Fig. 1a). This result was consistent with the fragmentation of 1-OHP reported previously,[20] indicating the peak at *m*/*z* 217 in Fig. 1a was due to the molecular ion of 1-OHP. For the other six non-polar solvents, the EESI-MS spectra obtained (Fig. S2†) were almost the same as that in Fig. 1a, indicating that the ionization of 1-OHP was achieved by using these non-polar organic solvents as the primary ESI solvent.

However, where 1-OHP was ionized due to the nebulization of the sample solution (*i.e.*, 1-OHP water solution), only the sample solution was introduced into the EESI source. The MS spectrum obtained (Fig. S3†) was different from the EESI-MS spectra discussed above (Fig. 1a and S2†). The peak intensity at *m*/*z* 217 was 21% of that of the base peak (*m*/*z* 61) and there were more peaks present over the *m*/*z* range of 50–250. This result confirmed that the molecular ion of 1-OHP observed in Fig. 1a stemmed from the extractive ionization of aqueous 1-OHP by using non-polar primary ESI solvent in EESI.

Moreover, when polar primary ESI solvents were used, *e.g.*, ethanol (EtOH), the peak intensity at *m*/*z* 217 was only 0.6% of that of the base peak (*m*/*z* 89) (Fig. 1b) although the MS/MS spectrum of the ion at *m*/*z* 217 was identical to the inset in Fig. 1a. Similar phenomena were also observed when the polar solvents water, methanol (MeOH), 1-propanol (PrOH), 1-butanol (BuOH), 1-pentanol (PeOH) and 1-hexanol (HeOH) were used as the primary ESI solvent (Fig. S4†). The lower normalized intensity in these spectra can be explained by the polar primary ESI solvents used being miscible with or soluble in the sample solvent of water, and thus the extraction is much less efficient compared with the non-polar primary ESI solvents, which are completely insoluble in water. As a result, the application of non-polar solvents rather than polar solvents as the primary ESI solvent in EESI can especially facilitate the extraction of low-polarity compounds.

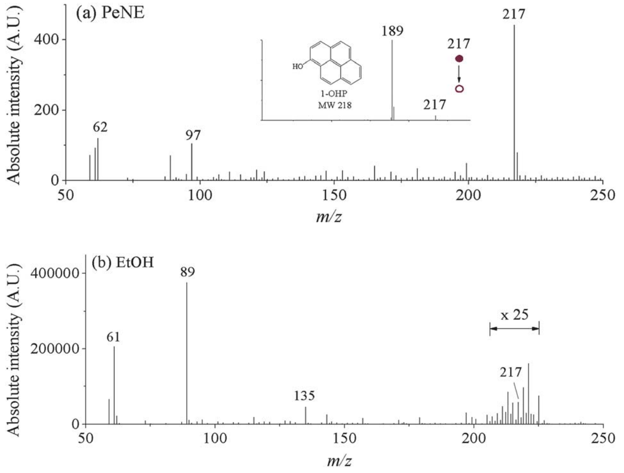

Fig. 1 EESI-MS spectra of 1-OHP in ultrapure water (50 μg L^{-1}) when PeNE (a) and ethanol (EtOH) (b) were used as the primary ESI solvent. The inset in (a) is the MS/MS spectrum of the deprotonated molecule ion $[M-H]^-$ (*m*/*z* 217) of 1-OHP; the peak at *m*/*z* 189 is the fragment ion of the 1-OHP molecular ion, which is produced through the loss of CO (28 Da).

Another interesting phenomenon observed when using non-polar primary ESI solvent in EESI was that a significant increase of TIC (over the *m*/*z* range of 50–250; Fig. 2a) or EIC (at *m*/*z* 217; Fig. 2b) was observed with the introduction of the sample solution into the EESI source. The average value of TIC (EIC) over 0.5–1.4 min was 2375 (435) whereas the TIC (EIC) value during 0.0–0.4 min or 1.8–2.2 min was only 1.32 (0) or 0.90 (0). This result implied that the sample solution also contributed to the ionization process. In addition, when non-polar solutions containing 1-OHP (50 μg L^{-1}) were directly analyzed by ESI-MS, no peak at *m*/*z* 217 was identified. Therefore, when a non-polar primary ESI solvent is used in EESI, 1-OHP cannot be ionized only in the presence of the primary ESI solvent but also with the assistance of the sample solution.

To investigate the effects of the sample solution on 1-OHP ionization efficiency, different sample solutions (containing 50 μg L^{-1} of 1-OHP) were analyzed by EESI-MS (PeNE was applied as the primary ESI solvent). According to the EESI-MS analysis results, the signal intensity at *m*/*z* 217 was strongly dependent on the composition of the sample solution injected (Fig. 3a). The highest signal intensity was obtained when EtOH was used as the sample solvent, which was followed by the sample solvents MeOH and PrOH. The signal intensity was further decreased when alcohols with longer carbon chains were applied as the sample solvent, *i.e.*, BuOH, PeOH and HeOH. As for water as the sample solvent, the signal intensity was lower than the values when alcohols were used as the sample solvent. For example, the value when water was adopted as the sample solvent was 2 orders of magnitude lower than that when EtOH was used. In the case of non-polar sample solvents (BeNE, ToNE, *o*-XyNE, *m*-XyNE, *p*-XyNE, PeNE and HeNE), no signal at *m*/*z* 217 was observed.

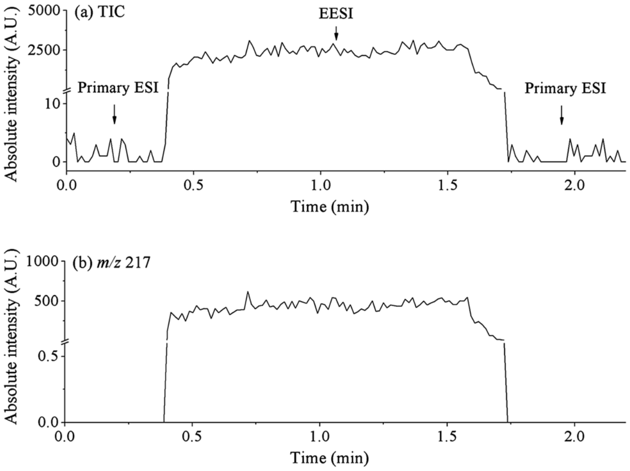

Fig. 2 Total ion current (TIC) (a) and extracted ion current (EIC) (b) chromatograms of 1-OHP water solution (50 μg L^{-1}) analyzed by EESI-MS when PeNE was used as the primary ESI solvent.

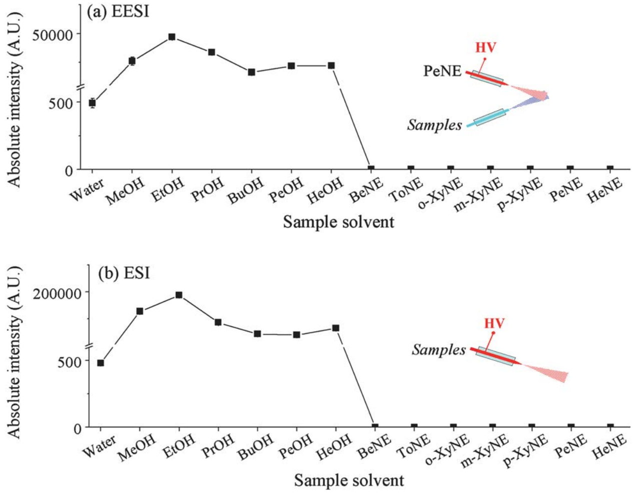

Fig. 3 Intensity variation of the signal at m/z 217 when different samples were analyzed by EESI-MS (a) and ESI-MS (b).

Interestingly, when the same sample solutions were analyzed directly by ESI-MS, the trend of signal variation (Fig. 3b) was identical to the EESI-MS result (Fig. 3a), suggesting that the ionization efficiency of 1-OHP in EESI was actually limited by the sample solution. Furthermore, when compared with the ESI-MS spectra, fewer peaks were observed in the EESI-MS spectra (Fig. S5–S11†), especially when the non-polar primary ESI solvent of PeNE was completely immiscible in the sample solution (*e.g.*, water, MeOH). This result indicated solvent–chemical noise can be greatly reduced by applying a non-polar primary ESI solvent in EESI.

In summary, by using non-polar organic solvents (*i.e.*, BeNE, ToNE, XyNE, PeNE and HeNE) as the primary ESI solvent in EESI, 1-OHP in polar sample solutions was successfully extracted and ionized. For the extractive ionization of 1-OHP in EESI, extraction was more efficient especially when the non-polar primary ESI solvent was completely immiscible in the sample solution, and 1-OHP ionization was strongly dependent on the sample solution. This is the first systematic investigation of the application of non-polar solvents in EESI to the extractive ionization of a low-polarity compound. This work can provide necessary information for the proper application of EESI-MS to the fast detection of 1-OHP (as well as other hydroxylated PAH metabolites) and also helpful insights into the EESI mechanism.

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