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COMMUNICATION

Analyte migration electrospray ionization for rapid analysis of complex samples with small volume using mass spectrometry

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Complex biological matrix can be effectively removed in the analyte migration process due to its weak solubility in organic solvents. This technique offers other potential capabilities including high-sensitivity and direct analysis of the components and their metabolites in small-volume raw samples without any pretreatment.

Mass spectrometry has been used as a diagnostic tool with success for the identification and quantification of target analytes in complex samples. In an attempt to improve the analysis sensitivity and selectivity, much effort has been made on the purification and separation of a raw sample prior to analysis with mass spectrometry.[1-3] Small volume samples, especially biological matrices, raise rigorous requirement to cut the loss of sample in the qualitative and quantitative analysis.

For simplifying the experimental procedures in a complex sample analysis, different ambient ionization strategies have been developed since the electrospray ionization source (ESI) was introduced in 1989.[4] Due to the simplicity, high throughput analysis, low-cost and without or with minimum sample pretreatment, many ambient ionization sources are available including desorption electrospray ionization (DESI),[5] direct analysis in real time (DART),[6] low temperature plasma (LTP)[7] and so on.[8-10] Although these techniques have been applied in many fields for rapid analysis of various compounds, the analysis of small volume samples still remains great challenge because of the low sensitivity. Nanoelectrospray ionization (NanoESI) is different from the ambient ionization sources above, and offers a good platform for the analysis of small volume samples based on its high sensitivity and low flow rate.[11] Recently, Huang et al introduced an induced nanoelectrospray ionization for matrix-tolerant and high-throughput analysis by applying a pulsed DC voltage on nanoelectrospray tips, which avoided the directly physical contact between electrode and spray solvent.[12] This technique allowed the rapid analysis of complex biological matrices with small volume including raw serum, whole urine, and concentrated salt solutions, and the limit of quantitation for propranolol in raw serum sample was around 2 ng mL^{-1}. Wei et al reported a step-voltage nanoelectrospray ionization to remove effectively the interference of matrices in the analysis of small-volume samples including Tris buffer solution and human tear samples, in which a 5.2 kV DC voltage was first applied on a nanoelectrospray capillary for 30 s followed by 2.4 kV voltage.[13]

However, the direct analysis of complex raw samples used the above methods would increase the background noise and lower the sensitivity in mass spectrometry analysis. So it is necessary to develop some methods with capabilities in effective removal of sample matrices that would offer potential opportunities for rapid qualitative and quantitative analysis of small-volume complex samples.

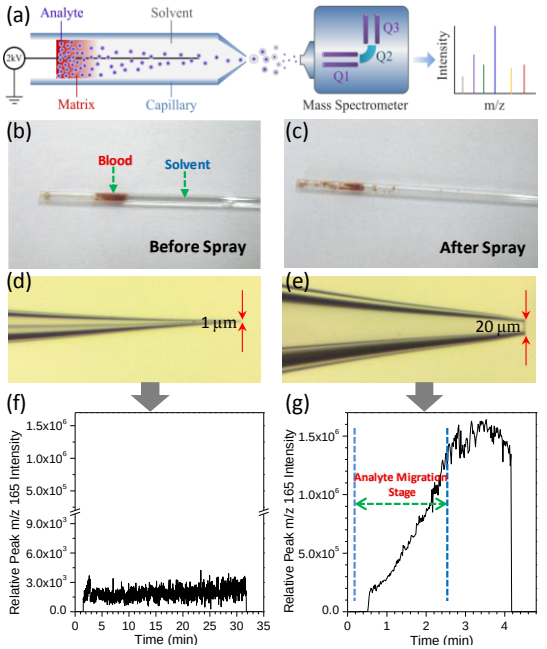

Fig. 1 (a) Analysis of fresh blood sample with analyte migration-electrospray ionization source. A spray solvent (e.g. 20 μL methanol) was first transferred into a capillary with 20 μm tip orifice, followed by adding 2 μL fresh blood containing 1 μg mL^{-1} verapamil. A DC voltage (2.0 kV) was then applied for direct analysis. (b) The capillary with 20 μm tip orifice containing 2 μL fresh blood and 20 μL methanol before spray, and the blood sample was added into the end of methanol. (c) The capillary after spray for several minutes. (d) Capillary with 1 μm tip orifice from an optical microscope. (e) Capillary with 20 μm tip orifice from an optical microscope. (f) Extracted ion current chronogram of the product ion m/z 165 by the MS/MS transition of verapamil m/z 455 using capillary with 1 μm tip orifice. (g) Extracted ion current chronogram of the product ion m/z 165 by the MS/MS transition of verapamil m/z 455 using capillary with 20 μm tip orifice.

In the present study, a novel ionization source named analyte

migration electrospray ionization (AM-ESI) has been developed for rapid analysis of complex biological samples with small volume as Fig. 1. This method effectively avoided the pretreatment of raw samples, and the sample matrix (e.g., red cells in blood sample) was suppressed or removed in the analyte migration procedure in a nanoelectrospray capillary containing organic solvent. Due to the insolubility or weak solubility of organic solvent with biological matrix, the sample matrix remains at the original position, whereas the target analytes such as the pharmaceutical drug verapamil soluble in methanol migrate to the tip of capillary for electrospray. This concept has been successfully applied in the quantitative analysis of trace amount of therapeutic drugs in fresh blood and in the qualitative analysis of some complex biological samples.

Fig. 1a shows the AM-ESI in which 20 µL solvent such as methanol was transferred into a capillary with 20 µm tip orifice using a micropipette, and then 2 µL of fresh blood was added to the end of the solvent in capillary. After applying 2 kV DC voltage on the solvent, the analytes in the raw sample will be extracted out and migrate with the solvent to capillary tip for spray. After the spray event completed and the solvent exhausted, the sample matrix remains almost constant (Fig. 1b and c and Fig. S-1 in the Supplementary Information), suggesting that the developed AM-ESI could effectively suppress or eliminate the interference of matrices in the analysis of raw samples.

This investigation demonstrated that the size of capillary orifice (Fig. 1d and e) had a pronounced effect on the analyte migration behavior. This effect was evidenced by the peak intensity of the product ion m/z 165 by the MS/MS transition of m/z 455 from verapamil with concentration of 1 µg mL^{-1} in fresh blood. As shown in Fig. 1f, the signal intensity kept almost constant with the conventional nanoESI tip (1 µm). On the contrary, the signal of the ion occurred at m/z 165 gradually increased in the period of Analyte Migration Stage, followed by maintaining with relatively stable signal with 20 µm orifice tip (Fig. 1g). The result illustrates that with the 1 µm capillary tip, verapamil in blood is hard to be extracted out and migrates to the capillary tip with solvent, probably resulting from its low flow rate.[14] By using the 20 µm tip (Fig. 1g), the solvent flow rate is high enough to carry the analyte to the capillary tip for electrospray in ca. 2.3 min.

After numerous experiments, the results illustrated that the performance of the AM-ESI is largely determined by the extraction efficiency of the target analytes and the analyte migration time. The extraction efficiency can be readily optimized by changing the extraction solvent (Fig. S-2 in the Supplementary Information). Because the analyte migration time is a crucial factor in determining the analysis speed, it was investigated in detail as below. To evaluate the migration time, linoleic acid m/z 279 in peanut oil was used as a target analyte. Fig. 2a shows the total ion chronogram of peanut oil with time. It is clear that in the first 2 min of the chronogram, the signal intensity appears a gradual increase trend followed by relatively stable one. This phenomenon can be well demonstrated by the extracted ion chronogram of linoleic acid m/z 279 (Fig. 2b). In the first stage as Stage I, the peak m/z 279 is hardly observed (Fig. 2c), just some peaks from methanol. With extension of the spray event, the linoleic acid peak gradually increased, and this period is named as Analyte Migration Stage. After about 2 min, the signal intensity of m/z 279 became dominant as shown in Fig. 2d.

We found that the analyte migration time is highly dependent on the capillary orifice size. Fig. 2e shows the effect of capillary orifice size on the analyte migration time. When the capillary with 1 µm orifice size was used, it is difficult to observe the peak m/z 279 even after a long time (Data not shown). With the increase of the capillary orifice size from 5-35 µm, the migration time demonstrated a decreasing trend followed by a gradual increasing. When the capillary size was 15-20 µm, the migration time was the shortest and was around 2 min. To our knowledge, the slow migration speed for the smaller capillary tips below 15 µm could be attributed to the higher resistance of the capillary orifice, resulting in the low solvent spray rate. With the further increase of capillary size above 20 µm, the surface tension at the capillary orifice also increases. The electric drive force from the electrode gradually becomes less and the solvent migration speed is lower and lower.

The migration time is also found to be closely related to the position of the loaded sample besides the capillary orifice size. When the fresh blood was loaded in methanol 4-6 mm away from the capillary tip rather than at the end of solvent, a rapid analyte migration event would occur (Fig. S-3 in the Supplementary Information). After a period of 10 min spray, the blood sample still kept there, demonstrating that a close distance between sample and capillary tip favors a fast analysis and would not cause the migration of sample matrix.

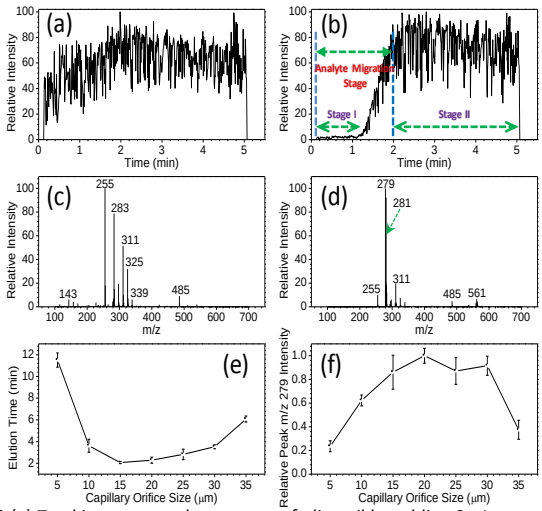

Fig. 2 (a) Total ion current chronogram of olive oil by adding 2 µL peanut oil sample into a 15 µm capillary containing 25 µL methanol (Applied voltage: -1.5 kV). (b) Extracted ion current chronogram of linoleic acid m/z 279 from the ion chronogram as shown in a). In the initial stage, analytes penetrate through the spray solvent and migrate to the spray tip as Stage I. When the concentration of analytes at the capillary tip is high enough, stable spray event occurs as Stage II. (c) Mass spectrum in Stage I as shown in (b). (d) Mass spectrum in Stage II as shown in (b). (e) Effect of capillary orifice size on the elution time of olive oil. The elution time was calculated from the spray event to the stable analyte signal as the period of Analyte Migration Stage in (b). (f) Effect of capillary orifice size on the peak intensity of linoleic acid m/z 279. The signal was evaluated by the average intensity as shown in Stage II of (b).

In the present experiment, we also found that the peak intensity of the characteristic ion m/z 279 (linoleic acid) in

peanut oil was related with the capillary orifice size. As shown in Fig. 2f, the peak intensity demonstrated a gradually increasing trend in the capillary orifice range of 5-20 μm followed by an almost constant signal in the range of 20-30 μm. Further increasing the orifice size will lead to the decreasing of the peak intensity. The variation of the peak intensity possibly has a close relationship with the solvent migration speed, spray plume efficiency as well as the evaporation performance of the solvent containing analytes. This issue remains to be resolved in the further study.

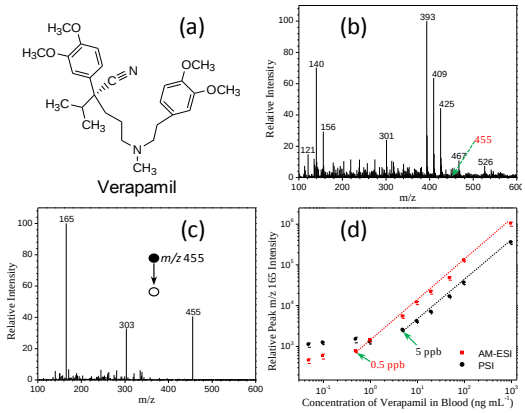

Fig. 3 (a) Molecular structure of verapamil. (b) AM-ESI full mass spectrum of whole blood spiked with 100 ng mL^{-1} verapamil. (c) AM-ESI tandem mass spectrum of 2.0 μL of whole blood sample containing 100 ng mL^{-1} verapamil identified and quantified by the MS/MS transition m/z 455 to m/z 165. (d) Comparison of the quantitative analysis of verapamil (0.05 ng mL^{-1} - 1,000 ng mL^{-1}) in blood sample between AM-ESI (red filled square) and PSI (black filled circle). For AM-ESI analysis, 2 μL whole blood was transferred into capillary with 20 μm tip orifice containing 25 μL methanol. For PSI analysis, 2 μL whole blood was deposited on Grade 1 chromatography paper, and analysis was carried out after overnight by applying 25 μL methanol as solvent.

The capability of the AM-ESI is demonstrated by the direct analysis of therapeutic drugs in fresh blood without any sample pretreatment. This point would play a significant role in the therapeutic clinic. For example, verapamil (Fig. 3a) is an L-type calcium channel blocker of the phenylalkylamine class, and has been used in the treatment of hypertension, cardiac arrhythmia, cluster headaches and others. It is efficacious in a therapeutic range over 50-250 ng mL^{-1}. The fresh bovine blood spiked with verapamil at concentrations ranging from 0.05-1000 ng mL^{-1} was directly added into a capillary with 20 μm tip orifice for analysis. Because there are many components soluble into methanol in the whole blood sample (e.g., m/z 616 $[Heme + H]^+$ and m/z 810 PC(18:0/20:4)), protonated verapamil occurred at m/z 455 is difficult to be identified in the full mass spectrum (Fig. 3b) although red cells, proteins such as albumin, gamma globulin, anti-hemophilic factor, and other clotting factors are removed with the developed AM-ESI resulting from their weak solubility in methanol (Fig. 1c). The most abundant peaks observed such as m/z 301, 393 and 409 could be assigned as the species from methanol (Fig. 3b). The tandem mass spectrum is much simpler, and the characteristic product ion m/z 165 can be easily distinguished from others (Fig. 3c). The quantitative analysis of verapamil was performed by the absolute peak intensity of m/z 165 versus the concentration of verapamil in the blood samples. As shown in Fig. 3d, the limit of quantitation (LOQ)[15] was 0.5 ng mL^{-1} with AM-ESI, which is one order of magnitude lower than the recently developed paper spray ionization (PSI).[16] Other therapeutic drugs including amitriptyline, clozapine, amisulpride, quetiapine and risperidone were also analyzed with AM-ESI (Fig. S-4 in the Supplementary Information) and PSI. The results demonstrated that the LOQ values from AM-ESI were 2-10–fold better than those from PSI (Tables S-1 and S-2 in the Supplementary Information). The experiments using AM-ESI were carried out with small volume samples, typically 2 μL, which could be further decreased by shortening the capillary length from the present 5 cm to less. Also, due to its high sensitivity and long period of spray, AM-ESI is a potential candidate for rapid quantitative analysis of therapeutic drugs or others in complex samples.

AM-ESI can also be used for fast identification of the fingerprint information in glutinous liquids such as oils and solid samples such as animal tissues and human hair, providing molecular information for metabonomics and pathology studies. Fig. 4a shows the full mass spectrum of olive oil (Table S-3 in the Supplementary Information). It is obvious that the abundant fingerprint information of olive oil was obtained with AM-ESI, which offers a good method to rapidly discriminate different oils (Fig. S-5 in the Supplementary Information).

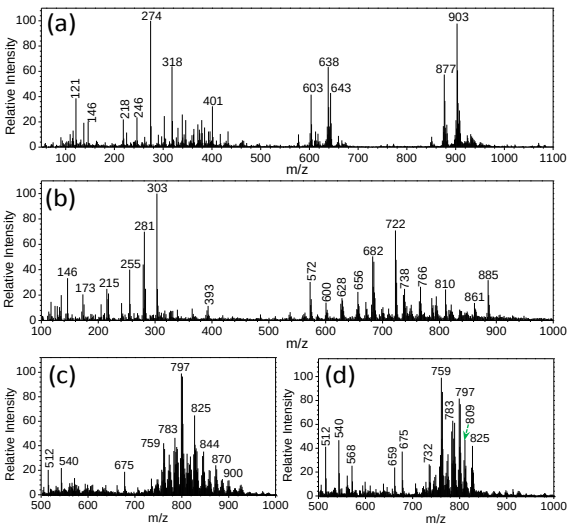

Fig. 4 (a) Mass spectrum of olive oil. For this analysis, 2 μL olive oil sample was added into a capillary containing 20 μL methanol, and then +2 kV DC voltage was applied. (b) Mass spectrum of porcine kidney. The sample was collected from 4 mm inside the kidney surface by using a disposable medical needle. The analysis was performed by applying -2 kV DC voltage onto a capillary containing 20 μL ethanol. (c) Mass spectrum of black hair root. (d) Mass spectrum of grey hair root. For (c) and (d) analysis, the samples were collected from a volunteer, and the hair root with 1 cm length hair was directly inserted into a capillary containing 20 μL methanol. After that, +2 kV DC voltage was applied for direct analysis.

This technique provides highly specific molecular information from biological samples as well. Fig. 4b shows the mass spectrum of a porcine kidney sample, in which different fatty acids (e.g. palmitic acid m/z 255, linoleic acid m/z 279, oleic acid m/z 281, and arachidonic acid m/z 303), phosphatidylserines (e.g. PS(38:4) m/z 810), glycerophosphoethanolamines (e.g. PE(36:4) m/z 738 and PE(38:4) m/z 766), plasmalogens (e.g. plasma-PE(36:4 m/z

722), and phosphatidylinositol (PI(36:2) m/z 861 and PI(38:4) m/z 885) can be detected and identified (Table S-4 in the Supplementary Information). The information is potential to distinguish diseased from healthy tissue.

Fig. 4c and d show the mass spectra of human black and grey hair roots from a volunteer using AM-ESI (Table S-5 in the Supplementary Information). It is interesting to note that in the range of m/z 500-700, m/z 568 and 659 were observed at much relative higher intensity in the grey hair root than those from the black hair root. In the m/z 700-900 range, the peak intensity of m/z 759 and 809 were much higher in the grey hair root than those in the black root. However, the peaks occurred at m/z 844, 856, 870, 884 and 900 in the black hair root were almost disappeared in the grey hair root. The molecular structure information probably will give an insight into the presence of a few grey hairs along the black hairs.

Conclusions

In summary, AM-ESI ionization source is demonstrated as a novel analysis method for matrix removal in the analysis of complex small-volume biological samples. This technique offers desirable capabilities for a broad application in many fields including therapeutic drug monitoring, food safety, and the detection and identification of various compounds (such as fatty acids, amino acids, phospholipids) in biological samples. These features pave the way for rapid and high-throughput analysis of complex biological samples without any pretreatment. The mechanism of ionization in AM-ESI is not completely understood at this time. However, our initial investigations suggest that it involves the extraction of the desirable analytes from raw samples, followed by migration to the capillary tip for electrospray. Due to the weak solubility of biological proteins and tissue samples in organic solvents, there are no clogging problems in the analysis.

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Notes and references

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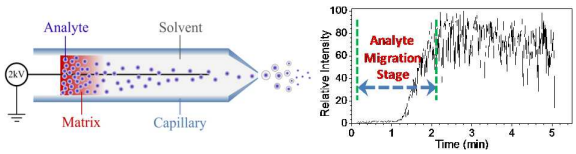

Novelty of the Work: Complex biological matrix can be effectively removed in the analyte migration process due to its weak solubility in organic solvents.