RSC Advances

COMMUNICATION

View Article Online
View Journal | View Issue

Cite this: *RSC Adv.*, 2015, **5**, 94315

Tissue paper assisted spray ionization mass spectrometry†

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Received 3rd September 2015
Accepted 29th October 2015

DOI: 10.1039/c5ra17878a

www.rsc.org/advances

Electrospray ionization mass spectrometry (ESI-MS) has been widely utilized in the analysis of analytes with a wide mass range. A metal ESI emitter, which is usually applied with a high voltage, is required in the setup of ESI-MS. Nevertheless, electrospray can be induced simply by the electric field provided by the mass spectrometer without applying electric contact on the ESI emitter. In this work, we demonstrate a simple and straightforward ionization technique for MS analysis by utilizing tissue paper as the sampling substrate and the ESI emitter. The fine fibers on the paper can work as ESI-like emitters to facilitate the generation of electrospray. When a small piece of tissue paper (diameter: ~4 mm) loaded with a few microliters of the sample solution was positioned in the proximity of the inlet of a mass spectrometer applied with a high voltage, the analyte ions were readily generated and obtained by the mass spectrometer. Although no electric contact was made on the paper, the high voltage applied at the inlet of the mass spectrometer induced the formation of electrospray for MS analysis. This approach is applicable to the analysis of various samples including organic compounds, peptides, proteins, and complex samples. Additionally, tissue paper is flexible and can be used to sample trace amounts of samples from surface of solid objects such as drug tablets simply through wiping. The tissue with a trace amount of samples then can be readily placed close in front of the inlet of mass spectrometer followed by solvent deposition for MS analysis. Additionally, we also examined the feasibility of using this approach for quantitative MS analysis.

In electrospray ionization mass spectrometry (ESI-MS), the ESI emitter made of metal capillary plays an essential role for generation of electrospray. When a sample solution is eluted from the sharp end of the ESI emitter applied with a high voltage, fine droplets can be generated from the eluted solution.[1,2] After solvent evaporation and Coulomb explosion, gas phase ions are generated for MS analysis. Although a sharp capillary emitter is usually employed to induce the formation of an electrospray, non-capillary emitters can also be utilized for the ionization process.[3–8] The first non-capillary type of the ESI emitter was patented by Fenn in 1998.[3] A wick element made of porous aggregates of fibers was used directly as the emitter. Sample liquid was supplied to the ESI ion source by a capillarity-induced flow through a wick element comprising of aggregated fibers wetted by the sample liquid. Additionally, solid emitters made of copper wires,[4] metal needles,[5–7] glass rods,[8] aluminum foil,[9] polyester,[10] paper,[11,12] wood,[13] optical fibers wired with a platinum coil,[14] metal wire inserted gel loading tip,[15] and nanostructured tungsten oxide[16] have been demonstrated to be ESI emitters for MS analysis of various organics and biomolecules. Substrates such as plants[17] or plant leaves[18] can act directly as the sample emitter upon application of a high voltage. When paper is used as the ESI emitter, it usually needs to have a sharp end for generation of electrospray. Furthermore, applying a high voltage directly on these emitters to induce the generation of an electrospray is required in these approaches.

Recently, we demonstrated a straightforward ionization method by eliminating the application of electric contact directly on the ESI emitter.[19–22] Electrospray derived from sample solutions can be readily induced by the high electric field applied on the mass spectrometer. When a short (~1 cm) and tapered capillary emitter is placed close to the inlet of a mass spectrometer, the sample solution eluted from the end of the tapered capillary can be readily ionized and detected by the mass spectrometer.[20,21] Static droplets[22] placed on a dielectric substrate and dynamic droplets[23] automatically ejected by an electronic pipette can be readily ionized to generate gaseous ions when placed very close to the inlet of the mass spectrometer applied with a high voltage. It is mainly due to the polarization effect induced by the electric field provided by the mass spectrometer. Nevertheless, the ionization mechanism for generating gas ions is similar to conventional ESI.

Carbon nanotubes (CNTs) are fibrous carbon materials. CNT impregnated paper[12] has been demonstrated to be effective ESI

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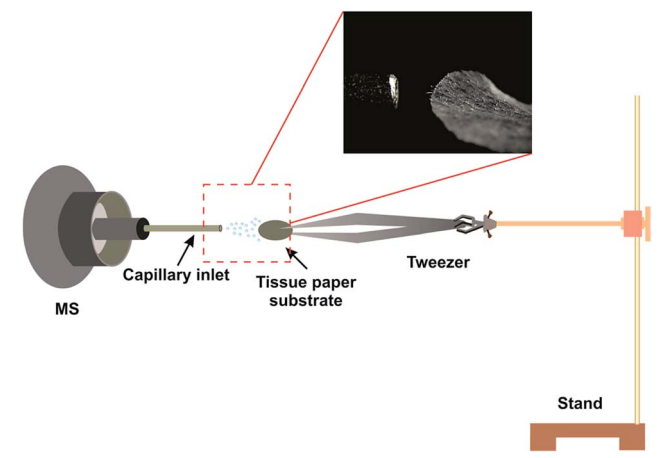
† Electronic supplementary information (ESI) available. See DOI: 10.1039/c5ra17878a

emitter owing to the presence of the nanostructured CNTs. Since the CNT impregnated paper is not easily available, we believed that tissue paper consisted of fine fibers can be an alternative. Thus, a small piece of tissue paper, *i.e.*, Kimwipe tissue, was used directly as the ESI emitter in this work. The sharp shape of the paper was essential to generate ESI in paper spray as emphasized in the previous report.[11] Since fine fibers in the tissue paper can act as the ESI emitters, the shape of the paper does not have an apparent influence on generation of electrospray. We found that an electrospray can be readily generated from the paper even with a round shape. Applying electric contact directly on the paper was unnecessary as demonstrated in previous appraohes,[22,23] so that this approach can be easily set up and operated.

Scheme 1 shows the experimental setup employed in this study. A piece of tissue paper with a diameter of ~4 mm was utilized as the sample substrate for loading sample solution. The tissue paper loaded with the sample solution (5–10 μL) was placed in front (2–4 mm) of the inlet of a Micro-Q-TOF II mass spectrometer (Bruker Daltonics, Bremen, Germany). The emitter was horizontally held in place by a pair of plastic tweezers. When operating in the positive ion mode, the voltage applied on the inlet of the mass spectrometer was −4500 V. The temperature of the ion transfer capillary was set to 220 °C. When the voltage on the mass spectrometer was switched on, the gas phase ions of arginine $[(M + H)^+ = 175]$ derived from the deposited sample droplet on the paper were readily acquired by the mass spectrometer (ESI video†). The inset in Scheme 1 shows a real image of the paper during MS analysis. Although the electrospray plume was too small to be observed, a thin layer of liquid on the top surface of the paper substrate was observed. Ultimately, this liquid emanated toward the MS inlet from the substrate in the form of fine droplets. Unless specified, the samples were prepared in a mixture of acetonitrile and deionized water (7 : 3, v/v). Formic acid (0.5%) was added to the solvent mixture during the preparation of the protein samples to enhance the ionization efficiency.



Scheme 1 Setup of the tissue paper assisted spray ionization mass spectrometry.

When a droplet (10 μL) of arginine (10^{-5} M) was placed over the tissue paper in close vicinity to the inlet of the mass spectrometer, the ions generated from the liquid droplet can be acquired by the mass spectrometer. Fig. 1A shows the resultant mass spectrum of arginine. A protonated arginine ion at *m*/*z* 175 appeared in the mass spectrum. This result indicated that the gas phase ions of the analytes can be successfully generated and detected by the mass spectrometer through the proposed approach. We further utilized large biomolecules, including peptides and proteins, to demonstrate the feasibility of using this approach in the analysis of large molecules. Fig. 1B shows the mass spectrum of bradykinin (10^{-5} M) obtained through this approach. Doubly charged ions of bradykinin (+2) at *m*/*z* 531 appeared in the mass spectrum. Fig. 1C shows the mass spectrum of cytochrome c (10^{-4} M). Multiply charged ions derived from cytochrome c dominated the mass spectra. Since we used a pair of plastic tweezers to hold the paper substrate, which was not made with any electric contact, placed close to the inlet of the mass spectrometer applied with a high voltage, the ionization was mainly directed by the electric field provided by the mass spectrometer. Additionally, the pressure difference between the atmospheric pressure and the inlet of the mass spectrometer also facilitated the transfer of the generated gaseous ions into the mass spectrometer. These results indicate that the Kimwipe paper could be used as the sample loading substrate and ESI-like emitter for the MS analysis of analytes with a wide mass range. When using a pair of metallic tweezers to hold the paper substrate loaded with large proteins such as human serum albumin (10^{-4} M, MW = ~66 kDa) (Fig. 1D), the multiply charged ions derived from human serum albumin

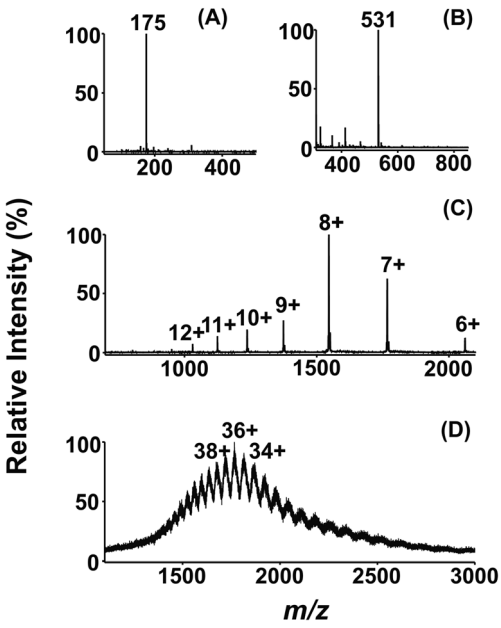


Fig. 1 Mass spectra of (A) arginine (10^{-5} M, 10 μL), (B) bradykinin (10^{-5} M, 10 μL), (C) cytochrome c (10^{-4} M, 10 μL), and (D) human serum albumin (10^{-4} M, 10 μL) using a piece of tissue paper as the sample loading substrate and ESI emitter. The spectra were obtained in the positive ion mode.

were observed. However, arcing could be observed if the metallic tweezer was placed too close to the inlet of the mass spectrometer. Arcing was not observed when the substrate was held by any plastic or dielectric holders. This approach is easy to be set up because no electric contact is required to make on the paper emitter, and the shape of the paper does not affect the analysis results. Although no apparent sharp emitter was made in this approach, the fine fiber structure on the tissue paper accompanied with the capillary action that can facilitate the sample solution flowing to the end of the tissue paper has assisted the ionization processes to occur. We observed that the position of the paper substrate with respect to the inlet of the mass spectrometer plays an important role for ion generation. In all the analyses, the paper substrate was placed at a distance of 2–4 mm from the orifice of the mass spectrometer. However, a small deviation from the position of substrate had a great impact on varying the ion intensity. Namely, the positioning of the substrate rather than the shape of the substrate has profound effects on varying the intensity of the generated ions.

Additionally, the approach is also suitable for conducting MS analysis in the negative ion mode. Fig. 2A and B show the mass spectra of retinoic acid (10^{-5} M, 10 μL) and ascorbic acid (10^{-5} M, 10 μL) prepared in a solvent containing ethanol and deionized water (7 : 3, v/v), respectively. A high percentage of organic solvent in the solution results in intense MS signals because of improved desolvation and the formation of fine droplets. Intense deprotonated pseudomolecular ion peaks at *m*/*z* 299 and *m*/*z* 175 derived from retinoic acid and ascorbic acid, respectively, were observed in the mass spectra. The total ion chromatograms (TICs) of the respective analytes in the insets show that the ion signal lasted for more than 60 s. The reason why the ion signal could not last long is that the electrospray was mainly generated through polarization induced processes.[22] When the charges in the droplet were depleted, the ion intensity declined quickly. Nevertheless, the time is long enough to allow for MS/MS analysis. Fig. 2C and D show the MS/MS spectra obtained by selecting the ion peaks at *m*/*z* 299 and 175 as the parent ions, respectively. Fragmentation was achieved by application of the collision energy of 20 eV in the collision cell for parent ions. Insets in Fig. 2C and D show the MS/MS spectra by selecting the ions at *m*/*z* 299 and 175 as the parent ions, giving rise to daughter ions at *m*/*z* 255 for retinoic acid and *m*/*z* 115 for ascorbic acid,[24] respectively. The results indicate that the ions resulting from paper substrate can last long enough for MS/MS analysis to be carried out.

The limit of detection (LOD) of the proposed tissue paper spray approach was also investigated. Fig. 3 shows the mass spectrum of cetyltrimethyl ammonium bromide (CTAB) (10^{-8} M, 10 μL). The ion peak at *m*/*z* 284 was derived from cetyltrimethyl ammonium cations. The signal-to-noise ratio (S/N) of the peak at 284 was ~321. Thus, the LOD was estimated to be $\sim 10^{-10}$ M based on the S/N of 3. Nevertheless, CTAB is a pre-charged ion; thus, LOD can be very low. The LODs for small organics (*e.g.*, arginine) and proteins (*e.g.*, cytochrome c) were $\sim 10^{-7}$ M and 10^{-6} M, respectively.

All the MS results shown above were obtained using a piece of Kimwipe paper (Kimberly-clark) as the sample loading substrate and the ESI emitter. We also evaluated the performance of different types of paper substrates. Fig. 4A shows the

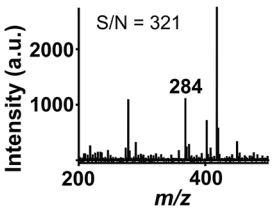


Fig. 3 Mass spectrum of cetyltrimethyl ammonium bromide (10^{-8} M, 10 μL) using a piece of tissue paper as the sample loading substrate and ESI emitter.

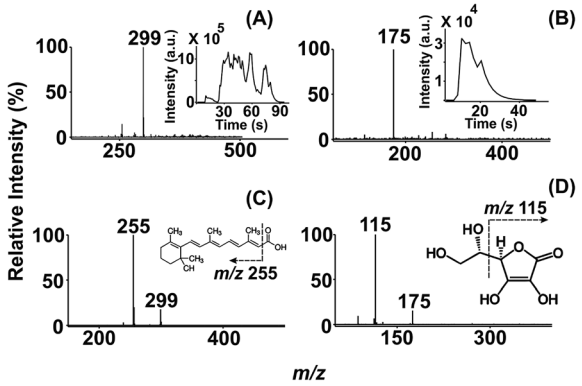


Fig. 2 Mass spectra of (A) retinoic acid (10^{-5} M, 10 μL) and (B) ascorbic acid (10^{-5} M, 10 μL) using a piece of tissue paper as the sample loading substrate and ESI emitter. The insets in Panels (A) and (B) show the corresponding TICs during MS analysis. (C) MS/MS spectrum of retinoic acid (10^{-5} M, 10 μL) by selecting the ion peak at *m*/*z* 299 as the parent ion. (D) MS/MS spectrum of ascorbic acid (10^{-5} M, 10 μL) by selecting the ion peak at the *m*/*z* 175 as the parent ion. The structures of the fragments are shown in insets in Panels C and D.

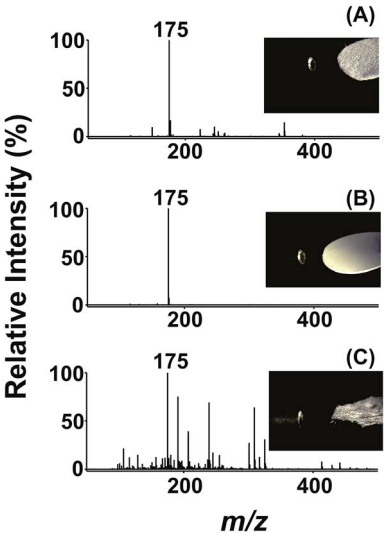


Fig. 4 Mass spectra of arginine (10^{-5} M, 10 μL) obtained using a piece of (A) Whatman filter paper, (B) Merck Millipore membrane filter, and (C) toilet tissue paper as the sample loading substrate and the emitter.

mass spectrum of arginine (10^{-5} M, 10 μL) obtained by using a piece of Whatman filter paper (GE Healthcare) as the sample loading substrate. The inset shows the image of the substrate during ion acquisition. Similar to the Kimwipe tissue paper, the Whatman filter paper possessed fibrous and capillary like structures. This filter paper is much thicker than the Kimwipe tissue paper. Thus, upon application of a sample droplet, the shape of the filter paper did not change significantly during the flow of the sample solution. Fig. 4B shows the mass spectrum of arginine obtained by using a piece of membrane filter (Merck Millipore), which is made up of polyvinylidene with a pore size of 0.1 μm, as the sample loading substrate and the ESI emitter. The inset shows the image of this filter paper, in which the surface of the paper looked quite smooth, and no apparent fibrous structures were observed. Ions could be readily generated in the beginning when a sample droplet was placed over this substrate. However, the ion signal could only last for <25 s. The ion generation initially was derived from polarization induced ESI owing to the charge accumulation on the thin layer of the sample liquid on the paper toward the mass spectrometer that was applied with a high voltage. Because this filter paper lacked fiber-like structures, analyte solution cannot efficiently flow through to the end of the paper through capillary action to generate ions continuously. Thus, the ion signals only lasted for a short period of time. Additionally, analyte ions such as protonated pseudomolecular arginine ions at *m*/*z* 175 can be readily observed (Fig. 4C) when a small piece of toilet tissue paper comprised of fibrous structures (inset in Fig. 4C) was used as the sample loading substrate and the ESI emitter. However, background ions generated from the tissue paper were observed in the resultant mass spectrum. The results indicated that paper substrates containing fibrous structures with limited additives are suitable sample loading substrates and emitters for this approach.

Additionally, tissue paper is soft and flexible, so it can be used as a sampling tool by simply wiping the surface of the sample. It is one of the advantages of using the tissue as the emitter in this approach. For example, when spraying deodorant directly over a tissue paper and then placing it in close proximity to the mass spectrometer followed by depositing ESI solvent (methanol, 5 μL), the protonated pseudomolecular ions derived from diethyl phthalate at *m*/*z* 223 can be easily observed in the mass spectrum (Fig. 5A). Additionally, the surface of plastic-made products may contain plasticizer residues, which are hazardous to human health. We used a piece of Kimwipe paper to wipe the surface of a plastic toy (inset in Fig. 5B) and then deposited 10 μL of methanol directly on the paper in front of the mass spectrometer. An intense ion peak at *m*/*z* 245, corresponding to the sodium adduct of diethyl phthalate [$(C_{12}H_{14}O_4 + Na)^+$], dominated the mass spectrum (Fig. 5B). This approach can also be employed to analyze the main composition of drug tablets by wiping the surface of a tablet with tissue paper. Fig. 5C shows the mass spectrum obtained by using Kimwipe tissue to wipe the surface of an acetaminophen tablet and then placing it in front of the inlet of the mass spectrometer followed by depositing with methanol (10 μL). An intense peak at *m*/*z* 152 corresponding to the protonated acetaminophen molecular ions dominated the mass spectrum (Fig. 5C). The results indicate that using the tissue paper as the sampling tool is convenient and mass spectral results can be easily obtained using this simple approach.

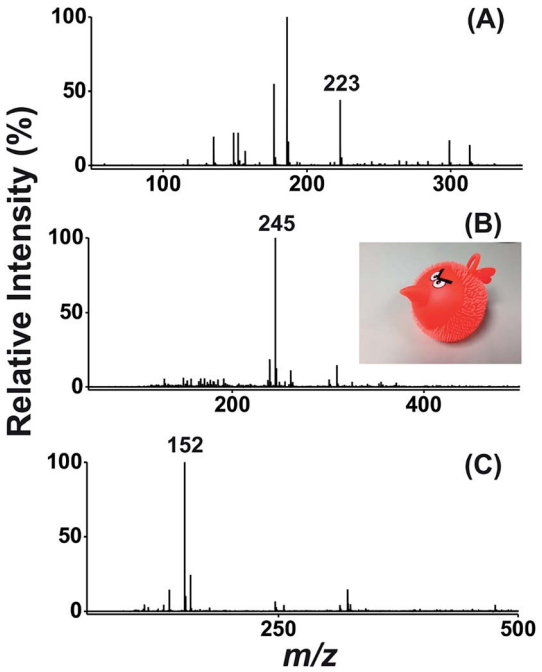


Fig. 5 Mass spectra obtained from (A) direct analysis of deodorant spray, (B) wiping over a plastic toy followed by depositing with 10 μL of ethanol, and (C) an acetaminophen tablet using a piece of tissue paper to wipe the surface of the samples. The tissue paper used for panel (B) was held by a pair of stainless steel tweezers, while the tissue paper used for obtaining panels (A) and (C) were held by a pair of plastic tweezers.

The analysis time is quite short using this approach. Thus, it only takes a short period of time to conduct multiple sample analyses. Next, we were wondering if our approach can be applied in quantitative MS analysis. The possibility of quantitative analysis using this approach was further examined. We selected arginine as the target analyte and spiked with an internal standard, *i.e.* atrazine. The samples were prepared in a solution of methanol and water (50 : 50, v/v). Fig. 6A–E show the representative extracted ion chromatograms (EICs) at *m*/*z* 175 (arginine) and *m*/*z* 216 (atrazine) obtained from the samples containing different concentrations of arginine. The analysis was repeated three times for each concentration. The calibration curve (R^2 = 0.981) (Fig. 6F) was obtained by plotting the ratio of peak area of the peak at *m*/*z* 175 derived from protonated arginine ions to that of the peak at *m*/*z* 216 derived from protonated atrazine ions. A good linearity was obtained. Nevertheless, the results may vary slightly from run to run. According to the results obtained from three replicates of experiments using the same samples, all the adjusted *R* squared values were >0.970. Although the linearity was in an acceptable range, it is worth of mentioning that the variation from three replicates of the same concentration of samples may vary to >20% in some analyses. That is, this approach may be suitable

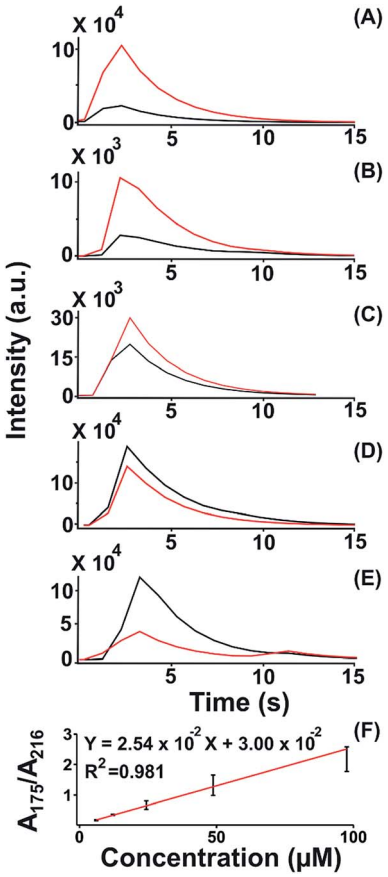


Fig. 6 Representative EICs of the ions derived from arginine and atrazine at *m/z* 175 (black) and 216 (red), respectively, obtained from the samples containing arginine with the concentrations of (A) 6.09 μM, (B) 12.19 μM, (C) 24.38 μM, (D) 48.75 μM, and (E) 97.50 μM. Atrazine (10.30 mM, 10 μL) was spiked to these arginine samples (1 mL) as the internal standard. The sample (10 μL) was deposited on a piece of paper for spray ionization MS analysis. The time scale on *X* axis in all the EICs had been adjusted and shifted to similar starting time point for ease of comparison. (F) Plot obtained from the ratio of the peak area at *m/z* 175 (A_{175}) to the peak area at *m/z* 216 (A_{216}) *versus* different concentrations of arginine. Three replicates were conducted for each sample.

for semi-quantitative analysis. Nevertheless, the quantitative analysis can be improved if analytes and selected internal standards have similar chemical structures and ionization efficiency. The variation of the ion intensity derived from the sample at the same concentration repeatedly conducted three replicates can be reduced to <20%. For example, when hydrochlorothiazide was selected as the target analyte and hydroflumethiazide was selected as the internal standard, the variation obtained from different analyses of the same sample was small. Fig. S1A–E† show the representative EICs of the deprotonated pseudomolecular hydrochlorothiazide ions and deprotonated pseudomolecular hydroflumethiazide ions at *m/z* 296 and *m/z* 330, respectively, obtained from the samples containing different concentrations of hydrochlorothiazide spiked with a fixed amount of internal standard, *i.e.* hydroflumethiazide. The corresponding calibration curve gave an adjusted *R* squared value of 0.978 (Fig. S1F†). Furthermore, the error bars shown in the curve were relatively small (<15%). Nevertheless, the setup requires further modifications to have improved reproducible results since the positioning of the paper substrate can greatly affect ion intensity. Also, to find a suitable internal standard that have a similar chemical structure and ionization efficiency to the target analyte is essential to obtain reliable quantitative MS analysis results.

In conclusion, we have demonstrated a straightforward approach involving the use of tissue paper with fibrous structures as the sample loading substrate, sampling tool, and ESI emitter for MS analysis. Tissue paper has been frequently used to wipe the surface of any substrate because of its softness and flexibility. Additionally, it is inexpensive, disposable, and can be easily obtained from most of laboratories. That is, the proposed approach has a simple setup and involves cheap accessories. Furthermore, it can be utilized to analyze analytes with a wide mass range. The main advantages of this approach include simplicity, speed, and low cost. Thus, it should be potentially useful for rapid screening of liquid sample and the presence of target species from the surface of suspicious solid samples. Additionally, it should be suitable for the analysis of forensic samples that are obtained from suspicious solid surface in crime scenes using tissue paper to sample trace residues.

Acknowledgements

We thank the Ministry of Science and Technology of Taiwan (MOST 102-2113-M-009-019) for financial support of this research. AKM thanks the National Chiao Tung University for providing him the International Student Scholarship.

References

1 M. Yamashita and J. B. Fenn, *J. Phys. Chem.*, 1984, **88**, 4451–4459.
2 J. B. Fenn, M. Mann, C. K. Meng, S. F. Wong and C. M. Whitehouse, *Science*, 1989, **246**, 64–71.
3 J. B. Fenn, US Patent 6,297,499 B1, 2001.
4 C. M. Hong, C. T. Lee, Y. M. Lee, C. P. Kuo, C. H. Yuan and J. Shiea, *Rapid Commun. Mass Spectrom.*, 1999, **13**, 21–25.
5 J. Liu, R. G. Cooks and Z. Ouyang, *Anal. Chem.*, 2011, **83**, 9221–9225.
6 M. Kanti Mandal and L. Chuin Chen, *Anal. Methods*, 2010, **2**, 1905–1912.
7 M. K. Mandal, S. Saha, Z. Yu, K. Yoshimura, S. Takeda and K. Hiraoka, *Anal. Methods*, 2015, **7**, 2630–2635.
8 J. Jeng and J. Shiea, *Rapid Commun. Mass Spectrom.*, 2003, **17**, 1709–1713.
9 B. Hu, P.-K. So and Z.-P. Yao, *Anal. Chim. Acta*, 2014, **817**, 1–8.
10 M. Y. M. Wong, H. W. Tang, S. H. Man, C. W. Lam, C. M. Che and K. M. Ng, *Rapid Commun. Mass Spectrom.*, 2013, **27**, 713–721.
11 Q. Yang, H. Wang, J. D. Maas, W. J. Chappell, N. E. Manicke, R. G. Cooks and Z. Ouyang, *Int. J. Mass Spectrom.*, 2012, **312**, 201–207.

12 R. Narayanan, D. Sarkar, R. G. Cooks and T. Pradeep, *Angew. Chem., Int. Ed.*, 2014, **53**, 5936–5940.
13 B. Hu, P.-K. So, H. Chen and Z.-P. Yao, *Anal. Chem.*, 2011, **83**, 8201–8207.
14 C.-P. Kuo, C.-H. Yuan and J. Shiea, *J. Am. Soc. Mass Spectrom.*, 2000, **11**, 464–467.
15 M. K. Mandal, K. Yoshimura, S. Saha, Z. Yu, S. Takeda and K. Hiraoka, *Anal. Chem.*, 2013, **86**, 987–992.
16 J. Jeng, C.-H. Lin and J. Shiea, *Anal. Chem.*, 2005, **77**, 8170–8173.
17 H. Zhang, K. Chingin, L. Zhu and H. Chen, *Anal. Chem.*, 2015, **87**, 2878–2883.
18 J. Liu, H. Wang, R. G. Cooks and Z. Ouyang, *Anal. Chem.*, 2011, **83**, 7608–7613.
19 T.-Y. Chen, J.-Y. Lin, J.-Y. Chen and Y.-C. Chen, *J. Am. Soc. Mass Spectrom.*, 2010, **21**, 1547–1553.
20 C.-H. Hsieh, C.-H. Chang, P. L. Urban and Y.-C. Chen, *Anal. Chem.*, 2011, **83**, 2866–2869.
21 C.-H. Hsieh, A. K. Meher and Y.-C. Chen, *PLoS One*, 2013, **8**, e66292.
22 A. K. Meher and Y. C. Chen, *J. Mass Spectrom.*, 2015, **50**, 444–450.
23 S. Y. Wong and Y. C. Chen, *J. Mass Spectrom.*, 2014, **49**, 432–436.
24 S. M. Wabaidur, Z. A. Alothman and M. R. Khan, *Spectrochim. Acta, Part A*, 2013, **108**, 20–25.

