analytical chemistry

Article

Cite This: *Anal. Chem.* 2019, 91, 7118−7124

pubs.acs.org/ac

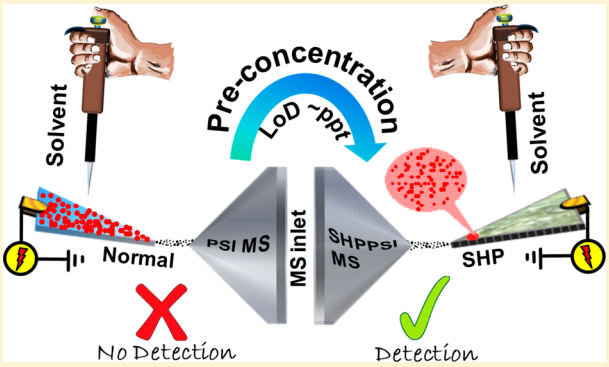
# Sub-Parts-per-Trillion Level Detection of Analytes by Superhydrophobic Preconcentration Paper Spray Ionization Mass Spectrometry (SHPPSI MS)

Pallab Basuri, Avijit Baidya, and Thalappil Pradeep*

DST Unit of Nanoscience (DST UNS), Thematic Unit of Excellence (TUE), Department of Chemistry, Indian Institute of Technology Madras, Chennai 600036, India

Supporting Information

**ABSTRACT:** A new kind of ambient ionization method named superhydrophobic preconcentration paper spray ionization mass spectrometry (SHPPSI MS) is introduced, where superhydrophobicity and paper spray mass spectrometry (PS MS) are coupled. The SHPPSI MS requires only microliter amounts of analyte solutions, allows easy sampling procedure, and provides high sensitivity for a diverse array of analytes. It can be used to detect food adulteration at extremely low concentrations. The experimental methodology involves modifying one of the surfaces of a triangularly cut filter paper to make it acquire low surface energy by drop casting a green and ecofriendly superhydrophobic coating material over it followed by drying. A micrometer scale defect was made at close proximity to one of the tips of the paper using a pin. Preconcentration of the sample was accomplished by allowing a 10 $\mu$L droplet of an aqueous solution of the analyte to stand at the defect followed by drying naturally. The dried paper was used as the substrate for paper spray mass spectrometry by eluting the analyte with a suitable solvent. This novel technique was used to detect melamine in adulterated milk, whose detection at the ppt level in milk normally needs sophisticated instruments, a larger amount of sample, and a complex sampling procedure, including further purification and separation. The SHPPSI MS detects melamine directly from milk at the sub-ppb level by simply putting a microdroplet of adulterated milk at the substrate and eluting the sample with methanol. This paper-based technique can be a promising tool for direct sensing of analytes such as drugs in body fluids, pesticides in water and soil, etc.




Ambient ionization mass spectrometry is one of the fast evolving areas of analytical mass spectrometry. Electrospray ionization (ESI),[1] desorption electrospray ionization (DESI),[2−4] low-temperature plasma ionization (LTP)[5−7] and atmospheric pressure chemical ionization (APCI)[8−10] are examples of ambient ionization techniques. Paperspray ionization (PSI),[11,12] where a sample in the form of a solution is ionized from the tip of a paper using an applied electric field in air, is one of the variants of the electrospray ionization method. Paper spray in diverse forms such as leaf spray,[13] cloth spray,[14] spray from polymer,[15] or glass[16] are used for the analysis of blood clots,[17,18] body fluids,[19] forensic specimens,[20] food stuff,[21] and catalytic reactions.[22] Inherent properties of the paper can be modified by incorporating nanostructures[23−25] leading to low-voltage ionization, and the technique has been used for the analysis of small molecules, such as drugs, pesticides, adulterating agents, etc., present in body fluids, food, and water.

While a paper modified by nanostructures[26,27] can enhance ionization as demonstrated by us previously, it can also be a platform for preconcentration. This can be done effectively by concentrating the analyte at the tip of a triangularly cut paper by drop casting droplets at the tip and evaporating the solvent leading to the deposition of the analyte and by repeating the process. The process of preconcentration can be achieved spontaneously on a superhydrophobic (SHP) paper. By doing this, preconcentration and ionization steps can be combined on the paper, making sample preparation simpler and rapid, which leads to improved limits of detection for a chosen analyte in solution.

The use of hydrophobic paper in paperspray ionization has already been demonstrated by Badu-Tawiah et al., which results in better ionization as well as lower detection limits. The method involves a silane coated hydrophobic paper spray technique to enhance the sensitivity of the detection. Going to the limit of detection in the range of the sub-ppt level is indeed a challenge.[28,29]

Herein, we demonstrate that the preconcentration technique can be used efficiently for mass spectrometric detection of an analyte from a complex mixture, at a low concentration, down

**Received:** January 9, 2019
**Accepted:** May 9, 2019
**Published:** May 9, 2019






DOI: 10.1021/acs.analchem.9b00144
*Anal. Chem.* 2019, 91, 7118−7124

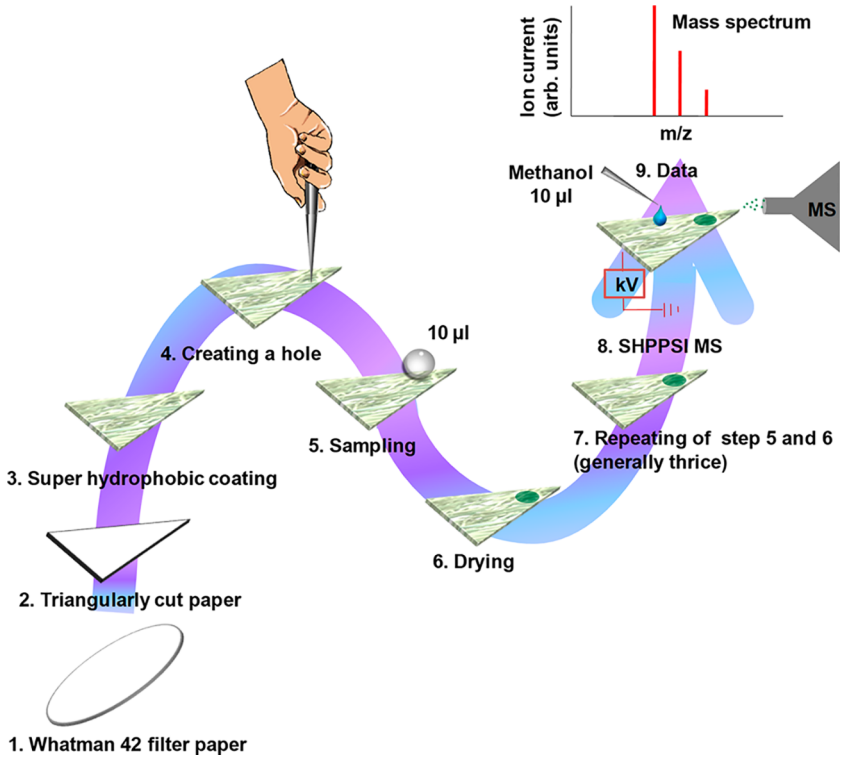



**Figure 1.** Schematic representation of the consecutive steps involved in the experimental setup for SHPPSI MS. The SHP coating can be made by any of the methods commonly used for making such surfaces.

to picomolar or sub-ppt levels. We combine selective localization and ionization of the analyte to achieve ultralow detection without sample preparation. The aforementioned modified paperspray ionization technique named superhydrophobic preconcentration paperspray ionization mass spectrometry (SHPPSI MS) is introduced here. In SHPPSI MS, the position of sample preconcentration over the substrate, which affects the ionization efficiency, can be manipulated depending upon the user's wish. Application of SHPPSI MS is demonstrated with analytes having different functionalities. The paper also illustrates application of the methodology for the detection of melamine, an adulterant in commercial milk. Melamine is a trimer of cyanamide which contains 66% nitrogen in its molecular mass. It is used as one of the major adulterants in commercially available milk to make false quantification of protein content in milk due to enhanced nitrogen, which increases the market value of the product. Detection of melamine in very low concentration needs sophisticated instruments, e.g., high-pressure liquid chromatography (HPLC),[30] gas chromatography/mass spectrometry (GC/MS),[31] nuclear magnetic resonance (NMR),[32] fluorescence polarization immunoassay (FPIA),[33] enzyme-linked immunosorbent assay (ELISA),[34,35] etc. It also requires complex sample preparation, purification, and pretreatment.

Prolonged melamine uptake leads to an adverse effect on human health. Renal dysfunction as well as kidney and bladder stones due to crystallization of melamine have been demonstrated in animals.[36−38]

## MATERIALS AND METHODS

**Chemicals and Materials.** Caffeine, rhodamine 6G, and methyl orange were purchased from Sigma-Aldrich, and HPLC grade methanol was purchased either from Sigma or Rankem. Diazepam was purchased as a medicine. All the chemicals were used without further pretreatment and purification. Milk was purchased from the local market for all the studies.

**Synthesis of the Superhydrophobic Material.** Synthesis protocol of the superhydrophobic material followed a recent publication from our group.[39] Briefly, a mixture of different functionalized silanes, aminosilane (0.92 v/v%) and fluorosilane (0.61 v/v%), was stirred in water in room temperature for 6 to 7 h. Finally, the well-dispersed composite suspension was used directly for the preparation of the substrate.

**Preparation of the Paper.** Superhydrophobic paper was prepared by the spray coating technique where the triangularly cut Whatman 42 filter paper was spray coated with the as-synthesized clay material. Finally, the coated paper was dried under ambient conditions and was used directly for SHPPSI MS.

**SHPPSI MS.** This method combines preconcentration and ionization on the substrate, useful in many analytical situations. Figure 1 schematically represents the steps involved. These steps include (1) preconcentration of analyte (steps 1−7) and (2) ionization leading to detection (steps 8 and 9).

**Preconcentration Steps.** To obtain preconcentration at a position of interest (1 mm away from the tip), a micrometer-sized point defect was made manually at the tip of the coated paper with a sharp pin. This designed paper was used for the experiments, where a 10 $\mu$L droplet of analyte solution was carefully drop-casted at the point defect using a micropipette keeping the paper on a planar surface. Due to the superhydrophobic property of the paper, the solution forms a perfect sphere and sits at the desired position. It was then dried in laboratory air at 30−35 °C by spontaneous evaporation of the solvent. The process was repeated thrice or more times to deposit more analyte. The same procedure


DOI: 10.1021/acs.analchem.9b00144
Anal. Chem. 2019, 91, 7118−7124

was also followed for the normal paper. Interestingly, while the sample was observed to spread over the normal paper, it stayed as a droplet over the modified paper and dried slowly. Hence, the droplet formation and slow evaporation of solvent led to the accumulation of analyte molecules at the micrometer-sized point defect. Figure 2 shows the schematic and photographic

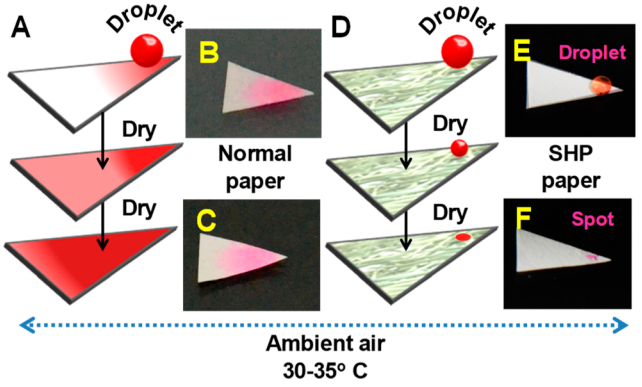



**Figure 2.** Liquid droplet behavior on (A) Whatmaan 42 filter paper and (D) superhydrophobic paper. B, C and E, F are optical images of the wet and dry paper samples.

representation of sampling over superhydrophobic and normal paper samples. The analyte used here was rhodamine 6G of 1 mM concentration to visually demonstrate the preconcentration technique in the picture. In Figure 2E, we can see a standing droplet which gets dried and accumulated in a smaller area shown in Figure 2F. However, Figure 2B,C shows that the dye got spread as soon as it was drop-casted over a normal paper. Similar images in Figure S1 show a distinct red dot on the superhydrophobic paper, which is a result of three times drop-casting of 25 $\mu$L of nanomolar concentration of the same dye. Only a faint color was visible on the normal paper due to the dispersion. In this case, a higher order of preconcentration was done for visual detection over the papers.

**Detection Step.** The sample loaded triangular paper was finally placed in front of a mass spectrometer, pointing the tip toward the inlet, at a distance of 1 cm apart. The paper was connected to a high voltage dc power supply for the experiments. The analyte was eluted by 10 $\mu$L of methanol.

The Thermo Scientific LTQ XL mass spectrometer was used for the mass spectrometric detection of analytes. The average size of the paper was chosen as 35 mm$^2$ with 7 mm at the base and 10 mm in height. The point defect was made approximately 0.5−1 mm away from the tip. The spray voltage was varied within the range of 3−5 kV depending on the analyte of interest. The capillary and tube lens voltages were set to ±45 and ±100 V in all the cases for positive and negative modes, respectively. The capillary temperature and the sheath gas pressure were set to be 275 °C and 0 psi, respectively. Collision induced dissociation (CID) was used in all cases to understand the fragmentation pattern of the molecules through a MS$^n$ study. Helium was used to effect collisions in CID. All these optimized conditions were chosen based on the trial and error method.

**Preparation of the Standard Samples.** Caffeine, rhodamine 6G, methyl orange, and melamine were chosen to characterize the system. All the samples were prepared in Milli Q water with different concentrations ranging from 1 mM to 1 pM using dilution methodology. Methanol was used to elute the analyte from the paper in all cases.

## RESULTS AND DISCUSSION

**Characterization of the SHP Coated Paper.** Coated filter paper was used directly without additional treatment. Figure S2A,B represents the scanning electron microscopy (SEM) image of normal Whatman 42 filter paper and the SHP coated paper at different magnifications. The fibrous nature of the paper remained intact after the coating. The fibrous nature of the substrate plays an important role to generate a large electric field between the fibers, which leads to the formation of electrospray from the tip.

**Characterization of SHPPSI MS.** Analysis of molecules having low concentrations such as the sub-ppt level is difficult to be detected in standard PSI mass spectrometry due to diffusion of the analyte over the paper. Hence, during elution of the analyte, only a small fraction of the total analyte gets detected. By changing the wettability of the paper surface, diffusion of the analytes can be restricted by a substantial amount that allows localization of the analyte molecules in a micrometer region at close proximity to the tip. Figure 3

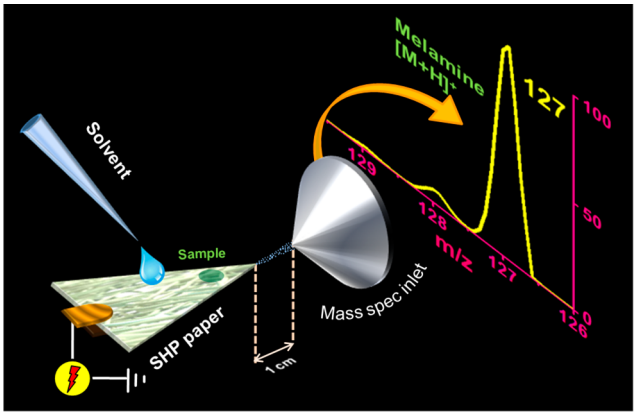



**Figure 3.** Schematic of the experimental setup used for superhydrophobic paper spray ionization mass spectrometry (SHPPSI MS). The triangularly cut SHP paper acts as the substrate to ionize the molecule. The inset shows the mass spectrum of melamine at 10 pM (1.2 ppt) concentration. The analyte containing the analyte is preconcentrated on the paper.

schematically represents the setup along with the mass spectrum of the analyte, melamine, at a concentration of 10 pM ($10^{-11}$ M or 1.2 ppt). The peak $m/z$ 127 corresponds to the protonated cation of the analyte in positive ion mode. This peak was further confirmed by the MS$^2$ data, which are shown in Figure S3. The peak at $m/z$ 127 (Figure S3a) upon CID gives a major peak at $m/z$ 85 (Figure S3b), which corresponds to the loss of the $H_2NCN$ fragment. This forms the $C_2N_4H_5^+$ cation. Further fragmentation of the peak at $m/z$ 85 gives $m/z$ 65 (Figure S3c), which corresponds to the $NH_3$ loss of the four member ring.

The same preconcentration technique was adopted for the sampling of other analytes to demonstrate the applicability of the methodology toward any analyte. The mass spectra of all the analytes are shown in Figure 4 and Figure S7. Figure 4 shows the mass spectra of three different analytes of interest, such as caffeine (positive ion mode), rhodamine 6G (positive ion mode), and methyl orange (negative ion mode) at


DOI: 10.1021/acs.analchem.9b00144
Anal. Chem. 2019, 91, 7118−7124

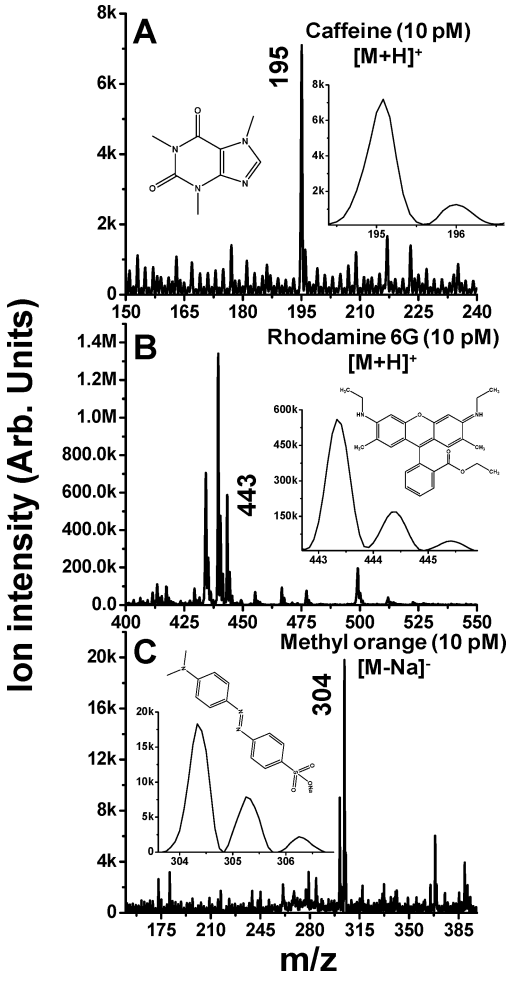



**Figure 4.** SHPPSI MS spectra of (A) caffeine, (B) rhodamine 6G, and (C) methyl orange in picomolar or ppt concentrations. Insets show the isotope distributions and structures of the analytes. The label, "k" represents $10^3$.

picomolar concentrations. The peaks $m/z$ 195, 443, and 304 present in Figure 4 correspond to the protonated cations of caffeine and rhodamine 6G and the deprotonated anion of methyl orange. From the ion intensity of the corresponding peaks, it is clear that SHPPSI MS has a capability to detect extremely low concentrations of analytes in solution and it works for different kinds of analytes irrespective of their functionality.

The $MS^2$ spectrum of caffeine is presented in Figure S4. Fragmentation of the peak at $m/z$ 195 (S4a) (also in Figure 4A) gives the major peak at $m/z$ 138 (S4b), which corresponds to the loss of one $CH_3NCO$. Fragmentation of $m/z$ 138 gives $m/z$ 110 due to the fragment shown in the structure S4c, as a result of the loss of one CO. In addition to this, two weak fragments at $m/z$ 85 and 68 are due to the loss of one CHN and CHN + $CH_3$ group from Figure S4c. Structures corresponding to the ions are presented in Figure S4d,e. The fragmentation patterns of rhodamine 6G and methyl orange are also presented in Figures S5 and S6.

Similarly, Figure S7 represents the mass spectra of isoleucine, adenine, and urea at concentrations of 1.3, 1.4, and 0.6 ppt. Mass peaks at $m/z$ 131, 135, and 61 correspond to the molecular ions of isoleucine, adenine, and the protonated peak of urea.

The fragmentation pattern of the peak at $m/z$ 131 is shown in Figure S8. Other peaks corresponding to adenine and urea could not be studied due to ion loss in the trap at that concentration. Additionally, we have analyzed diazepam, a well-known antianxiety drug at 10 pM in water (Figure S10). To show the stability of the ionization methodology, the total ion chronogram (TIC) and selected ion chronogram (SIC) are shown in Figure S11. It also provides the information about the background current. As we see in the TIC, the ion current is of the order of 8M, whereas the peak that corresponds to isoleucine has an intensity up to 40k. This is due to the fact that there are more ions in the background which either come from the paper or from the instrument and are shown in the inset of Figure S11A. However, from the SIC, it is clear that the ionization of the preconcentrated analytes at the ppt level is stable for a certain time interval until the maximum number of ion ejections has happened.

To compare with standard PSI MS, rhodamine 6G and glucose were chosen as potential candidates, as they can be detected in paper spray mode easily. The experiment was conducted by putting the same amount of analyte (10 $\mu$L each, thrice) on both the superhydrophobic paper and normal paper using similar sampling methodologies. Comparison of the mass spectrum of rhodamine 6G by SHPPSI MS and PSI MS are shown in Figure 5, where almost a 13-fold enhancement of the

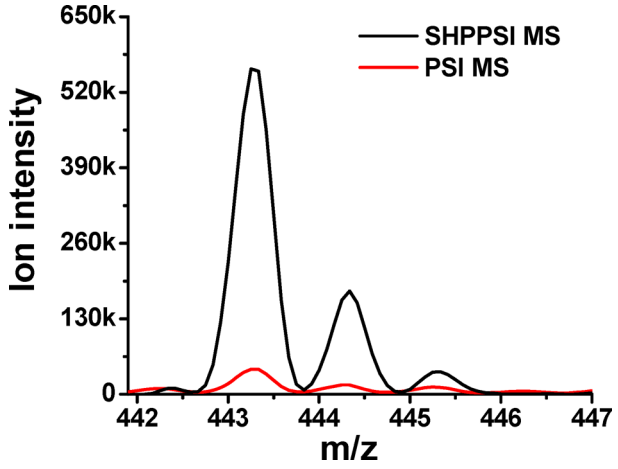



**Figure 5.** Comparison of the ion intensity for a chosen analyte (rhodamine 6G) in SHPPSI MS and by standard PSI MS. The same quantity of analyte was used in both the cases.

ion intensity was observed. The ion current of the selected peak at $m/z$ 443 in PSI MS is 43.26k, whereas in SHPPSI MS, the ion current for the same peak is 563.46k. The mass spectrum recorded for the two times preconcentrated sample has an ion current of 273.33k (Figure S12). In the case of glucose, the ion current in the normal paper is only 5.44k, whereas the superhydrophobic paper gives 48.14k for the peak at $m/z$ 181 (Figure S13).

We have also compared the ion current of the selected peak at $m/z$ 127, corresponding to protonated melamine in PSI, ESI, and SHPPSI, at 10 pM concentration. Figure S14 represents the comparative mass spectrum, in which we have not observed this peak at $m/z$ 127 in the case of PSI and ESI, whereas SHPPSI provides a distinct peak of melamine in the mass spectrum.

For further characterization of the method, five different concentrations of the analytes were chosen and the peak



DOI: 10.1021/acs.analchem.9b00144
Anal. Chem. 2019, 91, 7118−7124

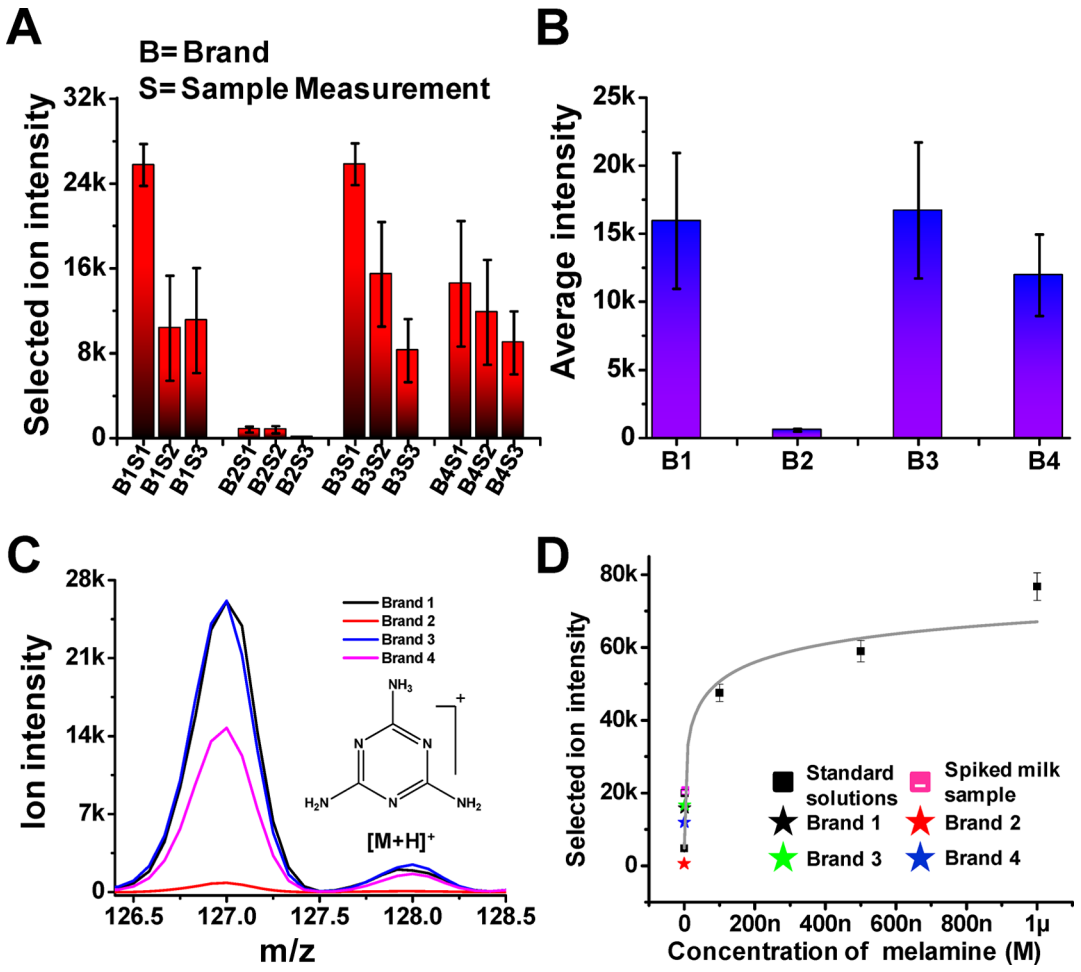



**Figure 6.** Mass spectrum of melamine found in commercial milk. (A) Selected ion intensity of each brand milk samples of three independent experiments, (B) avarage ion intensity of each brand, (C) mass spectra of the melamine in the commercially available milk brands, and (D) calibration curve based on the selected ion current for melamine in spiked milk, due to the peak at $m/z$ 127. The concentration of melamine in commercial milk was found to be in the nanomolar range for brands 1, 3, and 4. Brand 2 does not contain melamine.

intensity was compared. Figure S17 represents the SHPPSI MS spectra of five different concentrations of melamine ranging from picomolar to millimolar. The observed ion intensity change is from 3k to 1M. By correlating the ion intensity of the peak at $m/z$ 127, with the given concentration of the analyte in the solution, it can be concluded that the peak intensity gets saturated at higher concentration (Figure S18). Hence the methodology for preconcentrating a molecule and simultaneously detecting the same is both qualitative as well as quantitative.

**Detection of Melamine in Adulterated Milk.** To show the utility of the invention in an analytical context of societal relevance, we have chosen the detection of melamine in a sample of milk. Detection of melamine is important to know the actual nitrogen content of the milk. The commercial value of the milk is related to the total protein content, which is measured by quantifying the total nitrogen content in it. The melamine skeleton contains six nitrogen atoms. Hence by mixing it, the nitrogen content of milk can be enhanced.

The limit of daily melamine uptake is set to be 0.2 mg/kg or 1.6 $\mu$M by the World Health Organization (WHO) in 2008.[40] The reported melamine content in commercially available milk sample from the state Tamilnadu in India is 0.028−0.071 mg/kg or in the range of 0.2−0.58 $\mu$M.[41]

SHPPSI MS provides an easy and direct detection method of melamine in milk samples without having the sample processing step (Figure 6). The experiment was conducted by arbitrarily spiking a 5 nM (or 0.63 ppb) concentration of melamine in a melamine free milk sample, purchased from the local market. Figure S15 represents the mass spectrum of the milk before spiking which shows no melamine contamination. The prepared milk sample was directly used for SHPPSI MS analysis without further dilution. The intensity of the peak corresponding to melamine cation was 20.67k (Figure S16). Figure 6D shows the calibration curve of ion intensity vs solution phase concentration of standard samples of melamine in milk. Shape of the calibration curve shows a similar trend as shown in Figure S18. This seems to be related to the solubility of the analyte in the eluting solvent. At the lowest detectable concentration, almost all the analyte molecules can be eluted by methanol at the retention time scale, which subsequently gets ionized. Here, the retention time scale refers to the time taken for methanol to dissolve the analyte at that location and to get sprayed from the tip. With an increased concentration of melamine, part of it may get detected by one time drop casting of the methanol solution and the remaining part may stay at the groove and hence the overall intensity of the analyte become saturated after certain concentration. By correlating the ion intensity of the selected ion of $m/z$ 127 with the



DOI: 10.1021/acs.analchem.9b00144
Anal. Chem. 2019, 91, 7118−7124

calibration curve, we can conclude that the level of adulteration is in the nanomolar (or ppb) range. We have chosen four other brands to measure the level of contamination. Figure 6A represents the bar diagram of selected ion intensity vs brands. Each samples was tested three times to get an average ion intensity shown in Figure 6B. The average intensity was related to the fitted calibration curve. Brands 1, 3, and 4 had nanomolar concentrations of melamine, whereas brand 2 did not contain melamine. Figure 6C shows the mass spectra of all the brands. The data presented in Figure S17 also shows that analysis at even lower concentrations, down to the ppt, is possible. The minimum concentration of melamine in water with measurable ion count was 1.2 ppt (10 pM).

## CONCLUSION

In this article, we demonstrate a method of ionization in which ion intensity was increased by selective localization of analyte molecules at the tip of a superhydrophobic paper and simultaneously detecting it with an ion detector. This methodology named as superhydrophobic preconcentration paper spray ionization mass spectrometry (SHPPSI MS) is demonstrated for a variety of analytes with different functionalities. This technique requires less volume of sample and involves easy sample preparation. The actual amounts of the analytes used are at the femtogram level; e.g., 38, 18, 39.1, 40.5, 58.2 fg of melamine, urea, isolucine, adenine, and caffeine, respectively. We achieved detection at the 0.6 ppt level in water using SHPPSI MS for which urea was chosen as the analyte of choice. Detection of the 1.2 ppt (10 pM) level of melamine has been demonstrated here with standard samples in water. Qualitative and quantitative detection of melamine is also shown with a commercial milk sample.

SHPPSI MS can be utilized for the detection of many socially relevant analytes, e.g., pesticides, drugs, and toxic chemicals in an environment at lowest possible levels. This methodology can also be coupled with other excitation sources such as light, heat, etc. to enhance the ionization probability.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.9b00144.

> Part of the experimental data, optical images of the preconcentration technique, SEM of the normal and superhydrophobic papers, $MS^n$ data of the analytes, chronogram of the analytes, and mass spectra of standard melamine samples (PDF)

## AUTHOR INFORMATION


### Corresponding Author

*E-mail: pradeep@iitm.ac.in. Phone: +91-44-22574208. Fax: +91-44-2257 0509/0545.

### ORCID

Avijit Baidya: 0000-0001-5215-2856

Thalappil Pradeep: 0000-0003-3174-534X


### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

The authors thank the Department of Science and Technology, Government of India, for equipment support through the Nano Mission. P.B. acknowledges IIT Madras for providing the Institute Doctoral Fellowship.

## REFERENCES


(1) Yamashita, M.; Fenn, J. B. *J. Phys. Chem.* **1984**, *88* (20), 4451−9.

(2) Takats, Z.; Wiseman, J. M.; Gologan, B.; Cooks, R. G. *Science (Washington, DC, U. S.)* **2004**, *306* (5695), 471−473.

(3) Chen, H.; Talaty, N. N.; Takats, Z.; Cooks, R. G. *Anal. Chem.* **2005**, *77* (21), 6915−6927.

(4) Cotte-Rodriguez, I.; Takats, Z.; Talaty, N.; Chen, H.; Cooks, R. G. *Anal. Chem.* **2005**, *77* (21), 6755−6764.

(5) Harper, J. D.; Charipar, N. A.; Mulligan, C. C.; Zhang, X.; Cooks, R. G.; Ouyang, Z. *Anal. Chem. (Washington, DC, U. S.)* **2008**, *80* (23), 9097−9104.

(6) Spencer, S. E.; Santiago, B. G.; Glish, G. L. *Anal. Chem. (Washington, DC, U. S.)* **2015**, *87* (23), 11887−11892.

(7) Kuklya, A.; Engelhard, C.; Uteschil, F.; Kerpen, K.; Marks, R.; Telgheder, U. *Anal. Chem. (Washington, DC, U. S.)* **2015**, *87* (17), 8932−8940.

(8) Nagy, K.; Pollreisz, F.; Takats, Z.; Vekey, K. *Rapid Commun. Mass Spectrom.* **2004**, *18* (20), 2473−2478.

(9) van Bavel, B.; Geng, D.; Cherta, L.; Nacher-Mestre, J.; Portoles, T.; Abalos, M.; Saulo, J.; Abad, E.; Dunstan, J.; Jones, R.; Kotz, A.; Winterhalter, H.; Malisch, R.; Traag, W.; Hagberg, J.; Ericson Jogsten, I.; Beltran, J.; Hernandez, F. *Anal. Chem. (Washington, DC, U. S.)* **2015**, *87* (17), 9047−9053.

(10) Zuth, C.; Vogel, A. L.; Ockenfeld, S.; Huesmann, R.; Hoffmann, T. *Anal. Chem.* **2018**, *90*, 8816−8823.

(11) Wang, H.; Liu, J.; Cooks, R. G.; Ouyang, Z. *Angew. Chem., Int. Ed.* **2010**, *49* (5), 877−880.

(12) Ren, Y.; Wang, H.; Liu, J.; Zhang, Z.; McLuckey, M. N.; Ouyang, Z. *Chromatographia* **2013**, *76* (19−20), 1339−1346.

(13) Sarkar, D.; Srimany, A.; Pradeep, T. *Analyst (Cambridge, U. K.)* **2012**, *137* (19), 4559−4563.

(14) Jackson, S.; Swiner, D. J.; Capone, P. C.; Badu-Tawiah, A. K. *Anal. Chim. Acta* **2018**, *1023*, 81−88.

(15) Wang, T.; Zheng, Y.; Wang, X.; Austin, D. E.; Zhang, Z. *Anal. Chem. (Washington, DC, U. S.)* **2017**, *89* (15), 7988−7995.

(16) Jiang, J.; Zhang, H.; Li, M.; Dulay, M. T.; Ingram, A. J.; Li, N.; You, H.; Zare, R. N. *Anal. Chem. (Washington, DC, U. S.)* **2015**, *87* (16), 8057−8062.

(17) Espy, R. D.; Teunissen, S. F.; Manicke, N. E.; Ren, Y.; Ouyang, Z.; van Asten, A.; Cooks, R. G. *Anal. Chem. (Washington, DC, U. S.)* **2014**, *86* (15), 7712−7718.

(18) Yannell, K. E.; Kesely, K. R.; Chien, H. D.; Kissinger, C. B.; Cooks, R. G. *Anal. Bioanal. Chem.* **2017**, *409* (1), 121−131.

(19) Yang, Q.; Wang, H.; Maas, J. D.; Chappell, W. J.; Manicke, N. E.; Cooks, R. G.; Ouyang, Z. *Int. J. Mass Spectrom.* **2012**, *312*, 201−207.

(20) Amador, V. S.; Pereira, H. V.; Sena, M. M.; Augusti, R.; Piccin, E. *J. Am. Soc. Mass Spectrom.* **2017**, *28* (9), 1965−1976.

(21) Wang, Q.; Zheng, Y.; Zhang, X.; Han, X.; Wang, T.; Zhang, Z. *Analyst (Cambridge, U. K.)* **2015**, *140* (23), 8048−8056.

(22) Banerjee, S.; Basheer, C.; Zare, R. N. *Angew. Chem., Int. Ed.* **2016**, *55* (41), 12807−12811.

(23) Narayanan, R.; Sarkar, D.; Som, A.; Wleklinski, M.; Cooks, R. G.; Pradeep, T. *Anal. Chem.* **2015**, *87* (21), 10792−10798.

(24) Narayanan, R.; Pradeep, T. *Anal. Chem.* **2017**, *89* (20), 10696−10701.

(25) Narayanan, R.; Sarkar, D.; Cooks, R. G.; Pradeep, T. *Angew. Chem., Int. Ed.* **2014**, *53* (23), 5936−5940.

(26) Basuri, P.; Sarkar, D.; Paramasivam, G.; Pradeep, T. *Anal. Chem.* **2018**, *90* (7), 4663−4668.

(27) Sarkar, D.; Som, A.; Pradeep, T. *Anal. Chem.* **2017**, *89* (21), 11378−11382.




DOI: 10.1021/acs.analchem.9b00144
*Anal. Chem.* 2019, 91, 7118−7124

(28) Damon, D. E.; Davis, K. M.; Moreira, C. R.; Capone, P.; Cruttenden, R.; Badu-Tawiah, A. K. *Anal. Chem. (Washington, DC, U. S.)* **2016**, *88* (3), 1878−1884.
(29) Damon, D. E.; Yin, M.; Allen, D. M.; Maher, Y. S.; Tanny, C. J.; Oyola-Reynoso, S.; Smith, B. L.; Maher, S.; Thuo, M. M.; Badu-Tawiah, A. K. *Anal. Chem. (Washington, DC, U. S.)* **2018**, *90* (15), 9353−9358.
(30) Filazi, A.; Sireli, U. T.; Ekici, H.; Can, H. Y.; Karagoz, A. *J. Dairy Sci.* **2012**, *95* (2), 602−608.
(31) Li, J.; Qi, H.-Y.; Shi, Y.-P. *J. Chromatogr. A* **2009**, *1216* (29), 5467−5471.
(32) Harnly, J.; Bergana, M. M.; Adams, K. M.; Xie, Z.; Moore, J. C. *J. Agric. Food Chem.* **2018**, *66*, 8478−8488.
(33) Wang, Q.; Haughey, S. A.; Sun, Y.-M.; Eremin, S. A.; Li, Z.-F.; Liu, H.; Xu, Z.-L.; Shen, Y.-D.; Lei, H.-T. *Anal. Bioanal. Chem.* **2011**, *399* (6), 2275−2284.
(34) Cao, B.; Yang, H.; Song, J.; Chang, H.; Li, S.; Deng, A. *Talanta* **2013**, *116*, 173−180.
(35) Lei, H.; Su, R.; Haughey, S. A.; Wang, Q.; Xu, Z.; Yang, J.; Shen, Y.; Wang, H.; Jiang, Y.; Sun, Y. *Molecules* **2011**, *16*, 5591−5603.
(36) Lee, I.-C.; Ko, J.-W.; Park, S.-H.; Shin, I.-S.; Moon, C.; Kim, S.-H.; Kim, Y.-B.; Kim, J.-C. *Food Chem. Toxicol.* **2016**, *96*, 254−262.
(37) Bandele, O. J.; Stine, C. B.; Ferguson, M.; Black, T.; Olejnik, N.; Keltner, Z.; Evans, E. R.; Crosby, T. C.; Reimschuessel, R.; Sprando, R. L. *Food Chem. Toxicol.* **2014**, *74*, 301−308.
(38) Xu, C.-F.; Gao, X.-L.; Du, Y.-X.; Ren, S.-T. *Food Chem. Toxicol.* **2011**, *49* (12), 3013−3017.
(39) Baidya, A.; Ganayee, M. A.; Jakka Ravindran, S.; Tam, K. C.; Das, S. K.; Ras, R. H. A.; Pradeep, T. *ACS Nano* **2017**, *11* (11), 11091−11099.
(40) World Health Organization. *Melanie and Cyanuric Acid: Toxicity, Preliminary Risk Assessment and Guidance on Levels in Food*. October 30, 2008.
(41) Niraimathi, V.; Jerad Suresh, A.; Ananthakumar, T. *Indo Am. J. Pharm. Res.* **2015**, *5* (1), 271−276.

## NOTE ADDED AFTER ASAP PUBLICATION

This paper was originally published ASAP on May 22, 2019. Due to a production error, there was an error in the title. The corrected version was reposted on May 23, 2019.



DOI: 10.1021/acs.analchem.9b00144
*Anal. Chem.* 2019, 91, 7118−7124