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Conductive Polymer Spray Ionization Mass Spectrometry for Biofluid Analysis

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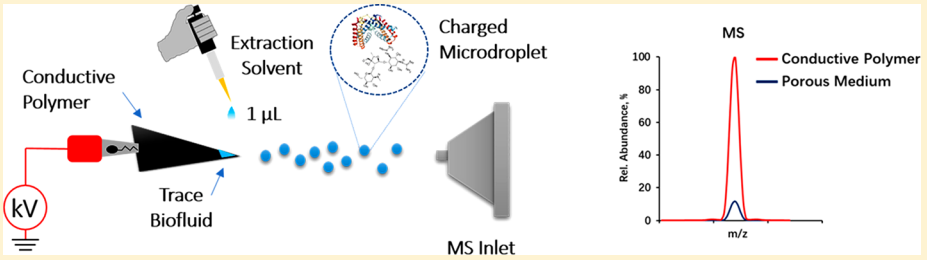
Supporting Information



ABSTRACT: We present a conductive polymer spray ionization (CPSI) method for the direct mass spectrometric analysis of hydrophilic drugs, saccharides, peptides, and proteins in biofluids. Carbon nanotubes (CNTs) were introduced into poly(methyl methacrylate) (PMMA) to fabricate a conductive composite substrate CNT/PMMA in the shape of a triangle (8 mm wide and 10 mm long) with its apex pointed toward the inlet of a mass spectrometer. In comparison with a traditional paper spray substrate, the conductive polymer absorbs less hydrophilic compounds owing to its hydrophobic nature. When aqueous biofluid samples are loaded, they also exhibit less diffusion on this nonporous surface. Only 1.0−2.0 μL solvent suffices to extract the components in a dried biofluid spot and to form charged microdroplets (4.5 kV high voltage applied). Furthermore, the hydrophobic polymer surface only needs to overcome weak surface tension to emit charged microdroplets, so that the signal has a typical duration of 7.5 min. For sunitinib, acarbose, melamine, and angiotensin II, the ion intensity of the target compound from the conductive polymer support is significantly higher than paper spray, typically by a factor of 20 to 100. These results suggest that the CNT/PMMA conductive polymer spray has great potential in the analysis of hydrophilic drugs, saccharides, peptides, and proteins in biofluids.

As one of the widely used ambient ionization methods, paper spray ionization (PSI)[1,2] had already shown its applicability for analysis of synthetic drugs,[3] reaction intermediates and products,[4] natural products,[5] food and environmental pollutants,[6] endogenous metabolites,[7] and proteins.[8,9] However, paper spray ionization has some drawbacks that limit its general use. Although high-quality filter paper is commonly employed, the paper substrate contains a range of impurities that yield interfering peaks in the mass spectrum. The paper's fibrous nature lacks structural integrity to support its repeated use. More importantly, the strong affinity between polar analytes and fibrous paper contributes to the loss of target molecules and inefficiency in ionization.[10] As a result, analysts struggle to achieve good sensitivity for a number of different hydrophilic compounds. Moreover, the concentration of target molecules becomes diluted by (1) biofluid diffusion when being loaded onto the porous paper support and (2) the need to introduce a continuous flow of solvent to keep the substrate conductive.

To overcome these challenges, one strategy is to coat paper with various materials to remove porosity and change its physiochemical properties. Among various additives, carbon nanotubes,[11,12] silica gel,[13] urea,[14] nanoparticles,[15] metal organic frameworks,[16] waxes,[17] and polystyrene microspheres[18] have been used. Another approach for improving the signal-to-noise ratio is to modify chemically the fibrous paper with different functional groups for target enrichment or solid phase microextraction. Examples are the use of a molecularly imprinted membrane,[19] a sorbent-coated blade,[20] and an aptamer-modified nanomaterial.[21] This procedure naturally increases the time and cost to prepare large amounts of parallel substrates to fulfill the task of large-scale sample determinations. A third approach is to replace the paper substrate. This procedure has been followed using a glass slide[22] or an organosiloxane polymer,[10] but both are nonconductive supports that require continuous liquid flow. We present here a variation in which we fabricate a conductive polymer support by embedding carbon nanotubes (CNTs) in poly(methyl methacrylate) (PMMA) polymer to form a composite we call CNT/PMMA.

Previous studies have shown that carbon nanotubes (CNTs) are an excellent paper-based coating material for detecting a wide variety of compounds.[23] It simultaneously shares hydrophobic and conductive properties. Nevertheless, the

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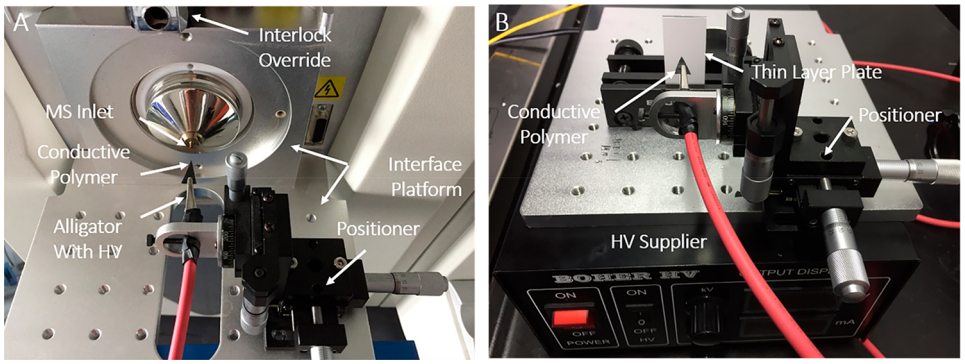

Figure 1. (A) Setup of conductive polymer spray ionization coupled with a mass spectrometer system and (B) the off-line sprayed spot tracing system employing a thin layer chromatography plate.

lack of appropriate mechanical integrity makes the conductive paper support not able to be cast into a certain shape without spiking with some binder inside. To the best of our knowledge, the use of a conductive polymer composite as the substrate has not been previously reported.

Unlike the paper-based substrate, the fabricated CNT/PMMA has elasticity and toughness. It is readily cleaned, and it can be used repeatedly owing to its smooth surface with no residue adhering to it. There is no sacrifice caused by biofluid diffusion and flow solvent dilution because of the nonporous nature of the composite. Furthermore, this approach makes aqueous extraction solvents much easier to overcome the surface tension to form charged microdroplets. Additionally, because of CNT/PMMA's native conductivity, we neither need to introduce continuous flow to move the components forward nor wet the whole substrate to keep the surface conductive. Only a very small volume of solvent (1.0−2.0 μL) is needed as the extraction and spraying agent, which dissolves target molecules from the dried biofluid spot. This trace solvent consumption generates much longer signal life than regular paper spray using the dropwise mode. Consequently, if the biofluid sample is very limited (such as cerebrospinal fluid), the fresh liquid sample could be loaded and directly tested without severe losses in sample signal. We report here the systematic investigation of the performance of this conductive polymer spray ionization (CPSI) in enhancing the detection of hydrophilic compounds, demonstrating a great advantage of this approach for the analysis of some analytes in biofluids.

EXPERIMENTAL SECTION

Reagents and Materials. Methanol, ethanol, acetonitrile, isopropanol, and formic acid were purchased from Fisher (Waltham, MA). Formic acid, ammonium acetate, and fluorescein were purchased from Sigma-Aldrich. Deionized water was prepared by Milli Q purification system (Millipore Advantage A10). Detail information for drug, saccharide, peptide, protein standards, and preparation of standard solution and biofluid sample are described in the Supporting Information. The tested blank plasma was collected from Vista mouse (Beijing Vital-Star Biotechnology Co., Ltd.). Poly(methyl methacrylate) (PMMA) was provided by Titan Scientific. Graphite powder and carboxylic multiwalled carbon nanotube (i.d. 2−5 nm, o.d. < 8 nm, length 10−30 μm) were purchased from Sangon Biotech and J&K Scientific, respectively. The chromatography paper grade 1 (0.21 mm thick) was manufactured by Whatman (GE Healthcare) and used without further chemical treatment.

Preparation of Conductive Polymer Composite. A square sample of PMMA (1.5 g) was added to 5.0 mL acetyl acetate followed by refluxing at 120 °C until it was fully dissolved. CNT (150.0 mg) was added to another 5.0 mL of acetyl acetate, followed by ultrasonication until homogeneously dispersed. Then equal amounts of CNT solution and heated PMMA solution were cast into a circular mold, one after another. After slightly stirring to make the above mixture uniform, the acetyl acetate was evaporated to dryness under ambient temperature, which created the CNT/PMMA composite. This raw conductive polymer (0.4 mm thickness) was cut into triangular shapes (10 mm long and 8 mm wide) and rinsed with methanol and water before use (see Figure S1).

SEM Characterization of Different Substrates. To evaluate the changes of microscale porosity on the substrate surface, images of the chromatography paper, CNT-coated papers, CNT-coated PMMA, and CNT-doped PMMA composite were recorded using a Nova NanoSem 450 field emission scanning electron microscope (SEM, FEI Company, Hillsboro, OR). The substrates were sputter-coated with platinum for 1.0 min before the analysis, and the accelerating voltage for the Everhart-Thornley detector (ETD, routine imaging) or through-the-lens detector (TLD, high magnification/resolution imaging) was 5 kV with a working distance of about 4.0 mm.

Substrate Comparison and Method Optimization. Graphite (G) and carbon nanotube (CNT) are two types of frequently used conductive materials in electrode fabrication. To find the optimal conductive substrate, we compared two coated-papers (G/Paper and CNT/Paper) with the two doped polymers (G/PMMA and CNT/PMMA). The filter paper was used as the contrast substrate. In this article, several clinical drugs, mono/oligo- saccharides, and peptides were selected as the model compounds (shown in Table S1). Furthermore, to achieve the best ionization efficiency, key parameters like tip angle, distance between tip and mass spectrometer (MS) inlet, solvent composition/ratio, applied high voltage, volume of loaded biosample, and spraying solvent were systematically optimized.

Conductive Polymer Spray Ionization. A copper alligator clip was used to apply high voltage (BOHER HV, Genvolt, U.K.) to the substrate. A combined XYZ-positioner (Beijing Optical Century Instrument Company, China) was used to set the 8.0 mm distance from the tip of triangular

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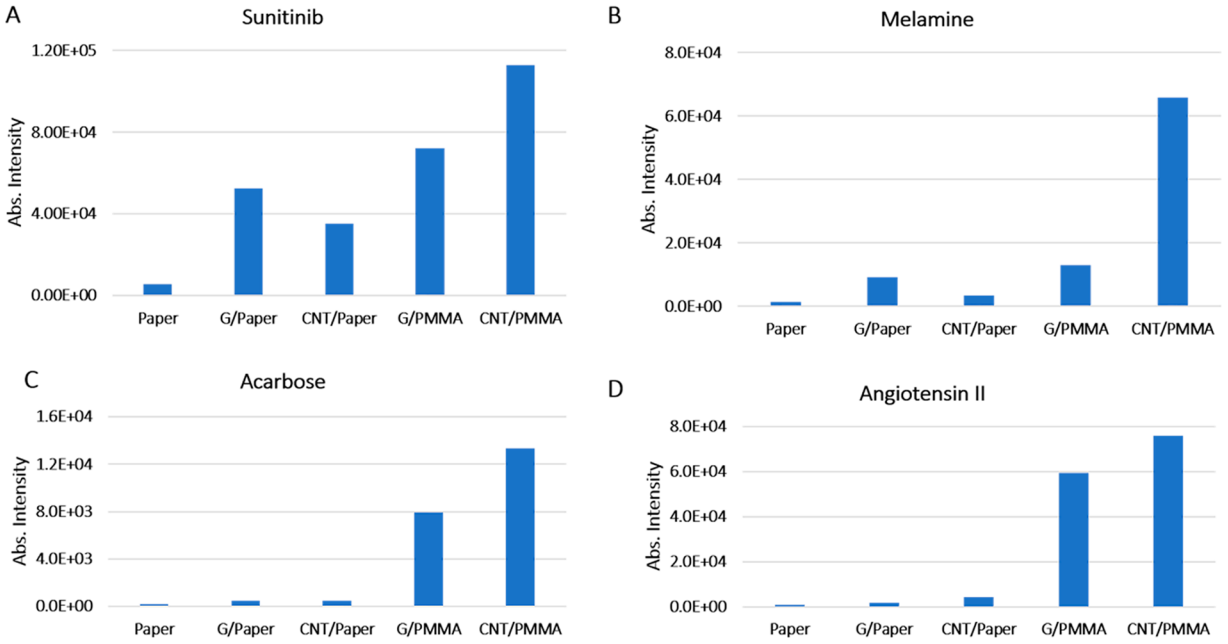

Figure 2. Ionization performance comparison among different spray substrates using sunitinib (A), melamine (B), acarbose (C), and angiotensin II (D) as the model compounds.

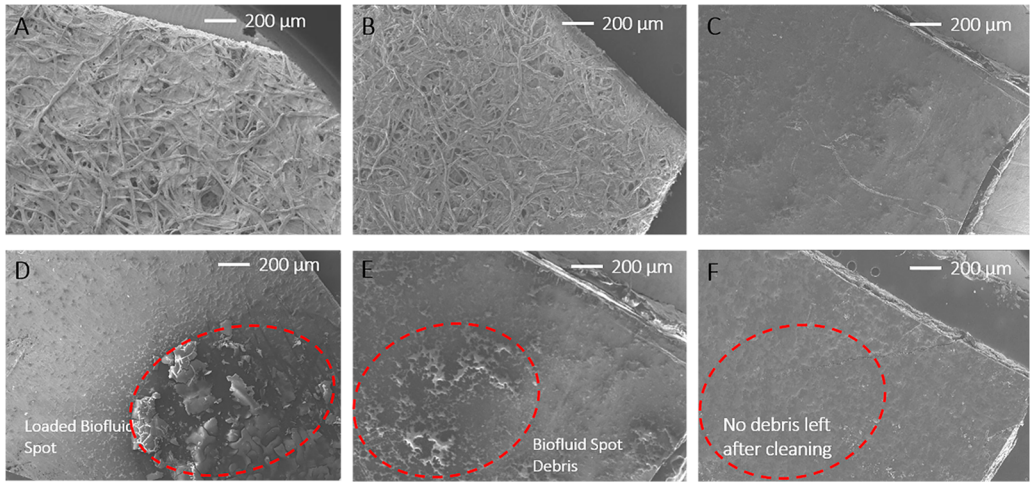

Figure 3. Microscale topography of different substrate surfaces characterized by SEM (A) filter paper, (B) CNT-coated paper, (C) CNT/PMMA, (D) CNT/PMMA with loaded biofluid spot, (E) sample-loaded CNT/PMMA after CPSI analysis, and (F) CNT/PMMA with washing after use.

polymer to the MS inlet. We carried out the conductive polymer spray ionization experiment after fully optimizing some key factors such as tip angle, tip-to-inlet distance, applied voltage, spraying solvent, etc. (Figure S2). After the substrate was loaded with 3 μL of biofluid positioned 1.0 mm away from the tip, a biofluid droplet was formed on the surface of the nonporous polymer. After the biofluid was fully dried and uniformly precipitated near the tip of substrate, it was extracted with 2.0 μL of spraying solvent (MeOH−water, 9:1 or 5:5, v/v, containing 0.1% formic acid), which formed a liquid layer. The direct MS analysis was carried out when the 4.5 kV high voltage was switched on. The experiments with paper spray and nanoelectrospray ionization (nESI) were similar to the reported procedure, which is illustrated in the Supporting Information.

CPSI-MS and Off-Line Thin Layer Spotting Setup. An LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, San Jose, CA) was employed to undertake the ambient MS analysis task. At the stage of conditions optimization, it was operated in the full scan mode. For drug, saccharide, or peptide quantitation, target selected ion monitoring (t-SIM) was used to detect the trace-level analytes in biofluids. The MS capillary temperature was set at 275 °C with the S-lens voltage set at 55 V. Figure 1A presents the whole setup for CPSI-MS. To further investigate the spraying trajectory of paper and conductive polymer, an off-line setup was established (illustrated in Figure 1B). Fluorescein was used as the model compound. Silica-coated thin layer plate for chromatography was employed to collect the sprayed mist under detection by a UV lamp at a wavelength of 365 nm. The Xcalibur software was employed for controlling the MS system and data analysis. Open-source software Bio-Loom (http://www.biobyte.com/index.html) was used to calculate the LogP value for each tested compounds. MATLAB 2017b was employed to make

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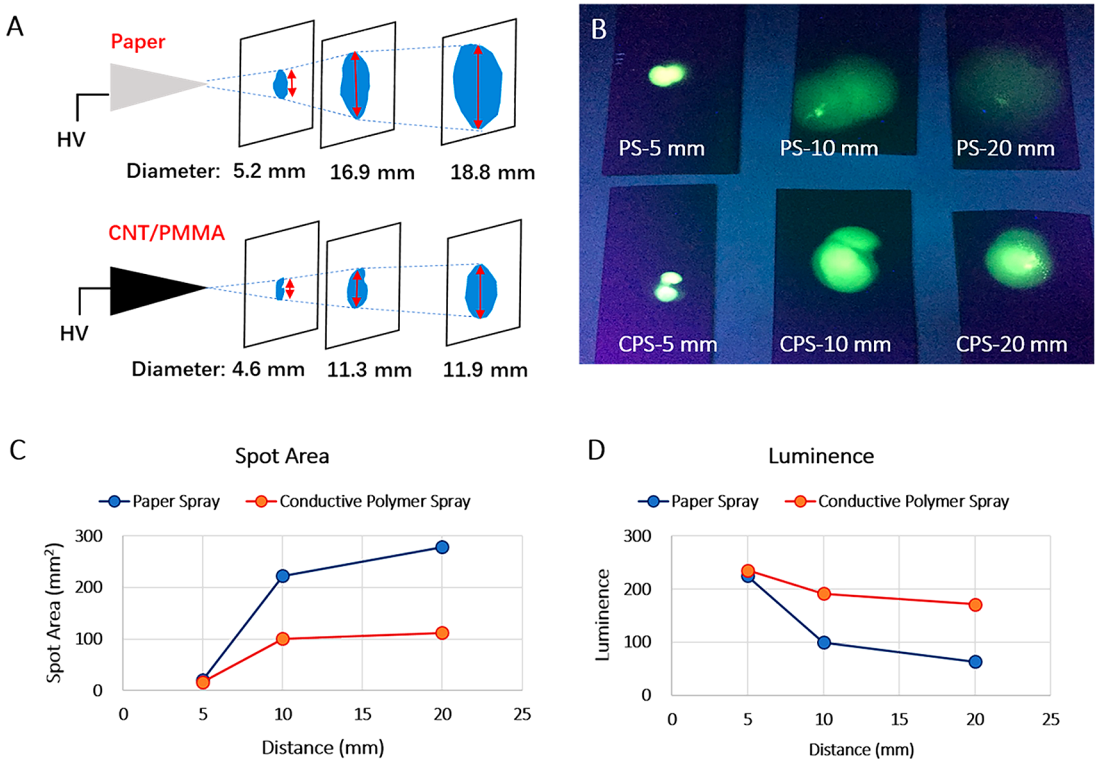

Figure 4. Narrowed charged droplet flow in conductive polymer spray compared with paper spray: (A) schematic illustration for change of sprayed spot size with the distance between tip and plate, (B) pictures about the sprayed spot traced by fluorescein under UV lamp detection, (C) spot area changes with the distance, and (D) fluorescent intensity changes with the distance.

principal component analysis, estimate the sample spot area, and process optical and fluorescent images.

RESULTS AND DISCUSSION

Comparison for Sensitivity Improvement. We initially evaluated the performance of CNT/PMMA and G/PMMA for improving the hydrophilic compounds' ionization efficiencies. CNT-/graphite-coated paper as well as traditional filter paper were compared. The standard solution of four hydrophilic compounds, sunitinib, acarbose, melamine, and angiotensin II was selected as representative drug, saccharide, illegal addictive, and peptide, respectively. It was surprising to find that the CNT/PMMA substrate significantly enhanced target ion intensities by 21- to 92-fold (Table S2). As for G/PMMA, it also gave an ideal signal enhancement of 8.7- to 72-fold. In comparison with paper spray ionization, the extent of signal enhancement using CNT/PMMA or G/PMMA was far greater than that in CNT- or graphite-coated paper (Figure 2).

SEM Characterization for Surface Porosity. The nature of the substrate is highly related with the retention of target analytes. Therefore, the surface features of these substrates were characterized by SEM. It was observed that there were no obvious microscale porous structures on the surface of our fabricated conductive polymer (Figure 3C) in comparison with filter paper (Figure 3A) and CNT-coated paper (Figure 3B). The strong absorption arising from hydroxyl groups in cellulose-composed paper are absent for these hydrophobic supports. Nonextractable components from biofluids are also easily washed away, leaving behind almost no residue (Figure 3D–F).

Surface Cleanliness. The surface cleanliness of substrate is another factor of importance. We used methanol–water (1:1, v/v) to wet the paper and polymer's surface. Then the constituents were sprayed into the mass spectrometer. The full scan mass range (m/z 100–900) was acquired and compared between the conductive polymer and paper substrates. We found that the total ion current in the conductive polymer was much weaker than paper (Figure S3A,B), demonstrating that there was less contamination in the polymer support than in the paper support. The background ions in the mass spectrum usually stemmed from the impurities in the substrate, which could cause severe suppression of ions of interest.

Repeated Utilization. As discussed above, the strong mechanical strength and surface cleanliness as well as no sample residue after cleaning enabled the conductive polymer to be repeatedly used. To prove this, we employed a single piece of conductive polymer to conduct the test sequence composed of five drug-spiked mixtures (sunitinib, imipramine, cimetidine, 100 μg/mL each dissolved in methanol) evenly inserted between six blank solvent samples. After each test cycle, the polymer was cleaned with water followed by being soaked in methanol for several minutes. Consequently, no drug ion signals were detected from those blank solvent samples. RSD% of the high-, medium-, and low-response drugs among five samples were 5.5%, 15.7% and 19.2%, respectively (Figure S4). We conclude that the fluctuation of five duplicates was low during repeated utilization with a single polymer substrate that was briefly cleaned between runs.

Sample Diffusion and Reconstitution. To further investigate the ionization mechanism, we manually added a model compound, fluorescein (100 μg/mL, 2 μL), onto the paper and CNT/PMMA substrates. The diameters of loaded sample spot on paper and CNT/PMMA was approximately 6.25 mm and 1.88 mm, respectively. The analyte was

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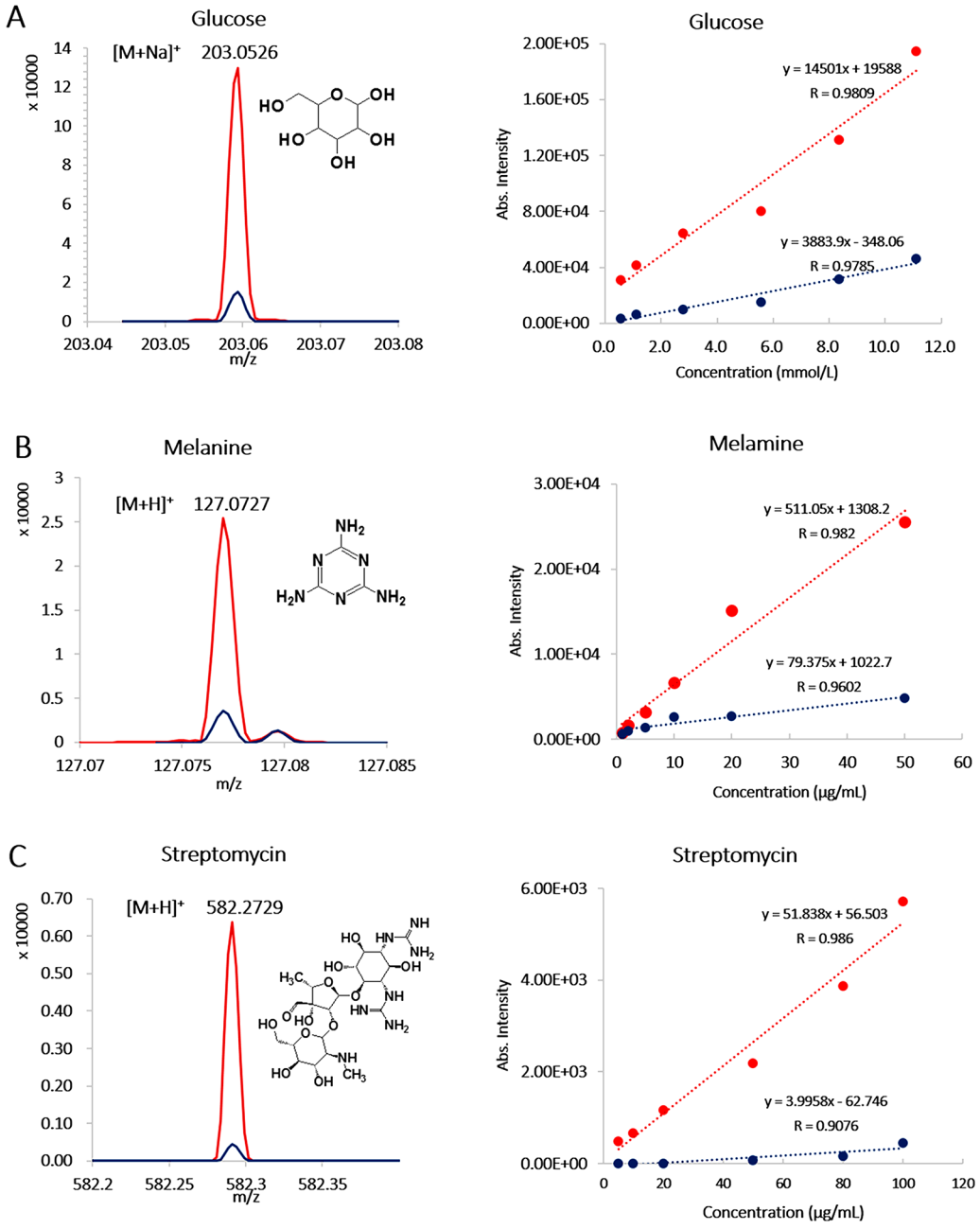

Figure 5. Comparison in quantitation of analyte in biofluid obtained with conductive polymer (red) versus paper (blue): (A) glucose in plasma, (B) melamine in milk, and (C) streptomycin in plasma.

precipitated within a relatively small area on polymer after dryness (Figure S5A,B). This behavior suggests that the initial distribution density of target molecule on CNT/PMMA (71.9 ng/mm^2) was about 11-fold higher than that on paper (6.52 ng/mm^2). This also illustrates that doping of conductive material into polymer is a more effective way to cover the porous structure than coating, causing the liquid sample to diffuse less on the conductive polymer.

Besides, we could also observe the more serious diffusion phenomenon in paper than CNT/PMMA during the ongoing spraying and ionization process (Figure S5C,D). In contrast, it is not necessary to introduce a large volume of solvent to continuously wet the whole substrate for keeping it conductive. The solvent would not spread throughout the whole polymer to make the analyte sample diluted again. This also avoids the cross-contamination to the copper alligator or in-house made cartridge system. Only 2.0 μL volume of solvent is enough to reconstitute the target molecules into aqueous phase with higher concentration. We suggest that, in general, less sample diffusion and higher reconstituted concentration are responsible for raising the target analyte's ion intensity.

Ion Signal Stability. In CPSI, although only 2.0 μL of solvent is loaded, we find that the target ion signal could last for a much longer period (7.5 min) than that of the acquired signal (2.0 min) when the same volume of solvent was dropped onto the paper substrate for spraying (Figure S6). When the charged microdroplet is formed and emitted from the tip, it needs to overcome the surface tension between solid−liquid interface. The existence of porous substrate surface will increase this energy barrier owing to its larger contacting

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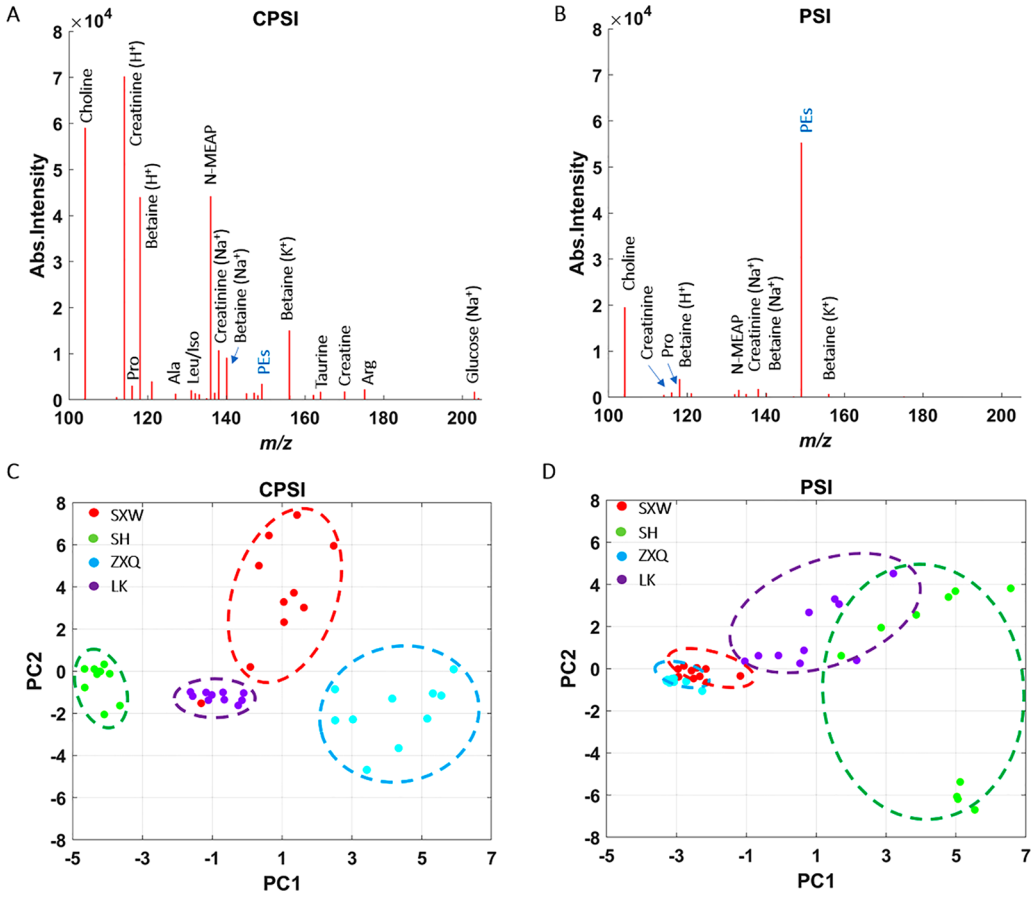

Figure 6. Average mass spectra of representative metabolites in saliva and clustering results comparison. The mass spectrum obtained by (A) conductive polymer spray ionization (CPSI) and (B) paper spray ionization (PSI). The PCA score plot of the saliva metabolomic profile PSI for four volunteers acquired by (C) CPSI and (D) PSI. PEs represent the commonly seen fragment ion (m/z 149.0233) originating from phthalate esters.

area. Therefore, the nonporous property of the conductive polymer will naturally lower this resistant force to yield a much more stable and persistent ion signal.

Focusing Charged Microdroplets. The conductivity of substrate is another key point for improving sensitivity, because it is highly related with the extent of surface polarization and the charge density across the substrate under high voltage. The charge density near tip, in turn, further influences the strength of the electrostatic force, which plays as the driving factor to emit charged microdroplets from the liquid surface in the PSI or CPSI process. Considering that the electric field around the triangular shape conductive polymer is hard to directly measure or simulate, we designed an off-line setup to record the sprayed spot area. Thus, the electric field line pattern could be indirectly characterized and compared. A silica-coated thin layer plate was used to collect the charged microdroplets emitted from tip. The model compound, fluorescein, was again used and detected under irradiation from a UV lamp. As a result, with the increase of tip-to-plate distance from 5.0 mm to 20.0 mm, the spot area extension in CNT/PMMA group was less than that in paper, whereas the fluorescence intensity achieved a relative slow decrease (Figure 4). Based on the diameters of sprayed spots with the increasing distances (5 mm, 15 mm, 20 mm), the spot areas in paper group are 1.28-fold, 2.24-fold, 2.50-fold of those areas using CNT/PMMA, respectively. We could estimate that the 3D cone of electric field line around polymer tip has less dispersion than paper. Besides, we suppose that the conductive polymer has stronger electric field strength, which serves as the driving force to enhance MS detection. As a result, the spray microdroplet beam is more focused in CNT/PMMA spray than in paper spray, which contributes to its increased sensitivity as well as its longer spray duration to yield a signal.

Comparison with PSI and nESI. After investigation of the underlying mechanism for CPSI, we further tested the sensitivity improvement for some other drugs, mono-/oligosaccharides and proteins in comparison with PSI. Among these analytes, the peptides and proteins like glutathione, amyloid β (1-14), cytochrome c, lysozyme, hemoglobin, and apomyoglobin were all successfully detected by CPSI whereas PSI failed to detect them (20 μM in water, Figure S7). This behavior showed the clear advantage of CPSI in peptide and protein analysis over PSI due to the reduced retention of analytes on the nonporous medium.

Furthermore, we selected three antibiotics, rifampicin, roxithromycin, and streptomycin as model compounds, which were spiked into blank plasma as the tested samples (20 μg/mL). After protein precipitation for 100 μL of plasma from the sample followed by addition of an equal amount of methanol, the supernatant was directly loaded into capillary emitter for nESI, the paper substrate for PSI and CNT/PMMA for CPSI. We found that there were no target analyte signals detected from the nESI group. In contrast, the SNR for rifampicin roxithromycin and streptomycin was 90.7, 441.7,

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and 100.2 in the CPSI group (Figure S8). Due to the strong viscosity and severe ion suppression by biofluid matrix, it was quite difficult to desorb and ionize the target analyte under the nESI mode. This further demonstrates that CPSI is advantageous in analyzing biofluid samples over nESI.

It should be also noted that the conductive polymer has an intrinsic electrical percolation threshold above which metabolites ion signals could be detected. For our studied CNT/PMMA, that threshold value is around 3.0 kV. In some way, it limits more versatile applications under the low-voltage operation, which of course need further development of novel conductive material and polymer medium.

Quantitative Analysis of Hydrophilic Compounds. Additionally, to prove the applicability of CPSI in biofluid analysis, three typical polar compounds with low oil−water partition coefficient (characterized by calculated LogP, cLogP), glucose (cLogP, −2.21), melamine (cLogP, −1.37), and streptomycin (cLogP, −3.46) were selected for quantitative study. Trace amounts of glucose and streptomycin were spiked into plasma. Series of melamine with different concentrations were added into milk. Native fucose, histidine, and externally spiked acarbose were used as the internal standard for these three analytes, respectively. The ideal linearities ($r > 0.99$) within the ranges (glucose, 0.56−11.1 mM; melamine, 1−50 μg/mL; streptomycin, 5−100 μg/mL) proved that the feasibility of CPSI in quantitation (Figure S9). To compare the response enhancement, the averaged absolute ion intensities were directly plotted with the concentration in biofluid without calibration by internal standard. The MS^2 spectra of target molecular ion were acquired to confirm the ion assignments (Figures S10 and S11). As shown in Figure 5, the slopes of three constructed content-response curves in CNT/PMMA gained 3.7-fold for glucose, 6.4-fold for melamine, and 12.97-fold for streptomycin. These study cases demonstrate the feasibility of CPSI for hydrophilic compounds analysis in biofluids, which are difficult to realize with the traditional paper spray ionization.

Saliva Metabolomic Profiling. Metabolomic profile contains important bioinformation to indicate the general status of physical condition for human body. In disease diagnosis or newborn disease screening, noninvasive biofluids like urine, saliva, or sweat are favorable due to its convenience and volunteer's adaptability during sample collection. In this research, we investigated whether this conductive polymer was also applicable for endogenous metabolites analysis. Saliva samples from four volunteers were collected into 1.5 mL Eppendorf tubes. After protein precipitation by 100 μL of methanol spiked into equal volume saliva, the samples were centrifuged at 10 000 rpm for 10 min. The supernatants (2.0 μL) were loaded 1.0 mm near the tip of CNT/PMMA polymer followed with CPSI-MS data acquisition (m/z 100−500). Principal component analysis (PCA) was used to distinguish the metabolomic pattern difference. Ten mass spectra acquired from each volunteer were taken as sample points.

As results, there was a relatively clear pattern difference observed in the mass spectra acquired by CPSI-MS other than PSI (Figure S11) for four volunteers. Unlike the endogenous metabolite ions obtained in paper spray ionization, some representative metabolites (like choline, creatine, creatinine, carnitine, valine, ornithine, taurine, etc.) were easy to achieve higher relative abundances (Figure 6A) rather than being buried by highly abundant background peaks (Figure 6B). Therefore, it would be much easier either to pick out directly these highly expressed metabolite markers or to classify samples into different groups from the profiling pattern. Further, from the PCA score plots, we can clearly see that in the CPSI group, the sample points from same volunteer could be well clustered whereas those sample points from different volunteers could be well separated (Figure 6C). In contrast, there were more nearly overlapping sample points between each two volunteers (Figure 6D) obtained by PSI. Figure 6 further demonstrates that CPSI provides more endogenous metabolomic features than PSI to make pattern recognition more efficient in biofluid profiling analysis.

CONCLUSIONS

Our studies indicate that conductive polymer spray ionization mass spectrometry (CPSI-MS) for direct biofluid analysis has many advantages over the use of paper spray or nESI. We found that a CNT/PMMA composite made an excellent spray substrate. With its natural conductivity, hydrophobicity, and lack of porosity, charged microdroplets could be easily formed not only with aid of strong electric field as driving force but also with weaker surface tension as a resistant force. It also significantly avoids the losses of target ions by focusing biofluid and concentrating molecule of interest. Due to the clean surface and flexible structure, it can be repeatedly used without cross contamination. Hydrophilic drugs, saccharides, peptides, and proteins in biofluids were selected as the study cases to evaluate the performance of CPSI-MS. Compared with traditional paper spray, the ionization efficiency for these compounds were enhanced for 2−25 times in biological fluid and 20−100-fold in standard solution. These observations proved that CPSI has great potential in bioanalytical application such as therapeutic drug monitoring or metabolomic profiling for disease diagnosis.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b03460.

Details on standard information, paper spray ionization and nESI, and cautious information about organic solvent compatibility (PDF)

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Wang, H.; Liu, J.; Cooks, R. G.; Zheng, O. *Angew. Chem., Int. Ed.* **2010**, *49*, 877−880.
(2) Espy, R. D.; Muliadi, A. R.; Ouyang, Z.; Cooks, R. G. *Int. J. Mass Spectrom.* **2012**, *325*−*327*, 167−171.
(3) Gaissmaier, T.; Siebenhaar, M.; Todorova, V.; Hullen, V.; Hopf, C. *Analyst* **2016**, *141*, 892−901.
(4) Banerjee, S.; Basheer, C.; Zare, R. N. *Angew. Chem., Int. Ed.* **2016**, *55*, 12807−12811.
(5) Guo, Y.; Gu, Z.; Liu, X.; Liu, J.; Ma, M.; Chen, B.; Wang, L. *Phytochem. Anal.* **2017**, *28*, 344−350.
(6) Poole, J. J.; Gomez-Rios, G. A.; Boyaci, E.; Reyes-Garces, N.; Pawliszyn, J. *Environ. Sci. Technol.* **2017**, *51*, 12566−12572.
(7) Tosato, F.; Correia, R. M.; Oliveira, B. G.; Fontes, A. M.; França, H. S.; Coltro, W. K. T.; Filgueiras, P. R.; Romão, W. *Anal. Methods* **2018**, *10*, 1952−1960.
(8) Zhang, Y.; Ju, Y.; Huang, C.; Wysocki, V. H. *Anal. Chem.* **2014**, *86*, 1342−1346.
(9) Zhang, C.; Glaros, T.; Manicke, N. E. *J. Am. Chem. Soc.* **2017**, *139*, 10996−10999.
(10) Dulay, M. T.; Zare, R. N. *Rapid Commun. Mass Spectrom.* **2017**, *31*, 1651−1658.
(11) Narayanan, R.; Sarkar, D.; Cooks, R. G.; Pradeep, T. *Angew. Chem., Int. Ed.* **2014**, *53*, 5936−5940.
(12) Sarkar, D.; Som, A.; Pradeep, T. *Anal. Chem.* **2017**, *89*, 11378−11382.
(13) Wang, Q.; Zheng, Y.; Zhang, X.; Han, X.; Wang, T.; Zhang, Z. *Analyst* **2015**, *140*, 8048−8056.
(14) Liu, J.; He, Y.; Chen, S.; Ma, M.; Yao, S.; Chen, B. *Talanta* **2017**, *166*, 306−314.
(15) Basuri, P.; Sarkar, D.; Paramasivam, G.; Pradeep, T. *Anal. Chem.* **2018**, *90*, 4663−4668.
(16) Wang, X.; Zheng, Y.; Wang, T.; Xiong, X.; Fang, X.; Zhang, Z. *Anal. Methods* **2016**, *8*, 8004−8014.
(17) Damon, D. E.; Maher, Y. S.; Yin, M.; Jjunju, F. P.; Young, I. S.; Taylor, S.; Maher, S.; Badu-Tawiah, A. K. *Analyst* **2016**, *141*, 3866−3873.
(18) Wang, T.; Zheng, Y.; Wang, X.; Austin, D. E.; Zhang, Z. *Anal. Chem.* **2017**, *89*, 7988−7995.
(19) Li, T.; Fan, L.; Wang, Y.; Huang, X.; Xu, J.; Lu, J.; Zhang, M.; Xu, W. *Anal. Chem.* **2017**, *89*, 1453−1458.
(20) Gomez-Rios, G. A.; Pawliszyn, J. *Angew. Chem., Int. Ed.* **2014**, *53*, 14503−14507.
(21) Zargar, T.; Khayamian, T.; Jafari, M. T. *Microchim. Acta* **2018**, *185*, 103−111.
(22) Yu, K.; Zhang, H.; He, J.; Zare, R. N.; Wang, Y.; Li, L.; Li, N.; Zhang, D.; Jiang, J. *Anal. Chem.* **2018**, *90*, 7154−7157.
(23) Narayanan, R.; Pradeep, T. *Anal. Chem.* **2017**, *89*, 10696−10701.

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