Electronic Supplementary Information

Ambient Electrospray Deposition Raman Spectroscopy (AESD RS) using Soft Landed Preformed Silver Nanoparticles for Rapid and Sensitive Analysis

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Fig. S1 (A) Optical photograph of the AESD RS set-up and (B) zoomed-in region of A, highlighted in a red-colored ellipse.



Fig. S2 A schematic representation of selecting four locations from the drop casted analyte for AESD.



Fig. S3 (A) UV-Vis absorption spectrum of the as-prepared AgNPs, and (B) DFM image of the preformed AgNPs immobilized on an ultra-clean glass slide.



Fig. S4 DFM images and their corresponding histograms of the particle size distribution of AgNPs, (A) before and (B) after electrospray.



Fig. S5 A comparative plot between conventional SERS and AESD SERS signals. (a) Blank ITO, (b) control SERS of only AgNPs electrospray without p-MBA, (c) SERS of p-MBA when AgNPs were drop casted, and (d) SERS of p-MBA when AgNPs were electrosprayed. Spectra were background corrected and vertically shifted for clarity, but no other manipulation was performed.

Peak position (cm ⁻¹)	Assignments ^[1-2]
717	γ(CCC) (aromatic)
842	$\delta(COO^{-})$
absent	δ (CSH)
1013	Ring deformation
1136	13β (CCC) + ν (C-S) + ν (C- COOH)
1186	δ (C-H)
1078-1086	v ₁₂ (ring)
1375-1380	v _s (COO⁻)
1585-1588	v _{8a} (ring)

 Table S1 Band assignments of SERS features of p-MBA

Table S2 Calculation of mean and standard deviation for calibration curve of p-MBA

Concentrati on (M)	Mean of intensity counts	Standard deviation of intensity counts
10 ⁻⁹	1628.277	26.106
10 ⁻⁸	2839.332	53.173
10 ⁻⁷	3504.938	294.986
10 ⁻⁶	5344.406	467.998
10 ⁻⁵	6769.224	441.697
10-4	8661.805	613.920



Fig. S6 (A) Stacked SERS plot of 2,4-DNT at different concentrations ranging from 3 to 0.5 μ M and (B) calibration curve between SERS intensity (1589 cm⁻¹ counts) and concentrations of 2,4-DNT.

Table S3 Calculation of mean and standard deviation for calibration curve of 2,4-	DNT
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Concentrati on (µM)	Mean of intensity counts	Standard deviation of intensity counts
0.5	1562.224	133.654
1	1818.565	78.055
1.5	2516.361	260.221
2	2632.293	227.489
2.5	2872.928	310.216
3	3006.579	132.228



Fig. S7 SERS spectra of 10 μ M p-MBA with electrosprayed AgNPs at different tip to collector distances.

Peak position (cm ⁻¹)	Assignments ^[3-4]
560-640	δ (COO) + carbohydrates
760-815	Tryptophan
950-1000	Membrane phospholipids
1080-1130	Proteins + lipids + carbohydrates
1370-1420	ν _{sym} (COO-) and δ(C-H) proteins
1530-1630	amide , ν(CN), γ(NH)

Table S4. Band assignments of SERS features of E. coli.



Fig. S8 Replica culture of ITO glass slides drop casted with *E.coli* on A) Nutrient agar before (left) and after electrospray (right), and B) MacConkey agar before (left) and after electrospray (right).



ig. S9 Fluorescence microscopic imaging of bacteria, (Ai) *E. coli* before electrospray, (Aii) zoomed-in view of (Ai), (Bi) *E. coli* after electrospray deposition, (Bii) zoomed-in view of (Bi), (Ci) fluorescence image of region of *E. coli* interacted with AgNPs, (Cii) zoomed-in view of (Ci)

shown in dotted square, (Di) optical DFM image corresponding to (Ci) to show AgNPs, and (Dii) zoomed-in view of (Di) shown in dotted square. Green and red dotted circles in (Cii) and (Dii) represent live and dead bacteria after their interaction with AgNPs.

Enhancement factor calculations:

Enhancement factor (EF) for SERS system can be described by the equation^{5,6} given below:

$$EF = (I_{SERS}/N_{surface})/(I_{NRS}/N_{bulk})$$
(1)

 I_{SERS} and I_{NRS} are the observed intensities arising from the interaction of drop casted analyte (here p-MBA) with the electrosprayed AgNPs and the normal Raman scattering intensity of analyte molecule in the absence of electrosprayed AgNPs (normal Raman signal). Parameters $N_{surface}$ and N_{bulk} are the number of analyte molecules excited under the laser spot interacted with sprayed AgNPs and the number of analyte molecules under the laser spot for the bulk specimen.

From experimental data, I_{SERS} = 8068 counts (obtained after the average of 24 spectra of 1 μ M concentration for 1586 cm⁻¹) and I_{RS} = 298 counts (52 mM).

$$\mathbf{N}_{\text{surface}} = 4\pi r^2.\text{C.A.N}$$
(2)

where r, C, A, N are average particle radius of the Ag nanoparticles in the spot, surface density of the analyte drop casted, area of the laser spot and the average number of particles per square micrometer area, respectively. The average particle radius *r* was taken (from ImageJ analysis of TEM images) as 20 nm, the density of analyte molecules (C) drop casted (1 μ M concentration for 40 uL volume) was calculated as 10⁶/ μ m², the area of the laser spot (10× objective, Numerical Aperture = 0.25) diameter was 7.6 μ m (A = ~ 45 μ m²), and the number of particles per square micrometer (N) from DFM measurement was 12.

N_{bulk} was calculated using the formula:

$$\mathbf{N}_{\text{bulk}} = \mathbf{N}_{\text{A}} \cdot \mathbf{A} \cdot \mathbf{h} \cdot \mathbf{\rho} / \mathbf{M}$$
(3)

where A is the area of the laser spot, h is the penetration depth of the laser, ρ is the density of the solid analyte (1.3 g/cm³ in case of p-MBA) and M is the molecular weight of the analyte (in

this work, 154.19 g/mol for p-MBA). The laser spot diameter was 7.6 μ m; penetration depth of laser h was calculated as ~1244.3 μ m [h = (2x3.14x ω^2)/ λ xn, where ω is laser spot size, λ is laser wavelength, i.e. 532 nm and n is the refractive index of Nd-YAG laser, i.e., 1.825]. Using these parameters and the previously quoted equations (1-3), the EF for the SERS system (p-MBA and electrosprayed AgNPs) was calculated to be 2 X 10⁸.

Comparison of enhancement factor values between electrospray versus drop casted SERS:

10 μ M p-MBA sample was treated with 0.3 uL of electrosprayed and drop casted AgNPs, respectivley.

In electrospray, the SERS intensity of 1586 cm⁻¹ peak was 11191 counts.

In drop cast method, the SERS intensity for the corresponding peak was 1084 counts.

Using EF equation described above, values for electrospray and drop casted SERS were 2.78x10⁸ and 5.37x10⁷, respectively.

Quantification of EF = EF of electrospray/EF of dropcast = $2.78 \times 10^8 / 5.37 \times 10^7 = 5.2$

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