Fluorescence and UV-vis spectrophotometry: a dual-mode competitive approach for selective sensing of dopamine

Somnath Bali[a], Arnab Halder[a*] and Avijit Mondal [a*]

[a]Department of Chemistry, Presidency University, Kolkata-700073, India.

EXPERIMENTAL SECTION:

Materials. Dopamine hydrochloride, epinephrine hydrochloride, DL-norepinephrine hydrochloride were purchased from Sigma-Aldrich (U.S). Silver nitrate, resorcinol, uric acid (Ura), ascorbic acid (Asb), aspartic acid (Asp), alanine (Ala), glycine (Gly), lysine (Lys), urea (Ura), glucose (Glc), sucrose (Suc), glutamic acid (Glu), sodium carbonate, and sodium hydroxide were obtained from Merck emplura. All chemicals were of analytical grade and used without any further purification. All solutions were prepared using deionized water.

Instrumentation. UV–vis absorption spectra of all samples were measured in Shimadzu UV-1900i UV visible spectrometer using quartz cuvettes with a 1 cm path length. The fluorescence measurements were performed on a HITACHI F-2500 fluorescence spectrophotometer equipped with a plotter unit and a 1 cm quartz cell. For transmission electron microscopy (TEM), samples were dried by putting a drop of particle dispersion on a carbon coated copper grid and imaged under FEI Tecnai G2 F20 microscope. The pH of the solution was measured using SESHIN ECM-712 pH meter. The high-resolution mass spectrometry was done with Q-TOF YA263 instruments (Water Corporation) using positive mode electrospray ionization.

DA Sensing and in-situ synthesis of AgNPs

The sensing technique is an in-situ process which is based on the combination methods, DA-resorcinol reaction and formation of resorcinol stabilized AgNPs in alkaline condition.[1-4] The aqueous $AgNO_3$ solution (90 µL; 10 mM) was added to 1.5 mL resorcinol solution (500µM) and followed by addition of various amounts of DA (1 or 10 mM). Then aqueous solution Na_2CO_3 (30 µL; 50mM) was mixed to make the solution slightly alkaline in nature and perform the reaction. The resulting solution displayed a bright yellow colour due to formation of resorcinol stabilized AgNPs and turns grey colour as the level of DA increases. The fluorescent intensity due to reaction of DA and resorcinol increases with the amount of DA in the reaction mixture. The final concentrations of resorcinol, $AgNO_3$, Na_2CO_3 and DA were 200 µM, 250 µM, 500 µM and 0 – 30 µM respectively. Also, the pH of the resulting solution was maintained below 8. Control experiments were done separately for the formation of AgNPs and blue fluorescent solution. All the UV-vis and fluorescence measurement were done after 30 min of reaction time.

DA detection in real samples:

The standard addition method was utilized to analyze DA in urine samples from healthy adult humans in order to assess the detection of DA in clinical applications. After adding a certain quantity of DA to the urine samples, spiked samples were examined using UV visible spectrometer as well as fluorescence spectrophotometry. To summarize, a 1 mL sample of spiked urine was combined with a 1mL resorcinol (600 µM), and a 1mL solution of Na_2CO_3 (2 mM) for a 10-minute reaction. For UV visible study 1 ml spiked urine sample was combined with 2 ml resorcinol stabilised AgNPs where resorcinol, Na_2CO_3 concentration were 200 µM and 500 µM respectively. All experiments were performed in compliance with the relevant guidelines and the study has been approved from the institutional ethical committee for research on human with approval no. PU-HEC-A/AMo/PU-Chemistry/2025/001/4 dated 6th March 2025. The consent from the participants were taken earlier for this study and they were all informed that this is strictly for scientific research and none of their personal identity will be revealed elsewhere.

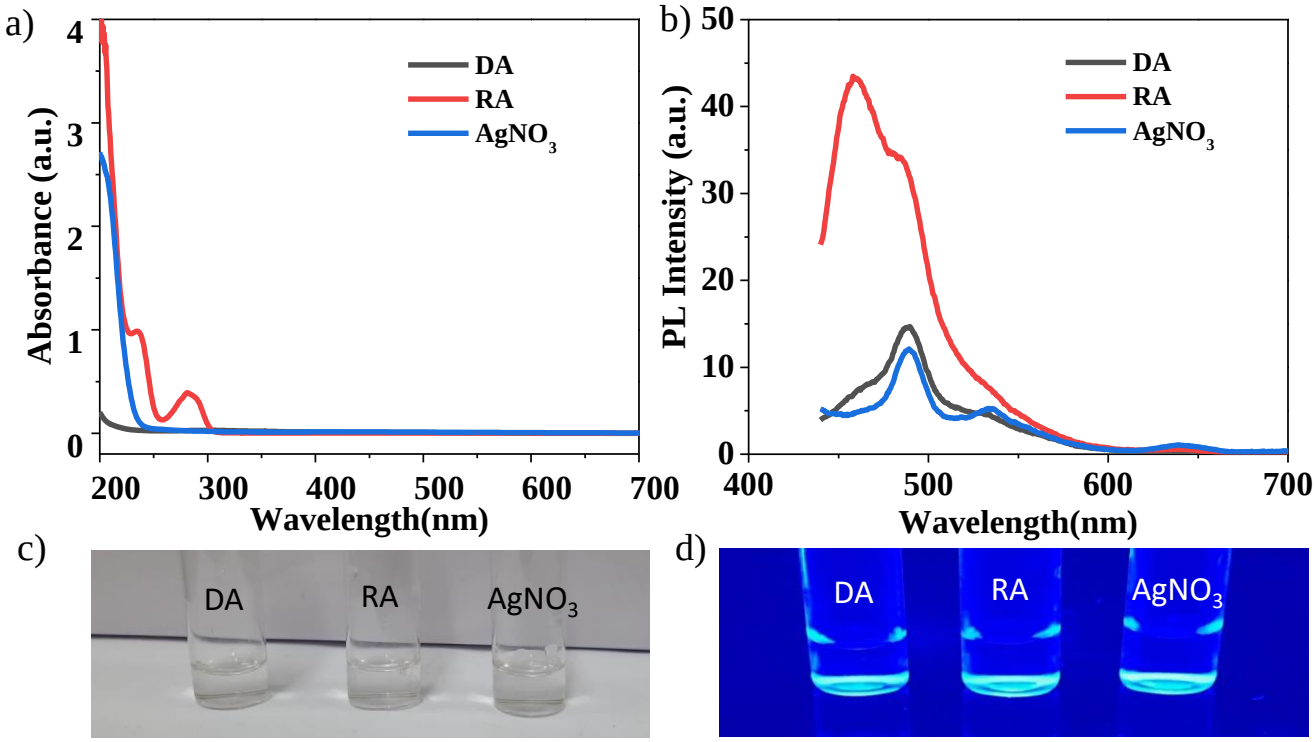

Figure S1. Absorbance spectra (a)and Fluorescence spectra (b) of DA, RA and $AgNO_3$ in Na_2CO_3 medium for control measurements. Only the RA has absorbance in the range of 200 -300 nm (n-π, π-π* transitions) and also has very less fluorescence intensity. The corresponding digital images of the solutions under ambient light source (c) and under UV-light treatment (d).

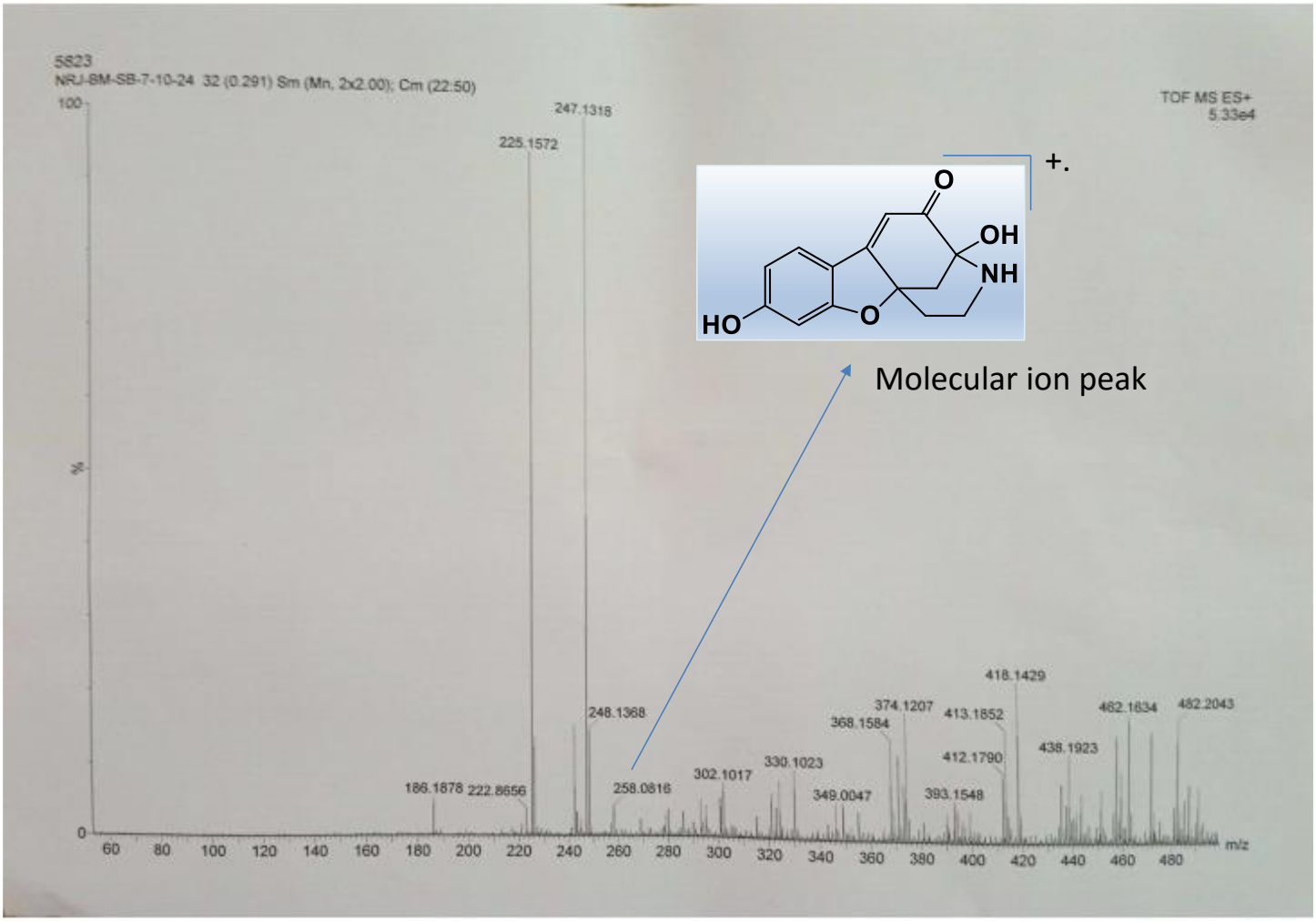

Figure S2. The mass spectra of products obtained due to the reaction between resorcinol and dopamine in Na_2CO_3 medium.

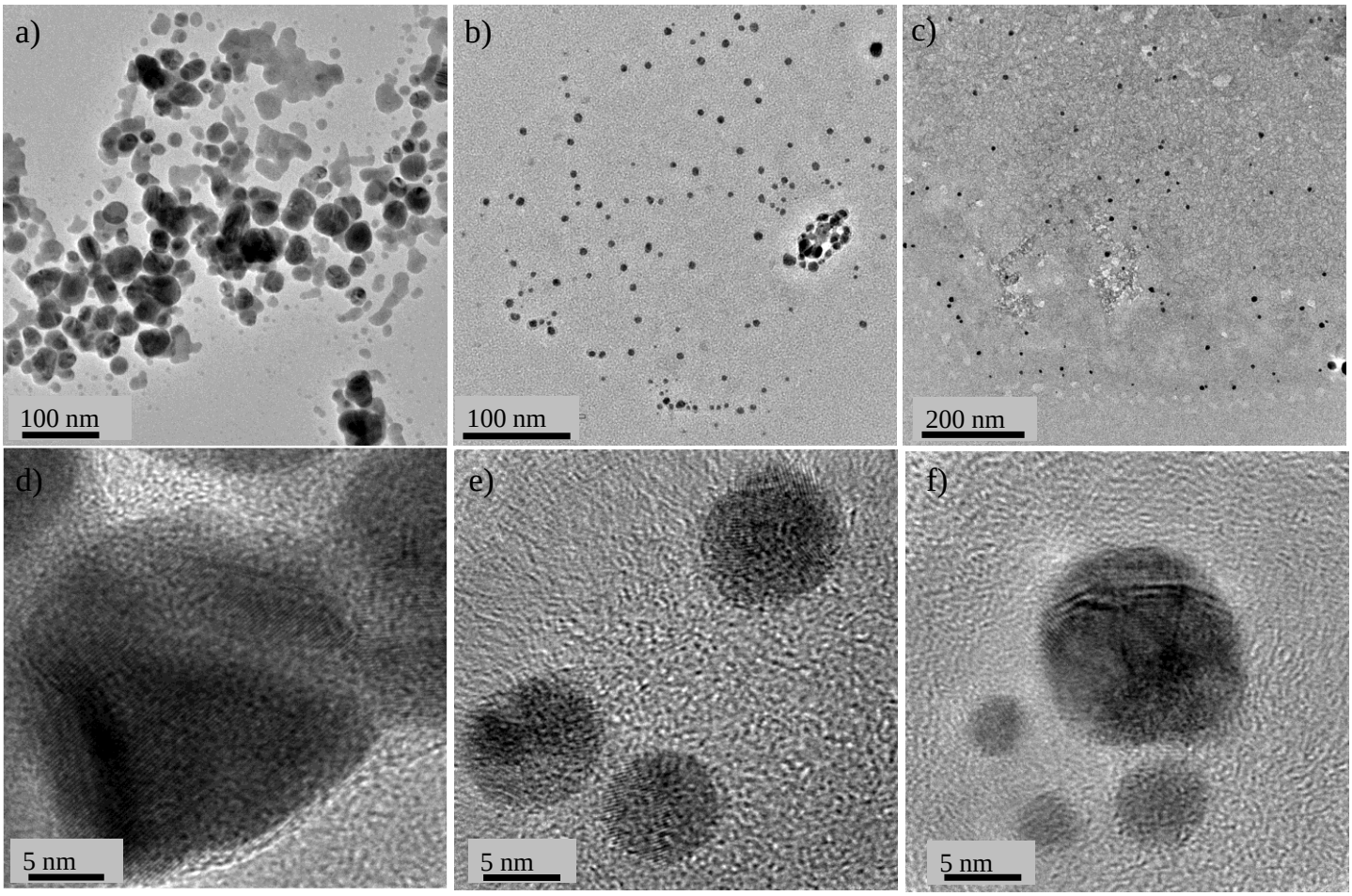

Figure S3. TEM images of RA stabilized AgNPs at three different concentrations of DA [*0.25μM (a,b); 5 μM (c,d); 20 μM (e,f)*]

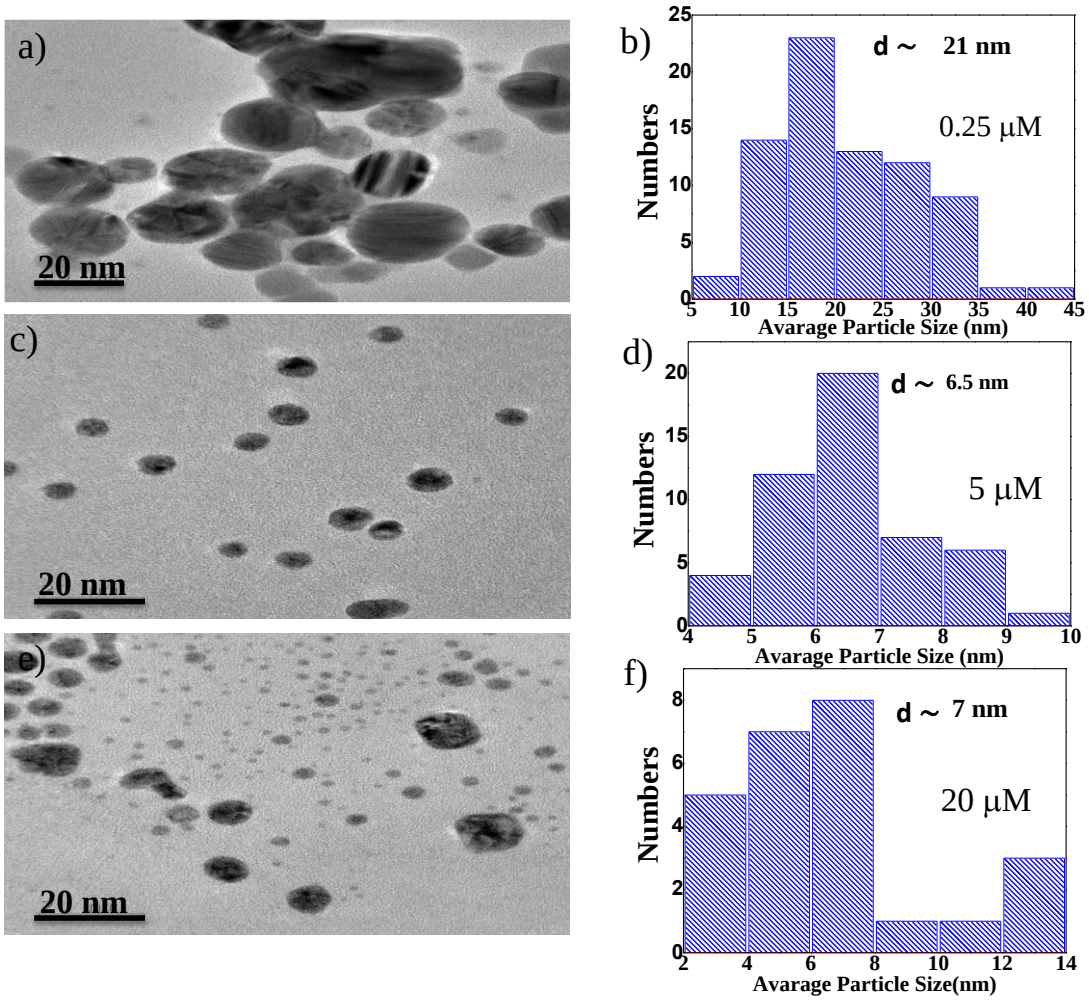

Figure S4. *The TEM images and TEM histogram study of AgNPs (considering all other TEM images at high magnification) in presence of DA [0.25μM (a,b); 5 μM (c,d); 20 μM (e,f)*]

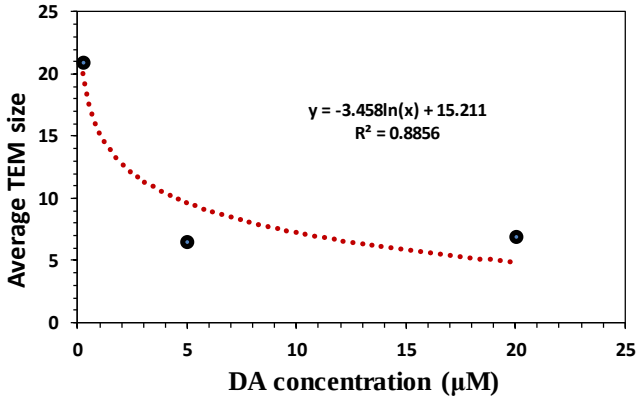

Figure S5. A plot of average TEM size vs DA concentration and equation is also given.

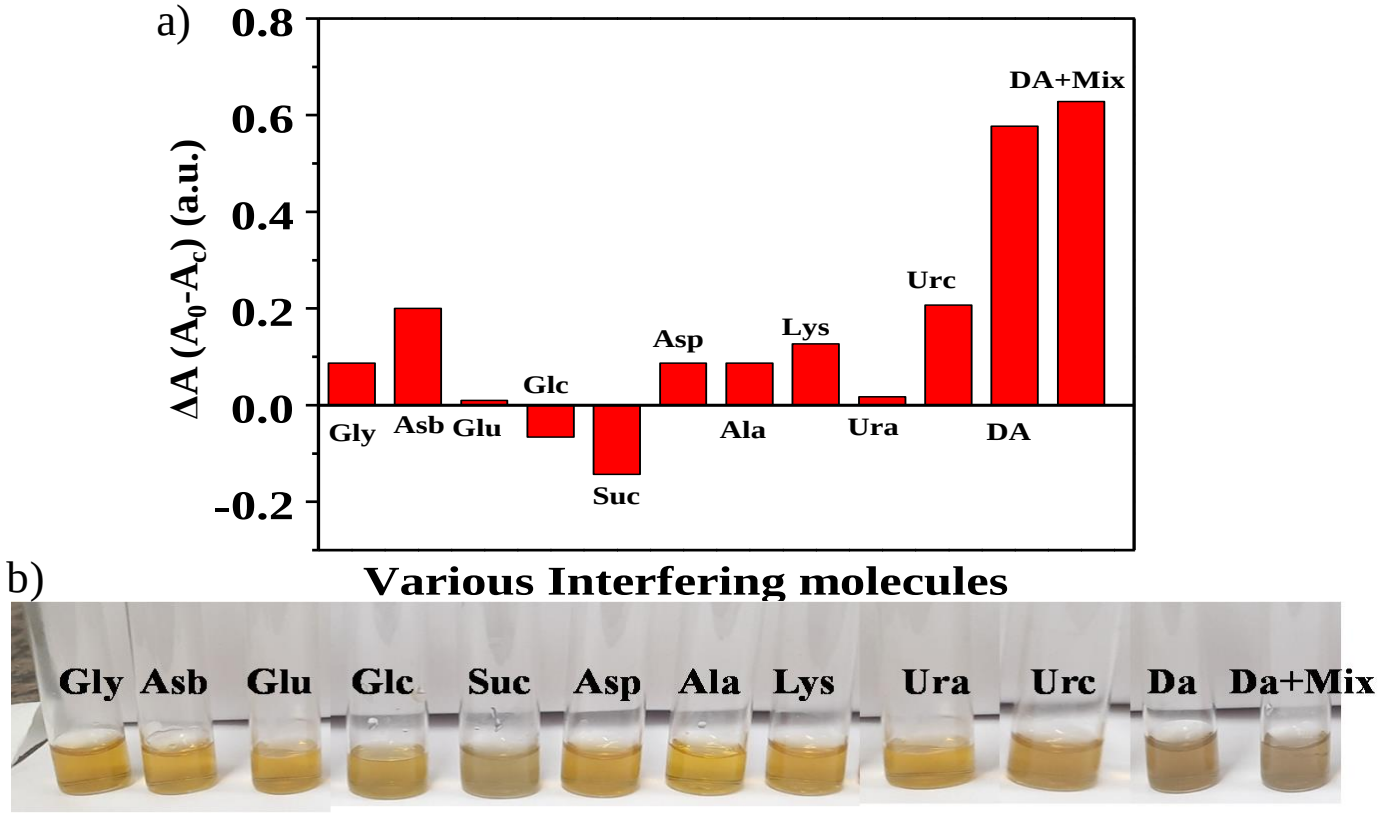

Figure S6. Interference study of dopamine using UV-vis spectroscopy (a) and corresponding digital image of selectivity assay (b). DA concentration was 5 μM and concentration of other substances were 100 μM.

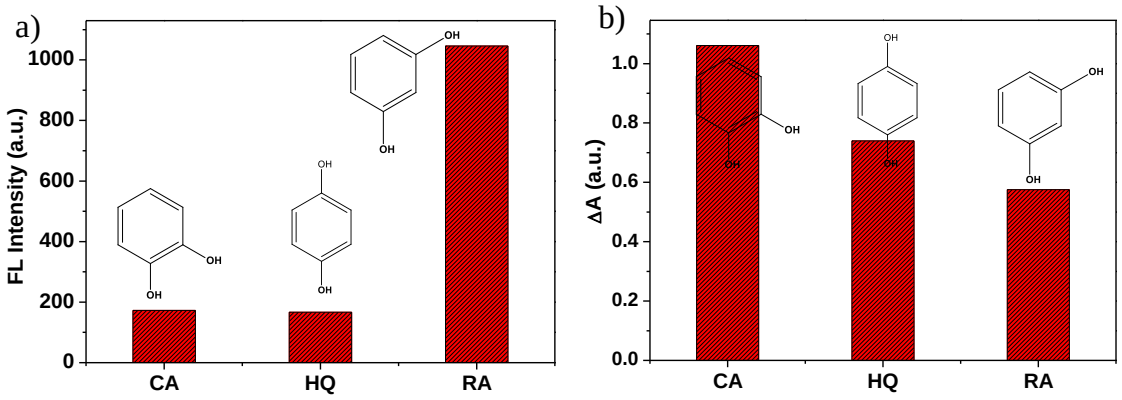

Figure S7. Study of DA detection catechol (CA), Hydroquinone (HQ) and resorcinol (RA) using two methods, fluorescence (a) and UV-vis techniques (b). DA concentration was 5 μM. This study signifies DA react with three isomers of dihydroxybenzene but measurable florescence intensity comes from resorcinol.

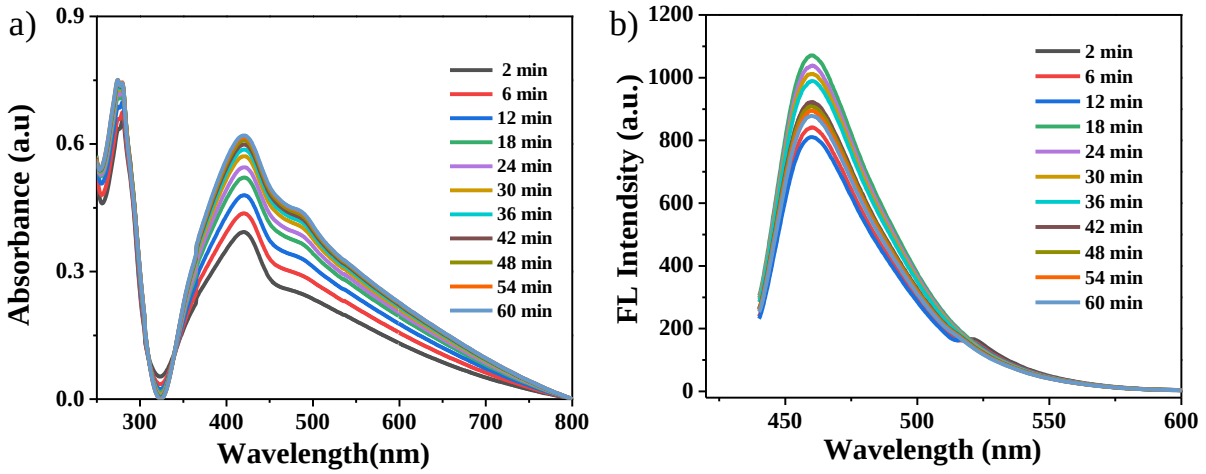

Figure S8. The stability test of reaction products with time using UV-vis (a) and fluorescence (b) spectrophotometry. The DA concentration was 5 μM. Smaller increment in intensity were observed after 30 min.

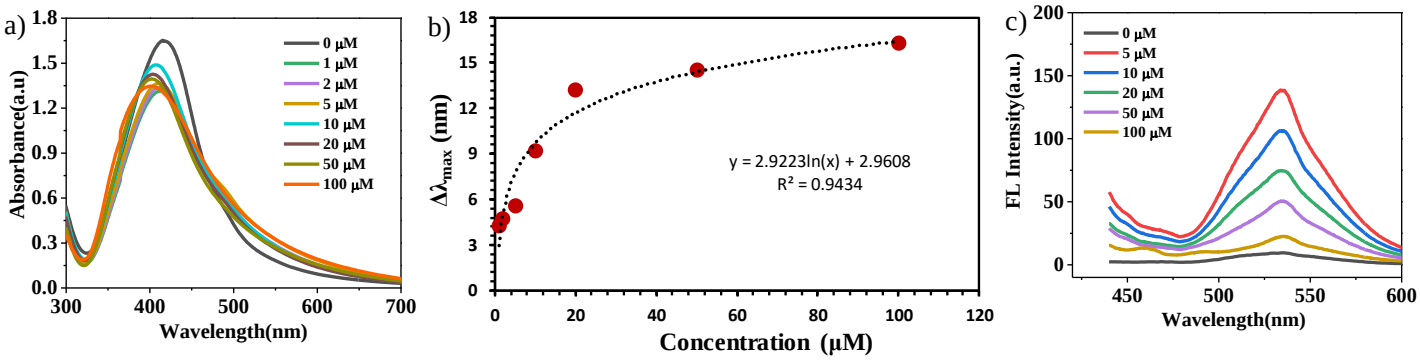

Figure S9. The details study of interference due to ascorbic acid (Asb) at various concentrations by UV-vis (a,b) and fluorescence techniques. There is minimum interference observed from Ascorbic by the both methods.

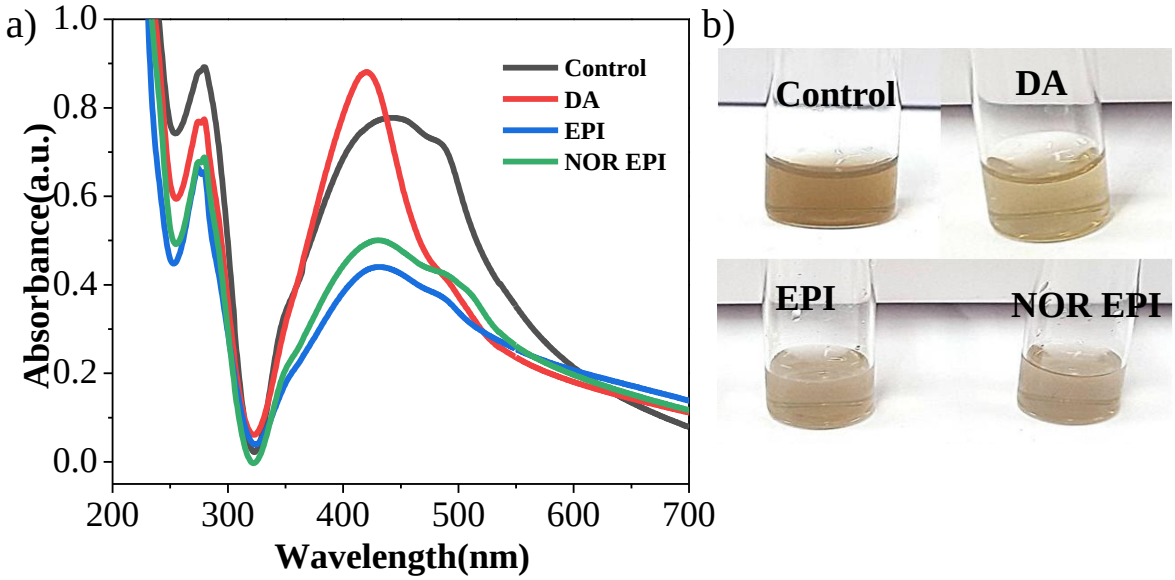

Figure S10. The effect of epinephrine (EPI) or nor-epinephrine (NOR EPI) against DA on the formation of AgNPs in the reaction mixture in bicarbonate medium using UV-Vis study (a) and digital images (b) at 5 µM concentration of each.

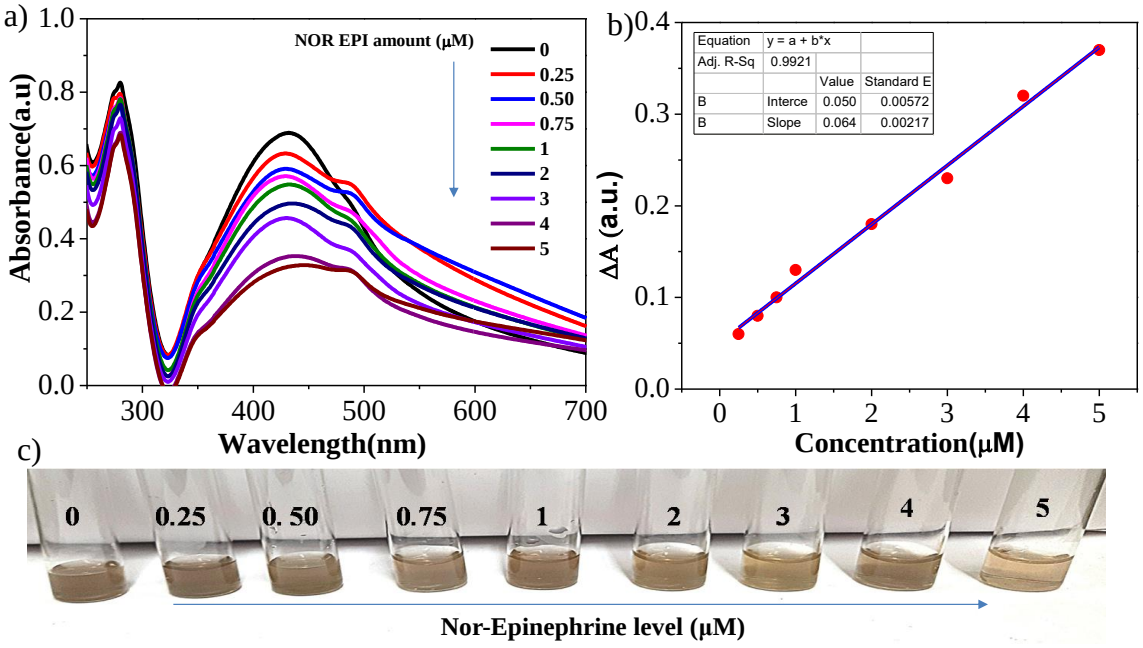

Figure S11. The similar preventive effect of NOR EPI on the formation of resorcinol stabilized AgNPs in bicarbonate medium using UV-vis spectrophotometer (a) and the corresponding linear plot of ΔA vs concentration was obtained along with digital images of the respective solutions. The LOD values calculated from the linear fitted curves were 0.25 µM.

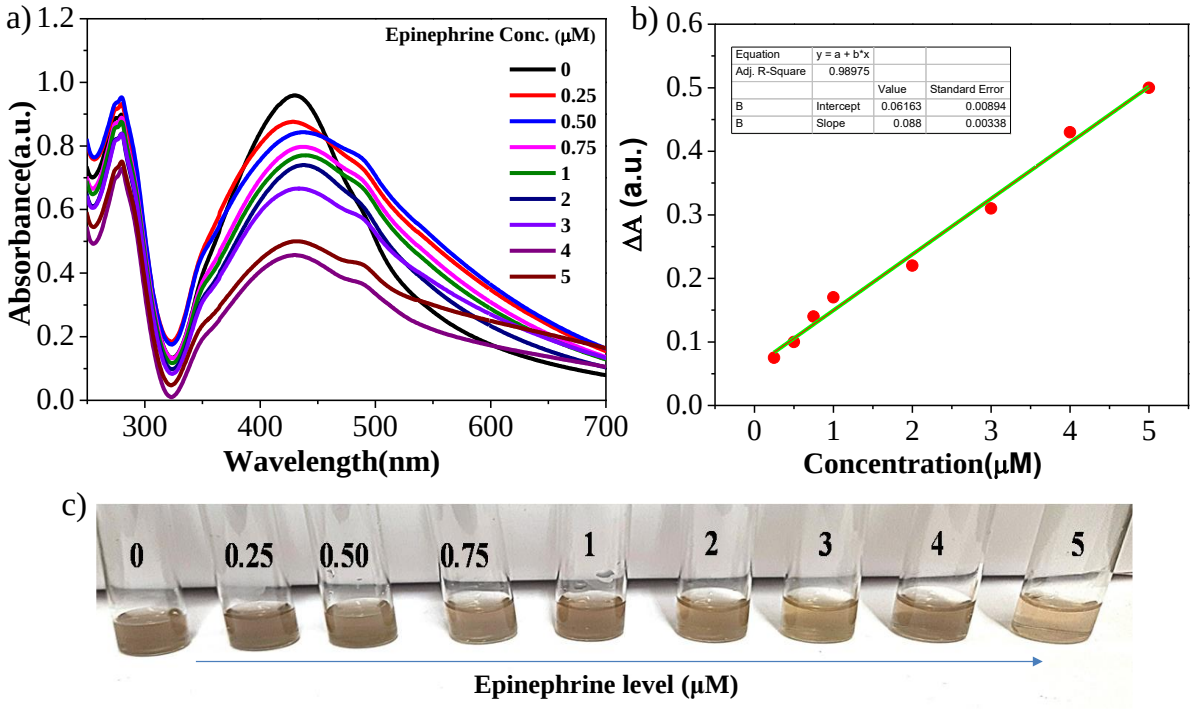

Figure S12. The similar preventive effect of EPI on the formation of resorcinol stabilized AgNPs in bicarbonate medium using UV-vis spectrophotometer (a) and the corresponding linear plot of ΔA vs concentration was obtained along with digital images of the respective solutions. The LOD values calculated from the linear fitted curves were 0.3 μM.

Table S1. The comparative study for DA detection with recent literature reports

Material	**Detection Technique**	**Dynamic Range**	**Limit of Detection / Quantification**	**Advantages/ disadvantages**	**Reference**
Hexagonal silver nanoparticles	colorimetric	0.1–7.5 μM	0.031 μM	Restricted to proper shape	5
Polydopamine (PDA) nanoparticles	Fluorescence Analysis	1.0-200 μM	0.3 μM	Intensity low at low level	6
Ionic liquid functionalized drug mediated AgNPs	colorimetric	0.01–3.6 μM	0.118 μM	Complex synthesis	7
Enhanced-oxidase mimicking activity of cerium(IV) using TMB	colorimetric	6.5–520 nM	2.88 nM	Selectivity issue	8
CoO Nanotubes Loaded on Graphene	colorimetric	0.2−10 μM	0.1391 μM	Complex synthesis	9

and Modified with Porphyrin Moieties					
Multifunctional lanthanide metal–organic framework (Ln-MOF)	Fluorescence Analysis	0.04–30 μM	0.01 μM	Complex synthesis, low detection level	10
Co_3O_4 Nanoplates	colorimetric	1.6 - 20 μM	0.82 μM	Restricted to proper shape	11
Boron and Sulfur co-doped graphene quantum dots (BS-GQDs)	Fluorescence Analysis	0–340 μM	3.6 μM	Complex synthesis and strategy	12
A rose-like CuS@PB/Pt composite	colorimetric	1- 60 μM	0.28 μM	Restricted to proper shape	13
Resorcinol-silver nanoparticle system	Colorimetric & Fluorescence Analysis	0.25-30 μM	0.25 μM	Simple synthesis chemistry and strategy; simultaneous detection by both methods	This work

Table S2. Dopamine detection in human urine sample using colorimetric and fluorescence analysis

	Colorimetric analysis				Fluorescence analysis				
Sample	Original Dopamine Content	Spiked dopamine[μM]	Measured[μM]	RSD(%)	Recovery (%)	Spiked dopamine[μM]	Measured[μM]	RSD(%)	Recovery (%)
U-1	ND	5	4.91	1.28	98.2	5	5.17	2.36	103.4
		10	10.17	1.19	101.7	10	9.95	0.35	99.5
U-2	ND	5	5.06	0.84	101.2	5	4.97	0.42	99.4
		10	9.94	0.42	99.4	10	10.13	0.91	101.3
U-3	ND	5	4.95	0.71	99	5	5.06	0.84	101.2
		10	10.25	1.74	102.5	10	9.88	0.85	98.8

*ND~ Not detected

Table S3. Statistical Comparison of Colorimetric and Fluorescence Methods for Dopamine Detection.

Spiked Concentration (µM)	Method A (Colorimetry) [µM]	Method B (Fluorescence) [µM]	t-Test p-value	f-Test p-value	Variance Equality (f-Test)	Mean Difference Significance (t-Test)
5	4.91, 5.06, 4.95	5.17, 4.97, 5.06	0.201	0.821	Variances are equal	No significant difference
10	10.17, 9.94, 10.25	9.95, 10.13, 9.88	0.305	0.394	Variances are equal	No significant difference

A comparative analysis was conducted to evaluate the accuracy and precision of colorimetric and fluorescence-based methods for quantifying dopamine in human urine samples. Samples collected from three healthy volunteers were spiked with known concentrations of dopamine (5 µM and 10 µM) and analyzed using both detection methods.

Statistical analysis included f-tests to assess equality of variances and two-tailed t-tests (assuming equal variances) to compare the means of detected concentrations across the two methods. At the 5 µM level, the f-test indicated no significant difference in variances ($p = 0.821$), and the t-test showed no statistically significant difference in mean dopamine concentrations between methods ($p = 0.201$). Similarly, for the 10 µM spiked samples, variances remained statistically equal (f-test $p = 0.394$), with no significant difference in mean values observed (t-test $p = 0.305$).

These findings demonstrate that both colorimetric and fluorescence assays produce statistically comparable results for dopamine quantification in human urine at low micromolar concentrations. The absence of significant differences in both precision and accuracy supports the interchangeable use of these methods for routine analysis of dopamine in biological matrices. The colorimetric method, in particular, may offer a cost-effective and accessible alternative to fluorescence-based techniques without compromising analytical reliability.

References:

1) X. Zhang, Y. Zhu, L. Xie, X. Guo, B. Zhang, X. Jia and B. Dai, *Anal. Chim. Acta*, 2016, **944**, 51–56.
2) A.Kumar, Swati Aerry and D. V. Goia, *J. Colloid Interface Sci.,* 2016, **470**, 196.
3) T. Long, J. Cheng, C. Peng, W. Xu, H. Luo, M. Ouyang, D. Xu, Q. Lin, J. Qu and X. Huang, *Anal. Biochem.*, 2022, **642**, 114562.
4) S. Panigrahi, S. Praharaj, S. Basu, S. Ghosh, S. Jana, S. Pande, T. Vo-Dinh, H. Jiang and T. Pal, *J. Phys. Chem. B.* 2006, **110**, 13436.
5) S.Rostami, A.Mehdinia, A.Jabbari, E. Kowsari; R. Niroumand and T. J. Booth, *Sens. Actuators B: Chem.* 2018, **271**, 64.
6) X. Wei, Z. Zhang and Z. Wang, *Microchem. J.,* 2019, **145**, 55.
7) U.Nishan, R. Gul, N. Muhammad, M. Asad, A. Rahim, M. Shah, J. Iqbal, J. Uddin, A.-H. A. Shah, and S. Shujah, *Microchem. J.,* 2020, **159**, 105382.
8) L. Liang, Z. Zhao, F. Ye, and S. Zhao, *New J. Chem.,* 2021, **45**, 6780.
9) X. Zhao, S. Zhao, S. Li, X. Yao, X. Zhu, W. Chen, G. Fan, Z. Liu, Q. Liu, and K. Yue, *ACS Appl. Nano Mater.* 2021, **4,** 8706.
10) L. Yu, L. Feng, L. Xiong, S. Li, Q. Xu, X. Pan, and Y. Xiao, *Nanoscale,* 2021, **13,** 11188.
11) Z. Tang, L. Zhang, S. Tang, J. Li, J. Xu, N. Li, L. Xu and J. Du, *Nanomat.,* 2022, **12**, 2990.
12) D. Yang, J. Ran, H. Yi, P. Feng and B. Liu, *Sensors,* 2023, **23,** 9029.
13) M. Chatterjee, P. Nath, S. Kadian, A. Kumar, V. Kumar, P. Roy, G. Manik and S. Satapathi, *Sci. Rep.,* 2022, **12**, 9061.