

Supporting Information

Electrochemical Sensing Platform for the Simultaneous Femtomolar Detection of Amlodipine and Atorvastatin Drugs

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1. Experimental setup for electrochemical characterization and analysis

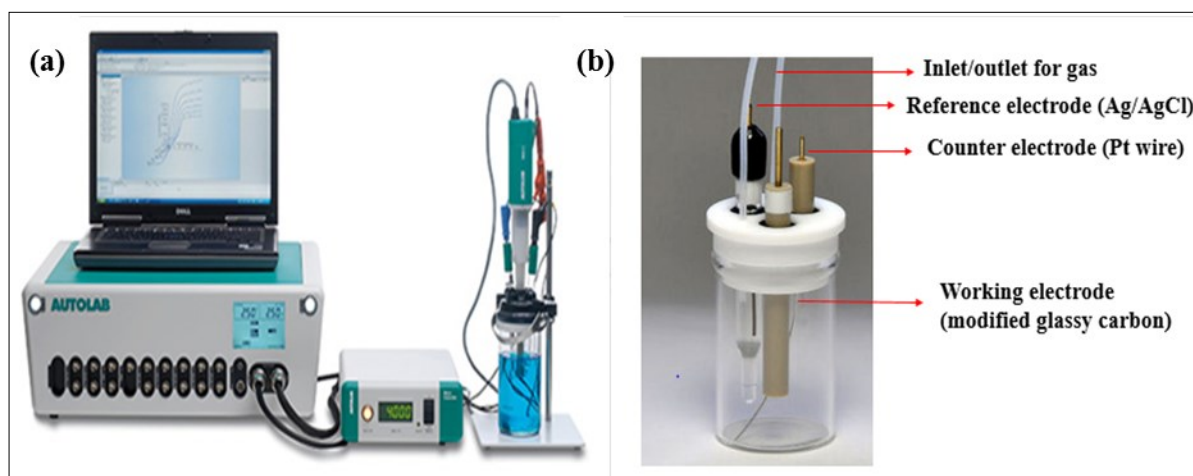


Figure S1. Design of experiments (a) Metrohm Autolab for electrochemical characterization and analysis of the designed sensor (b) An electrochemical cell setup.

2. UV-Vis Spectroscopy

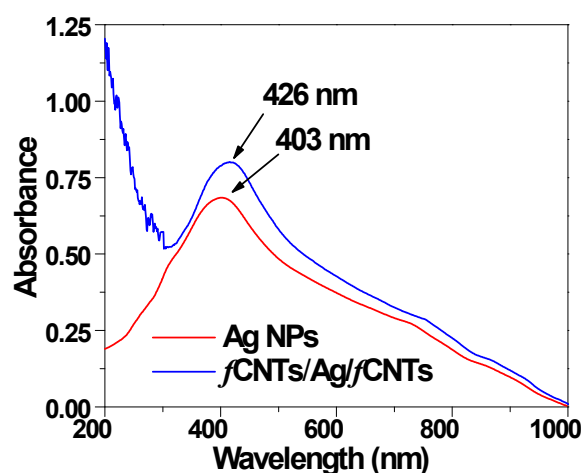


Figure S2 UV-Vis Spectra of Ag NPs and *f*CNTs/Ag/*f*CNTs.

3. Voltammetric parameters optimization

For the superlative performance of the designed at *f*CNTs/Ag/*f*CNTs/GCE sensor, the SWASVs method experimental conditions were optimized.

3.1. Effect of type and quantity of nanomaterials

As the sensor properties significantly depend on the type and quantity of modifier used, different types of metal and bi-metallic alloyed nanoparticles (NPs) such as Ag NPs, Au

NPs, ZnO NPs, Au-Ag (1:1) NPs, Au-Ag (2:1) NPs, Au-Ag (1:2) NPs, Au-Cu (1:3) NPs, Au-Cu (1:2) NPs, Au-Cu (1:1) NPs, Au-Cu (2:1) NPs, Au-Cu (3:1) NPs, and Ag-Cu (1:1) NPs, graphitic nitride (g-C₃N₄), metal doped g-C₃N₄ that are Au-g-C₃N₄ NPs, Ag-g-C₃N₄ NPs, Sn-g-C₃N₄ NPs, Fe-g-C₃N₄ NPs, Co-g-C₃N₄ NPs, Zn-g-C₃N₄ NPs, Mo-g-C₃N₄ NPs, and Ni-g-C₃N₄ NPs, pristine CNTs, functionalized CNTs like, COOH-*f*CNTs, and NH₂-*f*CNTs, and their various combinations for instance, CNTs/Ag-g-C₃N₄, Ag NPs/COOH-*f*CNTs, and NH₂-*f*CNTs/Ag NPs/COOH-*f*CNTs were fabricated on the GC electrode surface for reckoning the best sensor (**Table S1**).

Table S1: Modifiers type effect on the SWASVs response of AM (6 μM) and AT (9 μM) mixture in pH 6 PBS as stripping solvent, at sweep rate 100 mV/s, deposition potential 0 V and accumulation time of 5 s.

Modifier	<i>I</i>_p (μA) of AM	<i>I</i>_p (μA) of AT
Ag NPs/GCE	4.3	4
Au NPs/GCE	4.1	4.7
ZnO NPs/GCE	2.2	3
Au-Ag (1:1) NPs/GCE	3.2	4
Au-Ag (1:2) NPs/GCE	4.2	3
Au-Ag (2:1) NPs/GCE	4.4	4.1
Au-Cu (1:3) NPs/GCE	3	3.7
Au-Cu (1:2) NPs/GCE	2.2	4
Au-Cu (1:1) NPs/GCE	2.3	4.6
Au-Cu (2:1) NPs/GCE	2	4.1
Au-Cu (3:1) NPs/GCE	2.1	4.2
Ag-Cu (1:1) NPs/GCE	1.8	1.3
g-C ₃ N ₄ /GCE	4.1	4.6
Au-g-C ₃ N ₄ NPs/GCE	4	4.7
Ag-g-C ₃ N ₄ NPs/GCE	4.3	4
Sn-g-C ₃ N ₄ NPs/GCE	3	4.4
Fe-g-C ₃ N ₄ NPs/GCE	4.2	4.1
Co-g-C ₃ N ₄ NPs/GCE	3.2	2.9
Mo-g-C ₃ N ₄ NPs/GCE	3.7	3.9
Ni-g-C ₃ N ₄ NPs/GCE	2.8	3.6
Zn-g-C ₃ N ₄ NPs/GCE	3.1	3
CNTs/GCE	5.2	4.6
COOH- <i>f</i> CNTs/GCE	6.1	5.8
NH ₂ - <i>f</i> CNTs/GCE	6.5	6.1
CNTs/Ag-g-C ₃ N ₄ /GCE	5.1	5.4
Ag NPs/COOH- <i>f</i> CNTs/GCE	6.4	6.7
NH ₂ - <i>f</i> CNTs/Ag NPs/COOH- <i>f</i> CNTs/GCE	7.45	7.49

The NH₂-fCNTs/Ag NPs/COOH-fCNTs modified electrodes with the difference sequences of layer by layer (LBL) and dispersion fabrication techniques showed the highest current response for both analytes via LBL technique, thus, the NH₂-fCNTs/Ag NPs/COOH-fCNTs nanocomposite was selected as modifier among all the checked nanomaterials (**Figure S3**).

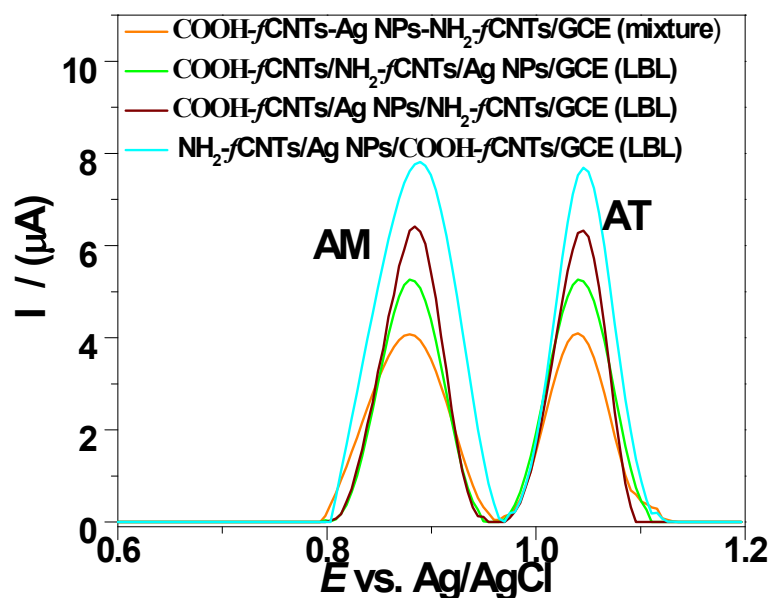


Figure S3. Performance tests of sensors fabricated by mixture and different sequences LBL methods for sensors fabrication

Furthermore, from EIS, CV, and SWASV results, it was confirmed that NH₂-fCNTs/Ag NPs/COOH-fCNTs modified GCE is suitable for developing into a highly sensing platform for the detection of AM-AT. All the techniques as mixing, or the sequence changing or layer-by-layer (LBL) were attempted. The best sensing response was achieved by adopting drop casting LBL fabrication process for NH₂-fCNTs/Ag NPs/COOH-fCNTs/GCE sandwiched matrix nanosensor. Afterward, the amount of the nanocomposite was then optimized by varying the composition ratios of NH₂-fCNTs: Ag NPs: COOH-fCNTs in LBL method in order to get the best oxidative response of AM-AT (**Figure S4**). The different ratios of modifiers were drop-casted by varying the ratio of one component while keeping fixed ratio of other two components. The intensification in AM-AT peak current on increase of modifiers ratios, can be attributed to the increase in effective surface area of GCE and electrocatalytic behavior of nanocomposite. At higher modifiers ratios AM-AT current signals were not uniform which may be due to sluggish electron transfer frequency as a result of surface blockage due to increase of film thickness on the electrode surface. Thereby, the

too thick or thin layers of the modifying agents were avoided for the electrochemical sensing. The 5 μL : 2 μL : 5 μL of $\text{NH}_2\text{-fCNTs}$: Ag NPs: COOH-fCNTs demonstrated the best results so further experiments were performed by using the 5 μL $\text{NH}_2\text{-fCNTs}$: 2 μL Ag NPs: 5 μL COOH-fCNTs .

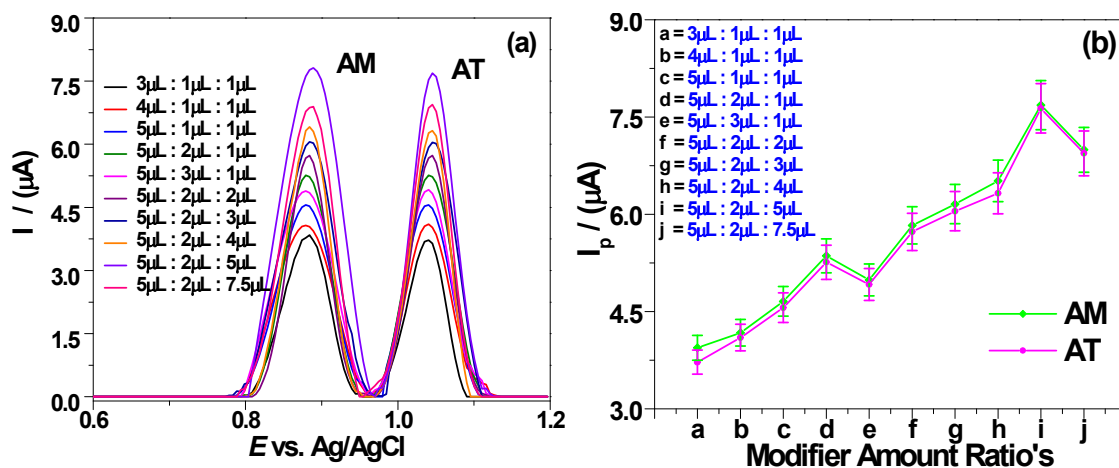


Figure S4. (a) Modifiers amount effect on the SWASVs response of AM (6 μM) and AT (9 μM) mixture in pH 6 PBS as stripping solvent, at sweep rate 100 mV/s, deposition potential 0 V and accumulation time of 5 s through LBL modification of GCE surface from different ratios of fCNTs : Ag NPs: fCNTs respectively (b) Plot of I_p of the AM and AT binary mixture vs modifiers fCNTs : Ag NPs: fCNTs ratios with error bars.

3.2. Optimization of pre-concentration step

The accumulation quantity of analytes and sensor sensitivity are primarily supervised by the analyte molecules properties and the sensor surface state. During pre-concentration step, firstly the analytes molecules diffused from the electrolyte to the sensor surface and then, become adsorbed on it. Thus, the impact of deposition potential and time of SWASVs on electrooxidation of AM (6 μM) and AT (9 μM) binary mixture was investigated at the $\text{fCNTs/Ag/fCNTs/GCE}$ under open-circuit and continuous stirring to avoid effect of mass transfer. It was found out that accumulation of AM and AT upsurge with increase of deposition potential and time, and consequently the voltammograms of AM-AT intensified that enhance the sensitivity. The effect of deposition potential recorded from -0.1 V to 0.7 V at accumulation time of 5 s to probe the maximum magnitude of the AM-AT current signal after their immobilization at the sensor surface. The deposition potential of 0 V presented highest current response for both analytes as illustrated in **Figure S5**. Likewise, the influence of the deposition time on the peak currents of AM and AT was investigated from 5 s to 160 s

at a fixed deposition potential of 0 V. The oxidation signals of AM-AT significantly increased with raised of the accumulation time from 5 s to 125 s. However, the signals after 125 s became a plateau due to complete coverage of electrode adsorptive sites and hindrance in the electron transfer process (**Figure S6**). Therefore, the 125 s accumulation time at 0 V was chosen for the electrooxidation of the AM-AT at designed sensor surface.

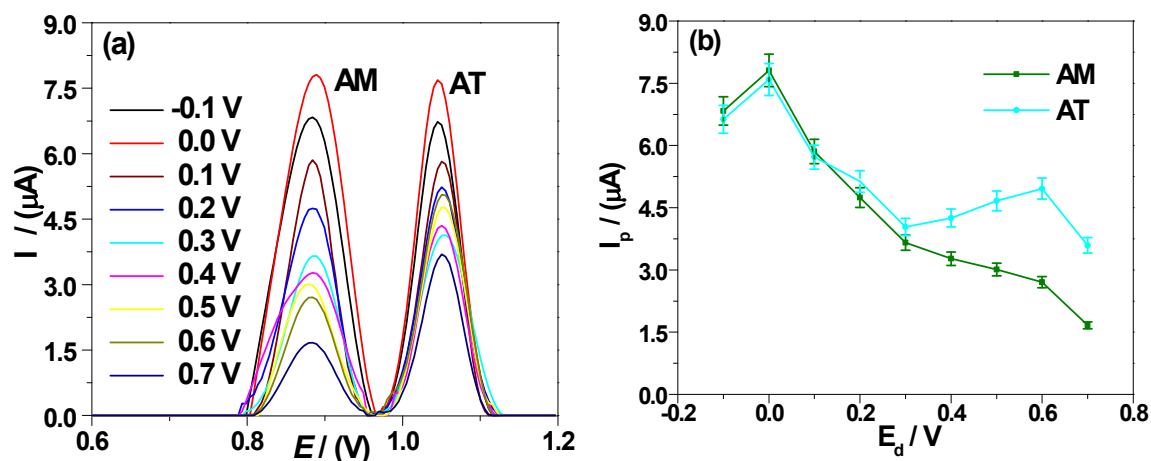


Figure S5. (a) Influence of accumulation potential on the SWASV current peak intensity of AM (6 μM) and AT (9 μM) mixture in pH 6 PBS, at scan rate 100 mV/s, and accumulation time of 5 s through LBL modification of GCE with 5 μL *f*CNTs/2 μL Ag NPs/5 μL *f*CNTs. (b) Plot of corresponding I_p vs E_d with error bar.

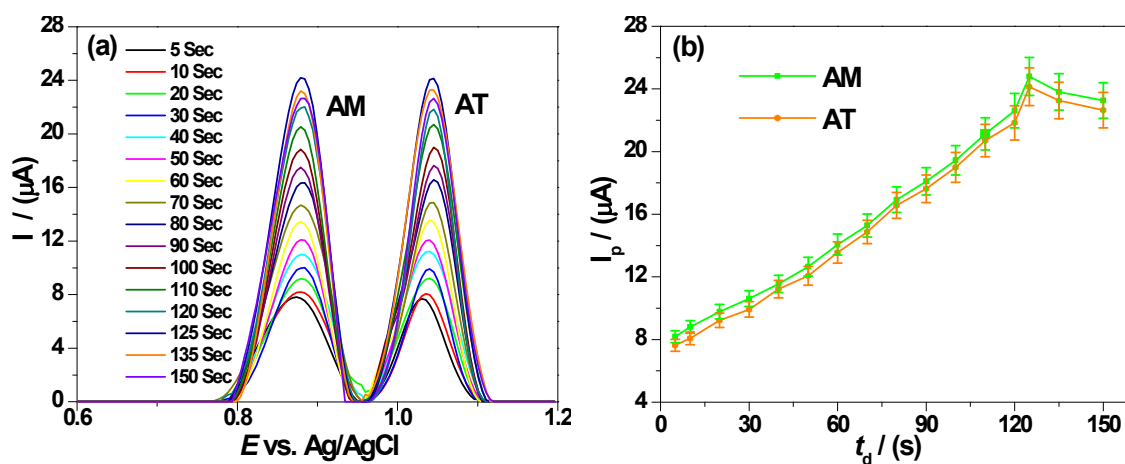


Figure S6. (a) The deposition time variation effect on the stripping current response of AM (6 μM) and AT (9 μM) mixture in pH 6 PBS, at scan rate 100 mV/s, at deposition potential of 0 V, through LBL modification of GCE with 5 μL *f*CNTs/2 μL Ag NPs/5 μL *f*CNTs. (b) Plot between I_p vs t_d with error bar.

4. Peak potential of AM and AT vs log (scan rate)

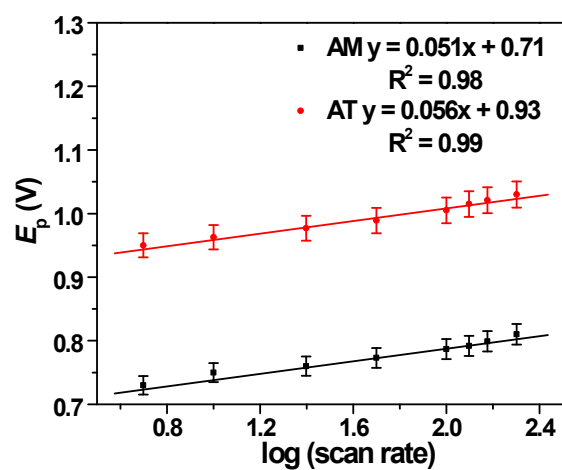


Figure S7. Cyclic voltammograms data of AM and AT at *f*CNTs/Ag/*f*CNTs/GCE in PBS of pH 6, scan rate varying from 5 mV/s to 200 mV/s at potential 0.2 V to 1.2 V for plot of peak potential of AM vs log (scan rate) and AT vs log (scan rate) with error bar and respective linear equation.

Table S2. Real sample analyses and standard addition recovery studies.

Drugs Analysis	Tablets		Serum		Urine		Artificial Sweat		Artificial Sweat		Drinking Water		Tap Water	
	AM	AT	AM	AT	AM	AT	AM	AT	AM	AT	AM	AT	AM	AT
Labeled claim (mg)	5	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	20	-	-	-	-	-	-	-	-	-	-	-	-
Amount found (mg)	5	10	-	-	-	-	-	-	-	-	-	-	-	-
	4.9	-	-	-	-	-	-	-	-	-	-	-	-	-
	-	20.2	-	-	-	-	-	-	-	-	-	-	-	-
% RSD	5.1	9.8	-	-	-	-	-	-	-	-	-	-	-	-
	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-
Spiked amount (nM)	-	0.7	-	-	-	-	-	-	-	-	-	-	-	-
	1.1	1.3	-	-	-	-	-	-	-	-	-	-	-	-
	2	-	2	3	2	3	2	3	2	3	2	3	2	3
Found amount (nM)	-	6	4	6	4	6	4	6	4	6	4	6	4	6
	6	9	6	9	6	9	6	9	6	9	6	9	6	9
	1.96	-	1.94	2.97	1.95	2.9	1.9	2.95	1.9	3.1	1.94	2.9	1.9	3.1
% Recovery	-	6.12	3.9	5.9	3.85	5.9	4.1	5.95	3.9	5.95	4.1	5.95	3.9	5.9
	5.8	9.1	5.7	8.95	6.1	9.1	6.15	8.95	6.0	9.1	6.15	9.1	5.8	8.95
	98	-	97	99	97.5	96.6	95	98	95	103	97	96.6	95	103
% RSD	-	102	97.5	98	96	98	102.5	99	97.5	99	102.5	99	97.5	98
	97	101	95	99	101.6	101	102.5	99	100	101	102.5	101	97	99
% RSD	1.1	-	1.2	1.1	2.2	1.7	1.2	2.1	1.5	2.4	2.1	1.1	1.1	2.2
	-	1.5	2.4	2.5	1.6	1.2	1.1	2.7	2.3	2.1	2.7	2.5	2.5	1.6
	1.3	2.2	2.7	3.3	1.3	2.3	1.5	3.1	1.9	2.9	3.1	3.3	1.3	1.3

5. Theoretical studies of AM and AT at the designed sensor

The HOMO (highest occupied molecular orbital), and LUMO (lowest unoccupied molecular orbital) of the AM and AT optimized molecular geometries (**Scheme 2**), with their band gap are portrayed in pictorial form in **Figure S8**. Likewise, the Ag NPs, Ag NPs/COOH-CNTs, and *f*CNTs/Ag/*f*CNTs merged AM-AT optimized systems are presented in **Figure S9**. The calculated quantum descriptors for the binding energies and chemical reactivities of the drugs at the modifier surfaces are computed and summarized in **Table S1**.

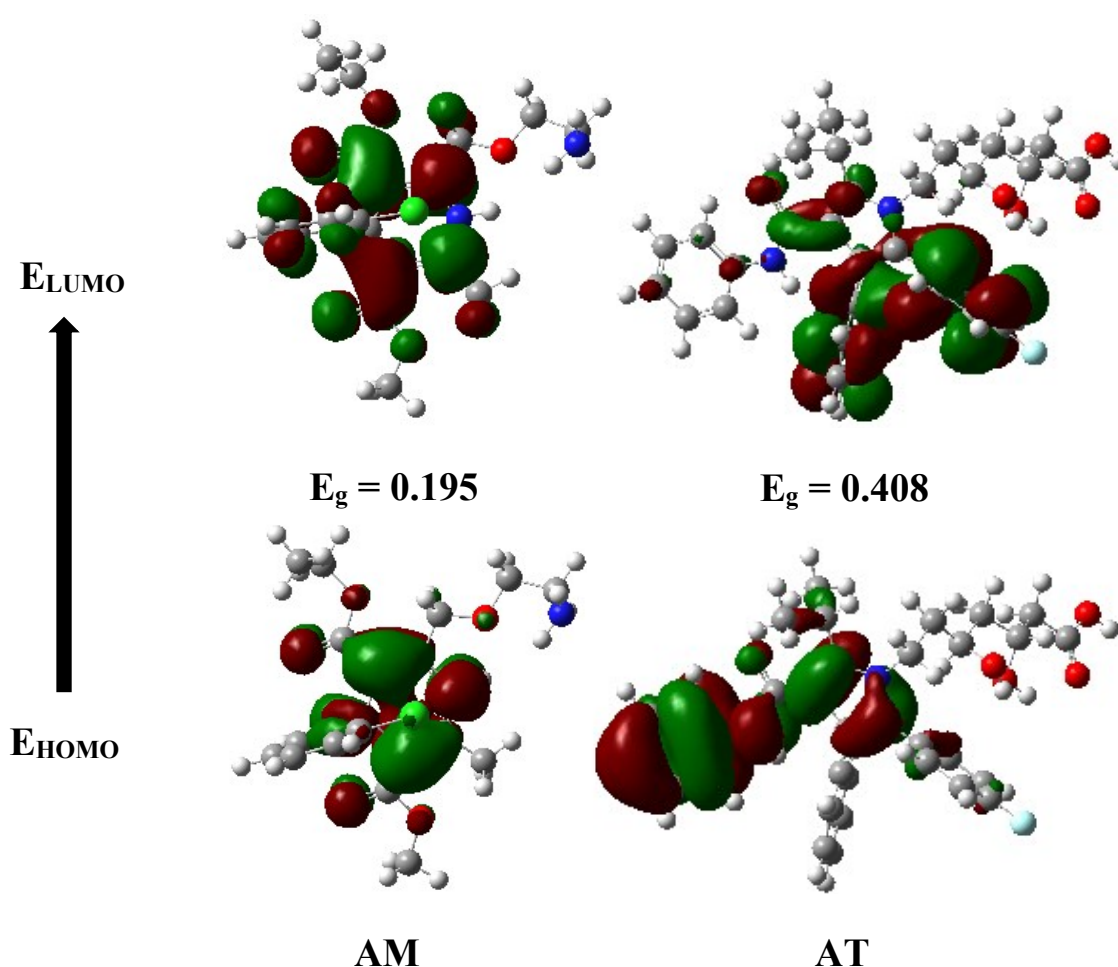


Figure S8. Pictorial representation of HOMO and LUMO of optimized structures of AM and AT along with their energy gaps by DFT on Gaussian 09 software B3YLP method with basis set 6-311G++ (d, p).

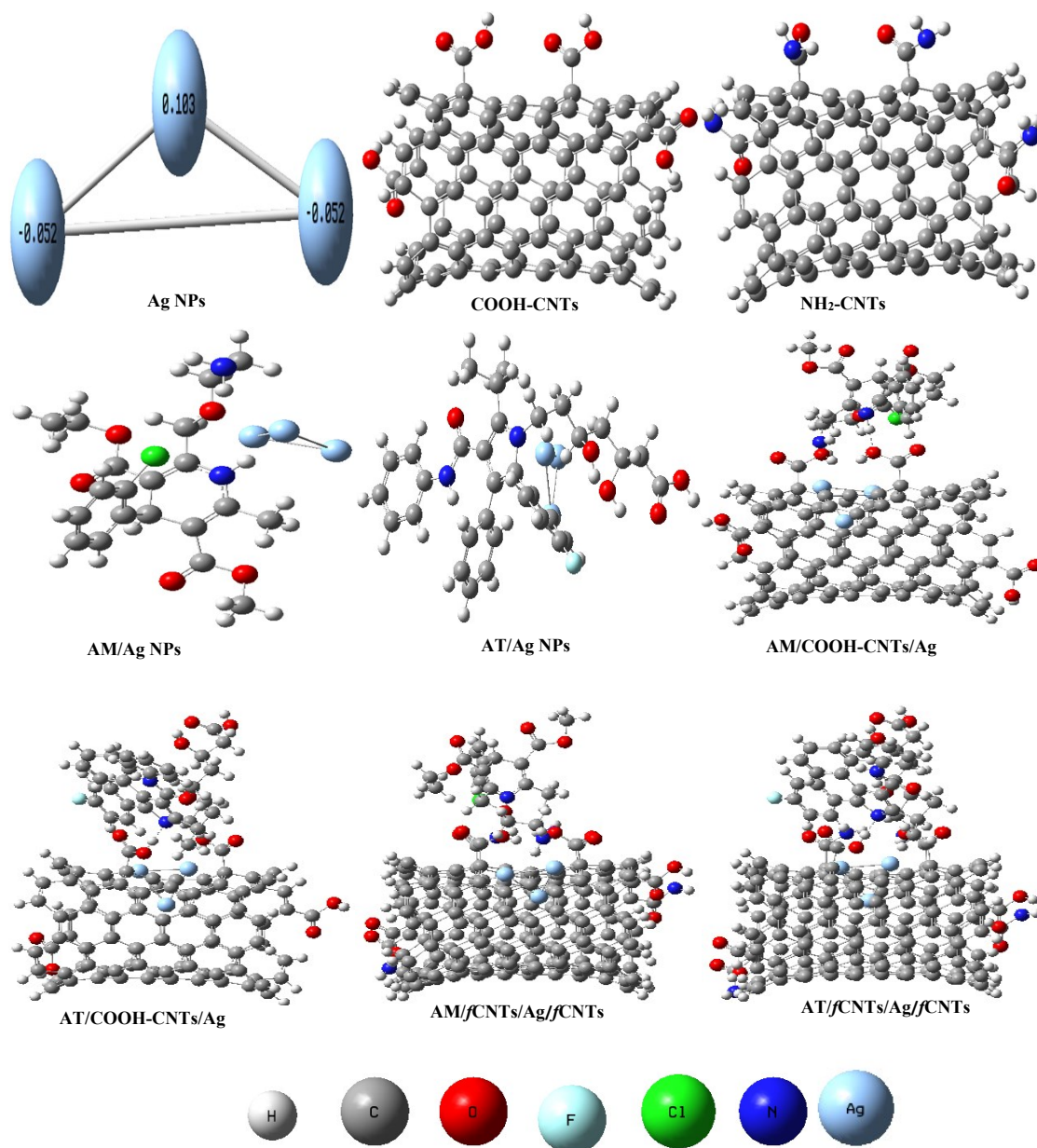


Figure S9. The optimized structures of Ag NPs (triangle),COOH-CNTs (armchair (5, 5) with four COOH groups), NH₂-CNTs (armchair (5, 5) with four CONH₂ groups) and their Ag /COOH-CNTs, and *f*CNTs/Ag/*f*CNTs merged systems with AM and AT by M06-2X method on Gaussian 09 software.

Table S3. Comparative data of the AM and AT with their Ag NPs, Ag NPs/COOH-*f*CNTs and *f*CNTs/Ag/*f*CNTs merged systems and quantum descriptors (in terms of Hartree units) calculated by M06-2X method.

Factors	Drugs		Ag NPs	COOH-CNTs	NH ₂ -CNTs	Ag NPs		Ag NPs/COOH-CNTs		<i>f</i> CNTs/Ag/ <i>f</i> CNTs	
	AM	AT				AM	AT	AM	AT	AM	AT
E	-1705.5	-1842.5	-437.4	-4198.4	-4083.5	-2142.1	-2279.4	-6340.2	-6477.6	-10420.3	-10558.2
D	4.42	5.16	0.37	5.30	6.60	4.54	5.57	-	-	-	-
ΔE	-	-	-	-	-	-0.9	-0.5	-1.2	-0.7	-4.5	-3.6
E_{HOMO}	-0.292	-0.286	-0.374	-0.154	-0.209	-0.106	-0.134	-0.225	-0.245	-0.219	-0.227
E_{LUMO}	-0.097	0.122	-0.084	-0.101	-0.168	-0.089	-0.087	-0.215	-0.214	-0.212	-0.212
E_g	0.195	0.408	0.290	0.053	0.041	0.017	0.047	0.010	0.031	0.007	0.015
IE	0.292	0.286	0.374	0.154	0.209	0.106	0.134	0.225	0.245	0.219	0.227
EA	0.097	-0.122	0.084	0.101	0.168	0.089	0.087	0.215	0.214	0.212	0.212
χ	0.195	0.082	0.229	0.128	0.189	0.098	0.111	0.220	0.230	0.216	0.220
μ	-0.195	-0.082	-0.229	-0.128	-0.189	-0.098	-0.111	-0.220	-0.230	-0.216	-0.220
η	0.098	0.204	0.145	0.027	0.021	0.009	0.024	0.005	0.016	0.004	0.008
σ	10.25	4.90	6.90	37.74	48.78	117.65	42.55	200.0	64.52	285.7	133.3
Ω	0.194	0.016	0.181	0.31	0.87	0.56	0.26	4.84	1.69	6.63	3.21