Electronic Supplementary Information

Naked-eye detection of Cysteine/Homocysteine through silver nano resonators and specific identification of Homocysteine through nanoresonators-thiosulphate conjugate.

Raksha Singh,^a Anurag Kumar Singh^a, Minu Yadav^a, Manish Sharma^b, Ida Tiwari^a and K. K. Upadhyay^{*a}

*Corresponding Author

^aDepartment of Chemistry (Centre of advanced Study), Institute of Science, Banaras Hindu University, Varanasi -221005, India. **E-mail**: <u>drkaushalbhu@yahoo.co.in</u>

^bDefence Institute of Physiological and Allied Sciences (DIPAS), DRDO, New Delhi-110054, India.

New Journal of Chemistry

Table of contents

S. No.	Fig. No.	Caption	Page No.
1	S1	Methodoloy for Synthesis of AgNPs	3
2	S2	image of AgNPs synthesized at 4 different temperature using	3
		same method. UV-Vis spectra of AgNPs synthesized at 40°C.	
3	S3	Image showing (1) blank solution containing Trisodium citrate +	4
		Silver nitrate, (2) image showing blank solution containing	
		Trisodium citrate + Hydrazine, (3) image showing blank solution	
		containg Hydrazine + Silver nitrate, (4) silver nanoparticles	
		solution(Trisodium citrate + Hydrazine + Silver nitrate).	
4	S4	Image showing tyndall effect.	4
5	S5	visible response of pH metric titration of AgNPs from 1-14 pH.	5
6	S6	UV-Vis response of pH metric titration from 1-14 pH.	5
7	S7	Visible response of pH metric titration with I-cysteine (from 1 to	6
		14 pH).	
8	S8	UV-Vis response of pH metric titration with I-cysteine from 1-	6
		14 pH.	
9	S9	Visible response of pH metric titration with I-homocysteine	7
		(from 1 to 14pH).	
10	S10	UV-Vis response of pH metric titration with I-homocysteine	7
		from 1-14 pH.	
11	S11	EDAX of AgNPs synthesized at 40°C.	8
12	S12	EDAX of AgNPs synthesized at 60°C.	8
13	S13	EDAX of AgNPs synthesized at 80°C.	9
14	S14	EDAX of AgNPs synthesized at 100°C.	9

15	S15	EDAX of AgNPs (synthesized at 40°C) after addition of I-	10
		cysteine.	
16	S16	DLS of AgNPs.	10
17	S17	DLS of AgNPs after addition I-cysteine.	11
18	S18	DLS of AgNPs after addition 1-homocysteine.	11
19	S19	Zeta potential of AgNPs.	12
20	S20	Zeta potential of AgNPs after the addition of I-cysteine.	12
21	S21	Zeta potential of AgNPs after the addition of I-homocysteine	13
22	S22	Zeta potential of AgNPs conjugate.	13
23	S23	Zeta potential of AgNPs conjugate + cysteine.	14
24	S24	Zeta potential of AgNPs conjugate + homocysteine.	14
25	S25	Table for Zeta Potential comparison.	15
26	S26	UV-Vis titration of AgNPs conjugate with I-homocysteine	15
27	S27	Image showing colorimetric and UV-Vis response of I-	16
		homocysteine (Hcys) with AgNPs conjugate in presence of I-	
		cysteine (Cys) and Glutathione.	
28	S28	Image showing colorimetric response of cysteine and	16
		homocysteine at different temperatures.	
29	S29	Image of musicnac 600 tablet containing 600mg of	17
		acetylcysteine.	
30	S30	Visible response of AgNPs with different ppm solution of	17
		acetylcysteine tablet	
31	S31	UV-Vis response of different ppm solution of acetylcysteine	17
		tablet (musinac 600) on interaction with silver nanoparticles.	
32	S32	Gel formation of AgNPs (at 4 different temp.) using agar-agar.	18
33	S33	IR spectrum of 3% agar-agar in millipore water (blank).	18
34	S34	IR spectrum of AgNPs at synthesized 40°C (3% agar-agar).	19
35	S35	IR spectrum of AgNPs synthesized at 60°C (3% agar-agar).	19
36	S36	IR spectrum of AgNPs synthesized at 80°C (3% agar-agar).	20
37	S37	IR spectrum of AgNPs synthesized at 100°C (3% agar-agar).	20
38	S38	IR spectrum of AgNPs(40°C) + Cys (3% agar-agar).	21
39	S39	IR spectrum of AgNPs(40 ^o C) + Hcys (3% agar-agar).	21
40	S40	IR spectrum of Trisodium Citrate (CA) (3% agar-agar).	22
41	S41	IR spectrum of Thiosulphate (3% agar-agar).	22
42	S42	IR spectrum of Nps conjugate (3% agar-agar).	23
43	S43	IR spectrum of Nps conjugate + Cys (3% agar-agar).	23
44	S44	IR spectrum of Nps conjugate + Hcys (3% agar-agar).	24
45	S45	IR of thiosulphate + cysteine (3% agar-agar).	24
46	S46	IR of thiosulphate + homocysteine (3% agar-agar).	25
47	S47	Table for FT-IR comparision.	25
48	S48	Probable mechanism for AgNPs formation and sensing.	26
49	S49	Stability check of AgNPs after 6 months.	27
50	S50	Matrix study of AgNPs.	27
51	S51	U.V-Vis response of matrix study.	28
52	S52	TEM image of AgNPs synthesized at 40°C.	28
53	S53	TEM image of AgNPs synthesized at 60°C.	29

54	S54	TEM image of AgNPs synthesized at 80°C.	29
55	S55	TEM image of AgNPs synthesized at 100°C. 30	
56	S56	Stability check through electrolyte addition (NaCl, .001M),	30
		repeated 8 UV-Vis readings at the interval of 5 min.	
57	S57	Stability check through electrolyte addition (NaCl, 0.1M),	31
		repeated 8 UV-Vis readings at the interval of 5 min.	
58	S58	Stability check through electrolyte addition (NaCl, 1M),	31
		repeated 8 UV-Vis readings at the interval of 5 min.	
59	S59	Stability check through electrolyte addition (NaCl, 2M),	32
		repeated 8 UV-Vis readings at the interval of 5 min.	
60	S60	Table for comparison of present work with previous works for	32
		sensing of cysteine and homocysteine through nanoparticles in	
		terms detection limit.	
61	S61	Table for comparision of specific detection of homocysteine	33
		with previous works in terms of detection limit.	
62	S62	SAED pattern of AgNPs after addition of I-cysteine.	33
63	S63	SAED pattern of AgNPs after addition of I-homocysteine.	34
64	S64	SEM images of AgNPs synthesized at different temperature.	34
65	S65	Interference study of AgNPs (synthesized at 40°C)	35

S1 Methodoloy for Synthesis of AgNPs:

Three different solutions were prepared; trisodium citrate $(1.0x10^{-2} \text{ M}, 30 \text{ mL})$, Hydrazine hydrate $(2.0x10^{-3} \text{ M}, 20 \text{ mL})$, and silver nitrate $(1.0x10^{-3} \text{ M}, 5 \text{ mL})$. To a round bottom flask maintained at 40°C, 60°C, 80°C and 100°C separately on an oil bath, 30 mL of tri sodium citrate and 20 mL of hydrazine solutions were added and stirred for 10 minutes. The silver nitrate solution (5 mL) was further added in drop wise fashion to the same round bottom flask under stirred conditions. The stirring was further continued for 15-16. Resulting AgNPs are shown below;



Fig. S2 image of AgNPs synthesized at 4 different temperature using same method. UV-Vis spectra of AgNPs synthesized at 40°C.



Fig. S3 Image showing (1) blank solution containing Trisodium citrate + Silver nitrate, (2) image showing blank solution containing Trisodium citrate + Hydrazine, (3) image showing blank solution containing Hydrazine + Silver nitrate, (4) silver nanoparticles solution(Trisodium citrate + Hydrazine + Silver nitrate).



Fig. S4 Image showing tyndall effect.



Fig.S5 visible response of pH metric titration of AgNPs from 1-14 pH.



Fig.S6 UV-Vis response of pH metric titration from 1-14 pH.



Fig.S7 Colorimetric response of pH metric titration with I-cysteine (from 1 to 14pH).



Fig.S8 UV-Vis response of pH metric titration with I-cysteine from 1-14 pH.



Fig.S9 Visible response of pH metric titration with I-homocysteine (from 1 to 14pH).



Fig.S10 UV-Vis response of pH metric titration with I-homocysteine from 1-14 pH.



Lsec: 30.0 0 Cnts 0.000 keV Det: Octane Plus Det

Fig.S11 EDAX of AgNPs synthesized at 40°C.



Fig.S12 EDAX of AgNPs synthesized at 60°C.



Lsec: 30.0 0 Cnts 0.000 keV Det: Octane Plus Det

Fig.S13 EDAX of AgNPs synthesized at 80°C.



Lsec: 30.0 0 Cnts 0.000 keV Det: Octane Plus Det

Fig.S14 EDAX of AgNPs synthesized at 100°C.



Lsec: 30.0 0 Cnts 0.000 keV Det: Octane Plus Det

Fig.S15 EDAX of AgNPs (synthesized at 40° C) after addition of cysteine.



Fig.S16 DLS of AgNPs.



Fig.S17 DLS of AgNPs after addition I-cysteine.



Fig. S18 DLS of AgNPs after addition l-homocysteine.



Fig.S19 Zeta potential of AgNPs.



Fig. S20 Zeta potential of AgNPs after the addition of I-cysteine.



Fig. S21 Zeta potential of AgNPs after the addition of I-homocysteine.



Fig. S22 Zeta potential of AgNPs conjugate.



Fig. S23 Zeta potential of AgNPs conjugate + cysteine.



Fig. S24 Zeta potential of AgNPs conjugate + homocysteine.

Fig. S25 Table for Zeta Potential comparison:

Sample	Peak 1st	Peak 2nd	Peak 3 rd
Nps	-71mV	-49mV	39mV
Nps + Cys	-	-31mV	-
Nps + Hcys	-	-33	-
Conjugate of Nps	-60mV	-49mV	45mV
Conjugate of Nps + Cys	-55mV	-	12.5mV
Conjugate of Nps + Hcys	-	-30mV	-



Fig. S26 UV-Vis titration of AgNPs conjugate with I-homocysteine (lod = $2.60 \times 10^{-6} M$).



Fig. S27 image showing colorimetric and UV-Vis response of I-homocysteine (Hcys) with AgNPs conjugate in presence of I-cysteine (Cys) and Glutathione (GSH).



Fastest
slowest

instant change in all solutions

Fig. S28 Image showing visible response of cysteine and homocysteine with AgNPs synthesized at different temperatures viz., $A = 40^{\circ}$ C, $B = 60^{\circ}$ C, $C = 80^{\circ}$ C and $D = 100^{\circ}$ C.



Fig.S29 Image of musicnac 600 tablet containing 600mg of acetylcysteine.



Fig.S30 Visible response of AgNPs with different ppm solution of acetylcysteine tablet i.e, (1) AgNPs, (2) AgNPs + 60 X 10³ppm, (3) AgNPs + 50 X 10³ppm, (4) AgNPs + 40 X 10³ppm, (5) AgNPs + 30 X 10³, (6) AgNPs + 20 X 10³ppm, (7) AgNPs + 10 X 10³ppm.



Fig.S31 UV-Vis response of different ppm solution of acetylcysteine tablet (musinac 600) on interaction with silver nanoparticles.



Fig.S32 Gel formation of AgNPs synthesized at different temperature using agar-agar; (1) 3% Agar-agar solution in millipore water (blank), (2) AgNPs (40°C) having 3% agar-agar, (3) AgNPs (60°C) having 3% agar-agar, (4) AgNPs (80°C) having 3% agar-agar, (5) AgNPs (100°C) having 3% agar-agar.



Fig.S33 IR spectrum of 3% agar-agar in millipore water (blank).



Fig.S34 IR spectrum of AgNPs synthesized at 40°C (3% agar-agar).



Fig.S35 IR spectrum of AgNPs synthesized at 60°C (3% agar-agar).



Fig.S36 IR spectrum of AgNPs synthesized at 80°C (3% agar-agar).



Fig.S37 IR spectrum of AgNPs synthesized at 100°C (3% agar-agar).



Fig.S38 IR spectrum of AgNPs (synthesized at40^o C) + Cys (3% agar-agar).



Fig. S39 IR spectrum of AgNPs(synthesized at 40^o C) + Hcys (3% agar-agar).



Fig. S40 IR spectrum of Trisodium Citrate(CA) (3% agar-agar).



Fig. S41 IR spectrum of Thiosulphate (3% agar-agar).



Fig. S42 IR spectrum of Nps conjugate (3% agar-agar).



Fig. S43 IR spectrum of Nps conjugate + Cys (3% agar-agar).



Fig. S44 IR spectrum of Nps conjugate + Hcys (3% agar-agar).



Fig. S45 IR of thiosulphate + cysteine (3% agar-agar).



Fig. S46 IR of thiosulphate + homocysteine (3% agar-agar).

Fig. S47 Table for FT-IR:

sample	Peaks (cm ⁻¹)	
AgNPs	1760, 1651, 1560, 1270	
AgNPs + Cys	1704, 1655, 1508, 1361	
AgNPs + Hcys	- , 1639, 1271	
AgNPs conjugate	- , 1640, 1286	
AgNPs conjugate + Cys	- , 1633, 1280	
AgNp Conjugate + Hcys	- , 1632, 1299	
Only Thiosulphate + Cys	- , 1636, 1299	
Only thiosulphate + Hcys	- , 1643, 1275	



Fig. S48 Probable mechanism for AgNPs formation and sensing.



Fig.S49 Stability check of AgNPs after 6 months.



Fig. S50 Matrix study; (1) AgNPs + mixture {solution} containing different amino acids i.e., Gly, Ala, Val, His, Arg, Leu, Lys, Gsh, Met, Phe, Thr, Trp. (2) AgNPs + solution containing obove amino acid + Cys. (3) AgNPs + solution containing above amino acids + Cys + Hcys. (4) AgNPs + solution containing obove amino acid + Hcys.



Fig. S51 U.V-Vis response of above matrix study.



Fig. S52 TEM image of AgNPs synthesized at 40°C.



Fig. S53 TEM image of AgNPs synthesized at 60° C.



Fig. S54 TEM image of AgNPs synthesized at 80°C.



Fig. S55 TEM image of AgNPs synthesized at 100°C.



Fig. S56 Stability check through electrolyte addition (NaCl, .00 1M), repeated 8 UV-Vis readings at the interval of 5 min.



Fig. S57 Stability check through electrolyte addition (NaCl, 0.1M), repeated 8 UV-Vis readings at the interval of 5 min.



Fig. S58 Stability check through electrolyte addition (NaCl, 1M), repeated 8 UV-Vis readings at the interval of 5 min.



Fig. S59 Stability check through electrolyte addition (NaCl, 2M), repeated 8 UV-Vis readings at the interval of 5 min.

Fig. S60	Table for comparison of present work with previous works for sensing of cysteine and
homocys	teine through nanoparticles in terms detection limit.

Analyte	Detection limit (lod)	References
1 Cysteine	0.16 μM	1
Homocysteine	0.25 μM	
2 Cysteine	0.05μΜ	2
3 Cysteine	0.260μM	3
Homocysteine	0.010μM	
4 Cysteine/Homocysteine	0.4µM	4
5 Cysteine	25.37μM	5
6 Cysteine	4nm	6
7 Cysteine	1.14 μM	Present study
Homocysteine	0.637μM	

- 1 S. Shariati and G. Khayatian, *RSC Adv.*, 2021, 11, 3295.
- 2 S. Chen, H. Gao, W. Shen, C. Lu and Q. Yuan, *Sens. Actuators B: Chem.*, 2014, *190*, 673-678.
- 3 Reference 34 main text.
- 4 H. Gao, W. Shen, C. Lu, H. Liang and Q. Yuan, *Talanta*, 2013, *115*,1-5.
- 5 A. D. Viana, E. T. Nobrega, E. P. Moraes, A. O. W. Neto, F. G. Menezes and L. H. Gasparotto, *Mater. Res. Bull.*, 2020, 124, 110755.
- 6 Reference 25 main text.

Fig. S61 Table for comparision of specific detection of homocysteine with previous works in terms of detection limit.

Analyte	Detection limit (lod)	reference
Hcys	0.2 μM	1
Hcys	2.6 μM	Present study

1. Reference 29 main text.



Fig. S62 SAED pattern of AgNPs after addition of L-Cysteine.



Fig. S63 SAED pattern of AgNPs after addition of I-homocysteine.



Fig. S64 SEM images of AgNPs synthesized at 4 different temperatures viz., (A) 40° C, (B) 60° C, (C) 80° C and (D) 100° C.



Fig. S65 Interference study of AgNPs (synthesized at 40°C) with various amino acids; blue bar: AgNPs + amino acids, red bars: AgNPs + amino acids + Cys, Control: Only AgNPs (blue bar) and AgNPs + Cys (Red bar).