NJC

Accepted Manuscript

This is an *Accepted Manuscript*, which has been through the Royal Society of Chemistry peer review process and has been accepted for publication.

Accepted Manuscripts are published online shortly after acceptance, before technical editing, formatting and proof reading. Using this free service, authors can make their results available to the community, in citable form, before we publish the edited article. We will replace this *Accepted Manuscript* with the edited and formatted *Advance Article* as soon as it is available.

You can find more information about *Accepted Manuscripts* in the Information for Authors.

Please note that technical editing may introduce minor changes to the text and/or graphics, which may alter content. The journal's standard Terms & Conditions and the Ethical guidelines still apply. In no event shall the Royal Society of Chemistry be held responsible for any errors or omissions in this *Accepted Manuscript* or any consequences arising from the use of any information it contains.

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/njc

NJC

PAPER

Naked eye sensing of Melamine: Aggregation induced recognition by Sodium D-gluconate stabilised Silver Nanoparticles

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Palanichamy Kaleeswaran,[a] Thanasekaran Nandhini,[a] Kasi Pitchumani[a,b*]

A highly selective, sensitive and low-cost colorimetric sensor for a biologically important analyte, namely melamine, is reported using sodium D-gluconate stabilised silver nanoparticles. Hydrogen bonding between sodium D-gluconate and melamine induces the silver nanoparticles to aggregate with consequent color change. The aggregation induced color change from yellow to red is monitored by UV-Visible spectroscopy and the structure is confirmed by HRTEM. The selective sensing of melamine by sodium D-gluconate stabilised silver nanoparticles is also confirmed by competitive binding studies. The detection limit is found to be 5 x 10^{-7} M (0.06 ppm) and this melamine sensor also finds applications in real sample analysis.

Introduction

Melamine, also known as tripolycyanamide, is used as fertilizer, fire-resistant resin and durable material for the manufacture of laminated surfaces such as formica, whiteboards, and flooring as well as kitchen utensils.[1,2] Eventhough melamine has been extensively used in the manufacture of plastics, and resins, melamine polymers are banned in food products. To increase the apparent crude protein value, melamine is added to infant formula and animal feeds. The addition of 1g of melamine to 1 litre of milk increases the apparent protein content by 0.4 %.[3] Customary Kjeldahl and Dumas methods fail to distinguish the protein nitrogen and non-protein nitrogen sources. Melamine is also an irritant when inhaled or in contact with the skin or eyes. Assimilation of melamine may lead to reproductive damage, bladder or kidney stones, which can lead to bladder cancer. Chronic exposure may cause cancer or reproductive damage. Excess of melamine in body results in the formation of insoluble melamine cyanurate crystals which are deposited in kidney and leads to renal failure.[4]

Apart from direct food, melamine can enter into the food chain through the use of the pesticide cyromazine on crops, use of nitrogenous fertilizers for growing food crops, consumption of cyromazine, melamine contaminated crops, crop residues by food producing animals, leaching of melamine from plastics used in food equipment, containers or packaging materials that come in contact with food, especially acidic foods at high temperatures. Foods may be adulterated with melamine to make the protein content of the food appear higher for economic gains. Due to this nephrotoxicity, a long-term melamine intake may result in illnesses and even deaths of infants and pets.[5,6]

Transition metal nanoparticles based colorimetric methods were developed to sense metal ions, nucleic acids, and small molecules. Unique optical properties of Ag and Au nanoparticles help in sensing various analytes, including biologically important molecules. The surrounding dielectric medium, the interparticle distance, size, shape were used to control the optical properties of nanoparticles.[7] Silver nanoparticles (Ag NPs) have been given more attention owing to their superior plasmon absorbance compared to Au NPs and their molecular recognition can be transformed into color change and high sensitivity.[8] Aggregation induced shifting of LSPR bands to longer wavelength, gets more attention in many Ag nanoparticle based sensors. Compared with Au NPs, Ag NPs have advantages, such as lower cost of preparation, higher extinction coefficients relative to Au NPs of the same size.[9]

Gluconic Acid and its derivatives are playing vital role in food products and cosmetics. Salts of gluconic acids are widely utilised in various food products as acids, coagulants, and mineral supplements. Gluconates are effectively utilised by bifidobacteria and contributes to increasing the number of faecal bifidobacteria.[10] Sodium gluconate is highly soluble in water, nontoxic and biodegradable. The hydroxyl groups present in gluconate can stabilise the nanoparticles and the carboxyl group has the tendency to form hydrogen bonds with melamine.

[a] Department of Natural Products Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai-625021
[b] Centre for Green Chemistry Processes, Madurai Kamaraj University, Madurai-625021

Electronic Supplementary Information (ESI) available: Linear calibration curve, HRTEM images, Competitive binding studies. See DOI: 10.1039/x0xx00000x

Recently many melamine sensors were developed using ellagic acid capped gold nanoparticles,[11] trisodium citrate stabilized Au nanoparticles,[12] thioglycolic acid capped CdS QD,[13] sodium citrate stabilized silver nanoparticles,[14] cystamine modified gold nanoparticles,[15] sulfanilic acid functionalised silver nanoparticles,[16] ellagic acid reduced gold nanoparticles,[17] 7-(benzylamino)-9,9-dibutyl-9*H*-fluorene-2-carbaldehyde modified gold nanoparticles,[18] bare gold nanoparticles by borohydride reduction,[19] silver/dopamine nanoparticles,[20] citrate capped gold nanoparticles based on size effect,[21] 1,4-dithiothreitol modified gold nanoparticles.[22] They, however, are associated with disadvantages such as high cost, low detection limit and tedious preparation. They also involve the use of analytical techniques such as HPLC,[23] LC-MS,[24] ELISA,[25] GC/MS,[26] electrochemical methods[27] and SERS[28] to detect melamine in food sample. In addition, the techniques are expensive and time-consuming. Consequently, the development of a cheap, simple, rapid and field-portable sensor to detect melamine is desirable. Our interest in developing chemosensors for biologically important cations,[29] anions,[30] and neutral molecules[31] has prompted us to develop a simple colorimetric sensor involving sodium-D-gluconate stabilized Ag NPs, for the selective sensing of melamine which involve aggregation of Ag NPs and the observed results are discussed below.

Experimental section

Materials and methods

Sodium D-gluconate (Sdglu), silver nitrate and melamine were purchased from Sigma Aldrich. Amino acids and other chemicals were purchased from Merck. All the chemicals were used as received without further purification. Deionised water was used for all the experiments.

UV-visible absorption spectra were recorded using the JASCO Spectra Manager (V-550) in a 1 cm pathlength quartz cuvette. High resolution transmission electron microscopy (HR-TEM) images of Ag NPs were obtained from a FEI-TecNai-T20G^2, operating at 200 kV. For TEM measurements, the sample was prepared by dropping 2 µL of a Glu-AgNP colloidal solution onto a carbon-coated copper grid.

Results and discussion

In the present work, a simple, cost-effective and biocompatible sodium D-gluconate stabilised Ag NPs were prepared. They were characterised using absorbance spectra and HRTEM. The sensing behavior of Sdglu-Ag NPs towards melamine was studied and the observed results and the relevant discussions are given below.

Preparation of sodium D-gluconate stabilised Ag NPs

The Sdglu-Ag NPs were synthesised using a modified procedure of a literature report.[32] Freshly prepared $AgNO_3$ (1 mM) was added slowly to a mixture of 20 mL of freshly prepared sodium borohydride (2 mM) and 5 mL of sodium D-gluconate (1 mM), with stirring of 2 hour at 25 °C. The colorless solution turns yellow indicating the formation of Ag nanoparticles. The prepared Sdglu-Ag NPs were utilised for further characterisation and sensing studies.

General procedure for melamine detection in real sample

Melamine with various concentrations was added to a pretreated milk sample.[20,33] The melamine added milk samples (1.0 mL) were added to 1.0 mL of Sdglu-Ag NPs and the absorption spectra were recorded.

Characterization of Sdglu-Ag NPs

The prepared colloidal Sdglu-Ag NPs show the LSPR (Localised Surface Plasmon Resonance) band at 405 nm in absorbance spectrum (Fig. 1a) and is yellow in color (Fig. 1b).

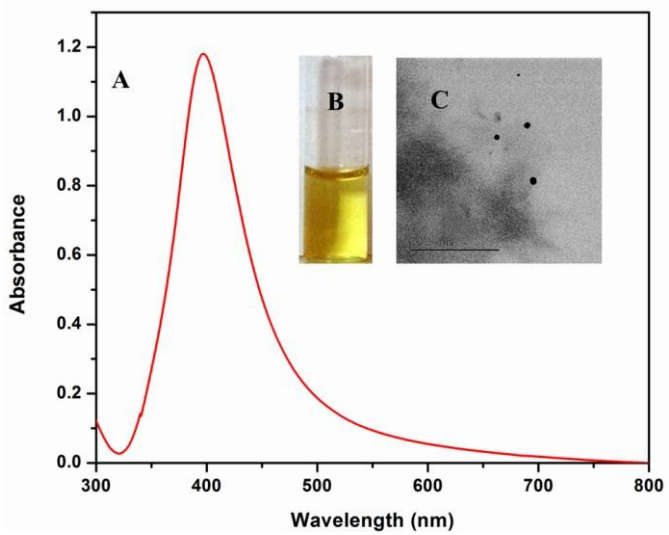

Fig. 1: (a) Absorbance spectrum of the Sdglu-Ag NPs; (b) Photograph of the Sdglu-Ag NPs

The color of the nanoparticles and the LSPR bands show the nanoparticles are well distributed and this was confirmed by HRTEM (Fig. 2a). The uniform distribution of the particle is evident in its HRTEM image and it is spherical in shape. The average particle size (100 particles were counted) is found to be 12 to 14 nm, evident from the particle size histogram (Fig. 2b).

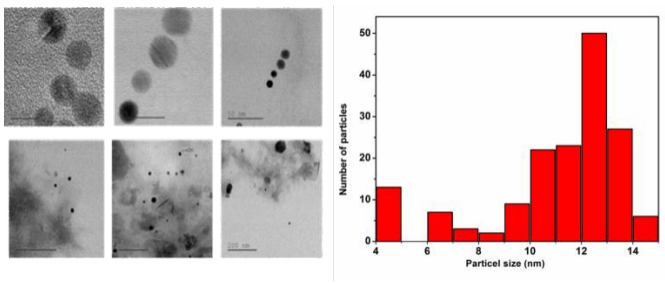

Fig. 2: (a) The HRTEM images of Sdglu-AgNPs; (b) Particle size distribution histogram of silver nanoparticles from HRTEM analysis

Sensor studies

The gluconate stabilised Ag nanoparticles are also quite stable and there is no LSPR shifting of Sdglu-Ag NPs upto two weeks. Thus it is clear that the negatively charged gluconate ion forms an electrostatic layer around Ag NPs, which keeps the nanoparticles stable in solution. While melamine is added, the intensity of the characteristic LSPR band at 405 nm decreased and a new peak was observed at 555 nm with a color change from yellow to red. This is rationalised by proposing that the hydroxyl group in gluconate interacts with melamine *via* hydrogen bonding which brings the silver nanoparticles closer leading to aggregation which supported by the appearance of a new band at 555 nm in its absorbance spectrum.

To verify the selective aggregation ability of Sdglu-Ag NPs only in presence of melamine, studies were also conducted with various other amino acids and organic molecules which possess similar structure to that of melamine. While aggregation was observed only by the addition of melamine, the other analytes did not show any aggregation (Fig. 3).

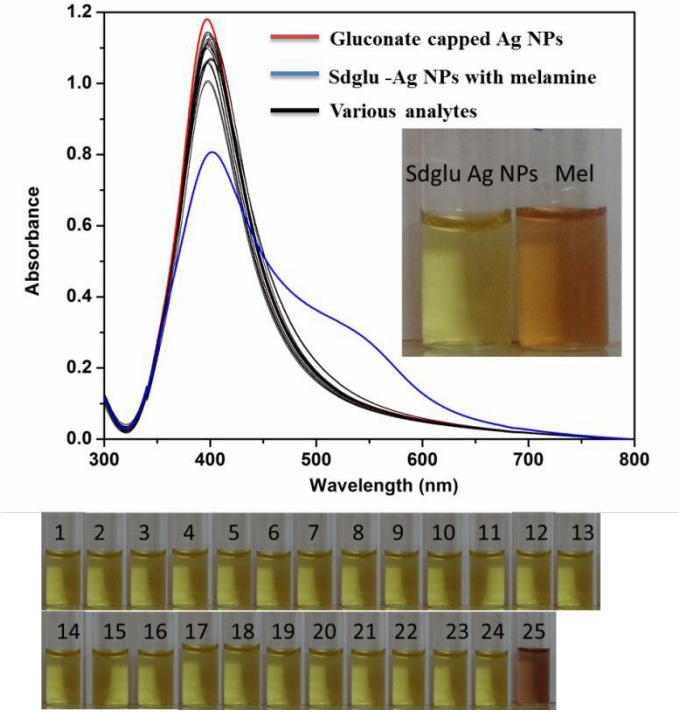

Fig. 3: (a) Absorbance spectra of Sdglu-Ag NPs in the absence of melamine (red line), with other analytes (5 x 10^{-5} M) (black lines) and with melamine (5 x 10^{-5} M) (blue line); (b) Photograph of colour change from yellow to red in melamine (5 x 10^{-5} M) induced aggregated Sdglu-Ag NPs; (c) Photograph of colour change of Sdglu-Ag NPs with various other analytes (5 x 10^{-5} M) (1. Sdglu-Ag NPs, 2. Gly, 3. Ala, 4. Leu, 5. Ile, 6. Phe, 7. Glu, 8. Arg, 9. His, 10. Trp, 11. Tyr, 12. Ser, 13. Lys, 14. Asp, 15. Met, 16. Cys, 17. Ascorbic acid, 18. Resorcinol, 19.Catechol, 20. Gallic acid, 21. Pyrogallol, 22. Na^+, 23. K^+, 24. Ca^{2+}, 25. Melamine)

The melamine induced aggregation was also monitored by absorption and colour change as a function of time. To study the aggregation time, 5 x 10^{-5} M melamine was added to Sdglu-Ag NPs and the absorption spectra was recorded. The aggregation occurs readily and a stable LSPR intensity is reached after 25 min (Fig. 4).

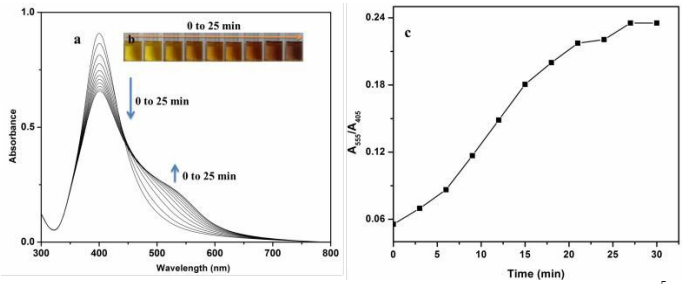

Fig. 4: (a) The UV-Vis spectra of Sdglu-Ag NPs in the presence of melamine (5 x 10^{-5} M) upto 25 min with a time interval of 3 min; (b) Photograph of colour change of Sdglu-Ag NPs in the presence of melamine (5 x 10^{-5} M) up to 25 min with a time interval of 3 min; (c) Corresponding calibration plot between A_{555}/A_{405} and time.

During aggregation, the colour change from yellow to red was also observed. Upon addition of melamine, the absorption band at 405 nm decreased and the peak at 555 nm increased gradually by increasing the reaction time. The absorbance reaches its stable LSPR intensity at 25 min. No further absorbance change is observed after 25 minutes.

Sensitivity of melamine detection

The sensitivity of melamine induced aggregation of Sdglu-Ag NPs was monitored by UV-Vis. spectroscopy. Whereas the absorption intensity decreases at 405 nm and a new peak appears at 555 nm and the colour of the mixture changed from yellow to deep red, with increase in concentration of melamine from 5 x 10^{-7} M to 5 x 10^{-4} M (Fig. 5). The value of the linearly dependent coefficient (R^2) was found to be 0.98 (Fig. S1) and the limit of detection is 5 x 10^{-7} M (0.06 ppm). It is relevant to note that the safety limit of melamine is 2.5 ppm in United States of America and the European Union; 1 ppm for infant formula in China.[34] In India, infant formula should not contain melamine more than 1 ppm and other foods not more than 2.5 ppm. The present Sdglu-Ag NPs detects melamine at a level less than the safety limits described above.

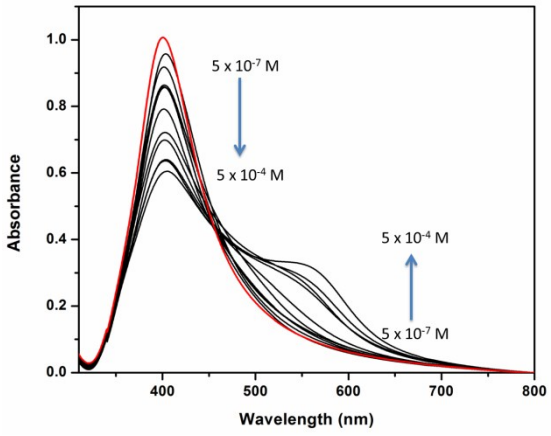

Fig. 5: (a) The change in UV-Vis absorption spectra of Sdglu-Ag NPs while increasing the concentration of melamine from 5 x 10^{-7} M to 5 x 10^{-4} M.

Selective sensing of melamine

To confirm the selectivity of melamine towards aggregation, studies are also carried out in presence of interfering species such as Na^+, K^+, Ca^{2+}, amino acids, ascorbic acid, resorcinol, gallic acid, pyrogallol and catechol.

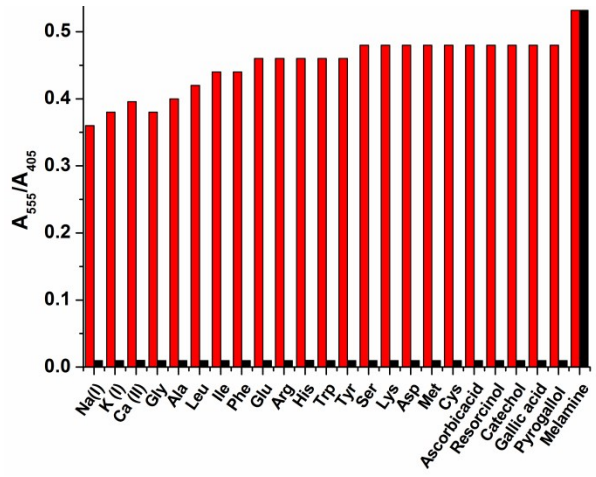

Fig. 6: Absorbance ratio (A_{555}/A_{405}) of Sdglu-Ag NPs with melamine (3 x 10^{-5} M) in the presence of various analytes (3 x 10^{-5} M) (Black bar: various analytes in the absence of melamine; Red bar: various analytes in the presence of melamine)

In the absence of melamine, they do not show any aggregation as well as color change. While melamine is added, ($3x10^{-5}$ M) significant absorption as well as color changes are observed. This confirms that melamine alone induces the aggregation even in the presence of other analytes indicating the good selectivity of melamine towards Ag NPs.

Sensing mechanism

The negatively charged gluconate ion forms an electrostatic layer around Ag NPs, which keep the nanoparticles stable in aqueous solution, and it shows an LSPR band at 405 nm. When melamine is added, the Ag NPs are aggregated with spectral variation in absorbance and colour change from yellow to deep red.

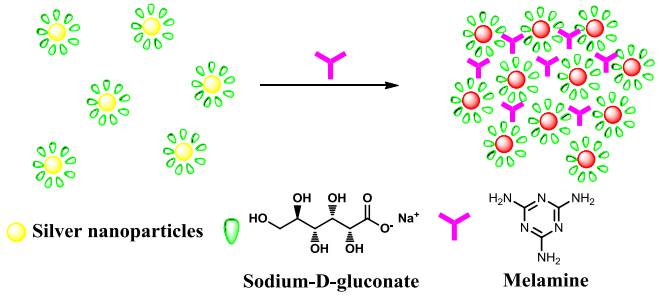

Fig. 7: Schematic representation of melamine induced aggregation of Sdglu-Ag NPs

The aggregation may be attributed to the formation of hydrogen bonding interaction between the hydroxyl groups present in gluconate and the amine groups/ring nitrogen present in melamine. A schematic representation of melamine sensing is given in fig. 7. The appearance of new peak at 555 nm is due to the coupling between plasmons of neighboring particles in the aggregates.[34] The proposed aggregation of silver nanoparticle is also supported by HRTEM images which are shown in fig. 8 and S2 – S4.

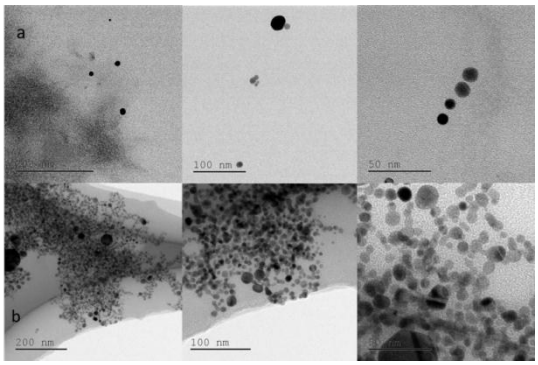

Fig. 8. Comparison of Sdglu-Ag NPs (a) in absence of melamine and (b) in presence of melamine (5 x 10^{-5} M) at different magnification

Real sample analysis

To evaluate the practical applicability of Sdglu-Ag NPs to real samples, analysis was also performed for the detection of melamine in pasteurised milk samples.[35] To 5.0 ml of raw milk, 10% of 2.0 ml trichloroacetic acid was added and shaken well to precipitate the protein in the sample. The process was repeated twice and it was then centrifuged at 10,000 rpm for 15 min to separate the deposits. The supernatant portion was neutralised with 1 M NaOH. All the samples with and without melamine were analysed using Sdglu-Ag NPs and the observed results are shown in table 1, which also includes recovery data.

Table 1: Real sample analysis using Sdglu-Ag NPs

Sample	Melamine spiked (μM)	Melamine found (μM)	Recovery (%)
Milk sample	5 μM	$4.9^a \pm 1.06^b$	98
	50 μM	$43^a \pm 0.79^a$	86
	500 μM	$450^a \pm 0.93^a$	90

Comparison with previous reported methods

Sensing ability of the present Sdglu-Ag NPs towards melamine was also compared with other reported literature methods (Table 2). It is evident that the present cost effective and simple colorimetric sensor for melamine and its LOD are superior to the potentiometric methods, SERS and magnetic nanoparticles reported earlier. The present method involves a simple naked eye sensing of melamine and avoids the need for sophisticated and costly instruments.

Table 2: Comparison of reported methods with Sdglu-Ag NPs

S. No.	Sensing Probe	Method	Detection limit	Reference
1	Fiber-optic biosensor	Fluorescence	5.14 μg/L	36
2	Polymer-capped CdTe quantum dots	Fluorescence	0.6 μM	37
3	Colorimetric/label free Ag NPs	Colorimetry	2.32 μM	14
4	SERS/Ag NPs	Raman	3.96 μM	38
5	Graphene Quantum Dots	Fluorescence	0.12 μM	39
6	Cyclodextrins capped silver NPs	Colorimetry	4.98 μM	40
7	Functionalized Fe/Fe_3O_4 nanoparticles	Nanoparticle based	~ 2 μM	41
8	Molecularly imprinted polymer	Potentiometric sensor	6.0×10^{-6} M	42
9	Tri-sodium citrate and dodecasodium of phytic acid (IP6) dual-functionalised gold nanoparticles modified filter paper	Raman	5×10^{-6} M	43
10	*p*-nitroaniline-modified silver nanoparticles	Colorimetry	0.1 ppm	44
11	Leaves' extract of *Jatropha gossypifolia* stabilized Ag NPs	Colorimetry	2 μM	45
12	Riboflavin stabilised gold nanoparticles	Colorimetry	0.1 μM	46
13	Cyclodextrin-decorated silver nanoparticles	SERS	3 μg/L	47
14	This method	Colorimetry	5×10^{-7} M (0.06 ppm)	--

Conclusions

A low cost, simple sodium D-gluconate stabilised Ag nanoparticle system was developed and its selectivity towards melamine was demonstrated by absorbance spectroscopy and visible colour change. The intensity of Localised Surface Plasmon Resonance band of silver nanoparticles at 405 nm decreased and a new peak is noticed at 555 nm while increasing the concentration of melamine. Hydrogen bonding between melamine and Sdglu-Ag nanoparticle is responsible for the aggregation of nanoparticles. The colour change from yellow to red was also visible by naked eye. The linearly dependent coefficient is 0.98 and the limit of detection is found to be 5×10^{-7} M (0.06 ppm). The reported sensing system detects melamine less than the safety limit prescribed in India, United States of America, Europe Union and China. Practical applications carried out in commercial milk samples, further indicate that the sensing system has potential application for facile real-time monitoring for melamine.

Acknowledgements

P.K. and K.P. gratefully acknowledge the Department of Science and Technology, New Delhi for financial support through DST – PURSE and UGC – UPE.

References

1 R. D. Hauck and H. F. Stephenson, *J. Agric. Food Chem.*, 1964, **12**, 147.
2 T. L. Fodey, C. S. Thompson, I. M. Traynor, S. A. Haughey, D. G. Kennedy and S. R. H. Crooks, *Anal. Chem.*, 2011, **83**, 5012.
3 A. K. Hau, T. H. Kwan and P. K. Li, *J. Am. Soc. Nephrol.*, 2009, **20**, 245
4 a) R. L. M. Dobson, S. Motlagh, M. Quijano, R. T. Cambron, T. R. Baker, A. M. Pullen, B. T. Regg, A. S. Bigalow-Kern, T. Vennard, A. Fix, R. Reimschuessel, G. Overmann, Y. Shan and G. P. Daston, *Toxicol. Sci.*, 2008, **106**, 251; b) C. A. Brown, K. S. Jeong, R. H. Poppenga, B. Puschner, D. M. Miller, A. E. Ellis, K. Kang, S. Sum, A. M. Cistola and S. A. Brown, *J. Vet. Diagn. Invest.*, 2007, **19**, 525; c) H. d'A. Heck and R. W. Tyl, *Regul. Toxicol. Pharm.*, 1985, **5**, 294.
5 J. L. Dorne, D. R. Doerge, M. Vandenbroeck, J. F. Gremmels, W. Mennes, H. K. Knutsen, F. Vernazza, L. Castle, L. Edler and D. Benford, *Toxicol. Appl. Pharmacol.*, 2013, **270**, 218.
6 C. M. E Gossner, J. Schlundt, P. B. Embarek, S. Hird, D. Lo-Fo-Wong, J. J. O. Betran, K. N. Teoh and A. Tritscher, *Environ. Health Perspect.*, 2009, **117**, 1803.
7 a) P. K. Jain and M. A. El-Sayed, *Nano Lett.*, 2008, **8**, 4347; b) J. Homola, *Chem. Rev.*, 2008, **108**, 462.
8 H. Wanga, D. Chen, L. Yu, M. Chang and L. Ci, *Spectrochim. Acta A*, 2015, **137**, 281.
9 J. S. Lee, A. K. R. L. Jean, S. J. Hurst and C. A. Mirkin, *Nano Lett.*, 2007, **7**, 2112.
10 T. Asano, K. Yuasa, K. Kunugita, T. Teraji and T. Mitsuoka, *Microb. Ecol. Health Dis.*, 1994, **7**, 247.
11 X. Zhang, Z. Wu, Y. Xue, Y. Zhang, H. Zhao, Y. He, X. Li and Z. Yuan, *Anal. Methods*, 2013, **5**, 1930.
12 N. Kumar, R. Seth and H. Kumar, *Anal. Biochem.*, 2014, **453**, 46.
13 G. L. Wang, H. J. Jiao, X. Y. Zhu, Y. M. Dong and Z. J. Li, *Talanta*, 2012, **93**, 398.
14 H. Ping, M. Zhang, H. Li, S. Li, Q. Chen, C. Sun and T. Zhang, *Food Control*, 2012, **23**, 191.
15 Y. Ma, L. Jiang, Y. Mei, R. Song and D. Tian, H. Huang, *Analyst*, 2013, **138**, 5338.
16 J. Song, F. Wu, Y. Wan and L. Ma, *Food Control*, 2015, **50**, 356.
17 X. Zhang, Z. Wu, Y. Xue, Y. Zhang, H. Zhao, Y. He, X. Li and Z. Yuan, *Anal. Methods*, 2013, **5**, 1930.
18 Q. Lu, J. Zhao, S. Xue, P. Yin, Y. Zhang and S. Yao, *Analyst*, 2015, **140**, 1155.
19 W. Chen, H-H. Deng, L. Hong, Z-Q. Wu, S. Wang, A-L. Liu, X-H. Lin and X.-H. Xia, *Analyst*, 2012, **137**, 5382.
20 Y. Ma, H. Niu, X. Zhang and Y. Cai, *Analyst*, 2011, **136**, 4192.

21 I. E. Paul, A. Rajeshwari, T. C. Prathna, A. M. Raichur, N. Chandrasekaran and A. Mukherjee, *Anal. Methods*, 2015, **7**, 1453.
22 C. Xiao, X. Zhang, J. Liu, A. Yang, H. Zhao, X. Li, Y. He and Z. Yuan, *Anal. Methods*, 2015, **7**, 924.
23 G. Venkatasami and J. R. Sowa Jr., *Anal. Chim. Acta*, 2010, **665**, 227.
24 J. V. Sancho, M. Ibanez, S. Grimalt, O. Pozo and F. Hernandez, *Anal. Chim. Acta*, 2005, **530**, 237.
25 W. W. Yin, J. T. Liu, T. C. Zhang, W. H. Li, W. Liu, M. Meng, F. Y. He, Y. P. Wan, C. W. Feng, S. L. Wang, X. A. Lu and R. J. Xi, *J. Agric. Food Chem.*, 2010, **58**, 8152.
26 H. Miao, S. Fan, Y. N. Wu, L. Zhang, P. P. Zhou, J. G. Li, H. J. Chen and Y. F. Zhao, *Biomed. Environ. Sci.*, 2009, **22**, 87.
27 Q. Cao, H. Zhao, Y. He, N. Ding and J. Wang, *Anal. Chim. Acta*, 2010, **675**, 24.
28 X. Du, Chu, Y. Huang and Y. Zhao, *Appl. Spectrosc.*, 2010, **64**,781.
29 a) P. Kaleeswaran, I. A. Azath, V. Tharmaraj and K. Pitchumani, *ChemPlusChem*, 2014, **79**, 1361; b) P. Suresh, I. A. Azath and K. Pitchumani, *Sensors Actuat., B*, 2010,**146**, 273; c) K. Kanagaraj, K. Bavanidevi, T. J. Chow and K. Pitchumani, *RSC Adv.*, 2014, **4**, 11714; d) V. Tharmaraj and K. Pitchumani, *Anal. Chim. Acta*, 2012, **751**, 171; e) V. Tharmaraj, S. Devi and Kasi Pitchumani, *Analyst*, 2012, **137**, 5320; f) V. Tharmaraj and K. Pitchumani, *J. Mater. Chem. B*, 2013, **1**, 1962; g) V. Tharmaraj and K. Pitchumani, *Nanoscale*, 2011, **3**, 1166; h) A. Affrose, S. D. S. Parveen, B. S. Kumar, K. Pitchumani, *Sensors Actuat. B*, 2015, **206**, 170.
30 a) I. A. Azath, P. Suresh and K. Pitchumani, *Sensors Actuat. B*, 2011, **155**, 909; b) K. Kanagaraj and K. Pitchumani, *Chem. Asian J.*, 2014, **9**, 146; c) V. Tharmaraj and K. Pitchumani, *RSC Adv.*, 2013, **3**,11566.
31 a) I. A. Azath and K. Pitchumani, *Sensors Actuat. B*, 2013, **188**, 59; b) K. Kanagaraj, A. Affrose, S. Sivakolunthu and K. Pitchumani, *Biosens. Bioelectron.*, 2012, **35**, 452.
32 J. A. Creighton, C. G. Blatchford and M. G. Albrecht, *J. Chem. Soc. Farady Trans. II*, 1979, **75**, 790.
33 L. Guo, J. Zhong, J. Wu, F. Fu, G. Chen, X. Zheng, S. Lin, *Talanta*, 2010, **82**, 1654.
34 a) K. Ai, Y. Liu and L. Lu, *J. Am. Chem. Soc.*, 2009, **131**, 9496; b) L. Zhu, G. Gamez, H. W. Chen, K. Chingin and R. Zenobi, *Chem. Commun.*, 2009, 559; c) R. K. Bera, C. R. Raj, *Analyst*, 2011, **136**, 1644.
35 Y. Ma, H. Niu, X. Zhang and Y. Cai, *Analyst*, 2011, **136**, 4192.
36 H. Xiu-juana, Z. Xiao-hong, Z. Yan, L. Lan-hua, L. Feng, S. Lei and S. Han-chang, *Sensors Actuat. B*, 2014, **204**, 682.
37 B. T. Huy, M. Seo, X. Zhang and Y. Lee, *Biosens. Bioelectron*, 2014, **57**, 310.
38 X. F. Zhang, M. Q. Zou, X. H. Qi, F. Liu, X. H. Zhu and B. H. Zhao, *J. Raman Spectrosc.*, 2010, **41**, 1655.
39 L. Li, G. Wu, T. Hong, Z. Yin, D. Sun, E. S. A. Halim and J. J.Zhu, *ACS Appl. Mater. Interfaces*, 2014, **6**, 2858.
40 S. S. J. Xavier, C. Karthikeyan, G. Gnana kumar, A. R. Kimb and D. J. Yoo, *Anal. Methods*, 2014, **6**, 8165.
41 J. Shen, Y. Zhang, H. Yang, Y. Yang, Z. Zho and S. Yang, *Sensors Actuat. B*, 2014, **203**, 477.
42 R. Liang, R. Zhang and W. Qin, *Sensors Actuat. B*, 2009, **141**, 544.
43 H. Wang, X. Guo, S. Fu, T. Yang, Y. Wen and H. Yang, *Sensors Actuat. B*, 2014, **193**, 630.
44 C. Han and H. Li, *Analyst*, 2010, **135**, 583.
45 H. P. Borase, C. D. Patil, R. B. Salunkhe1, R. K. Suryawanshi, B. K. Salunke and S. V. Patil, *Biotechnol. Appl. Biochem.*, 10.1002/bab.1306.
46 B. Roy, A. Saha and A. K. Nandi, *Analyst*, 2011, **136**, 67.
47 P. Ma, F. Liang, Y. Sun, Y. Jin, Y. Chen, X. Wang, H. Zhang, D. Gao and D. Song, *Microchim. Acta*, 2013, **180**, 1173.

A simple, cost-effective sodium D-gluconate stabilized Ag NPs system was developed and its sensing behavior towards melamine was studied.