Analyst

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

A paper based microfluidic device for easy detection of uric acid using positively charged gold nanoparticles

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Received (in XXX, XXX) XthXXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

A paper based microfluidic device is fabricated that can rapidly detect very low concentration of uric acid (UA) using 3,5,3',5'-tetramethyl benzidine (TMB), H_2O_2 and positively charged gold nanoparticles ((+)AuNPs). In presence of (+)AuNPs, H_2O_2 reacts with TMB to produce bluish-green colour which becomes colourless on reaction with UA. This colorimetric method can detect as low as 8.1 ppm of UA within <20 minutes on a white filter paper. This technique provides an alternative way of UA detection.

Uric acid (UA) is the primary end product of purine metabolism. Determination of serum UA is a regular practice in clinical laboratories since abnormalities in UA level is associated to several diseases.[1, 2] An elevated level of UA is a sign of gout, hyperuricemia, Lysch-Nyhan syndrome. It is also related to other physical abnormalities like obesity, diabetes, high cholesterol, kidney disease and heart disease.[3-6] Common methods for serum UA detection are: (a) the photometric method that is based on the reduction of phospho-tungstic acid by UA to give tungsten blue[7, 8], (b) high performance liquid chromatography on reversed phase columns along with detection by either UV absorbance or mass spectrometry,[9-11] and (c) uricase based method utilizing the specific enzymatic oxidation of UA by oxygen to produce hydrogen peroxide, allantoin and carbon dioxide.[12, 13] All these analytical methods are well established and exhibit large range of advantages. However, these methods also have weaknesses such as they are time consuming, expensive, cannot be performed outside the laboratory and need very highly skilled technicians. On the other hand simplistic early warning systems for detecting these clinically important molecules have been the need of the hour.

Recently, gold nanoparticles (AuNPs) have been extensively used for sensing various clinically important biomolecules and environmental contaminants.[14-18] Good optical property, easy surface conjugation and controlled synthesis make AuNPs as attractive materials for sensing purpose. On the other hand, paper based microfluidic devices have attracted the research attention due to its capillary based self-pumping ability, low cost and ease of availability. The embedded microstructures of the paper substrate helps to pump the fluid due to capillary action. It is worth noting that such paper based materials have extensively been investigated in microfluidic research on biosensor and electronic applications and widely used in point-of-care (POC) diagnostics.[19-32]

In this present work, we have fabricated an inexpensive, portable, easy to fabricate and user friendly paper based microfluidic device embedded with positively charged AuNPs for reliable and sensitive detection of UA. Here we have considered colorimetric redox reaction of 3,5,3',5'-tetramethyl benzidine (TMB) for UA analysis. Colourless solution of TMB gets oxidised in presence of H_2O_2 and positively charged gold nanoparticles ((+)AuNPs) to a bluish-green colour.[33] This bluish-green colour solution when comes in contact with UA becomes colourless due to the reduction of oxidised TMB (oxTMB). Reaction scheme is presented in Fig. 1.

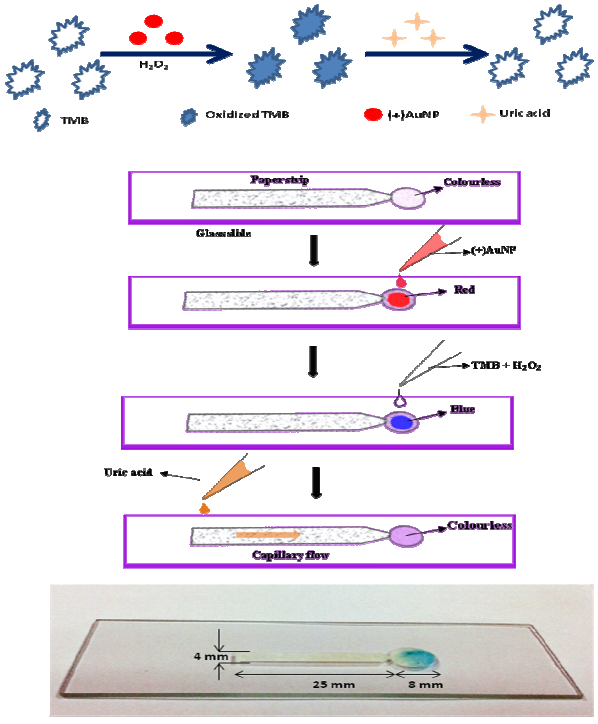

Fig. 1 Reaction scheme for uric acid detection by colorimetric redox reaction of TMB and H_2O_2 in presence of positively charged gold nanoparticles. Bottom image shows the dimension of the paper based microfluidic device.

This (+)AuNPs-TMB-H_2O_2 based detection process of UA can be carried out on a paper based microfluidic device which would be similar to chromatographic type low cost, safe, disposable sensing device generally used for glucose and malaria detection. To the best of our knowledge such gold nanoparticle based UA detection on paper strip has not been reported earlier.

Positively charged gold nanoparticles were synthesized via reduction of sodium chloroaurate ($NaAuCl_4$) by ascorbic acid. The resulting (+)AuNPs were characterized by particle analyzer and average size and charge of the particles were found to be 82 nm and +20 mV respectively in aqueous medium. Details of the size and charge distribution profile along with relevant FESEM images are shown in Fig. S1 and Fig. S2 of supporting information. The red colour (+)AuNPs solution was analysed by UV-Vis spectrophotometry and the maximum absorption peak was observed at 540nm (as shown in Fig. S3 of supporting information). A mixture of 0.02 (M) TMB (in methanol) and 8.82 (M) H_2O_2 solution was freshly prepared (in 5:1 volumetric ratio) and added with (+)AuNPs solution where TMB gets oxidised to a bluish-green solution. Saturated solution of UA is prepared in 0.01(N) NaOH at room temperature and its solubility was found to be around 1.62 mg/ml. The pH of this UA solution was found to be 7.3 and it was diluted with HPLC water to prepare different concentrations for further analysis.

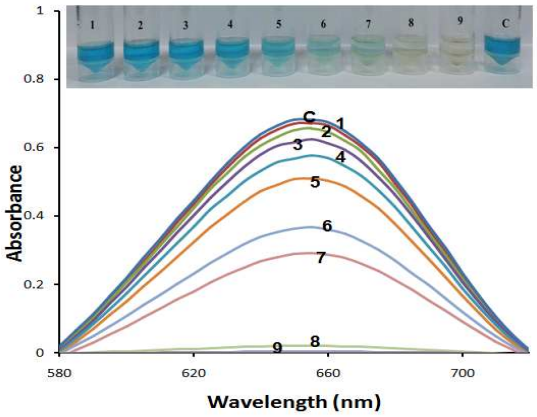

Fig. 2 Differential UV-Vis absorption response (at 654 nm) of the (+)AuNPs-TMB-H_2O_2 treated with various concentrations of uric acid solutions. Uric acid concentrations (in ppm) are 0 (1), 8.1 (2), 24.3 (3), 40.5 (4), 56.7 (5), 72.9 (6), 81.0 (7), 121.5 (8), 162.0 (9) respectively. Control curve (c) denotes the case when a solution of equimolar (0.01N) NaOH and (0.01N) HCl is treated with AuNP-TMB-H_2O_2. Inset: Visual color changes of (+)AuNPs-TMB-H_2O_2 for different concentration of UA solutions.

Initially, UV-Vis technique was employed to monitor the colour change for the detection purpose in liquid phase. Fig. 2 demonstrates the UV-Vis spectra of the bluish-green coloured TMB solution and post UA treated solutions. In all the cases, 1.0 ml of bluish-green solution produced from the oxidation of TMB was taken and another 1.0 ml of HPLC water and UA mixture solution was added to it. Ratio of HPLC water and UA varies in 1.0 ml mixture for different cases. In Fig. 2, curve 1 corresponds to zero concentration of UA and shows maximum intensity of bluish-green colour. From the intensities of curve 2 to 9, it is clear that as the concentration of UA is increased, the intensity of peaks at 654 nm is lowered. The visual colour changes in different cases are presented in inset of Fig 2. It shows that the intensities of bluish-green colour is gradually decreasing (from 1 - 9). 'C' denotes a system where 1 ml of bluish-green solution is mixed with 1 ml of equimolar solution of NaOH (0.01N) and HCl (0.01N). The resultant bluish-green solution shows maximum intensity similar to curve 1 and this retention of colour reveals that Na^+ ion does not interfere in the process of UA detection. Based on the intensities of the UV-Vis-spectrum, a calibration curve was plotted and presented in Fig. 3. It shows the variation of absorbances with the concentration of UA solutions along with the respective standard deviations. A best fit linear curve was obtained along with its mathematical expression. It indicates that the present method can estimate UA level upto 8.1 ppm. Normal range of serum UA level of human blood lies between 2.5 - 8.0 mg/dl (25 - 80 ppm) for male and 1.5 - 6.0 mg/dl (15 - 60 ppm) for female[34] which suggests that above method can be safely used for clinical determination of UA level. The inset of Fig. 2 indicates that there are significant differences in colour intensities between case 1(blank, 0 ppm of UA) and case 7 (81.0 ppm of UA) which can be differentiated by naked eye. The determination of lower level by visual observation is difficult since there is slight colour difference between case 1 and case 3 (24.3 ppm).

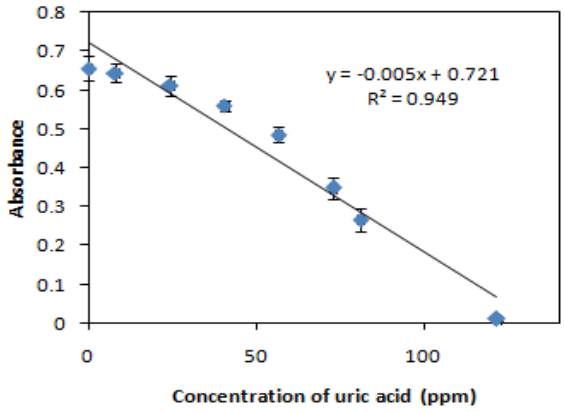

Fig. 3 Linear calibration plot between the absorbance intensities at 654 nm and uric acid concentration in the range 0 – 81 ppm.

Though, the above solution based UA analysis can give accurate result on UV-Vis spectrophotometry, it can only be carried out in a laboratory set-up. To make the detection process more user friendly and sensitive, we have implemented this concept through paper based microfluidics. Generally, microchannel flows are low Reynolds number flows and associated with high amount of wall friction which increases the flow resistance. As a result, pumping the liquid through microchannel becomes difficult. To overcome this limitation, a paper based microfluidic device similar to malaria and glucose detection kit has been used. The advantage of paper substrate is that it contains an embedded network of microchannels through which liquid can pass automatically due to capillary flow. This self-pumping property of paper substrate

along with its low cost and easy availability makes it an attractive substrate for the present study.

Here, the filter paper was cut into the shape of straight strip with a circular head at one end. Shape and dimensions of the paper strip is shown in Fig. 1. The paper strips were washed with hot HPLC water to remove unwanted impurities and dried. The dried strips were then coated with 0.5% poly-vinyl alcohol (PVA) which helps the paper to retain aqueous sample that would otherwise spread more rapidly on hydrophilic paper. The PVA coating on paper also helps biomolecules to maintain their functional characteristics which are well-known in literature.[25, 35] Moreover PVA coating has very little effect on the morphology and porosity of the paper. FESEM images in Fig. S4 of supporting information also show that PVA coating does not affect the physical structure of the paper substrate as demonstrated by Zhao et. al.[25]

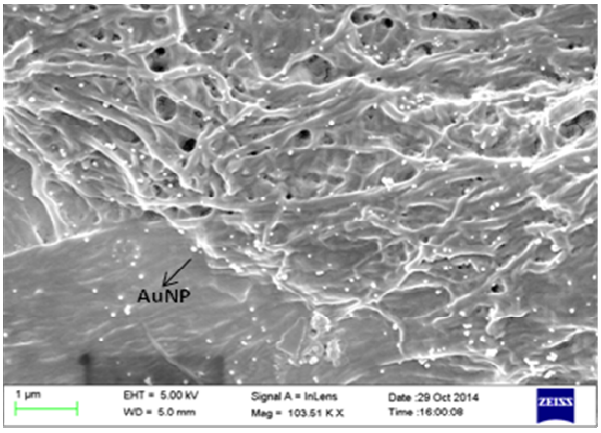

Fig. 4 FESEM image of gold nanoparticle embedded filter paper. White dots represent the image of the nanoparticles.

The circular heads of the PVA coated paper strips were then soaked in a solution of (+)AuNPs. It is found that 10 µl of (+)AuNPs solution (~1.93 X 10^9 particles/ml concentration) shows detectable color change for the present purpose (as shown in Fig. S5 of supporting information). The deposition of nanoparticles on cellulose fibres of paper was checked by field emission scanning electron microscopy (FESEM). The FESEM image of gold nanoparticles embedded paper strip is presented in Fig. 4 which clearly shows the presence of AuNPs on the paper matrix. After drying, a drop of freshly prepared TMB and H_2O_2 solution mixture was added on the (+)AuNPs embedded circular paper head. Within few minutes, bluish-green colour is appeared on the paper head due to the oxidation reaction mentioned in the reaction scheme in Fig. 1. The initial bluish-green colour of the paper head is shown in Fig. 5(a). The feet of the paper strip were then kept vertically in various petridishes in such a way that it remain submerged in UA solutions of different concentrations. Due to capillary action, UA solution moved up and reached to the circular head of the paper strip within moments and reduction of oxidised TMB was initiated. The narrow zone of the paper strip helps to deliver the UA solution to the reactive head in a controlled fashion so that colour change of the reaction becomes clearly visible. In absence of this narrow zone, excess solution will be pumped to the strip head causing a local flooding which will hamper the visibility of the colour change. After 20 minutes the bluish-green colour of the paper heads became faded to different extents depending on concentration of UA solution. Final states of the faded colour of the respective cases are presented in Fig. 5(b) - 5(e). Like liquid phase study, the paper based systems also show a gradual colour change with increasing UA concentrations. Fig. 5(a) (extreme left) shows the blank study where no UA was added. From cases 5(b) - 5(e), UA concentration gradually increases from 33.2 to 100.0 ppm.

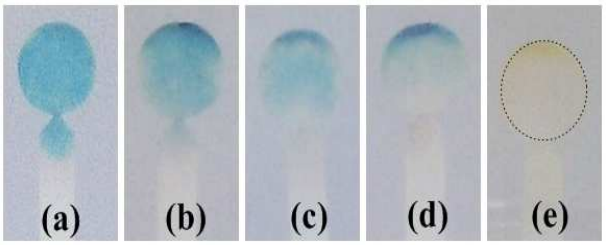

Fig. 5 Paper strip assay of gold nanosensor of uric acid solutions of various concentrations (ppm), i.e. 0 (a), 33.2 (b), 49.8 (c), 83.0 (d) and 100.0 (e) respectively after 20 minutes of uric acid addition. (a) shows the final state of the control (0 ppm of UA) which does not change with time. Dotted circle in (e) shows boundary of the paper strip head.

We further confirmed this gradual change with concentrations on paper strip using solid state UV-Vis analysis. UV-Vis absorption spectrum of four cases are shown in Fig. 6. The top most spectrum shows the maximum intensity of the bluish-green colour where no UA is added. In the following cases, it shows that with the increasing UA concentration peak intensity gradually decreases. Right side column of Fig. 6 shows the respective images of paper strip-heads which indicate the visual

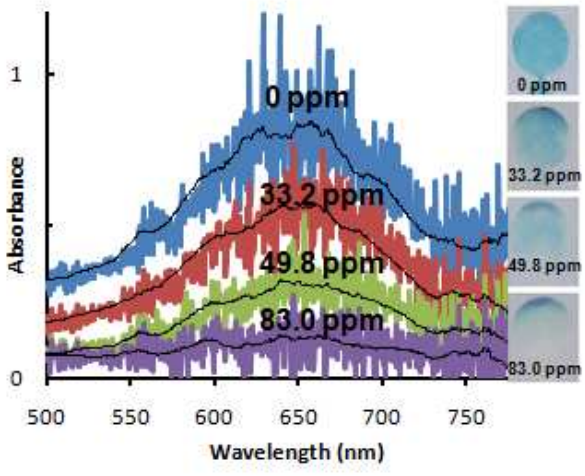

Fig. 6 Solid state UV-Vis absorption response of the (+)AuNP-TMB-H_2O_2 treated with various concentrations of uric acid solutions on paper strip. Uric acid concentrations (in ppm) are 0 (1), 33.2 (2), 49.8 (3), and 83.0 (4) respectively. Right side column shows the visual color changes of (+)AuNP-TMB-H_2O_2 on paper for the respective cases.

colour changes with varying concentration of UA. The colour difference between 0 ppm and 83.0 ppm is quite significant and can easily be detected by naked eye. This can help in UA estimation in human blood serum since higher level of UA in serum is around 80 ppm. So, this paper based chip can offer a simple and alternative user-friendly method for routine check up of UA level.

In conclusion, we have demonstrated a microfluidic paper based device embedded with positively charged gold nanoparticles as an alternative method for UA detection. This paper based device is cost effective, easy to fabricate, operationally simple and can detect low level concentrations of UA (up to 8.1 ppm in liquid phase) and thus has the potential to be employed for commercial use. However, the selectivity of this method has not been investigated in detail in present work. This selectivity test along with real serum sample analysis with this method is kept as a scope of future research.

Acknowledgements

The authors thank, Director, CSIR-CMERI, Durgapur and Dr. Nagahanumaiah, Head, Micro System Technology Labs, CSIR-CMERI, Durgapur for their encouragement. Support from CSIR 12th FYP project no. ESC0112 is gratefully acknowledged. The authors also thank to the institute CRF facility for the SEM study.

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† Electronic Supplementary Information (ESI) available:
See DOI: 10.1039/b000000x/

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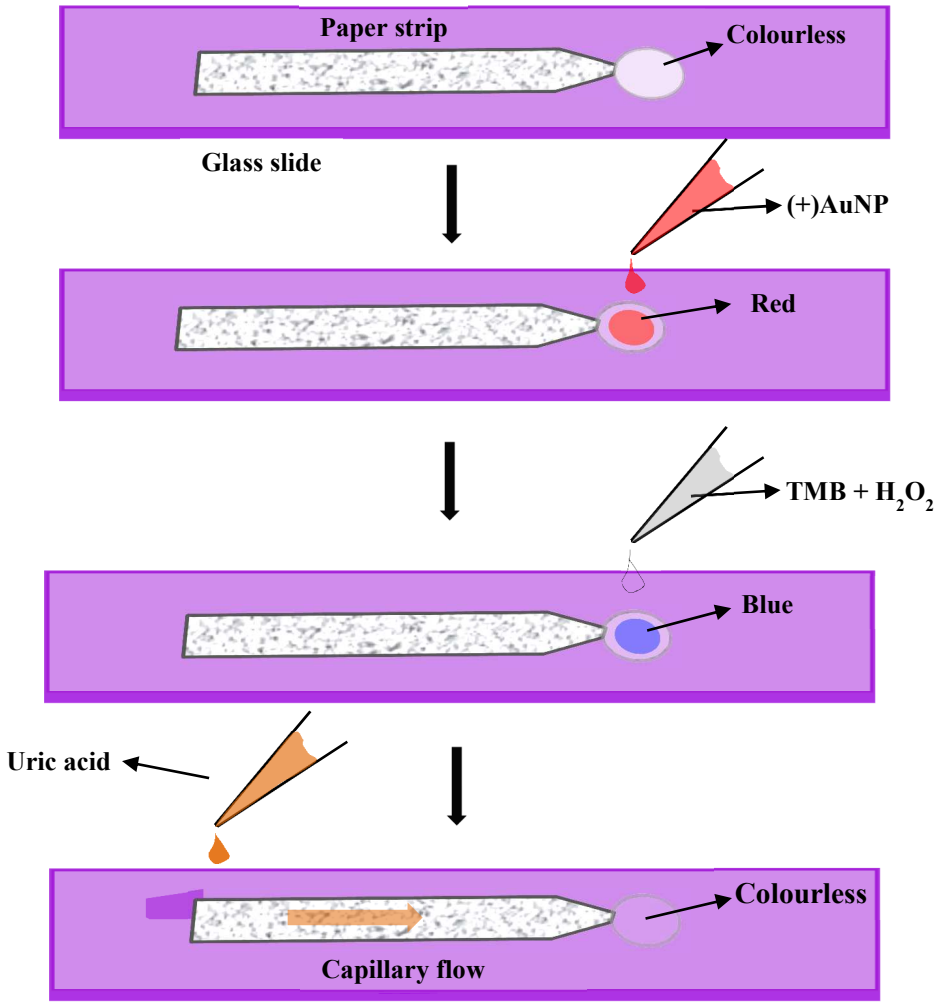

A paper based microfluidic device is fabricated that can rapidly detect very low concentration of uric acid (UA) on a simple white filter paper.