A paper based selective and quantitative detection of uric acid using citrate capped Pt nanoparticles (PtNPs) as a colorimetric sensing probe through a simple and remote based device

(Supplementary Information)

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1.1. Reagents and Solutions

All reagents and chemicals used were analytical grade and consumed without further purification. 3,3',5,5'-tetramethylbenzidine (TMB) was purchased from Sigma-Aldrich Korea, Uric acid (UA) was acquired from MPI biomedical. Chloroplatinic acid hexahydrate H_2PtCl_6. $(H_2O)_6$, Hydrogen peroxide H_2O_2 (30% w/w), Sodium borohydrate ($NaBH_4$) and Trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) were obtained from Sigma-Aldrich Korea. Cellulose fiber used as a paper strip, was provided from Surewick. Solutions and dilutions were made using deionized water (18.2 MU cm) obtained from a Milli-Q system (Millipore).0.09g of TMB was dissolved in 20 mL of methanol and a fresh solution was prepared, H_2O_2 (9.0 M) was also prepared and mixed with TMB in (1:5 volumetric ratio). Standard solution of UA was prepared in 0.05 M NaOH (Sigma-Aldrich) and stored at 4˚C when not in use. UA is insoluble in PBS but soluble in NaOH, the solubility of UA is 50 $mgmL^{-1}$@ 20ºC has been described in our previous work also [10]. Aliquots were prepared in DI water for analysis different concentration of UA on the cellulose strip.

1.2. Instruments

To obtain the morphological, chemical and electrical properties of materials used and sensor development, the following instruments and sensor components were used. Ultraviolet -visible (UV-vis) absorption spectra of PtNPs and various solutions have been recorded by a UV–vis, Lambda 25UV/VIS/NIR PerkinElmer spectrophotometer, available in Jeju National University. Transmission electron microscopy (TEM) was used to analyze PtNPs. SEM images of cellulose fiber and XRD mapping of PtNPs were taken by FESEM. White LED and a photodiode [YwRobot] Brightness sensor (TEMT6000) were used for the measurement setup along with a smart phone in which the android based app for Point of care testing has already been installed. For 3D printing of the required module, Creality CR-20 3D Printer was used to fabricate the setup according to design.

1.3. Synthesis of PtNps

The synthesis of PtNps includes a four step procedure: (a) A precursor solution of Chloroplatinic acid hexahydrate $H_2PtCl_6.(H_2O)_6$ was prepared by dissolving 7 mg of $H_2PtCl_6.(H_2O)_6$ in DI water (40mL), upon which the pH of the solution was adjusted by the addition of 1 N NaOH solution under dynamic stirring. (b) Trisodium citrate dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$) solution (100 mM) was prepared by dissolving 0.148 g in 5 mL DI water, has been used as a capping agent to preclude the particles from growing beyond the nanometric size of interest. (c) Afterwards, a portion of 400 µL was vigorously mixed with TCD and kept it on magnetic stirrer to make it properly mixed with it. The mixture was maintained at room temperature (25˚C) for 30 min. (d) eventually, a few drops of a freshly prepared solution (0.2 mL, 50 mM) of Sodium borohydride ($NaBH_4$) has been added and stirred it until a uniform color change was observed. The colorless reactant mixture immediately turned light brown. A colloidal solution of Pt nanoparticles was prepared, stored at 4˚C and out of reach of light until further use.

1.4. Colorimetric detection of Uric acid

A protocol has been followed for the detection of Uric acid as shown in the Fig. 1. First, cellulose substrate was cut in the form of circular shaped strip of 8 mm diameter, which could be handled easily. Circular area of the strip was treated with polyvinyl alcohol (PVA) and let it dry at room temperature. A mixture of TMB and H_2O_2 (5:1 v/v) was prepared, 80 µL was added to the strip. Subsequently, followed by adding 40 µL solution of citrate capped Pt Nps, where colorless solution of TMB gets oxidized in the presence of H_2O_2 and bluish green color was observed on the strip. Details of the concluded concentration PtNPs has shown in Fig. S2. TMB act as a hydrogen donor for the reduction of hydrogen peroxide in the presence of Pt Nps (peroxidase like activity), producing diimine (RCH=NR') causes the substrate to take on a blue color as shown in Fig.1 and this color can be read on spectrophotometer at the wavelength of 650nm. Now this bluish green color strip can be used for the detection of target analyte (UA). By the introduction of UA on the surface of strip causes the substrate turn to yellow. This change is due to the reduction of oxidized TMB. Depends upon the concentration of UA the bluish green color gradually declined to yellow as shown in Fig. 1(b), and this color variety has been observed by a portable colorimetric detection setup which has facilitated wirelessly and gives quantitative information of the target analyte.

1.5. Design of POCT device

Some off the shelf electronics components along with an Arduino microcontroller and Bluetooth (HC-06) slave module have been used for the measurement setup and placed inside 3D printed assembly (designed in Creo 3.0 and printed using Creality CR-20 3D Printer) to complete the portable POCT unit as shown in Fig. S1. The device works on the principle of colorimetric quantification. For this purpose a white LED and a photodiode were used in the measurement setup. A cellulose strip loaded with sample and dried as described previously is inserted inside the portable setup opening and is illuminated using a white LED. From the other side a photodiode measures the light intensity after absorption through the sample. This colorimetric quantification approach allows the better estimation in programming the controller for measurement as the light intensity changes with the change in color of the strip (full thickness) due to change in analyte concentration. For through transmission of light the LED and Photo diode (PD) have been placed in close proximity (3mm) to the absorbance strip area for maximum response and minimum loss.

For remote monitoring an android application was developed in MIT app inventor 2 platform online to receive the data from the portable setup via Bluetooth communication. Once the POCT setup is turned on, it is required to open the application in an android phone. In the two step process, first it is required to connect the portable setup by pressing the Bluetooth symbol which displays the different Bluetooth options. The required Bluetooth module is selected and now the mobile is connected wirelessly to the POCT setup. In the second step when sample strip is placed inside the portable setup the application asks for pressing start to get the measurement. The measurement is processed inside the POCT measurement setup and sent to the application using Bluetooth connection and is displayed in the application, worked in 10 m range. This Portable setup has been used to measure the different concentration samples of uric acid.

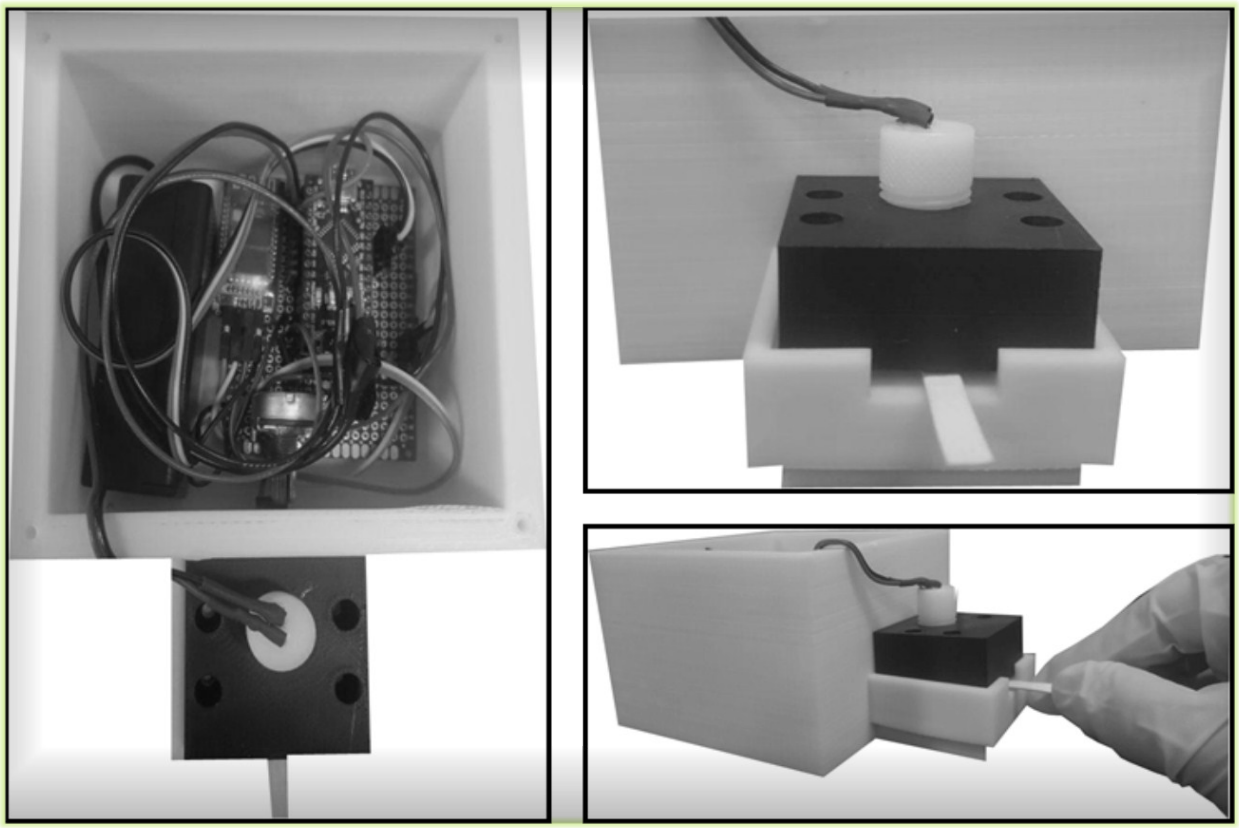


Fig. S1 POCT device images (front, side and top view)

Table S2 Comparison of the developed PtNPs based detection (colorimetric stimulated) with the other reported uric acid analysis

Biosensor composition	**Real sample**	**Sensor type**	**Linear Range**	**LOD**	**Ref.**
OD	urine	colorimetric	1.0 - 5.0 mM	0.15 mM	[1]
4-AAP/DHBS	urine	colorimetric	1.0 – 5.0 mM	0.3 mM	[2]
2-thiouracil (2-TU) tailored Au nanoparticles	serum	colorimetric	_	0.5 µM	[3]
Uricase/MIL-53(Fe)	urine	colorimetric	4.5 – 60 µM	1.3 µM	[4]
Uricase/HRP-CdS quantum dots	urine	colorimetriic	125 – 1000 µM	125 µM	[5]
TMB–Cu^{2+} uricase	urine	colorimetry	1 – 1000 µM	0.64 µM	[6]
Ag nanoprism/uricase	serum	colorimetry	1 – 40 µM	0.7 µM	[7]
TMB/g-C3N4 /uricase	Not reported	colorimetry	1 – 100 µM	8.9 µM	[8]
TCPO/H2O2/rubrenee	serum	chemiluminescence	10 – 1000 µM	5.0 µM	[9]
CdTe nanoparticles	serum	Fluorimetry	0.22 – 6 µM	0.1 µM	[10]
TMB+H2O2 and PtNPs	urine	colorimetric	0 – 8 mM	4.2±5 µM	This work

1.6. Effect of the amount of Pt nanoparticles and color variation of substrate:

In the figure below, shows the color intensity with the varying amount of PtNPs. A sample of uric acid concentration (8 mM) has used to check the variation of color change by changing the concentration of PtNPs. A series concentration of PtNPs ranging from 0 to 40 µl has been tested to check the color intensity of paper strip by introducing uric acid. A mixture of TMB and H_2O_2 (5:1 v/v) was prepared, 80 µL was added to the strip. Hence, it can be observed that a detectable color change for 40 µl is sufficient for the present purpose.

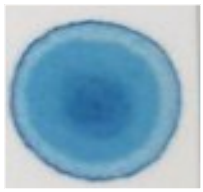
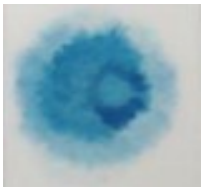
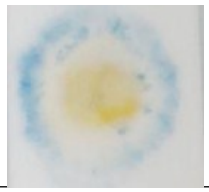
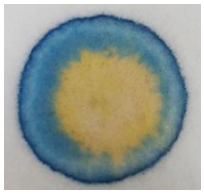
	0 µl
	10 µl
	20 µl
	30 µl
	40 µl

Fig. S2 Color variation of paper strips with changing the amount of PtNPs.

1.7. Effect of uric acid concentration vs. Simulation results:

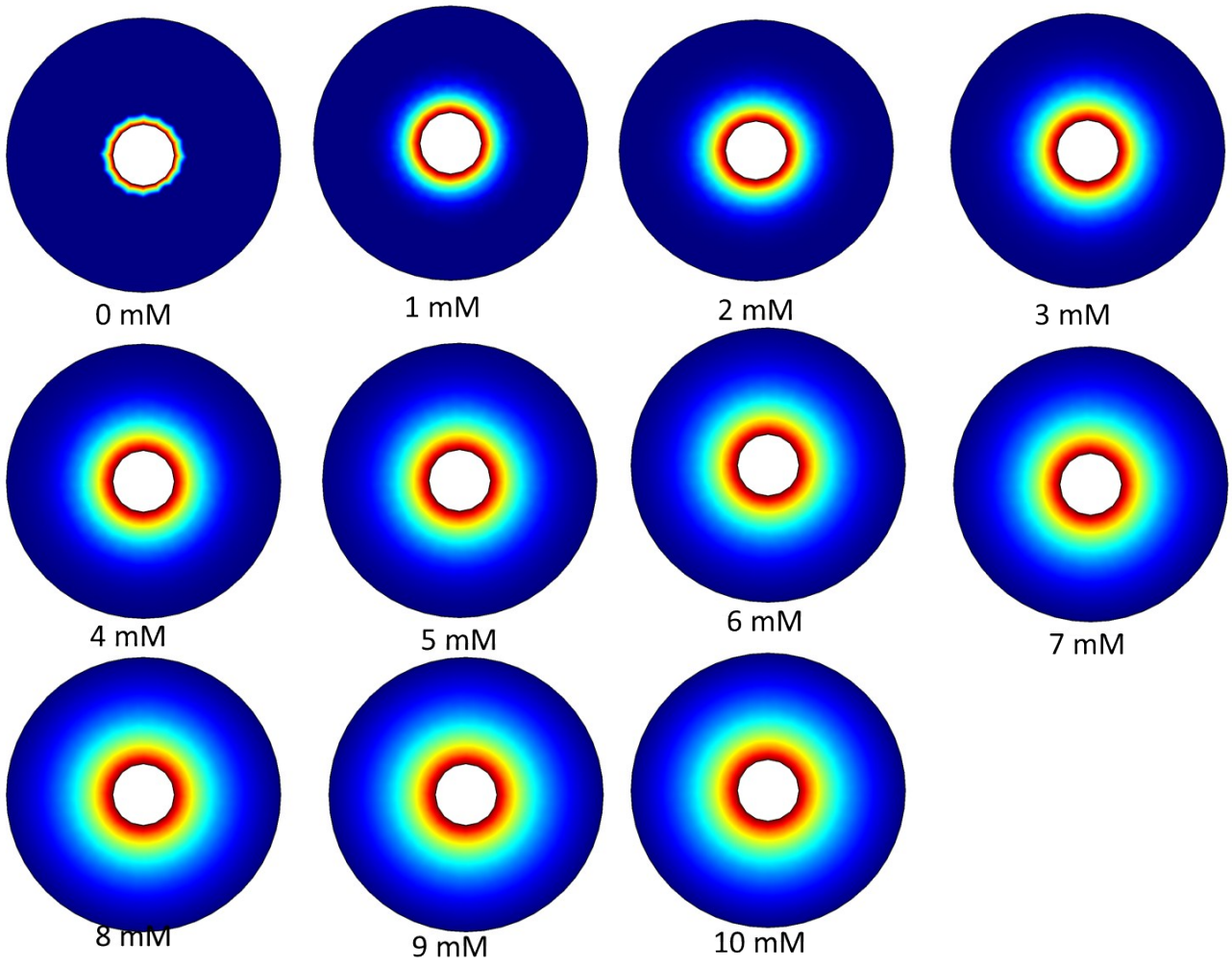


Fig. S3 This figure illustrates the simulation results of paper strips color by changing the concentration of uric acid ranging from 0 to 10 mM.

1.8. FESEM images of Paper substrate embedded PtNPs:

FESM images were taken of paper strips modified with PtNPs as sown below. PtNPs (8 – 10 nm) are tiny sized particles which cannot be located under this resolution so they were characterized by high resolution Transmission Electron Microscopy (TEM) as the image has shown in the manuscript.

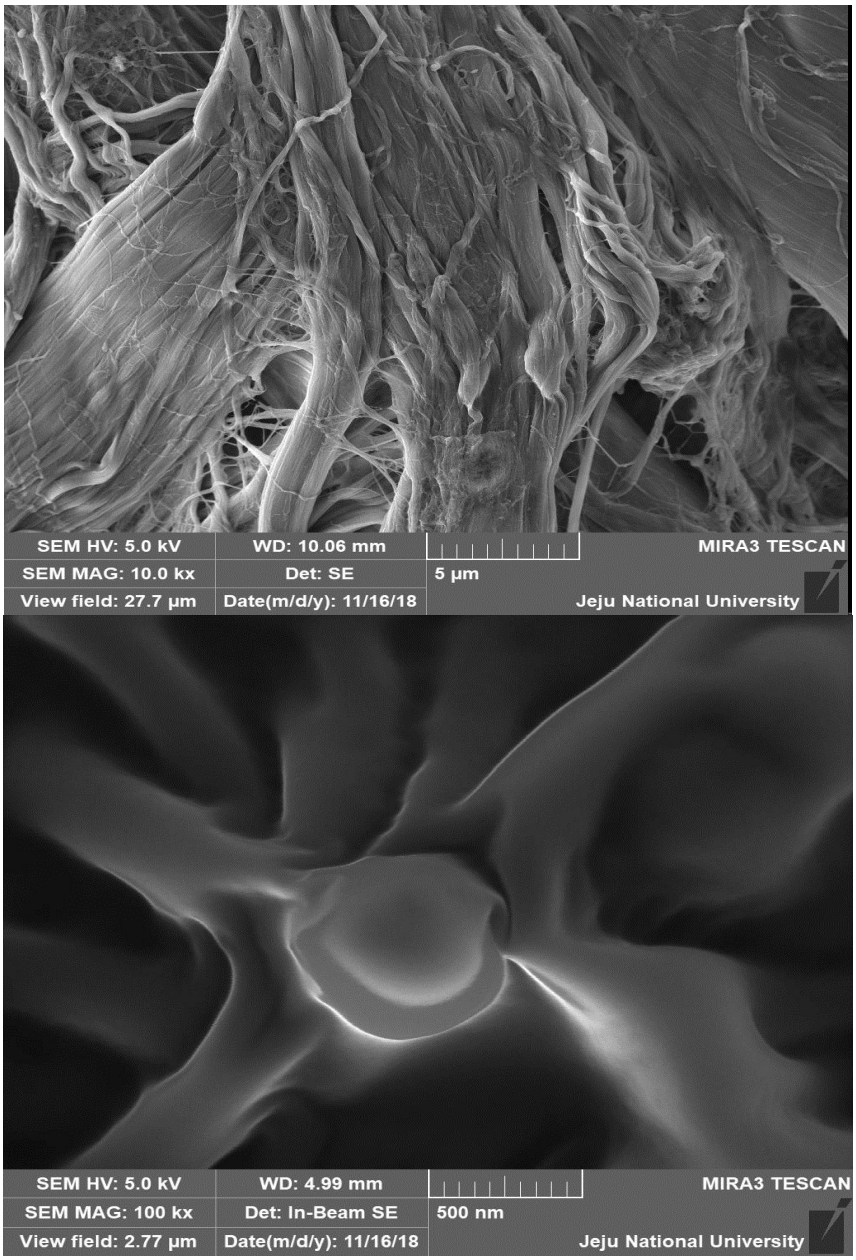


Fig. S4 FESEM images of paper substrate modified with PtNPs and (TMB and H_2O_2) mixture under the resolution of 5 μm and 500 nm

1.9. Reaction Kinetics:

To know the reaction kinetics, the Michaels constant (Km) values of these NPs have been analyzed. Km is an important parameter used to measure binding affinity of the enzyme for the substrate as shown in Fig. 5a and can also be used here to study the enzyme mimic–substrate interaction. The Km values of citrate capped–PtNPs were obtained from the Line weaver Burk plot. The apparent Km value of citrate–PtNPs with TMB as a substrate is 0.055 mM. The Michaels–Menten behavior of citrate capped–PtNPs with H_2O_2 as a substrate is illustrated in Figure 5b. The apparent Km value of citrate capped–PtNPs for H_2O_2 was 63 mM.

catalyst	**Substrate**	**K_m (mM)**	**V_{max} (mM sec^{-1})**
Citrate-capped PtNPs	TMB	0.055	0.00035
	H_2O_2	63	0.00032

2.0. XRD patterns of as synthesized Pt nano particles:

As shown in figure XRD pattern is showing 2θ values of 40, 46.9 and 67.5 which match perfectly with the (111), (200), and (220) crystalline planes respectively face centered cubic shape.

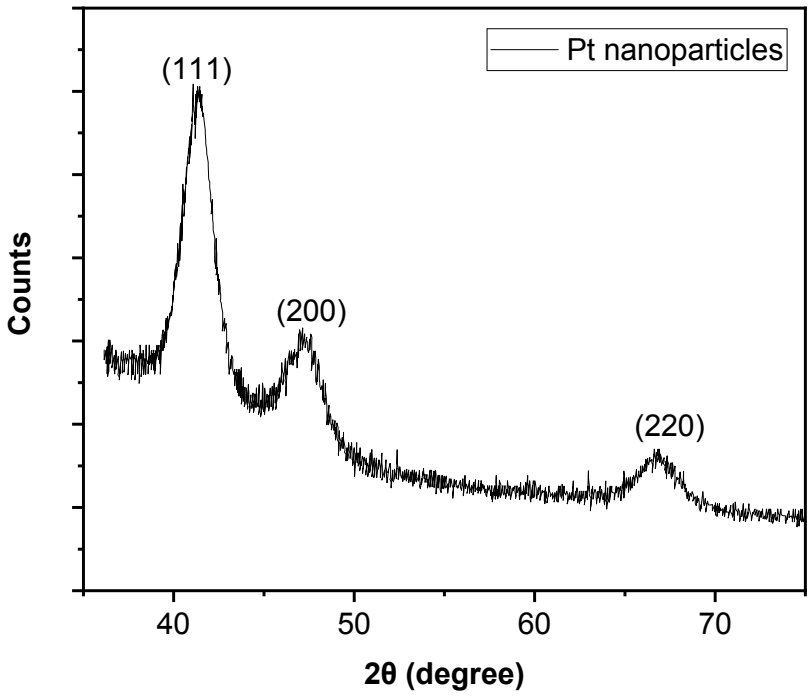


Fig. S5 XRD pattern of as synthesized Pt nanoparticles by the reduction method

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