

## Research Article

**Smartphone-based Portable Wireless Optical System for the Detection of Target Analytes**Shreedhar Gautam<sup>1</sup>,Bhagwan S Batule<sup>1</sup>,Hyo Yong Kim<sup>1</sup>,Ki Soo Park<sup>1</sup>,Hyun Gyu Park<sup>1</sup>

<sup>1</sup>Department of Chemical and Biomolecular Engineering (BK 21+ program), Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea

**Correspondence:** Prof. Hyun Gyu Park, Department of Chemical and Biomolecular Engineering (BK 21+ program), Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea.

**E-mail:** hgpark@kaist.ac.kr

**Keywords:** Smartphone; Multimeter; Colorimetric Detection; Biosensor; Melamine; Hydrogen Peroxide

**Abbreviations:** PAWS, Portable and Wireless Optical System; HRP, horse radish peroxidase; POCT, point-of-care testing; GPS, Global Positioning System; GNP, gold nanoparticles; TMB, 3,3',5,5'-tetramethylbenzidine; LDR, light dependent resistor; LED, light emitting diode; LOD, limit of detection.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/biot.201600581.

Submitted: 23-Sep-2016  
Revised: 22-Nov-2016  
Accepted: 30-Nov-2016

This article is protected by copyright. All rights reserved.

**Abstract**

Rapid and accurate on-site wireless measurement of hazardous molecules or biomarkers is one of the biggest challenges in nanobiotechnology. We herein describe a novel smartphone-based Portable and Wireless Optical System (PAWS) for rapid, quantitative, and on-site analysis of target analytes. As a proof-of-concept, we employed gold nanoparticles (GNP) and an enzyme, horse radish peroxidase (HRP), to generate colorimetric signals in response to two model target molecules, melamine and hydrogen peroxide, respectively. The colorimetric signal produced by the presence of the target molecules is converted to an electrical signal by the inbuilt electronic circuit of the device. The converted electrical signal is then measured wirelessly via multimeter in the smartphone which processes the data and displays the results, including the concentration of analytes and its significance. This handheld device has great potential as a programmable and miniaturized platform to achieve rapid and on-site detection of various analytes in a point-of-care testing (POCT) manner.

## 1 Introduction

Point-of-care testing (POCT) is defined as medical testing conducted near the location of a patient whenever medical care is necessary [1]. In recent years, POCT devices have attracted a great deal of attention because they facilitate near-patient testing, broaden convenient access to diagnostics, and provide immediate results with simple and cost-effective instrumentation [2, 3]. POCT systems are normally based on electrochemical [3, 4], optical [3, 5], thermal [6] or piezoelectric [7] detection. Among these techniques, optical platforms have been widely utilized due to their simplicity [8, 9], visual response capability [10, 11], accuracy [9], and high sensitivity [9]. Currently, various types of optical POCT devices are commercially available and most of the popular ones are being produced by leading companies such as Hach [12], LaMotte [12], Roche Diagnostics [12], and Abbott Diagnostics [3]. They are portable and comprised of cheaper components compared to bulky lab-based conventional spectrophotometers. However, the devices have several remaining drawbacks. First, they are still expensive. Second, they do not possess the advanced functionalities found in most portable smart devices, which routinely include computing power, wireless and internet connectivity, intuitive touch-based display and Global Positioning System (GPS) tagging, all of which can facilitate several key activities, such as analyzing the measured data and then informing relevant authorities of the results.

With the goal of overcoming the drawbacks of the aforementioned systems, many researchers are trying to develop POCT devices by integrating smartphones, which harness their advanced computational ability and communication functions. Most of the smartphone-integrated POCT devices include software that can analyze the data obtained from the sensing system. Representatively, Pelegris et al. [13] reported an application that checks the heartbeat of a user based on the color change of a finger placed on the smartphone's camera. Shen et al. developed a pH sensor utilizing a smartphone to take images of pH test strips [14]. Recently, Ozcan et al. developed a novel strategy to detect mercury ions [15] by analyzing a photo captured by the smartphone camera; similar strategies have been presented by S.K Vashist et al. and A. Garcia et al. for the detection of c-reactive protein (CRP) [16] and potassium [17], respectively. Priye et al. developed a smartphone-based platform to detect nucleic acids that can be deployed for the inflight detection [18]. The core of their strategies

involves a target-specific colorimetric reaction followed by quantification of the resulting color by analyzing a photo of the solution. These strategies, unlike conventional techniques, are applicable to POCT instrumentation to achieve on-site detection. However, these approaches have limitations in terms of repeatability and detection sensitivity, both of which depend on undesirable variations in ambient light conditions (e.g., day Vs night), alignment and/or user operation (angle of taking photo) while capturing the photo. Moreover, their detection results vary according to the smartphone chosen because the detection accuracy depends on the quality of the smartphone camera (e.g., 2 megapixels versus 10 megapixels).

We herein report a lightweight (~350 g), standalone, cost-effective, field-portable, wireless and smartphone-based device for the sensitive and quantitative on-site detection, taking melamine and hydrogen peroxide as the proof-of-concept target analytes by promoting the colorimetric reactions of the respective colorimetric substrates, gold nanoparticles (GNP) and 3,3',5,5'-tetramethylbenzidine (TMB). The smartphone is installed with a custom-modified application that analyzes and displays the detection result and its significance.

## **2. Materials and methods**

### **2.1 Construction of PAWS**

To construct the PAWS circuit, a transistor (MPS 2222a), LDR (light dependent resistor) (GL55), LED (light emitting diode) (3 mm, White), resistors (EPX34LM4 and EPX34LNG), battery (AAA), capacitor (100  $\mu$ F), and red laser pointer (emission at 650 nm) were purchased from eLePARTS, South Korea. The wireless multimeter (Mooshimeter, Bluetooth: 4.0) was purchased from Mooshim Engineering. The LED works as an indicator to check the functioning of the circuit. The wireless multimeter was

connected in parallel to the LED to measure the changes in voltage drop according to the resistance of the LDR. Darlington transistors were employed to amplify the current from the LDR, and batteries (6 V) were the power source of the circuit. The capacitor was connected parallel to the power source to reduce voltage fluctuations in the circuit. The multimeter was connected to the circuit, which measures the voltage wirelessly and sends the signal to the smartphone. The smartphone (Samsung Galaxy S4 mini) detects the wireless signal and displays the voltage and measurement details. Disposable cuvettes were used to load samples and a red laser pointer was used as the light source. The laser is placed ~10 mm away from the cuvette and the cuvette is ~16 mm away from LDR. The sizes of the cuvette, multimeter, circuit and battery are approximately 10 mm × 10 mm, 105 mm × 40 mm, 25 mm × 25 mm, and 50 mm × 25 mm, respectively.

We modified the application of the multimeter to detect the target analytes. We first measured the voltage change in response to the concentration of the analyte, which was then used to plot a standard curve of voltage change versus concentration of the analyte. Finally, we calculated the equation correlating the analyte concentration with the voltage change, which was saved in the application. Based on the equation of the curve, the application automatically calculated the unknown concentration of target analytes from the measured voltage. In order to detect other analytes, standard curves of the analytes can be constructed and stored in the application. When a specific target analyte under measurement is selected, it refers to the corresponding curve and calculates the unknown concentration. The equations for standard curves used to quantify melamine and hydrogen peroxide were  $y = -0.0034x + 1.9562$  and  $y = -0.0042x + 1.9213$ , respectively, where “x” is the concentration of target analyte and “y” is the voltage measured by PAWS. We used these equations to calculate the unknown concentrations of melamine and hydrogen peroxide. We further programmed the application to show the significance of the measured concentrations of the analytes, so that users can determine whether the milk is edible, inedible or to be reported to the relevant authorities.

## 2.2 GNP-based melamine detection

GNP was synthesized based on the procedure of Katherine et al. with slight modifications [19]. Fifty  $\mu\text{L}$  of  $\text{HAuCl}_4$  (1 mM) was prepared in a flask, which was then stirred continuously until boiling. 5 mL of sodium citrate (38.8 mM) was added to the boiling solution resulting in a color change from pale yellow to blue and slowly to red. Boiling was continued for 7 minutes; the hot plate was removed but stirring was maintained for 15 more minutes. Finally, GNP with 13 nm in diameter was prepared and used for the detection of melamine in milk.

Milk and melamine were bought from the local supermarket in Daejeon, South Korea, and Sigma Aldrich, respectively. Melamine was spiked in the whole milk followed by dilution with milk to prepare the different concentrations of melamine in milk. Melamine-spiked milk was then centrifuged down in Millipore Amplicon centrifugal tubes (3K) for 10 minutes at 14,000 rpm. The filtrate was mixed with the GNP solution in a ratio of 1:10 (vol/vol) and incubated for 10 minutes to promote the colorimetric reaction, followed by 3 times dilution with distilled water, followed by the absorbance measurement. Absorbance was measured by Tecan Infinite 200 Pro and PAWS.

## 2.3 TMB-based hydrogen peroxide detection

HRP (P8735) and TMB were bought from Sigma Aldrich. Typically, 20  $\mu\text{L}$  of TMB (5 mM), 10  $\mu\text{L}$  of HRP (1 U/mL), and 50  $\mu\text{L}$  of hydrogen peroxide spiked with different concentrations in lake water were added into 20  $\mu\text{L}$  of Tris-acetate (25 mM, pH 4). Then, the final mixture was incubated at 40 °C for 30 minutes and the measurement was done via PAWS and UV-Vis spectrophotometer.

### 3 Results

#### 3.1 Design and working principle of PAWS

The PAWS system consists of four major components (Fig. 1a): a red laser pointer as a light source, cuvette holder to hold the sample, a circuit including LDR to convert the optical signal to an electrical signal and wireless multimeter to send the electrical signal to the smartphone. The light from the laser pointer first passes through the sample and a certain amount of the light is absorbed by the sample, depending on the concentration of the target analyte. The target analyte with higher concentration would lead to higher absorption of light, reducing its intensity. After absorption, this reduced intensity of light then hits the LDR in the circuit, whose resistance is inversely proportional to the intensity of the light (Fig. 1b). The small round shaped LDR has a high spectral response at a wavelength similar to that of red light from a laser pointer (Fig. S1). Next, the current from the LDR passes through an amplifier and is amplified exponentially, consequently increasing the voltage in the circuit (Fig. 1b). Thus, the small change in the intensity of the incoming light leads to a large change in voltage, which achieves the enhanced sensitivity of our system. We employed an advanced multimeter, which is capable of wirelessly measuring the voltage and displays in the smartphone (Fig. 1b). The custom-modified application installed in the smartphone finally utilizes the voltage signal, analyzes it, and displays the measurement results on the screen.

As a proof of concept, GNP and TMB were employed as colorimetric substrates to detect two model targets, melamine and hydrogen peroxide, respectively. We exploited the feature that GNP, which is originally red in color, becomes blue (absorbance around 625 nm) [20] in the presence of melamine, and TMB forms oxidized TMB (oxy-TMB) with absorbance around 650 nm by HRP in the presence of hydrogen peroxide. Therefore, a red laser pointer was employed as a light source which yielded red light at the wavelength analogous to the absorption wavelengths of the two substrates.

In our prototype, all the components were combined in an opaque cuboidal box as shown in Fig. 1a, which is drawn to approximately the same scale as the real setup (165 mm × 115 mm, Fig. 1c). The size and weight of the system can be further reduced by using button cells and a smaller cuvette, and

arranging the components more compactly. The device has a lid (Fig. 1a) on top of the cuvette holder. Closing the lid is very crucial during the measurement because the resistance of the LDR will be reduced by stray light, which might lead to incorrect results. In this work, we have used a Samsung Galaxy 4 mini (Fig. 1c); however, other phones can be also used for the analysis and display of the data. Of note, a smartphone can easily detect the wireless signal from up to 50 meters away, which eases the detection process.

### **3.2 Android-based smartphone application for the analysis and display of the data**

We developed a custom-designed android application which analyzes the voltage measured in response to the analyte concentration, and displays the results. The android application can be installed on smartphones available after mid 2013 (we checked that the Samsung Galaxy S4 mini, S4, S3, and Note 3 could be employed for our system.). After installation, the smartphone needs to be connected to the system via Bluetooth network by pressing the “Connect” button on the starting screen (Fig. 2a).

In order to detect a target analyte, users put a sample into a cuvette in the cuvette holder, close the lid, and run the application. Pressing the “Detection” button on the main screen of the application (Fig. 2b) will show the voltage obtained from the circuit and direct the application to the next page (Fig. 2c), where users are supposed to select the target analyte. Since “Melamine” and “Hydrogen Peroxide” were employed as the model analytes in this study, the screen shows these two corresponding buttons (Fig. 2c). Finally, the application will calculate the concentration of the target analyte by using a previously uploaded equation, and display the determined concentration together with the proper instructions (Fig. 2d and 2e). The instructions will instruct users about the significance of the results by informing them, for example, of the desired action for that particular analyte concentration.

### 3.3 GNP-utilized detection of melamine

In order to prove the detecting capability of PAWS, melamine was first employed as a target analyte. Melamine is an organic compound widely used in polymer industries; however, it is illegally added to adulterate protein rich foods due to its high nitrogen content (66% nitrogen by mass [21]). It is estimated that more than 50,000 babies and 300,000 victims were hospitalized and hundreds of pets died of renal failure by consuming melamine-adulterated milk products in 2007 and 2008 [21]. Until now, various techniques such as capillary electrophoresis [22], gas/mass chromatography [23], ionization mass spectroscopy [24], electrochemical methods [25], ELISA-based methods [26], and colorimetric sensors [27] have been reported to identify the presence of melamine, but none of them provides miniaturized and on-site sensing interfaced with wireless connectivity.

To achieve a novel system capable of the on-site sensing of melamine, coupled with an intuitive touch-based display and wireless connectivity, GNP was employed as a colorimetric substrate. GNP has unique colorimetric properties which can tune by particle size; it is originally red in color, but changes to blue after aggregation. This property of GNP has been intensively studied [28-32], and utilized in various colorimetric detection systems including those for DNA [33-36], proteins [33, 37], small molecules [33, 38], heavy metal ions [33, 39-41], hazardous materials [42, 43], disease biomarkers [44], bacteria [45], and cancerous cells [46]. Melamine has electron rich amine and ring nitrogen groups that bind onto the electron deficient surfaces of metal nanoparticles [47, 48]. The three exocyclic amine groups within a melamine molecule can bind to three GNPs at the same time and crosslink them, consequently inducing the aggregation of GNPs (Fig. 3a) [49].

In this study, we prepared GNP with about 13 nm particle diameters (Fig. S2), which exhibited little variation in size, by  $\pm 2$  nm, and a narrow absorbance peak at 520 nm, and then incubated the GNP with milk spiked by melamine. As a result, we observed the color change from wine red to purple or blue depending on the concentration of melamine (Fig. 3b). Since the target samples were melamine-adulterated milk products, it was possible that the results might be affected by interfering compounds in the milk, including amino acids such as glycine and L-alanine, small molecules such as glucose, and salts such as  $\text{Na}^+$  and  $\text{K}^+$ . Therefore, we examined the selectivity of our system by employing 100 fold

higher concentrations of these compounds compared to melamine. As a result, as shown in Fig. 4, a quite significant signal was observed only from the presence of the target melamine, which confirms the excellent selectivity of our system.

We next checked the sensitivity of our system. The limit of detection (LOD) for the detection of melamine in whole milk was determined to be 6.12  $\mu\text{M}$ , and the linear range was observed from 10 to 90  $\mu\text{M}$  (Fig. 5a). According to the Food and Drug Administration of United States and the European Union, the safety limit of melamine in milk and dairy products is 20  $\mu\text{M}$  [49], which is much higher than our LOD, and lies within the linear range of our system. Therefore, PAWS could actually be employed for the on-site quantification of melamine in whole milk.

We next evaluated the detection precision of our system by analyzing three independent milk samples spiked with melamine. The recovery rates were in the range from 96.18% to 106.4% and the CVs of all datasets were less than 7.05%, indicating the high precision of our system (Table 1a).

We also examined the performance of a typical large benchtop UV-Vis spectrophotometer, a Tecan Infinite 200 Pro, and compared it with that of our system (Fig. 5a). The absorbance observed from the benchtop system showed almost the same pattern and linear range from 10 to 90  $\mu\text{M}$  (Fig. 5a), and its LOD was determined to be 3.24  $\mu\text{M}$  (Fig. 5a) which is comparable to that of our system. These results confirm that our system is as sensitive and accurate as the commercially dominant benchtop spectrophotometers.

### 3.4 TMB-utilized detection of hydrogen peroxide

To further demonstrate the detecting capability of our system, we applied the PAWS system to the detection of our second model analyte, hydrogen peroxide, by employing TMB as a colorimetric substrate. TMB forms a stable and insoluble blue colored product after oxidation (Fig. 3c). Since hydrogen peroxide is produced as a byproduct of various metabolic and enzymatic reactions including the signal transduction pathways [50] and oxidative reactions[51-53], it's detection could be used to monitor these reactions and identify the corresponding substrate molecules, such as glucose [54], galactose [55], choline [56], and acetylcholine [57]. We examined the sensitivity of PAWS for the

detection of hydrogen peroxide in the same manner as the first model analyte, melamine, and the LOD was determined to be 0.70  $\mu\text{M}$  (Fig. 5b), which is even slightly lower than that of a standard benchtop UV-Vis spectrophotometer (0.81  $\mu\text{M}$ , Fig. 5b and Fig. 3d). All the absorbance measured by PAWS were quite consistent with those obtained with the benchtop spectrophotometer. The recovery rates were in the range from 100.52% to 102.27% and the CVs of all the datasets were less than 1.85%, indicating the high precision of our system (Table 1b).

### 3.5 Stability of PAWS

For our system to be actually applied for the on-site detection of target analytes, it should be stable enough for a certain period of time and reproduce results consistently. Our system employs a battery, and measures the circuit's voltage to quantify the analyte. Since a battery has a certain lifetime and voltage might fluctuate during the measurement, it is necessary to confirm the stability of the device when measuring the circuit's voltage. We examined the stability of our device and confirmed that the circuit's voltage, measured by PAWS, was quite consistent for up to two months (Fig. S3a) and that the time dependent fluctuation of the voltage was negligible during the measurement (Fig. S3b, green number on the right), assuming that clicking the button does not take more than 90 seconds.

## 4 Discussion

In summary, we have developed a simple, portable, cost effective and smartphone-based PAWS platform capable of wirelessly sending the electrical signal resulting from the colorimetric assay to a smartphone, which finally quantifies the amount of target analytes. We demonstrated the capability and utility of our PAWS prototype by successfully detecting melamine spiked in milk and hydrogen peroxide spiked in lake water, and demonstrated the PAWS prototype was as sensitive as a bulky, expensive and highly advanced benchtop spectrophotometer. Notably, PAWS is very cheap compared with the currently available representative portable optical devices and the typical benchtop spectrophotometer, Tecan Infinite 200 Pro (Table S1), and its measurement is very fast (within a few seconds).

Our system can be further improved with advanced system hardware and software. Our ongoing endeavour to enhance the system's detection sensitivity includes utilization of a photodiodes array, and optimization of amplification with respect to voltage for an enhanced signal. Moreover, our device can be also extended to quantify other target molecules, including hazardous molecules [58], proteins [58], bacteria [58], and cancerous cells [59] by labelling the GNP with target-specific ligands [58] or by employing a laser with a different wavelength and the corresponding LDR.

PAWS is an excellent example of building a new system by utilizing pre-existing individual technical components, including multimeter-based measurement and wireless signal transfer to the smartphone, and the computing and communication capability of the smartphone. By taking advantage of its computational capability and internet connectivity, our device could be used to detect hazardous materials and dangerous pathogens while simultaneously conducting their spatial mapping. Furthermore, PAWS could be also applied to continuously monitor harmful chemicals and pathogens found in different parts of the world, and to upload that information to the internet to warn people.

In fact, mobile phone subscriptions by 2013 reached more than 7 billion worldwide and smartphone penetration was expected to be more than 60%, 45%, and 25% in North America, Europe, and Africa, respectively, at the end of 2015 [15]. Therefore, smartphone-based bioanalytical applications can be widely deployed and used quite effectively even in resource-limited regions, including third world countries, where on-site measurement of hazardous materials or food additives is very important because of deep-rooted problems such as famine and lack of clean drinking water. To date, in these places, samples such as water or food need to be sent away to a laboratory in order to verify their purity, despite the immediate necessity. Therefore, we believe that the PAWS system could be widely utilized for the on-site sensing of various target analytes and serve as a good model to construct a novel POCT device capable of computational works and communication functions.

**Acknowledgements**

*The financial support was provided by Basic Science Research Program through the NRF funded by the Ministry of Education [No. 2015R1A2A1A01005393]. This research was also supported by the Bio-Synergy Research Project [NRF-2015M3A9C4070484] of the Ministry of Science, ICT and Future Planning through the National Research Foundation.*

**Conflict of interest**

*The authors declare no financial or commercial conflict of interest.*

## 5 References

- [1] Kost, G. J. Guidelines for point-of-care testing. improving patient outcomes. *Am. J. Clin. Pathol.* 1995, *104*, 111-127.
- [2] Lippa, P. B., Muller, C., Schlichtiger, A., Schlebusch, H. Point-of-care testing (POCT): current techniques and future perspectives. *Trends Anal. Chem.* 2011, *30*, 887-898.
- [3] Vashist, S. K., Lippa, P. B., Yeo, L. Y., Ozcan, A., Luong, J. H. Emerging technologies for next-generation point-of-care testing. *Trends Anal. Chem.* 2015, *33*, 692-705.
- [4] Lu, J., Ge, S., Ge, L., Yan, M., Yu, J. Electrochemical dna sensor based on three-dimensional folding paper device for specific and sensitive point-of-care testing. *Electrochim. Acta.* 2012, *80*, 334-341.
- [5] Baldini, F., Bolzoni, L., Giannetti, A., Kess, M., et al., A new procalcitonin optical immunosensor for poct applications. *Anal. Bioanal. Chem.* 2009, *393*, 1183-1190.
- [6] Louie, R. F., Sumner, S. L., Belcher, S., Mathew, R., et al., Thermal stress and point-of-care testing performance: suitability of glucose test strips and blood gas cartridges for disaster response. *Disaster Med. Public Health Prep.* 2009, *3*, 13-17.
- [7] Jeong-Beom, I., Fu-Kuo, C. Detection and monitoring of hidden fatigue crack growth using a built-in piezoelectric sensor/actuator network: i. diagnostics. *Smart Mater. Struct.* 2004, *13*, 609.
- [8] Jung, Y. L., Jung, C., Parab, H., Cho, D. Y., Park, H. G. Colorimetric snp genotyping based on allele-specific pcr by using a thiol-labeled primer. *ChemBioChem.* 2011, *12*, 1387-1390.
- [9] Kim, H. N., Ren, W. X., Kim, J. S., Yoon, J. Fluorescent and colorimetric sensors for detection of lead, cadmium, and mercury ions. *Chem. Soc. Rev.* 2012, *41*, 3210-3244.
- [10] Park, K. S., Kim, M. I., Cho, D. Y., Park, H. G. Label-free colorimetric detection of nucleic acids based on target-induced shielding against the peroxidase-mimicking activity of magnetic nanoparticles. *Small* 2011, *7*, 1521-1525.
- [11] Suksai, C., Tuntulani, T. Chromogenic anion sensors. *Chem. Soc. Rev.* 2003, *32*, 192-202.

- [12] Schaefer Samuel. Colorimetric water quality sensing with mobile smartphones. (Masters dissertation) 2014, Retrieved from University of British Columbia library database.
- [13] Pelegris, P., Banitsas, K., Orbach, T., Marias, K. In a novel method to detect heart beat rate using a mobile phone. *Proc. Conf. IEEE Eng. Eng. Med. Biol. Soc.* 2010, 5488-5491.
- [14] Shen, L., Hagen, J. A., Papautsky, I. Point-of-care colorimetric detection with a smartphone. *Lab Chip* 2012, 12, 4240-4243.
- [15] Wei, Q., Nagi, R., Sadeghi, K., Feng, S., et al., Detection and spatial mapping of mercury contamination in water samples using a smart-phone. *ACS nano* 2014, 8, 1121-1129.
- [16] Vashist, S. K., van Oordt, T., Schneider, E. M., Zengerle, R., et al., A smartphone-based colorimetric reader for bioanalytical applications using the screen-based bottom illumination provided by gadgets. *Biosens. Bioelectron.* 2014, 67, 248-255.
- [17] García, A., Erenas, M. M., Marinetto, E. D., Abad, et al., Mobile phone platform as portable chemical analyzer. *Sens. Actuator B-Chem.* 2011, 156, 350-359.
- [18] Priye A, Wong S, Bi Y, Carpio M., et al., *Anal Chem.* 2016, 88, 4651-4660.
- [19] Grabar, K. C., Freeman, R. G., Hommer, M. B., Natan, M. J. Preparation and characterization of au colloid monolayers. *Anal. Chem.* 1995, 67, 735-743.
- [20] Hazarika, P., Ceyhan, B., Niemeyer, C. M. Reversible switching of dna-gold nanoparticle aggregation. *Angew. Chem. Int. Ed.* 2004, 116, 6631-6633.
- [21] Chan, E. Y. Y., Griffiths, S. M., Chan, C. W. Public-health risks of melamine in milk products. *Lancet* 2008, 372, 1444-1445.
- [22] Li, B., Du, Y., Li, T., Dong, S. Investigation of 3, 3', 5, 5'-tetramethylbenzidine as colorimetric substrate for a peroxidatic dnazyme. *Anal. Chim. Acta.* 2009, 651, 234-240.
- [23] Xu, X.-m., Ren, Y.-p., Zhu, Y., Cai, Z.-x., et al., Direct determination of melamine in dairy products by gas chromatography/mass spectrometry with coupled column separation. *Anal. Chim. Acta.* 2009, 650, 39-43.

- [24] Zhu, L., Gamez, G., Chen, H., Chingin, K., Zenobi, R. Rapid detection of melamine in untreated milk and wheat gluten by ultrasound-assisted extractive electrospray ionization mass spectrometry (EESI-MS). *Chem. Commun.* 2009, 5, 559-561.
- [25] Cao, Q., Zhao, H., Zeng, L., Wang, J., et al., Electrochemical determination of melamine using oligonucleotides modified gold electrodes. *Talanta* 2009, 80, 484-488.
- [26] Yin, W., Liu, J., Zhang, T., Li, W., et al., Preparation of monoclonal antibody for melamine and development of an indirect competitive elisa for melamine detection in raw milk, milk powder, and animal feeds. *J. Agri. Food. Chem.* 2010, 58, 8152-8157.
- [27] Liang, X., Wei, H., Cui, Z., Deng, J., et al., Colorimetric detection of melamine in complex matrices based on cysteamine-modified gold nanoparticles. *Analyst* 2011, 136, 179-183.
- [28] Félidj, N., Aubard, J., Lévi, G. Discrete dipole approximation for ultraviolet-visible extinction spectra simulation of silver and gold colloids. *J. Chem. Phys.* 1999, 111, 1195-1208.
- [29] Yang, W. H., Schatz, G. C., Van Duyne, R. P. Discrete dipole approximation for calculating extinction and raman intensities for small particles with arbitrary shapes. *J. Chem. Phys.* 1995, 103, 869-875.
- [30] Storhoff, J. J., Lazarides, A. A., Mucic, R. C., Mirkin, et al., What controls the optical properties of dna-linked gold nanoparticle assemblies? *J. Am. Chem. Soc.* 2000, 122, 4640-4650.
- [31] Lytton-Jean, A. K., Mirkin, C. A. A thermodynamic investigation into the binding properties of dna functionalized gold nanoparticle probes and molecular fluorophore probes. *J. Am. Chem. Soc.* 2005, 127, 12754-12755.
- [32] Novak, J. P., Brousseau, L. C., Vance, F. W., Johnson, R. C., et al., Nonlinear optical properties of molecularly bridged gold nanoparticle arrays. *J. Am. Chem. Soc.* 2000, 122, 12029-12030.
- [33] Xia, F., Zuo, X., Yang, R., Xiao, Y., et al., Colorimetric detection of dna, small molecules, proteins, and ions using unmodified gold nanoparticles and conjugated polyelectrolytes. *Proc. Natl. Acad. Sci. USA* 107, 10837-10841
- [34] Cao, Y. C., Jin, R., Thaxton, C. S., Mirkin, C. A. A two-color-change, nanoparticle-based method for dna detection. *Talanta* 2005, 67, 449-455.

- [35] Elghanian, R., Storhoff, J. J., Mucic, R. C., Letsinger, R. L., Mirkin, C. A. Selective colorimetric detection of polynucleotides based on the distance-dependent optical properties of gold nanoparticles. *Science* 1997, 277, 1078-1081.
- [36] Li, H., Rothberg, L. Colorimetric detection of dna sequences based on electrostatic interactions with unmodified gold nanoparticles. *Proc. Natl. Acad. Sci. USA* 2004, 101, 14036-14039.
- [37] Wei, H., Li, B., Li, J., Wang, E., Dong, S. Simple and sensitive aptamer-based colorimetric sensing of protein using unmodified gold nanoparticle probes. *Chem. Commun.* 2007, 3735-3737.
- [38] Lee, J. S., Ulmann, P. A., Han, M. S., Mirkin, C. A. A DNA-gold nanoparticle-based colorimetric competition assay for the detection of cysteine. *Nano Lett.* 2008, 8, 529-533.
- [39] Hung, Y. L., Hsiung, T. M., Chen, Y. Y., Huang, Y. F., Huang, C. C. Colorimetric detection of heavy metal ions using label-free gold nanoparticles and alkanethiols. *Phys. Chem. C.* 2010, 114, 16329-16334.
- [40] Xu, X., Daniel, W. L., Wei, W., Mirkin, C. A. Colorimetric  $\text{Cu}^{2+}$  detection using DNA-modified gold-nanoparticle aggregates as probes and click chemistry. *Small* 2010, 6, 623-626.
- [41] Xue, X., Wang, F., Liu, X. One-step, room temperature, colorimetric detection of mercury ( $\text{Hg}^{2+}$ ) using DNA/nanoparticle conjugates. *J. Am. Chem. Soc.* 2008, 130, 3244-3245.
- [42] Ai, K., Liu, Y., Lu, L. Hydrogen-bonding recognition-induced color change of gold nanoparticles for visual detection of melamine in raw milk and infant formula. *J. Am. Chem. Soc.* 2009, 131, 9496-9497.
- [43] Daniel, W. L., Han, M. S., Lee, J.-S., Mirkin, C. A. Colorimetric nitrite and nitrate detection with gold nanoparticle probes and kinetic end points. *J. Am. Chem. Soc.* 2009, 131, 6362-6363.
- [44] Neely, A., Perry, C., Varisli, B., Singh, A. K., et al., Ultrasensitive and highly selective detection of alzheimer's disease biomarker using two-photon rayleigh scattering properties of gold nanoparticle. *Acs Nano* 2009, 3, 2834-2840.
- [45] Baptista, P. V., Koziol-Montewka, M., Paluch-Oles, J., Doria, G., Franco, R. Gold-Nanoparticle-Probe-Based Assay for Rapid and Direct Detection of Mycobacterium Tuberculosis DNA in Clinical Samples. *Clin. Chem.* 2006, 52, 1433-1434.

- [46] Medley, C. D., Smith, J. E., Tang, Z., Wu, Y., et al., Gold nanoparticle-based colorimetric assay for the direct detection of cancerous cells. *Anal. Chem.* 2008, *80*, 1067-1072.
- [47] Braun, G., Pavel, I., Morrill, A. R., Seferos, D. S., et al., Chemically patterned microspheres for controlled nanoparticle assembly in the construction of sers hot spots. *J. Am. Chem. Soc.* 2007, *129*, 7760-7761.
- [48] Chi, H., Liu, B., Guan, G., Zhang, Z., Han, M.-Y. A simple, reliable and sensitive colorimetric visualization of melamine in milk by unmodified gold nanoparticles. *Analyst* 2010, *135*, 1070-1075.
- [49] Guo, L., Zhong, J., Wu, J., Fu, F., et al., Visual detection of melamine in milk products by label-free gold nanoparticles. *Talanta* 2010, *82*, 1654-1658.
- [50] Giorgio, M., Trinei, M., Migliaccio, E., Pelicci, P. G. Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals? *Nat. Rev. Mol. Cell Biol.* 2007, *8*, 722-728.
- [51] Jv, Y., Li, B., Cao, R. Positively-charged gold nanoparticles as peroxidase mimic and their application in hydrogen peroxide and glucose detection. *Chem. Commun.* 2010, *46*, 8017-8019.
- [52] Okuda, T., Matsuda, Y., Yamanaka, A., Sagisaka, S. Abrupt increase in the level of hydrogen peroxide in leaves of winter wheat is caused by cold treatment. *Plant Physiol.* 1991, *97*, 1265-1267.
- [53] Räisänen, S. R., Lehenkari, P., Tasanen, M., Rahkila, P., et al., Carbonic anhydrase III protects cells from hydrogen peroxide-induced apoptosis. *FASEB J.* 1999, *13*, 513-522.
- [54] Su, L., Feng, J., Zhou, X., Ren, C., et al., Colorimetric detection of urine glucose based  $\text{ZnFe}_2\text{O}_4$  magnetic nanoparticles. *Anal. Chem.* 2012, *84*, 5753-5758.
- [55] Kim, M. I., Shim, J., Li, T., Woo, M.-A., et al., Colorimetric quantification of galactose using a nanostructured multi-catalyst system entrapping galactose oxidase and magnetic nanoparticles as peroxidase mimetics. *Analyst* 2012, *137*, 1137-1143.
- [56] Song, Z., Huang, J.-D., Wu, B.-Y., Shi, H.-B., et al., Amperometric aqueous sol-gel biosensor for low-potential stable choline detection at multi-wall carbon nanotube modified platinum electrode. *Sens. Actuator B-Chem.* 2006, *115*, 626-633.

- [57] Godoy, S., Leca-Bouvier, B., Boullanger, P., Blum, L. J., Girard-Egrot, A. P. Electrochemiluminescent detection of acetylcholine using acetylcholinesterase immobilized in a biomimetic langmuir-blodgett nanostructure. *Sens. Actuator B-Chem.* 2005, *107*, 82-87.
- [58] Jans, H., Huo, Q. Gold nanoparticle-enabled biological and chemical detection and analysis. *Chem. Soc. Rev.* 2012, *41*, 2849-2866.
- [59] Lu, W., Arumugam, S. R., Senapati, D., Singh, et al., Multifunctional oval-shaped gold-nanoparticle-based selective detection of breast cancer cells using simple colorimetric and highly sensitive two-photon scattering assay. *ACS Nano* 2010, *4*, 1739-1749.

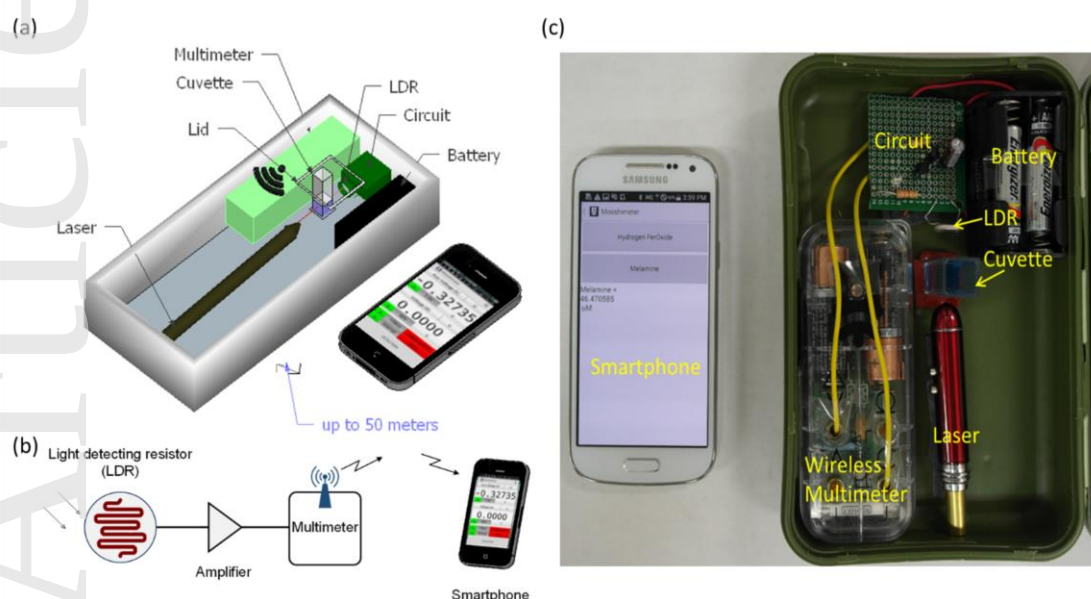
**Table 1.** Detection precision of PAWS for (a) GNP-based detection of melamine in whole milk and (b) TMB-based detection of hydrogen peroxide in lake water. Recovery (%) = measured value/expected value  $\times$  100.

(a)

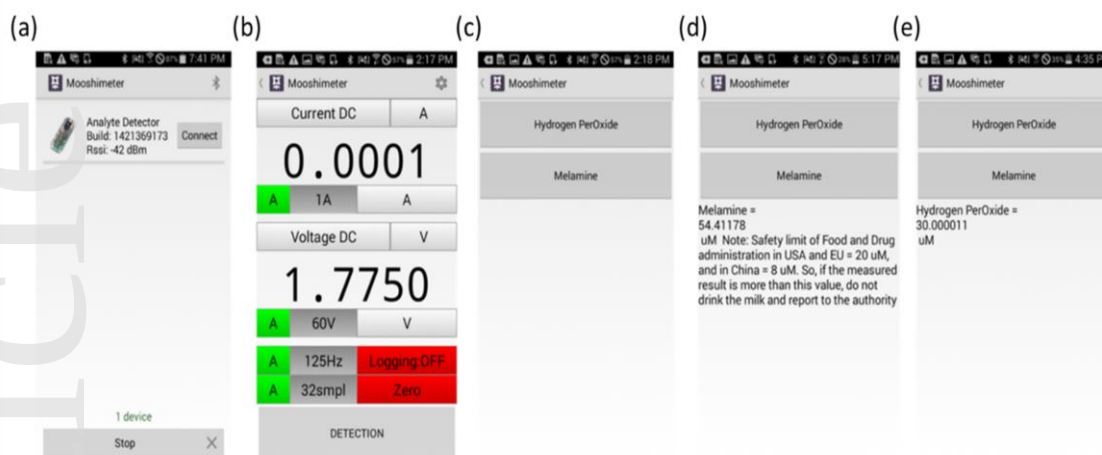
| Sample | Melamine added ( $\mu\text{M}$ ) | Melamine recovered ( $\mu\text{M}$ ) | CV (%) | Recovery (%) |
|--------|----------------------------------|--------------------------------------|--------|--------------|
| 1      | 0                                | 0                                    | -      | -            |
| 2      | 25                               | $25.5 \pm 1.4$                       | 5.40   | 102.1        |
| 3      | 40                               | $42.5 \pm 3.0$                       | 7.05   | 106.4        |
| 4      | 55                               | $52.9 \pm 1.9$                       | 3.60   | 96.18        |

(b)

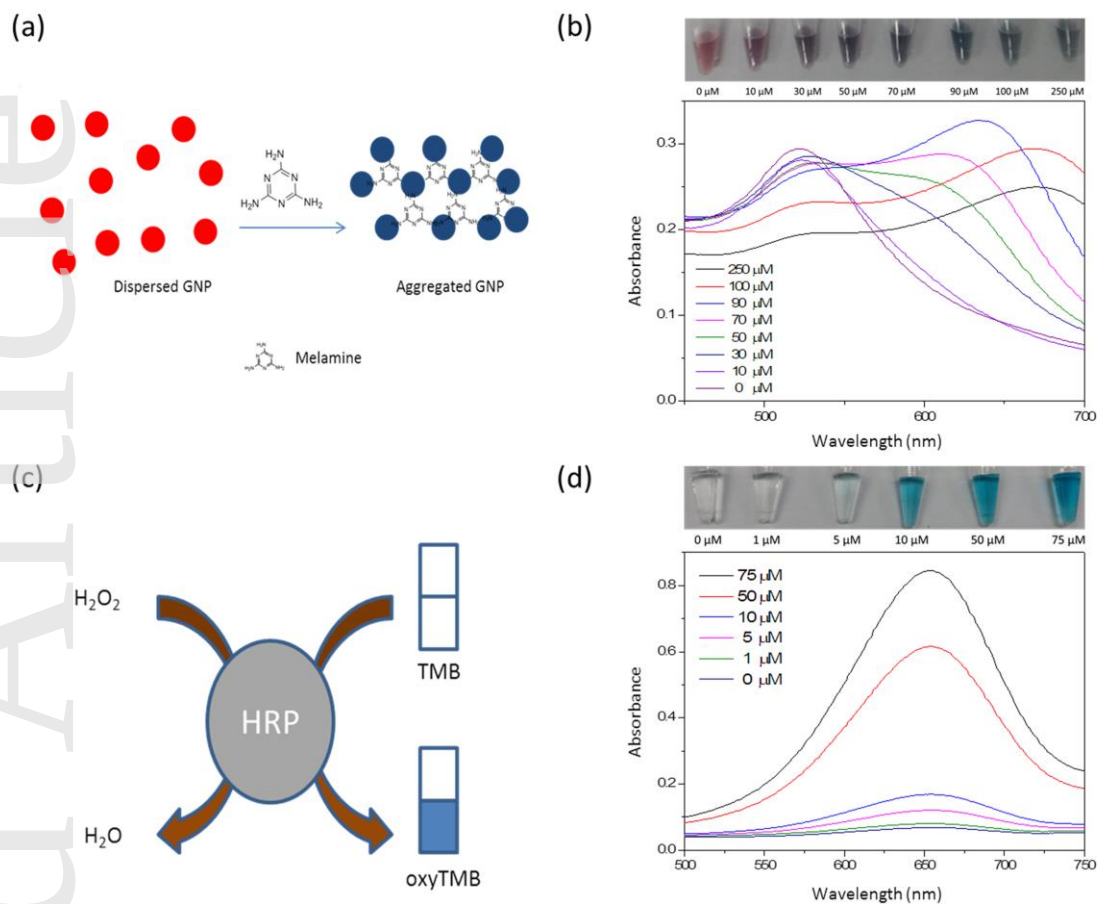
| Sample | H <sub>2</sub> O <sub>2</sub> added ( $\mu\text{M}$ ) | H <sub>2</sub> O <sub>2</sub> recovered ( $\mu\text{M}$ ) | CV (%) | Recovery (%) |
|--------|-------------------------------------------------------|-----------------------------------------------------------|--------|--------------|
| 1      | 0                                                     | 0                                                         | -      | -            |
| 2      | 30                                                    | $30.17 \pm 0.11$                                          | 0.37   | 100.52       |
| 3      | 35                                                    | $35.79 \pm 0.54$                                          | 1.53   | 102.27       |
| 4      | 55                                                    | $55.39 \pm 1.02$                                          | 1.85   | 100.71       |

**Figure legends**

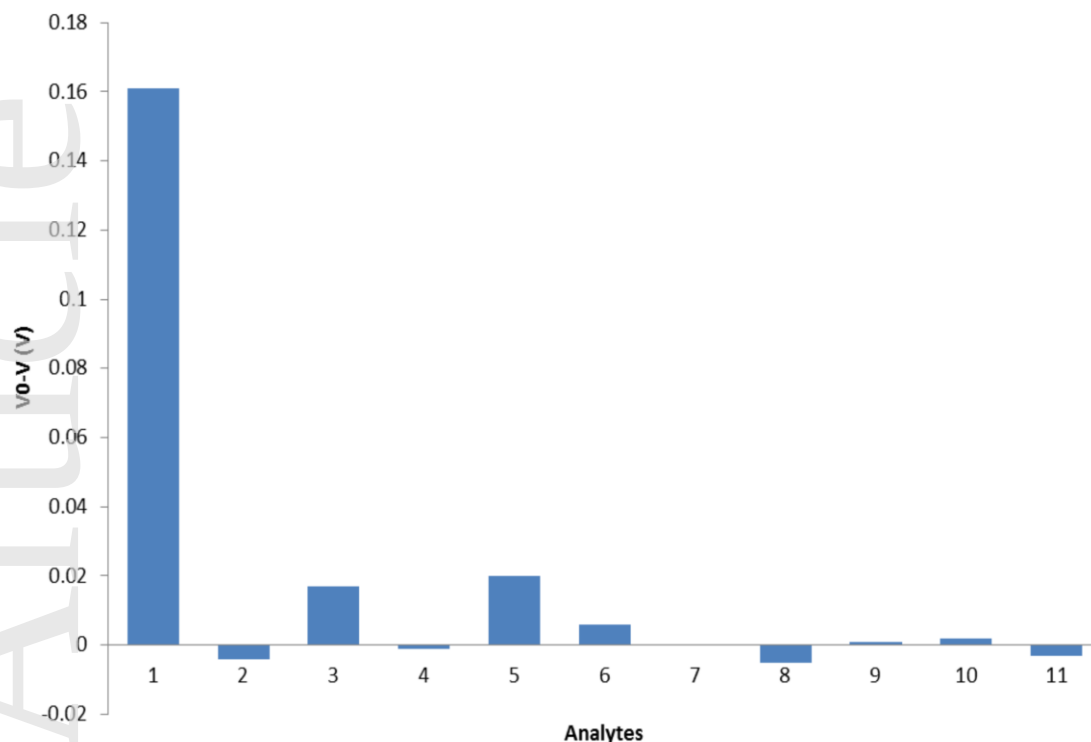
**Figure 1.** Design of PAWS. (a) 3D schematic illustration of the internal structure of PAWS. The upper cover of the system is transparent to illustrate the internal structure (in reality, it is opaque to block the ambient light.). (b) Procedure for wireless signaling on the smartphone. The LDR converts the optical signal to an electrical signal and the multimeter measures the voltage wirelessly in the smartphone, which analyses it and shows the result. (c) Photograph of the actual PAWS device.



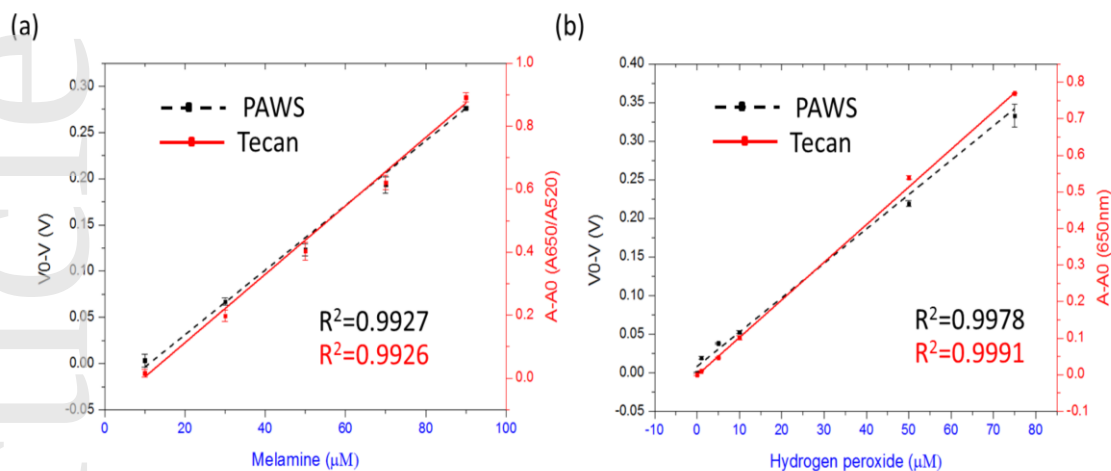
**Figure 2.** Screenshots of our android-based application. (a) Start screen on the smartphone after installation of the android application. (b) Main screen of the application. The number in the second lane is the voltage measured in response to the analyte concentration, while the number in the first lane is the current. (c) Detection menu. Screen displayed after pressing the “Detection” button in the main menu. (d) Melamine detection menu. The detected concentration of melamine and its significance are displayed on the screen. (e) Hydrogen peroxide detection menu. The detected concentration of hydrogen peroxide and its significance is displayed on the screen.



**Figure 3.** Schematic illustrations of colorimetric reactions. (a) Aggregation of GNP in the presence of melamine. (b) UV-Vis absorption spectra of GNP incubated with milk spiked by melamine at varying concentrations. Inset shows the corresponding photographs. (c) Oxidation of TMB in the presence of hydrogen peroxide. (d) UV-Vis spectra obtained from the HRP-catalyzed oxidation product of TMB in the presence of hydrogen peroxide at varying concentrations. The final concentrations of TMB and HRP were 1 mM and 0.1 U/mL, respectively. Inset shows the corresponding photographs.



**Figure 4.** Selectivity of GNP-based melamine detection in whole milk. The voltage change ( $V_0-V$ ) measured by PAWS in the presence of different analytes, where  $V_0$  and  $V$  are the voltages measured in the absence (control) and presence of analytes, respectively. Analytes: 1, melamine; 2, glycine; 3, L-alanine; 4, L-aspartic acid; 5, L-histidine; 6, cyanuric acid; 7, glucose; 8, galactose; 9, L-ascorbic acid; 10, sodium chloride; 11, potassium hydroxide. Concentrations of melamine and other analytes were  $10\ \mu\text{M}$  and  $1\ \text{mM}$ , respectively.



**Figure 5.** Sensitivity and accuracy of the PAWS system. The detecting performance of the PAWS system was compared with that of the commercial UV-Vis spectrophotometer, Tecan Infinite 200 Pro, for the detection of (a) melamine and (b) hydrogen peroxide using GNP and TMB, respectively, as the colorimetric substrates.