

SIMULTANEOUS PHOTOMETRIC AND FLUORESCENCE BASED SENSING FOR DETECTION OF ZINC IONS IN WATER

This chapter discusses the development of a dual mode smartphone-based sensing system to estimate zinc ions (Zn^{2+}) concentration in water samples. The proposed system has been designed for simultaneous sensing in photometric and fluorescence mode on a single platform. Two inbuilt optical sensors of the phone, namely, the ambient light sensor (ALS) and the rear camera, have been utilised to develop the proposed sensing system. For photometric-based sensing, the ALS receives the transmitted modulated signal from the sample, while the rear camera records the fluorescence signal emitted from the sample at right angle to the direction of the excitation signal. Using the designed platform, Zn^{2+} ions in field-collected water samples has been estimated successfully. Sensor's performance of the designed platform has been compared with the standard instrument. The limit of detection and the percentage recovery of the proposed sensor are found to be 0.1 ppm and 103.10% respectively.

6.1 Introduction

Zinc is one of the key parameters that has been often estimated while assessing the quality of water. It has a great physiological importance which assists in the proper functioning of the immune system, cell division, cell growth, wound healing and breakdown of carbohydrates inside the human body [1]. According to the Environmental Protection Agency – United States (EPA-US), the permissible limit of zinc in drinking water should not be more than 5 ppm [2]. Usually, zinc is found in all kinds of water resources as it is one of the most abundant elements on earth. Moreover, mining of natural resources, purification of metal ores, burning of coal also releases zinc to the nearby water bodies [3]. Excess content of zinc in water may change the taste, odour and turbidity level of water. Consumption of water containing excessive level of zinc may cause health issues such as vomiting, stomach ache and nausea [4]. Zinc in supply water also causes corrosion on the pipe lines. Thus proper estimation of zinc in water is critical as far as human health and the environmental ecosystem are concerned. A good number of spectrometric techniques are available in the literature to estimate zinc ion concentration in water. These techniques mostly use various organic and inorganic chemo-sensors for selective detection of zinc in water samples. Fluorescent-dyes and nanoparticle (NP) based probes are also used to detect trace amount of zinc ions in water samples [5–7].

Smartphone-based analytical platforms are generally designed for a single mode of analytical application [8–10]. Very limited works have been reported where smartphone-based sensing has been utilised for multi-modal sensing applications [11]. Furthermore, simultaneous sensing is not possible with these multi-modal systems. In the present work, the working of a smartphone-based analytical platform has been demonstrated which can be used as a photometer and a fluorescence spectrometer at the same time. The designed tool evaluates the absorbance and fluorescence spectra from a test sample with fluorescence property by utilising two different inbuilt sensors of a smartphone. Here, the ALS of the phone has been employed to collect the transmitted intensity while the phone's rear camera is used to detect the fluorescence signal. A custom-developed Android application transforms the light signal captured by the ALS into the corresponding absorbance value of the sample. Again, the same application evaluates the sample's fluorescence spectra from the emitted light signal captured by the rear camera. Simultaneous evaluation of absorbance and fluorescence spectra on a single platform using two different embedded optical sensors of a smartphone is the first-ever demonstration in the field

of smartphone sensing.

As a proof-of-concept, detection of zinc ions of field-collected water samples has been performed using the designed tool. Recently, Tira et al. has reported a frequency-resonance-energy-transfer (FRET) based assay which shows variation in both absorbance and fluorescence signal intensities according to the zinc ion concentration present in the medium [12]. Current study exploits this particular sensing principle to demonstrate the working of the proposed system. With the designed platform the signal intensity variations in both photometric and fluorescence modes have been recorded by the designed smartphone sensor. The limit of detection (LoD) of the designed tool was calculated to be 0.13 ppm for the photometric mode. For the fluorescence mode, this value was found to be 0.10 ppm.

6.2 Design of the proposed analytical platform for simultaneous sensing in photometric and fluorescence mode

Figure 6.1 (a) shows the schematic representation of the proposed analytical platform that has been designed for simultaneous detection of both the transmitted and emitted light signals from the test sample. In the present work, two different smartphone's embedded sensors have been used to develop the sensing platform. The ALS collects the transmitted light signal whereas the rear camera records the sample's fluorescence signal at an angle 90° to the incident light. For both the sensing mode, a single light-emitting diode (LED) has been incorporated in the platform as an optical source. To obtain a collimated beam from the optical source, a plano-convex (Lens 1) lens has been placed in front of the LED. Upon passing the light beam through the sample solution, the transmitted signal has been coupled to an optical fiber (*Edmund optics*, Product No: 02-534) by using another plano-convex lens (Lens 2). Finally a third plano-convex lens (Lens 3) has been used to collect the light coming out of the optical fiber and focus on the ALS of the phone. The light signal received by the ALS of the phone has been utilised to evaluate the optical density (OD) of the test sample.

Furthermore, a fourth focusing lens (Lens 4) has been placed in the optical setup to collect the fluorescence signal at right angle to the incident signal. While

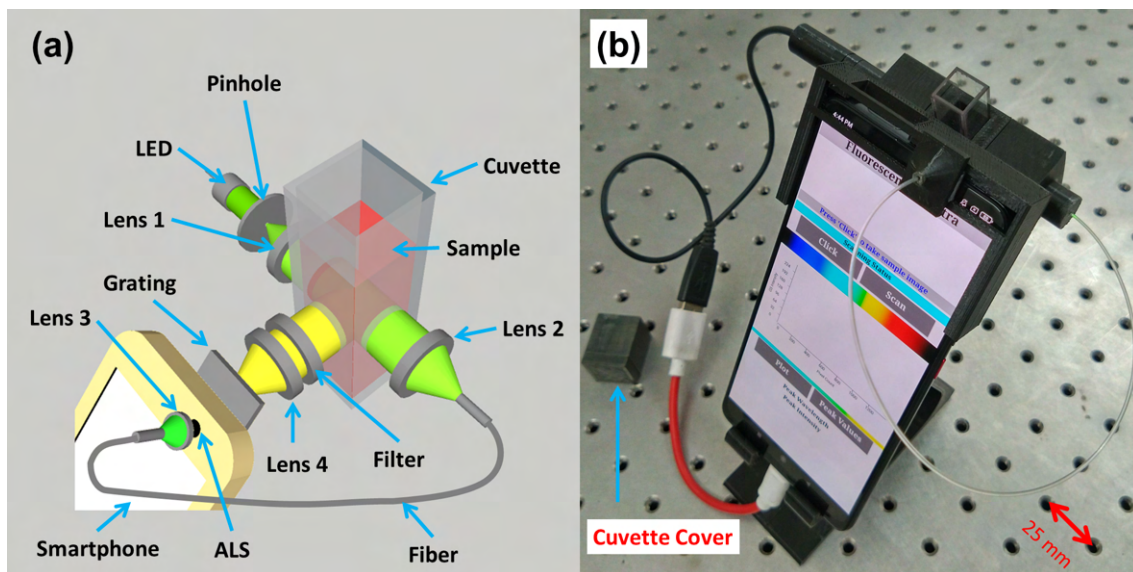


Figure 6.1: (a) optical layout design and (b) photo-image of the 3D printed dual mode smartphone-based analytical platform.

measuring the fluorescence signal, a long-pass optical filter (*Edmund Optics*, Part No: 62-977) with the cut-off wavelength of 550 nm has been placed to eliminate the excitation wavelength. The phone camera captures a dispersed spectra of the emitted signal produced by a transmission diffraction grating with 1200 grooves/mm (*Edmund Optics*, Part No: 49-578) which has been placed in front of the imaging sensor. Further, the captured image is analysed to evaluate the fluorescence spectra of the test samples.

The specifications of the plano-convex lenses used to develop the present setup are identical to each other with focal length of 11 mm and diameter of 6 mm (*Edmund Optics*, Product No: 02-534). During the analysis, the test sample has been placed in a 10×10 mm fluorescence quartz cuvette (*Erma*, Part No: RE-019). While recording the emitted signal, the ISO and the camera's exposure time have been set at 400 and 500 milliseconds respectively. All the optical components were mounted in a 3D-printed plastic setup shown in Figure 6.1 (b). The overall dimension of the designed setup is 60×35×35 mm in length, width and height, and the net weight is estimated to be 300 g, including the weight of the smartphone.

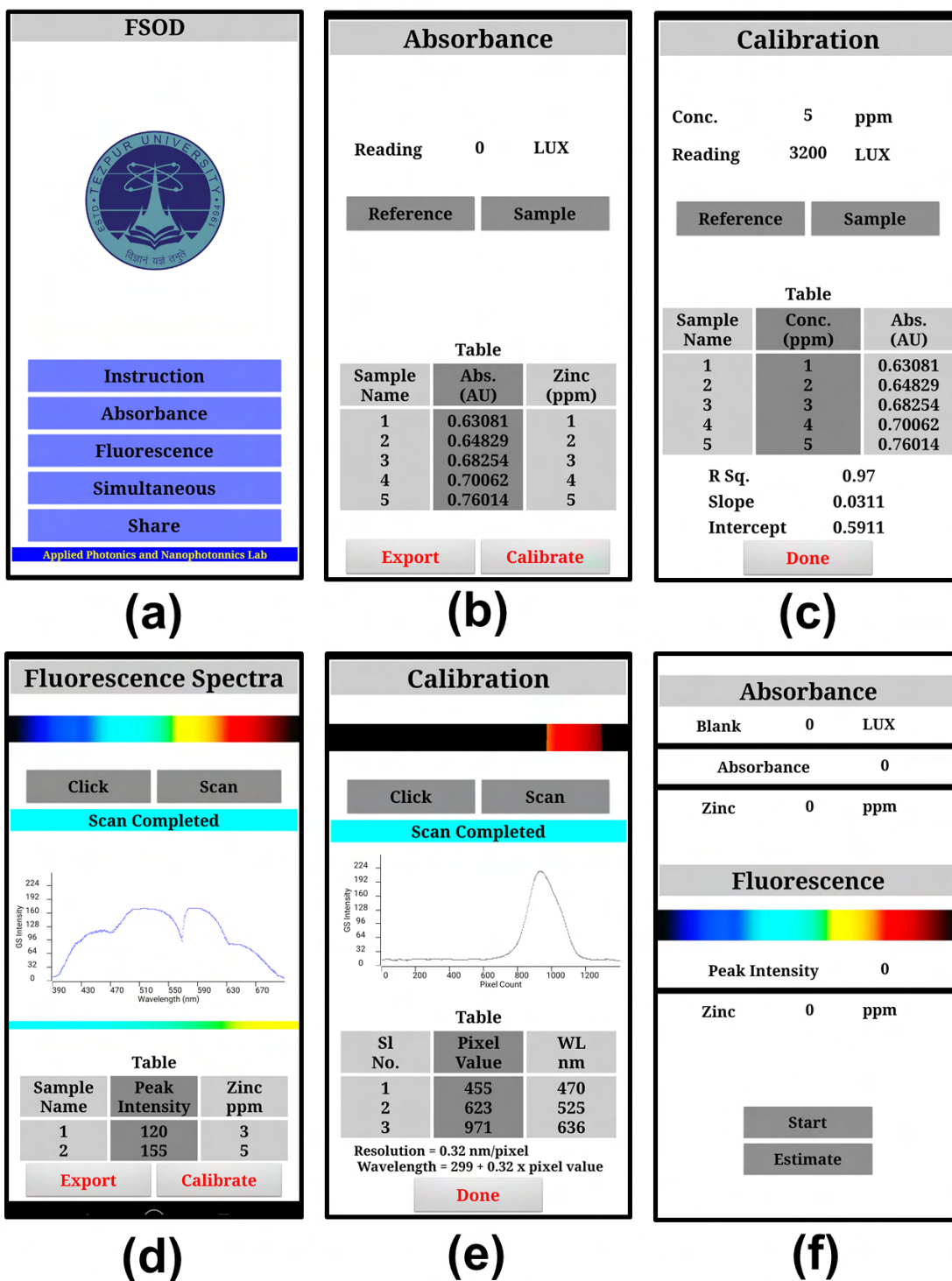


Figure 6.2: Screenshot images of the custom developed Android application (a) home screen of the app, (b) & (d) pre-calibrated settings for estimation of absorbance and fluorescence spectra, (c) & (e) calibration settings for estimation of absorbance and fluorescence spectra, (f) simultaneous spectrophotometric and fluorescence based detection of zinc ions.

6.3 Development of the Android application for simultaneous measurement of absorbance and fluorescence spectrum

A custom designed Android application has been developed to convert the recorded data in both the photometric and fluorescence modes into a readable form. As shown in Figure 6.2 (a), it initiates with a straightforward user-interface (UI) that contains various features of the developed application. The ‘*Absorbance*’ and ‘*fluorescence*’ tab on the screen, enables a user to choose the absorption or fluorescence mode of operation separately for any complementary investigations. Figures 6.2 (b) and (d) show the steps involved in the measurement of the absorbance and fluorescence spectra for a sample solution. The ‘*Calibrate*’ button provides access to re-calibrate the designed sensor. The calibration screen for photometric analysis (Absorbance mode) is shown in the Figure 6.2 (c). To evaluate the fluorescence spectra, a user needs to capture an image of the test sample by hitting the ‘*Click*’ button shown in Figure 6.2 (d). Then, the application automatically crops out a region of pixel dimension 1500×20 from the original image and displays on the screen. The cropping of the captured images helps in acquiring the experimental data within a limited time. Upon pressing the ‘*Scan*’ button, the application will generate a graphical plot between the gray-scale intensities (Y-axis) and the corresponding pixel numbers (X-axis). This plot can be converted to the corresponding wavelength values by following the wavelength calibration process shown in the Figure 6.2 (e). The prime objective of the Android application is to estimate zinc ions concentration using two different optical methods simultaneously. Figure 6.2 (f) shows the simultaneous determination of zinc ion in water samples on the designed sensing platform. The user needs to click on the ‘*Start*’ button followed by the ‘*Estimate*’ button to measure the analyte concentration in both the sensing modes.

6.4 Wavelength calibration

The designed platform acquires the fluorescence spectrum of a test sample by recording the emitted signal using the rear camera of the phone. The designed Android application then processes these images and evaluates the transverse profile of the pixel information. More specifically, the application plots the gray scale intensities against each pixel of the captured image. To obtain a corrected emission

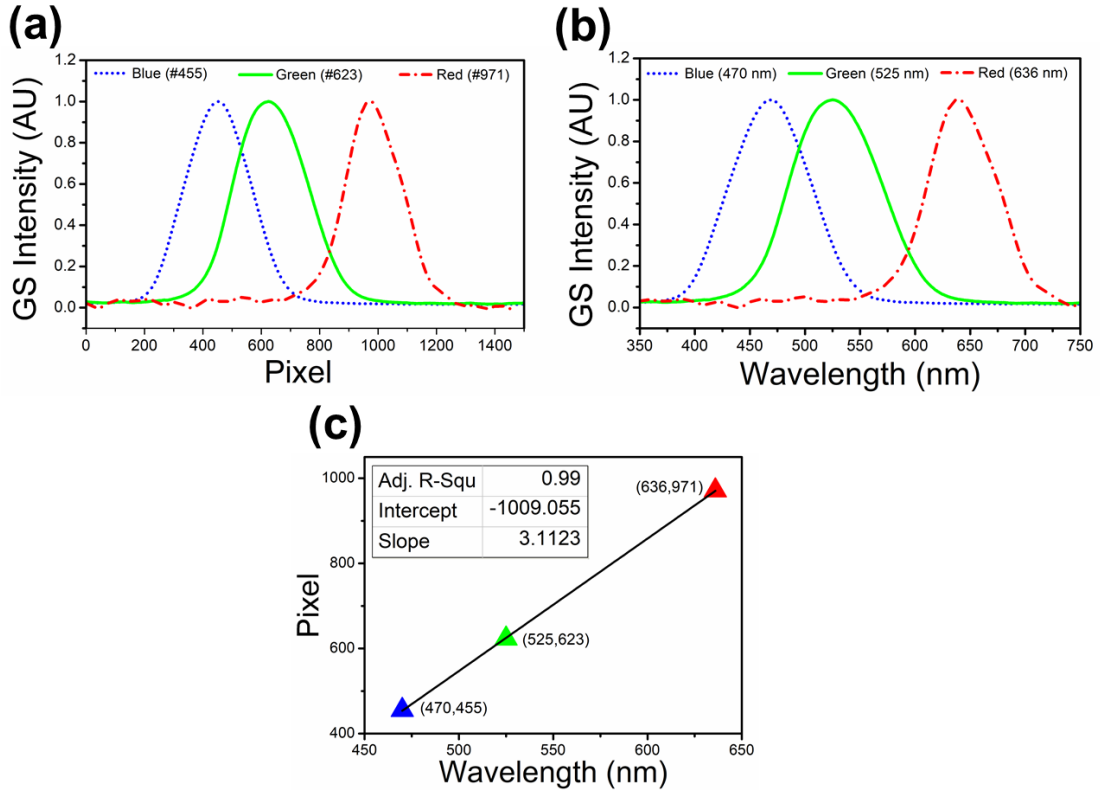


Figure 6.3: Pixel to wavelength conversion of the designed sensing platform. (a) uncorrected spectra, (b) corrected spectra after estimating the correction factor of the smartphone spectrometer, and (c) pixel vs. wavelength plot for three LEDs having peak emission wavelengths at 470 nm, 525 nm and 636 nm.

spectrum, the pixel values should be converted into the corresponding wavelength values through an appropriate wavelength calibration. Here, wavelength calibration has been carried out by analysing the emission spectra of three known LEDs using the fluorescence mode of the smartphone platform. The peak emission wavelengths of the three LEDs are measured to be at 470 nm, 525 nm and 636 nm. At first, the raw spectra of the LEDs have been recorded by the designed platform that contains only the grayscale intensities and the corresponding pixel values. Figure 6.3 (a) shows the respective emission spectra of the considered LEDs as recorded by the smartphone system. In the next step, the pixel values corresponding to the peak emission intensities have been compared with peak emission wavelength of the LEDs to evaluate the correction factor. The correction factor for the present optical arrangement is found to be 0.32 nm/pixel. Figure 6.3 (b) shows the corrected spectra of the considered LEDs after applying the correction factor to the pixel values. The relationship between the pixel and wavelength values of the three LEDs' emission

spectra is found to be linear with a regression coefficient (R^2) of 0.99 shown in the Figure 6.3 (c).

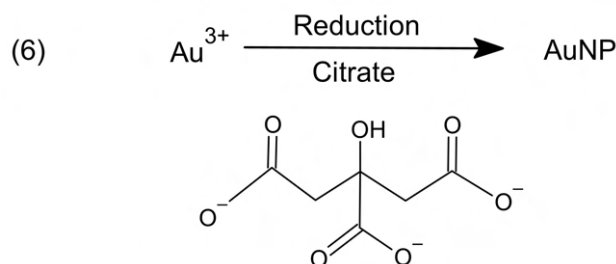
6.5 Materials and methods

For the present sensing investigation, following chemicals have been procured from different manufacturers and were used as received without further processing. Zinc chloride (ZnCl_2), iron chloride (FeCl_3), mercury chloride (HgCl_2), copper sulphate (CuSO_4), arsenic tri-iodide (AsI_3) and sodium citrate ($\text{C}_6\text{H}_5\text{O}_7 \cdot 3\text{Na}$) were procured from *Merck*, India. Rhodamine B ($\text{C}_{28}\text{H}_{31}\text{ClN}_2\text{O}_3$) and aluminium nitrate ($\text{Al}(\text{NO}_3)_3$) were procured from *Loba chemicals*, India. Gold (III) chloride tri-hydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was procured from *Himedia*, India.

6.5.1 Preparation of the water sample for selective detection of zinc ions

Utilising the designed platform, zinc ions in water samples were estimated following the protocol reported by Tira et al. [12]. Here, rhodamine B (RhB) and gold nanoparticles (AuNPs) were used as a FRET pair to detect zinc ions in water. The electrostatic interaction between the considered FRET pair results in the quenching of the fluorescence property of RhB in the final mixture. The assay shows variation in absorbance and fluorescence emission intensity upon varying the zinc concentration in the solution. This change is attributed to the attachment of Zn^{2+} ions to the AuNPs and gradually replacing the RhB molecules from the FRET pair. The release of RhB molecules increases the overall absorbance and fluorescence emission of the sample solution.

Following the standard Turkevich–Frens method, AuNPs have been synthesized in the laboratory, where $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ was reduced to spherical nanoparticles in the presence of sodium citrate [13] as shown in the chemical reaction 6. 0.05 mM Stock solution of RhB has been prepared by adding 23.95 mg of the reagent in 1000 ml of distilled water. Zinc stock solution (10 ppm) was prepared by mixing 20.84 mg of ZnCl_2 in 1000 ml of distilled water. For the present sensing study, 1 ml of each RhB (0.05 mM) and AuNPs (1 mM) solution were mixed together to prepare the considered FRET assay. 2 ml of the prepared assay was then added to 1 ml of zinc solution for the analysis.



Preparation of goldnanoparticles (Turkevich-Frens method)

6.5.2 Spectrometric analysis of the prepared standard samples with varying zinc concentration

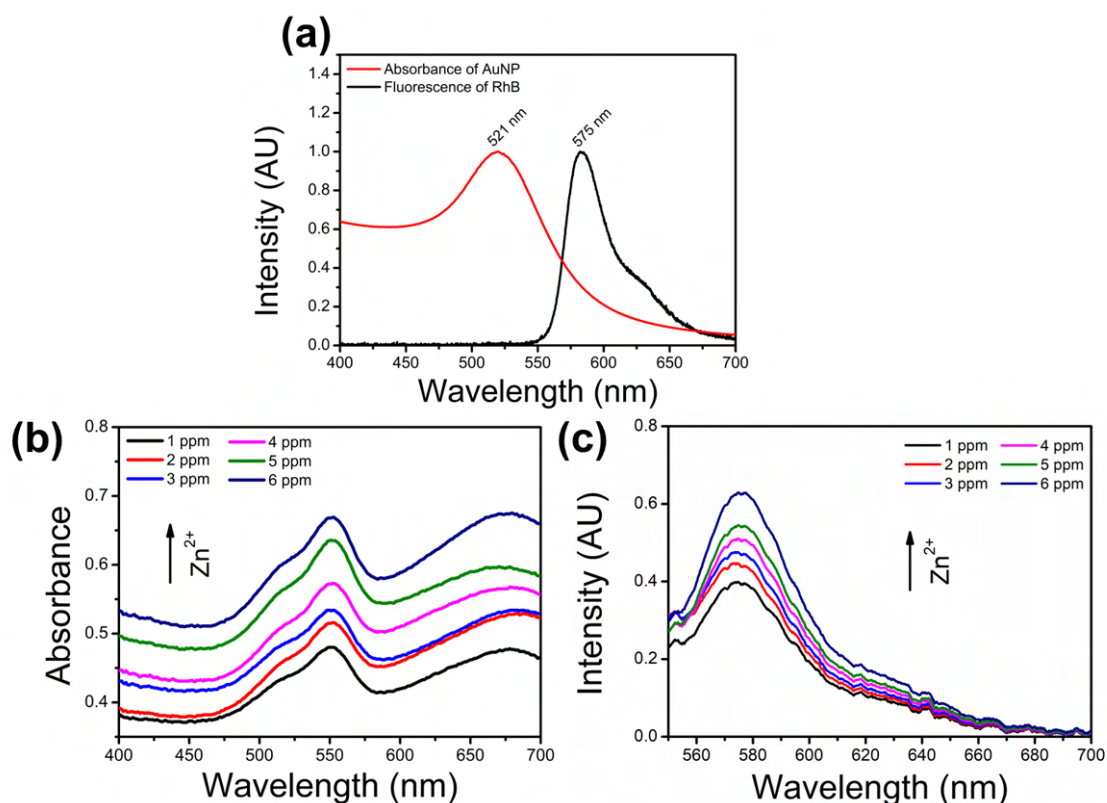


Figure 6.4: (a) Absorption spectra of gold nanoparticles (1 mM) and rhodamine B (0.05 mM), (b) absorption and (c) fluorescence spectra of AuNP-RhB mixture at varying concentrations of zinc ion in the medium.

At first, the spectrometric analyses of the standard solutions have been evaluated using a UV-VIS spectrophotometer (*Systronics India*, AU 2603) and a compact spectrometer (*CCS200*, Thorlabs, USA). The UV-VIS spectrophotometer records the absorption characterises of the samples for a wavelength range of 400 nm to

700 nm. On the other hand, the compact spectrometer records the fluorescence emission of the same at 525 nm excitation. Figure 6.4 (a) shows the absorption spectrum of bare AuNPs and fluorescence spectrum of RhB solution. AuNPs show the peak absorbance at 521 nm while RhB exhibits peak emission at 575 nm. The overlapping of the absorbance and fluorescence spectra manifests the effectiveness of the considered FRET assay.

The spectrometric analyses of the FRET assay with standard Zn^{2+} solutions were then studied. The analyses were carried out for multiple zinc samples with varying concentrations (1 ppm to 6 ppm). Figure 6.4 (b) shows the UV-VIS spectra of the final zinc solutions and Figure 6.4 (c) illustrates the emission spectra of the same. From the characteristic curve it has been observed that upon increasing the Zn^{2+} ions concentration, the absorbance and the fluorescence emission increases gradually. The gradual increments in absorbance and fluorescence emission have been quantified by the designed platform to evaluate the zinc ion concentration in the sample.

6.6 Sensor calibration

The designed sensing platform has been initially calibrated for standard zinc samples. Six different samples of concentration ranging from 1 ppm to 6 ppm have been considered for the present study. Calibration of the photometric and the fluorescence mode of the designed tool are illustrated in section 6.6.1 and 6.6.2 respectively.

6.6.1 Calibration in the photometric mode of the designed sensor

For the photometric mode, the designed sensor estimates the absorbance of a given sample utilising the equation 6.1. Five consecutive responses of each standard sample were recorded by the ALS of the phone. Standard deviation of a blank sample is estimated to be 0.002 for the considered mode of operation. Figure 6.5 (a) represents the least square fitted plot of the sensor responses against the analyte concentrations. The R^2 value is found to be 0.98 for the plotted data. Equation 6.2 is the calibration equation that has been used to calculate Zn^{2+} ions concentration of an unknown sample. The estimated sensitivity, LoD and LoQ for the photometric mode

of the designed platform are found to be 0.05 AU/ppm, 0.13 ppm and 0.45 ppm respectively. The correlation between the designed tool's responses and an UV-VIS spectrophotometer data is found to be linear which is shown in Figure 6.5 (b). For the photometric mode of sensing, the absorbance of the test sample is calculated as

$$A = \log_{10}(I_0/I) \quad (6.1)$$

where, A represents the absorbance of the sample, I refer to transmitted light signal intensity and I_0 represents the sensor response for blank sample (distilled water).

$$C = (18.84 \times A) - 0.49 \quad (6.2)$$

where, C represents the analyte concentration in the sample and A represents the absorbance of the test sample.

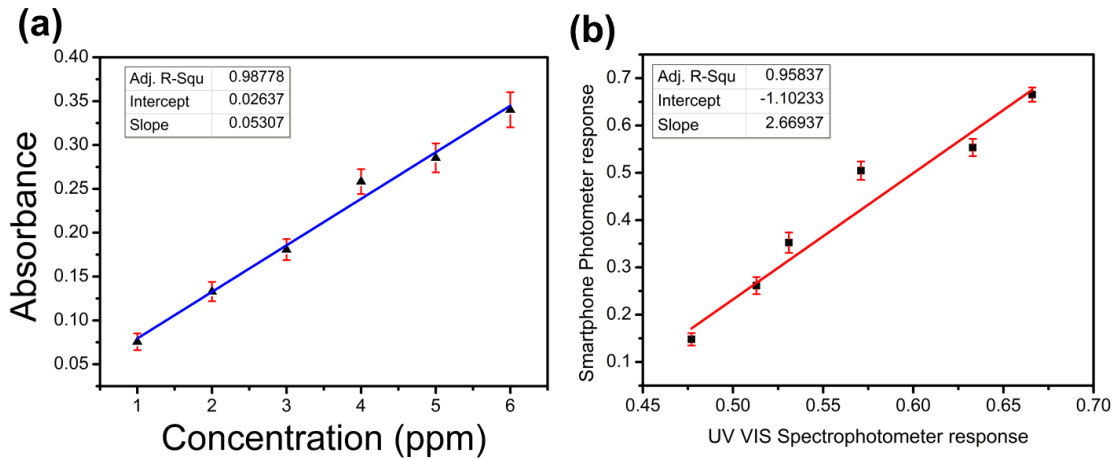


Figure 6.5: (a) Calibration curve of the photometric mode of the designed platform for standard Zn^{2+} samples mixed with RhB (0.05 mM) and AuNPs (1 mM) assay, (b) absorbance measured in the designed tool in comparison with a standard UV-VIS spectrophotometer data.

6.6.2 Calibration in the fluorescence mode of the designed sensor

A similar approach has been adopted to calibrate the fluorescence mode of the designed sensing platform. In the present investigation, the sample has been excited with a LED source of peak emission wavelength 525nm. For each sample, five consecutive images were rapidly captured by the designed system. Figure 6.6 (a)

shows the corrected fluorescence spectra of standard Zn^{2+} samples recorded by the designed tool. The system shows maximum sensitivity at 575 nm and the gray scale intensities corresponding to this specific wavelength have been considered for evaluating the calibration equation 6.3. Equation 6.3 has been used to estimate Zn^{2+} ions concentration of unknown samples in fluorescence mode for the proposed tool. The sensitivity, LoD and LoQ for the fluorescence mode of the designed platform are estimated to be 10.5 AU/ppm, 0.1 ppm and 0.35 ppm respectively. Figure 6.6 (b) illustrates the comparison of fluorescence responses measured in the designed system and a standard spectrometer.

$$C = (0.33 \times GS) - 1.61 \quad (6.3)$$

where, C represents the analyte concentration in the sample and GS represents the grayscale intensity of the captured image by the proposed system.

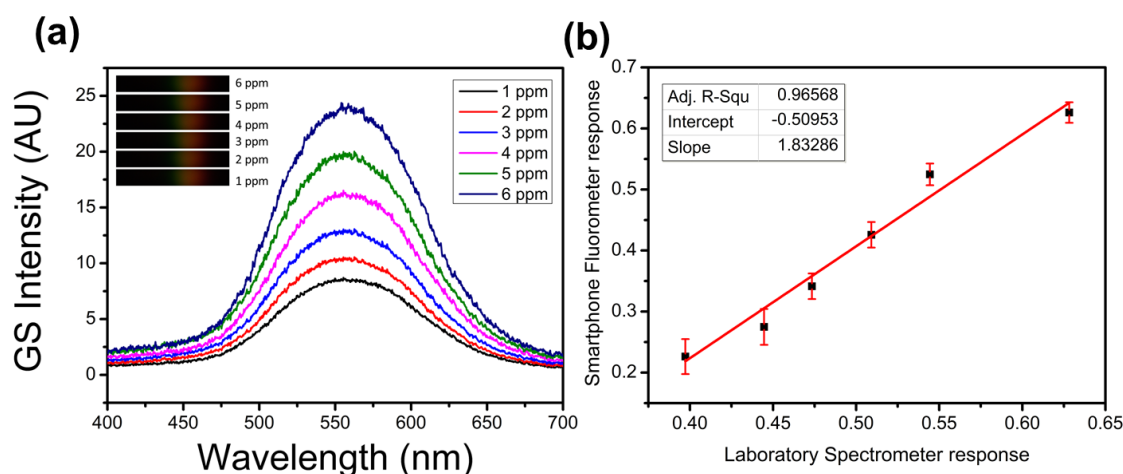


Figure 6.6: (a) Fluorescence spectra of standard Zn^{2+} samples with RhB (0.05 mM) and AuNPs (1 mM) mixture as obtained from the designed smartphone platform and (b) fluorescence responses of the designed tool in comparison with the standard laboratory spectrometer data.

6.7 Performance evaluation of the sensor in presence of other interfering elements

In the next step, the performance of the designed sensing platform has been studied in presence of different metal ions. Seven metal ions copper (Cu^{2+}), manganese

(Mn^{2+}), aluminum (Al^{3+}), sodium (Na^+), magnesium (Mg^{2+}), mercury (Hg^{2+}) and arsenic (As^{3+}) have been considered to evaluate the sensor characteristic. Standard Zn^{2+} solution of 0.5 ppm was prepared in the laboratory. The interfering elements were taken in the concentration of 5 ppm each. 1 ml of each of these interfering elements was added separately to equal volume of zinc solution. Figure 6.7 shows the characteristic histogram representation of the sensor responses in presence of the other elements. The figure clearly shows that the response of the sensor is unaffected due to the presence of the other elements.

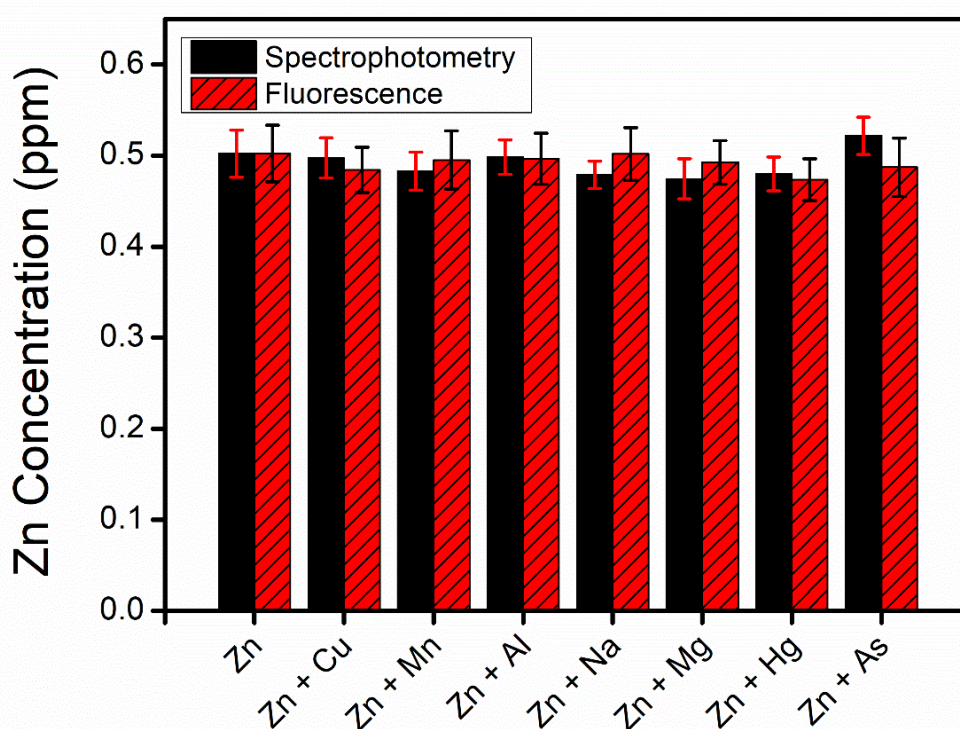


Figure 6.7: Responses of the designed smartphone platform were recorded under both the sensing modes after adding Cu^{2+} , Mn^{2+} , Al^{3+} , Na^+ , Mg^{2+} , Hg^{2+} and As^{3+} in the standard Zn^{2+} samples.

6.8 Performance evaluation of the sensor for field-collected water samples

In the final step, the performance of the sensor was evaluated for field-collected water samples. Water samples from five different locations of Assam namely–Bharolu river water, Guwahati (SW1), Brahmaputra River water, Sonitpur (SW2), public water

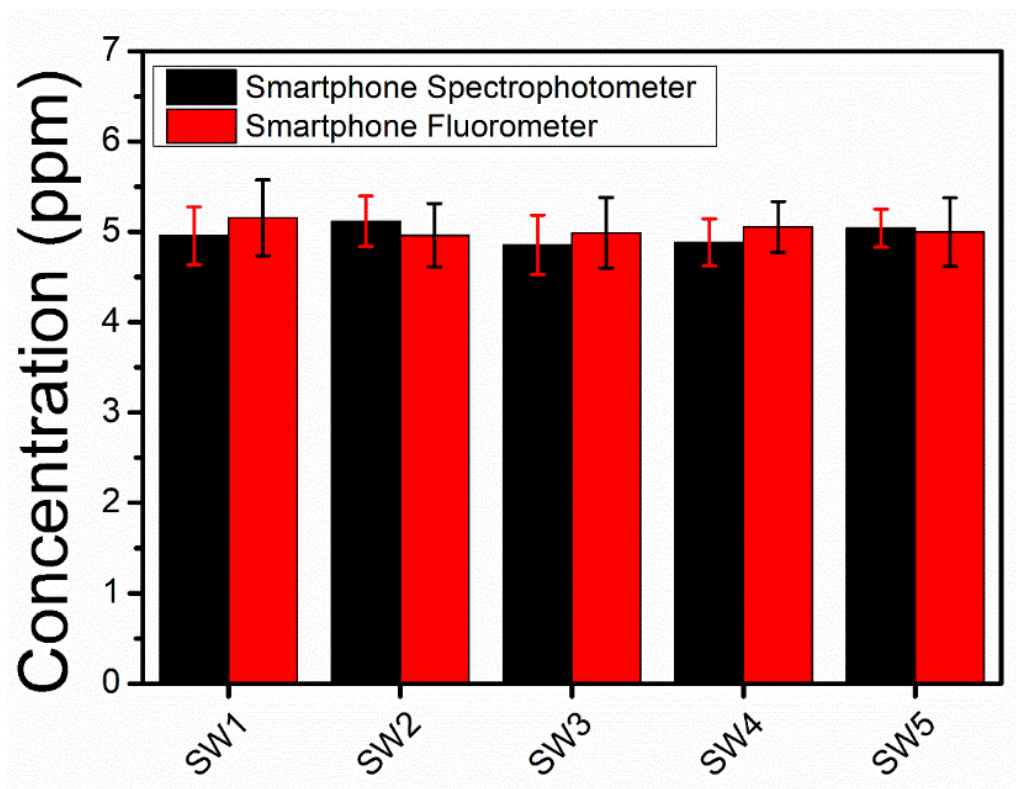


Figure 6.8: Estimation of Zn^{2+} ions concentration in field-collected water samples using both the sensing modes (absorbance and fluorescence) of the designed smartphone tool.

supply of Napaam Village, Sonitpur (SW3), pond water of Tezpur University (SW4), and tap water of Tezpur University (SW5) have been collected for the study. The water samples were initially filtered with grade-1 *Whatman* paper for three times until a clear solution is obtained with zero turbidity reading. For estimation of dissolved Zn^{2+} ion concentration, the chemical treatment process has been carried out following the procedure described in the material and method section 6.5.1. All the samples show negative responses for Zn^{2+} ions when analysed using the designed smartphone platform. The negative response indicates the low presence of Zn^{2+} in the field-collected water samples. The actual concentrations of Zn^{2+} ions in all the field-collected samples were confirmed from Atomic Absorption Spectrometer (*Thermo Scientific iCE 3000 Series*) data which are found to be in the range of 0.05 ppm to 0.09 ppm. The actual concentrations are found to be far below the detection limit of the designed smartphone sensing system. Hence, Zn^{2+} ions have been added externally to all the field-collected water samples for the present investigation. The final Zn^{2+} ion concentration of each sample was made to 5 ppm. Figure 6.7 shows the characteristic responses of each sensing mode (photometric and fluorescence)

of the designed platform. A good degree of correlation between the experimental data obtained from both the sensing modes was observed. The maximum variation between the data recorded by each sensing mode was found to be 8.9%. The recovery percentage (%Recovery) of the designed platform is shown in tabular form in Table 6.1.

Table 6.1: Estimation of %Recovery while investigating the field-collected water samples under both the sensing modalities of the designed smartphone sensor

Sample	Actual concentration (ppm)	Photometric mode		Fluorescence mode	
		Estimated value (ppm)	% Recovery	Estimated value (ppm)	% Recovery
SW1	5	4.95	99.14	5.15	103.10
SW2	5	5.11	102.34	4.96	99.27
SW3	5	4.85	97.14	4.99	99.80
SW4	5	4.88	97.67	5.05	101.10
SW5	5	5.04	100.84	4.99	99.90

6.9 Summary

The prime focus of the present chapter entails the development of a smartphone based analytical platform with two modes of sensing abilities. Two embedded sensors of the phone- the ALS and the rear camera have been used to design the proposed platform. It is the first ever demonstration of simultaneous detection of photometric and fluorescence signals on a single sensing platform using two inbuilt sensor of a smartphone. Development of a custom designed Android application makes the designed platform a truly standalone system. The designed tool allows simultaneous estimation of absorbance and fluorescence of any test samples on a single platform. The applicability of the platform has been demonstrated through detection of Zn^{2+} ion in field-collected water samples. The LoDs of the designed tool for the photometric and fluorescence sensing modes are found to be 0.13 ppm and 0.1 ppm respectively. It is also possible to perform other photometric and fluorescence based studies to detect and analyse other chemical and biological sample by suitably selecting the optical source and the filter in the present sensing set-up.

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