Chapter 3

SMARTPHONE-BASED FLUORESCENCE SENSING FOR ACCURATE ESTIMATION OF MERCURY IN WATER

This chapter discusses the development of a fluorescence-based sensing system to measure the mercury ion (Hg^{2+}) concentration in water samples. The working principle of the proposed analytical system is based on the detection of fluorescence signal intensities emitted by rhodamine 6g (R6G) solutions in the presence of Hg^{2+} ions. The emitted fluorescence signal is captured by the rear camera and further analysed on a custom-developed Android application. The application extracts the V channel values in HSV colour space and correlates them with the analyte concentrations. The designed platform has a detection limit of 32 ppb. The experimental values on comparing with Atomic Absorption Spectrometer (AAS) data, a good degree of correlation of sensor responses has been observed.

3.1   Introduction

From environmental and human health point of view, the estimation of heavy metals in water resources is critical. Heavy metals are not easily decomposed in human body and thus only accumulate inside the various organs [1]. Heavy metal in water

resources may possess severe threat to human health. Mercury (Hg) is one of the many toxic heavy metals alongside cadmium, lead, arsenic and zinc that can be found in different types of water resources. Some of these are very lethal even at a minute level of concentration. According to the World Health Organization's (WHO) water quality guideline, the permissible limit of Hg^{2+} concentration in drinking water should not be above 2 ppb [2]. Generally, three forms of mercury compounds are found in water: elemental, organic and inorganic mercury. These different forms of Hg have unique toxicity profiles for human health, exposure to which can lead to severe damage of the kidney and gastrointestinal tract. Excess accumulation of Hg in the brain may also cause serious neurological diseases such as memory loss, mental disturbances and mood swings [3, 4]. Therefore, to prevent any diseases related to Hg contamination, water resources should be monitored regularly.

Water monitoring laboratories all around the world make use of various sophisticated instrumental methods to determine metal contaminations in the water resources. Among these methods, Atomic Absorption Spectrometry (AAS), Atomic Emission Spectrometry (AES), High-Performance Liquid Chromatography (HPLC) and Mass Spectrometry (MS) are some of the notable standard laboratory methods that have been used around the globe [5–7]. In India, the availability of such water laboratories is very limited and hence monitoring of water qualities in natural and wastewater at regular intervals is a very tedious job. For this, the developments of field-portable and decentralised laboratory facilities are of prime concerns in recent times. In this regard, smartphone-based sensing of heavy metals can be applied as an alternative tool to assess water qualities in remote and resource-poor areas of the country [8–10].

Recently, various fluorescence-based sensing schemes have been used for estimation of heavy metals in water [11, 12]. This type of optical sensing is found to be the most time saving and sensitive one while measuring the trace amount of the analyte. As an addition to the existing sensing systems and need for a truly handheld sensing tool, a simple fluorescence-based smartphone sensing system has been demonstrated in this chapter by investigating the presence of heavy metal in water samples. The designed system is compact and has been created for in-field studies. While developing the platform, 3D printing technology has been utilised to fabricate a custom-designed optical attachment that can be coupled to a smartphone. It uses the rear camera (CMOS sensor) as a photo-detector and captures the incoming light signal from the test sample. Nevertheless, the custom-designed 3D printed setup houses all the required optical components, including a sample holder and an optical

source. As a proof-of-concept, Hg^{2+} ion concentration in water samples has been measured by the designed platform. The performance of the designed sensing system has been then evaluated by comparing the experimental results with a standard laboratory-grade Atomic Absorption Spectrometer (AAS). Furthermore, the sensor characteristic of the proposed tool has also been evaluated in presence of different interfering elements. The responses of the proposed system are found to be reliable while estimating the Hg^{2+} ion concentration, which suggests that the device can be used as a handheld heavy metal detector in field-collected water samples.

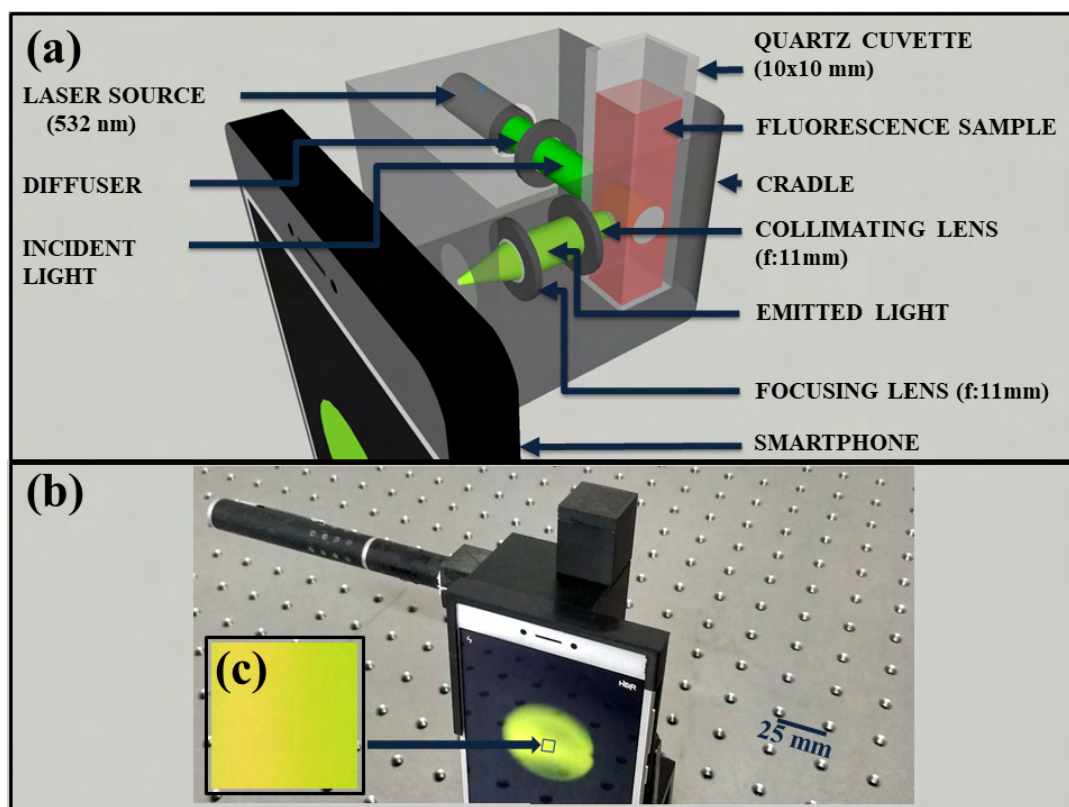## 3.2 Design and development of the smartphone-based fluorometer

Figure 3.1: (a) schematic and (b) photo-image of the designed smartphone fluorescence platform, and (c) cropped region of interest (200×200 pixel) of the captured image.

Figure 3.1 (a) shows the optical layout design of the smartphone-based fluorescence sensor. The primary objectives of the proposed system are to capture

and quantify the fluorescence signal emitted by a fluorophore. As mentioned earlier, the optical setup has been printed using a 3D printer (*Raise 3D N2*), with a bio-degradable polymer (*Polylactide Acid* (PLA)). The plastic setup houses all the optical components required to develop a compact fluorometer. The system utilises a diode laser pointer as an excitation source. A plastic diffuser has been placed in front of the laser source to uniformly illuminate the sample cuvette filled with the test sample. After that, the emitted fluorescence signal from the sample is captured by the rear camera, which is positioned at the right angle to the direction of the incident signal. The collection of the emitted light signal is done by a pair of plano-convex lenses, each with a focal length of 11 mm and a diameter of 8 mm. The first lens in this combination collects the signal and then focuses on the camera through the second lens. In the present sensing tool, a *Xaiomi Redmi Note 4* smartphone, which is equipped with a 13 MP CMOS image sensor, has been used to construct the compact fluorometer. The detail technical specifications of the phone can be found elsewhere [13]. While capturing the fluorescence images, the ISO of the considered camera phone has been set at 400 for all the investigations accomplished in the present work. The overall dimension of the designed sensor is measured to be 50 mm×40 mm×40 mm in length, breadth and height, and the net weight including the phone is estimated to be 275 g. The whole setup is optically shielded so that ambient light cannot perturb the sensing signal. Figure 3.1 (b) shows the photo-image of the complete setup of the designed tool attached with a smartphone.

The rear camera of the phone captures the fluorescence signal emitted from the sample followed by further analysis via image processing. Figure 3.1 (c) depicts the cropped region of the captured image. Image processing involves the extraction of digital information like the various colour channel values from the cropped images. After the extraction, these values are correlated with the analyte concentrations to obtain the calibration equation. In the present study, the V channel value in HSV colour space has been utilised to estimate Hg^{2+} ions in water samples. The reason for considering V channel value for the present sensing study is that the plot between V channel values and Hg^{2+} concentrations shows the most linear trend with an optimal R^2 value. The HSV is a perceptual colour space with three distinct colour channels hue (H), saturation (S) and value (V) respectively. Here, H represents a specific colour, where S and V give the purity and brightness of that colour, respectively. HSV colour channels can be computed by the non-linear transformation of RGB colour channel values [14, 15]. Using the following equation 3.1, the V channel value of HSV colour space can be derived from RGB channel values.

$$V = \frac{\left(\frac{1}{3} \times (R + G + B)\right)}{255} \tag{3.1}$$

where, V is the V Channel value of the captured images in HSV colour space and R, G and B represents red, green and blue channel values in RGB colour space respectively [16, 17].

3.3 Development of an Android application

Alongside the 3D printed optical setup, a custom-designed Android application has been developed to capture and analyse the fluorescence signal. Development of the Android applications transforms the designed tool into a standalone analytical device. This application has been developed by using the cloud-based app developer platform, namely, '*MIT App Inventor-2*' [18]. The application captures the image of the test sample, crops it down to a 200×200 pixels image and evaluates the mean V channel value for the cropped region. The app has been pre-calibrated with a standard data set to estimate Hg^{2+} concentration in unknown water samples. Standard data are produced from the pre-recorded fluorescence signals of standard Hg solutions with varying concentrations. In case of any discrepancy in the sensor responses, the application has the feature to re-calibrate itself with a fresh set of standard samples.

Figure 3.2 (a) shows the screenshots of the steps involved while estimating Hg^{2+} ion concentration in water samples using pre-calibrated data. On running the application, a home screen will pop up that will contains several tabs. To estimate Hg^{2+} ion concentration, a user needs to insert the sample into the sample holder of the designed setup. Upon inserting the sample, it is required to press the '*Determine*' tab on the screen. After that, a new screen will pop up, which will contain two tabs, namely '*Click*' and '*Estimate*'. On pressing the '*Click*' tab, the application will capture the photo-image of the fluorescence signal and will crop it down to a 200×200 pixels image. The right-hand side of Figure 3.2 (a) shows the cropped image which is captured by the designed smartphone sensor. After this step, the user will need to press the '*Estimate*' tab, and the application will quickly calculate the Hg^{2+} ion concentration of the unknown sample.

Figure 3.2 (b) shows the steps involved when the designed smartphone sensor requires an on-field calibration. Usually, re-calibration of the tool has been carried out to eliminate any interference that arises due to the ambient conditions such
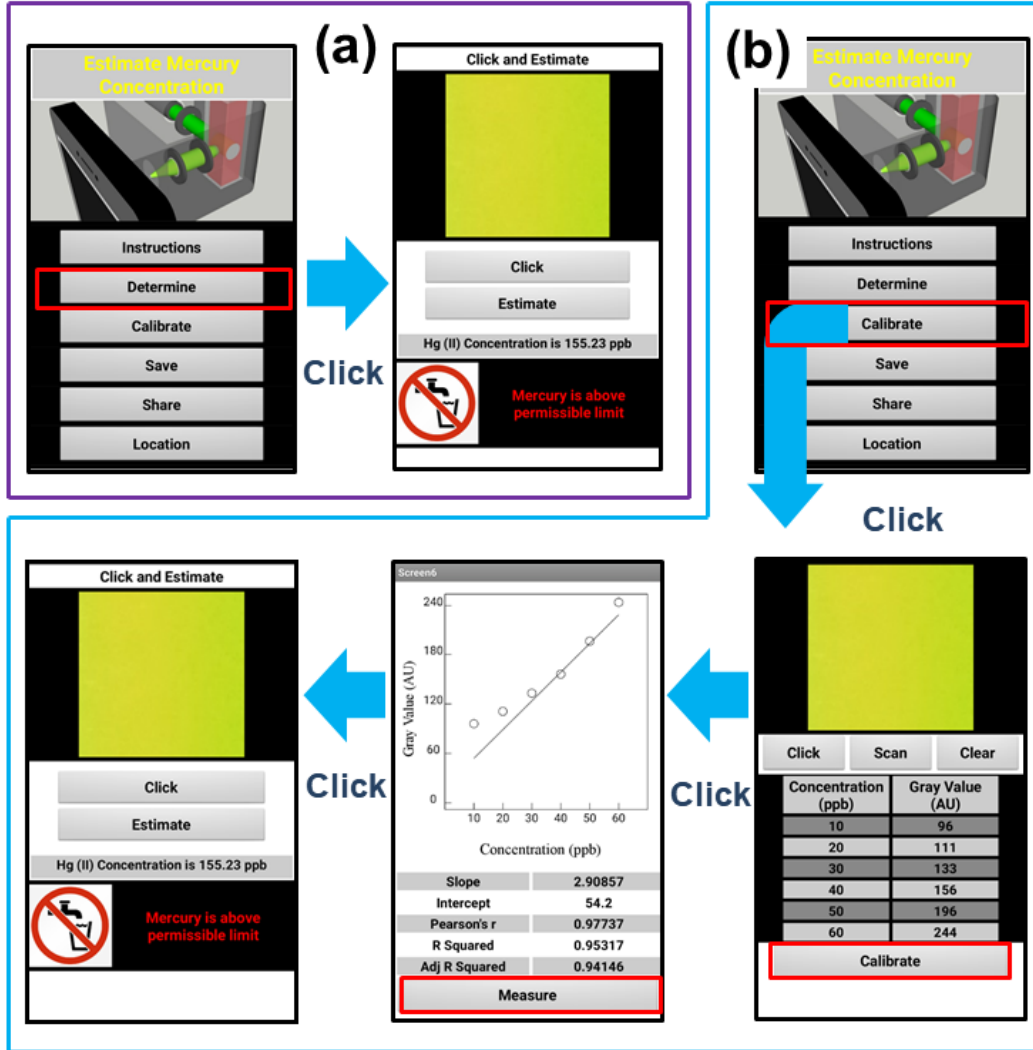
Figure 3.2: Screenshot of (a) pre-calibrated mode of operation and (b) in-field calibration mode of operation of the developed Android application.

as temperature, light, humidity and more. As shown in the top right figure, the user needs to press the '*Calibrate*' tab on the screen of the application to perform the calibration. On pressing this tab, a new panel will pop up where the user can insert the sensor responses of standard Hg^{2+} samples alongside the respective concentrations. Upon obtaining the desired responses, the user can proceed further by passing the '*Calibrate*' tab present below the data table. On hitting this tab, another new screen will pop up which shows the calibration curve of the new data set along with the regression analysis and calibration equation therein. At this point, the platform becomes ready to be used and on hitting the '*Measure*' tab, it will redirect to a window where unknown samples can be analysed with a fresh

calibration equation.

3.4 Materials and methods

To validate the designed smartphone platform, a previously reported chemical protocol to detect trace amount of Hg^{2+} in water samples reported by Ramakrishna et al. has been adopted for the present investigation [19]. According to this protocol, a solution of mercuric chloride ($HgCl_2$) is allowed to react with an iodide (KI) buffer to form a complex ion of tetra-iodomercurate (II) (HgI_4^{2-}). The interactions between various reagents have been illustrated in chemical equations 3.1 and 3.2. The prepared complex ion is then treated with R6G to form a complex compound. The formation of the complex compound in the final solution alters the fluorescence emission of the final solution. The level of variation in fluorescence emission occurs depending upon the concentration of Hg^{2+} ions in the sample. Hence by quantifying the fluorescence signal, the concentration of Hg^{2+} ions can be estimated in a given water sample. The details of the chemical procedure and the necessary chemicals are discussed below.
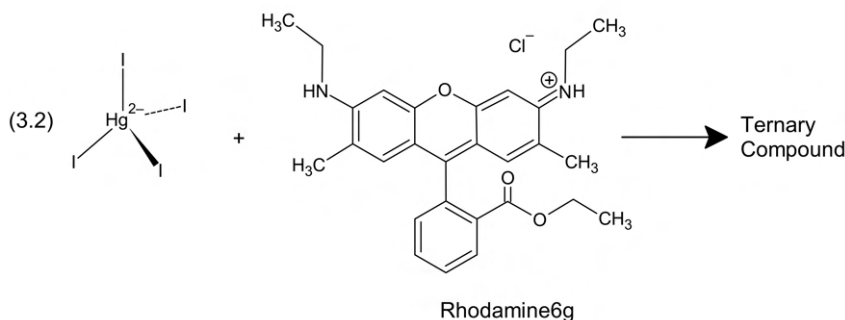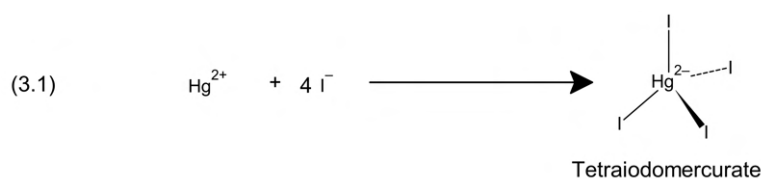
Method for detection of mercury (II) ions in water

3.4.1 Preparation of the standard solutions

All necessary chemicals were procured from certified suppliers and have been used as received without further purification. Laboratory grade rhodamine 6G (R6G),

mercury (II) chloride ($HgCl_2$), potassium iodide (KI), sodium thiosulfate ($Na_2S_2O_3$) and potassium hydrogen phthalate ($C_8H_5KO_4$) were procured from *Merck India*; gelatin powder was purchased from *Loba chemicals*, India.

To prepare the mercury stock solution of 1 ppm, 0.3385 g of $HgCl_2$ was dissolved in 250 ml of distilled water. Standard mercury solutions of different concentrations have been prepared by diluting the stock solution with a proportionate amount of distilled water. The iodide buffer solution has been prepared by mixing 5 g of KI, 5 g of $C_8H_5KO_4$ and 5 g of $Na_2S_2O_3$ in 250 ml distilled water and stirring the mixture until all the reagents dissolved completely in the solution. R6G solution of 0.005% (w/v) has been prepared by dissolving 0.05 g of it in 1 litre of distilled water. Finally, 1% (w/v) of gelatin solution has been prepared by adding 5 g of the reagent powder to 50 ml of distilled water and continued to stir it for 15 minutes at 60°C.

Upon preparing the above standard solutions, 5 ml of iodide buffer was mixed with 10 ml of standard Hg solution in a 25 ml volumetric flask. This was followed by the treatment with 5 ml of R6G solution. To ensure the stability of the fluorescence emission from the resultant mixture, 1 ml of gelatin solution was added to it. The final mixture was diluted to 25 ml through the addition of 4 ml of distilled water to the solution.

3.4.2 Spectroscopic analysis of the standard solutions

Spectroscopic analysis of the standard sample solutions synthesized in the laboratory has been performed to ensure the reliability of the chemical protocol. Figures 3.3 (a) and (b) show the spectroscopic characterisations of the sample solutions measured by a UV-VIS spectrophotometer (*Systronics India*, AU 2603) and a compact spectrometer (*CCS200*, Thorlabs, USA) respectively. The absorbance spectra of the solutions are recorded within the visible EM range of 400 to 700 nm. Again, the fluorescence spectra are recorded after exciting the prepared solutions using a light source with a peak emission wavelength of 532 nm. It has been observed that the absorbance values at 532 nm and fluorescence emission intensities at 556 nm are varying in accordance with the concentration of Hg^{2+} ions in the sample solutions. With the increasing concentration of Hg^{2+} ions in the sample solution, both absorbance and fluorescence spectra of the standard solutions are observed to be decreasing gradually. This implies that the considered chemical assay is reliable to detect Hg^{2+} ions in water samples.
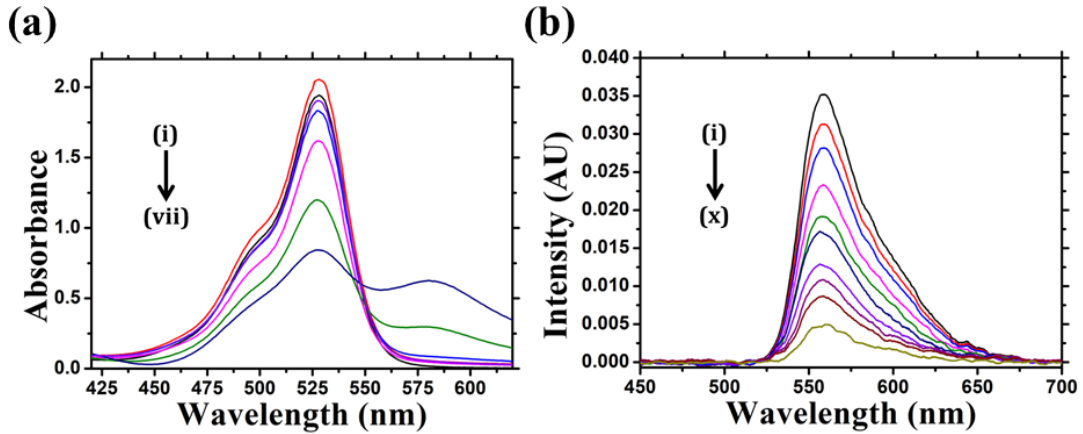
Figure 3.3: (a) Absorbance spectra of standard Hg^{2+} test samples with concentration 0.00 ppm, 0.05 ppm, 0.10 ppm, 0.15 ppm, 0.30 ppm, 0.50 ppm and 0.80 ppm [(i) to (vii)] measured with the standard UV-VIS spectrometer. (b) fluorescence spectra of the standard Hg^{2+} test samples with concentration 0.00 ppm, 0.05 ppm, 0.10 ppm, 0.20 ppm, 0.25 ppm, 0.30 ppm, 0.40 ppm, 0.45 ppm, 0.50 ppm and 0.60 ppm [(i) to (x)] recorded by the standard spectrometer.

3.5   Results and Discussions

Prior to engagement with the unknown water samples towards estimation of Hg^{2+} ions concentration, an absolute regression analysis of the designed platform's responses using standard samples has been evaluated. Standards samples of varying Hg^{2+} ions concentrations (0-0.80 ppm) have been prepared by utilising the chemical protocol as described in section 3.4.1. The fluorescence signal of each standard sample has been recorded by the smartphone fluorometer for five consecutive times. After that, all the captured images were cropped down to 200×200 pixels, and the mean V channel values were estimated by the Android application. Figure 3.4 (a) shows the characteristic calibration curve of the designed sensing platform as obtained for the prepared standard Hg^{2+} solutions. The figure also includes the linear fitted line of the experimental data where the co-efficient of regression (R^2) is estimated to be 0.98. It indicates a good degree of linearity between V channel values and the analyte concentrations. Figure 3.4 (b) depicts the collection of cropped images that have been captured against each Hg^{2+} sample solution to perform the current study. From the above regression analysis, a calibration equation of the designed sensing system can be obtained and is shown in equation 3.2. Using the equation 3.2, one can easily estimate the concentration of Hg^{2+} ions of an unknown water sample. The important figure of merits of the proposed sensing platform have

also been evaluated and are summarised in tabular form in Table 3.1.

$$C = \frac{(V - 1.007)}{(-0.20)} \tag{3.2}$$

where, C is the Hg^{2+} concentration and V is the V channel value in HSV colour space of the captured image.
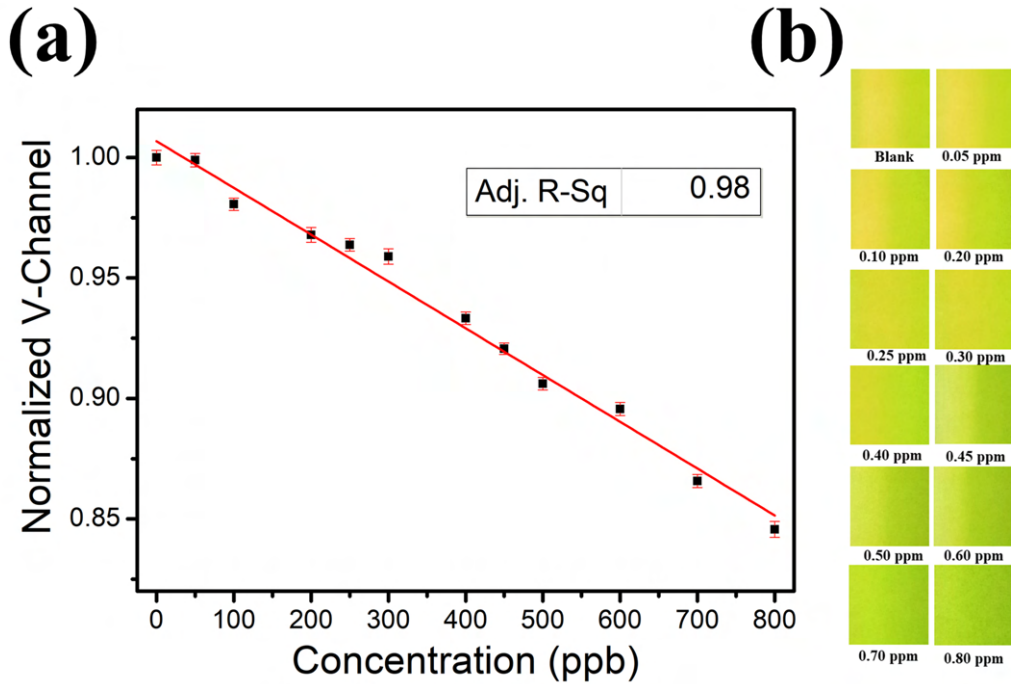

Figure 3.4: (a) Calibration curve of the proposed platform obtained for standard Hg^{2+} ion concentrations in water sample. (b) Cropped images of the fluorescence emission signals recorded by the designed sensor for different concentration of Hg^{2+} ion in water samples. The error bar has been obtained upon recording the signal response for 5 consecutive measurements of each sample.

The responses recorded by the developed smartphone platform have been subjected to linear regression analysis to formulate the above equation 3.2. Linear regression is an important statistical approach that has been widely used to predict outcomes based on some input variables. Here, the outcome and input variables are called the dependent and independent variables respectively. In this study, the analyte concentrations have been considered as independent variables, while the V

channel values of the captured images are considered as the dependent variables. These variables are plotted against each other and try to obtain a line that suitably fits all the data points with minimum errors using the least square fit method. Let, x_n's represents the concentrations of the analyte, while y_n's represents the V channel values, where n represents the number of observations (n=1, 2, 3 …. n). From the given dataset, a linear line of the form, y = mx + c can be obtained where m gives the slope and c gives the y-intercept. The following formula can be used to get the value of the slope m and the intercept c.

$$m = \frac{(n\Sigma xy - \Sigma x\Sigma y)}{(n\Sigma x^2 - (\Sigma x)^2)} \qquad (3.3)$$

$$c = \frac{(\Sigma y - m\Sigma x)}{n} \qquad (3.4)$$

Table 3.1: Characteristic parameters of the designed smartphone fluorometer.

Sl. No.	Figure of merit	Numerical value
1	Standard deviation	1.87×10^{-3}
2	Sensitivity	0.2 AU/ppm
3	LoD	32 ppb
4	% Bias	0.71
5	% RSD	0.12

3.6 Performance evaluation of the tool in presence of other elements in the test sample

In the next step, the performance of the proposed sensing platform has been evaluated by measuring the sensor responses in presence of interfering elements such as Fe^{2+}, Cu^{2+}, Zn^{2+} and Mg^{2+} ions. 1 mM solution of each element has been added separately to 0.1 ppm Hg^{2+} solution. 1 mM of Fe^{2+}, Cu^{2+}, Zn^{2+} and Mg^{2+} ions are equivalent to 56 ppm, 63 ppm, 65 ppm and 24 ppm respectively which have been added separately to the 0.1 ppm of Hg^{2+} samples. Also, a mixture of all the interfering elements of the same concentration has been added to the 0.1 ppm Hg^{2+} sample solution. A total of six samples including the reference sample which contain
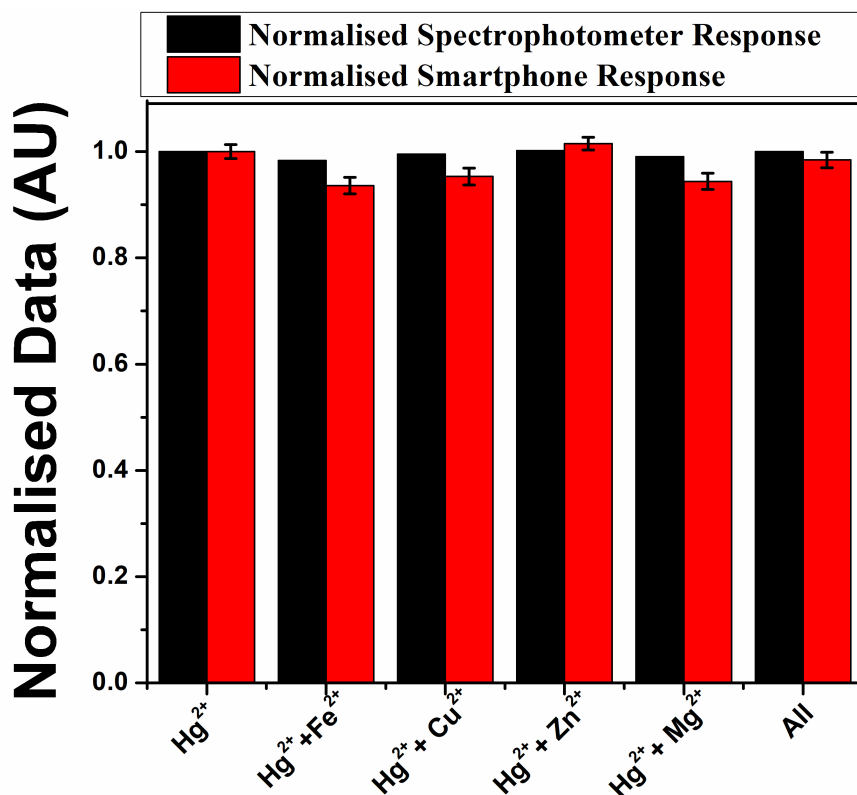
Figure 3.5: Histogram representation of developed smartphone platform and standard spectrometer responses in presence of Fe^{2+}, Cu^{2+}, Zn^{2+} and Mg^{2+} ions with concentration 56 ppm, 63 ppm, 65 ppm and 24 ppm respectively in Hg^{2+} test sample. The error bar has been obtained upon recording the signal response for 5 consecutive measurements for each sample.

only Hg have been prepared to evaluate the performance of the proposed sensor in the presence of the interfering elements. Figure 3.5 illustrates the histogram representation of the sensor responses for all the considered samples. The figure also includes the spectrophotometer data of the same solutions measured by a standard spectrophotometer. Clearly, the figure shows that the smartphone sensor response has not been perturbed by the presence of other elements in the sample solutions. Clearly, a good degree of correlation has been noticed between the smartphone sensor data and the spectrophotometer data for all the sample solutions which again suggest that with the considered protocol the smartphone sensing platform is suitable to estimate Hg^{2+} ion even in presence of the interfering elements in the sample solution.

3.7 Study of the sensor response for field-collected water samples

In the final step, performance of the designed smartphone senor has been evaluated by measuring the Hg^{2+} ion concentration for field-collected water samples. Water samples from five different water sources of Assam, India, namely, pond water of Tezpur town (S1), Brahmaputra river water of Tezpur (S2), pond water of Tezpur University campus (S3), Bharolu river water of Guwahati (S4), drain water near Downtown hospital, Guwahati (S5) have been collected. To eliminate the suspended particles, all the field-collected samples were initially filtered thrice with grade-1 *Whatman* papers. Upon filtering the water samples, 50 ppb concentration of Hg^{2+} ion has been added separately to each field-collected water samples. This has been followed by treatment with KI buffer and R6G to the sample solutions. Figure 3.6 illustrates the histogram representation of sensor response for different Hg^{2+} ion concentrations present in the filed-collected water sample. For comparison, the figure also includes Atomic Absorption Spectrometer (AAS) data of the same samples recorded by the standard tool (*Thermo Scientific iCE 3000 Series*). The maximum difference of the sensing data recorded by both tools was found to be 11.25%. This suggests that the designed smartphone sensor can be reliably used to measure Hg^{2+} ion concentration for field-collected water samples. For field-collected water samples, the maximum %Recovery was observed to be 107.23% corresponding to the sample S3. Table 3.2 summarizes the %Recovery for all the field-collected water samples when the 50 ppb of Hg^{2+} has been added to the samples before analysis.

Table 3.2: Analysis of field samples by the proposed smartphone sensor collected from different location in Assam, India.

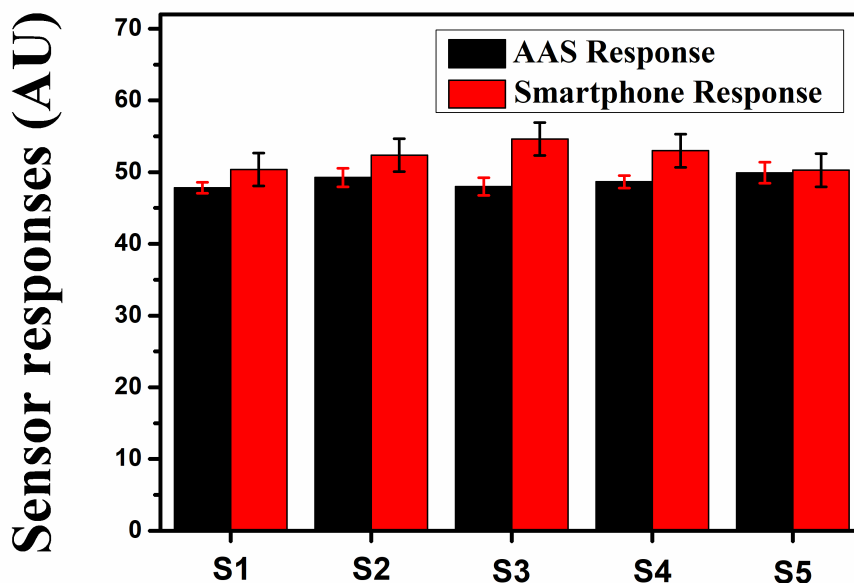| Water sample	Mercury added (ppb)	Mercury Estimated (ppb)	% Recovery
S1	50	49.36	98.79
S2	50	51.37	102.74
S3	50	53.61	107.23
S4	50	41.99	103.99
S5	50	49.27	98.55

Figure 3.6: Histogram representation of Hg^{2+} concentration in field collected water sample measured by both smartphone platform and Atomic Absorption Spectrometer (AAS). The error bar represents the signal response for 5 consecutive measurements of each sample measured by both the tools.

3.8 Summary

The present chapter describes the working of a smartphone-based Hg^{2+} sensor by using commonly used optical components and relatively low-cost 3D printed plastic setup. The proposed sensing system is found to be inexpensive, light-weight and compact. Furthermore, the custom-designed application developed for this proposed sensor makes the setup truly user-friendly analytical tool. With the present chemical protocol, Hg^{2+} ion concentration up to 32 ppb can be reliably measured by the designed platform. This limit is above the permissible level set by the US-EPA and WHO. Present work describes only the proof-of-concept of the fluorescence-based sensing scheme using which Hg^{2+} ion concentration in water can be estimated with the proposed smartphone platform. There exit several other fluorescence-based sensing schemes that use different chemical routes [20–22] using which Hg^{2+} ion concentration well below the permissible limit can be detected. Such studies can be carried out with the designed sensor in the future course of work. It is envisioned that the proposed sensing system will emerge as an alternative platform for monitoring other toxic heavy elements as well, which will be detected by measuring the fluorescence signal intensities emitted from the analyte sample.

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