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An improved simple, robust, cheap and potential diagnostic device for Lead(II)

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Environmental pollution and its consequences are of global concern. There is an urgent need of development of simple, low-cost detection or diagnostic point of care devices to detect, analyze and determine the pollutants in various environmental, food and biological samples. Such development will be critically important for improvisation of human health and quality of life. Toxic heavy metals potential contaminates and has lethal effects on deterioration of environment and thereby on human health. Paper based analytical sensors are an efficient diagnostic point-of-care devices being cheap, robust, user friendly and environmental-friendly. We have proposed a paper-based sensor for detection and quantitative determination of Lead by immobilization of potassium iodide and ninhydrin over paper platform. A bright yellow colour of varying intensity develops on coming in contact with samples contaminated with lead. The coloured area is then scanned and images are transferred to image processing tool of MATLAB software to read their RGB values. The effective intensities are then calculated mathematically. The developed sensor determines the contamination of lead up to 0.01 ppm. Thus, the proposed paper based analytical sensor is potential diagnostic point –of-care device.

Keywords: Analytical device, Lead detection, MATLAB assisted paper-based sensor, Point-of-care diagnostics

Environmental pollution is drastically exploiting the resources of nature and hence is a global concern. Heavy metals considerably account for this problem, because of their widespread use and tendency of biomagnification. Lead (Pb) is one of the most hazardous toxic metals which cause lethal effects when it enters the food web. It is used widely and is a versatile and persistent poison. Its significant presence in environment leads to deformities in young children.

Sensing toxic entities on paper-based devices is comparatively recent and a new alternative technology. These analytical devices are simple, low-cost, portable and disposable. These can be applied for clinical diagnosis (health diagnostics)[1-19], food quality control[8,13,20] as well as for environmental monitoring[13,21,22,23,24] The main advantages of using paper as a sensing platform are its uniqueness to allow passive liquid transport and compatibility with chemicals/biochemicals. The paper which is cellulose fiber allows liquid samples to penetrate into its hydrophilic fiber matrix and does not need an active pump or external source[25]. These paper-based devices can be functionalized by bringing changes in hydrophilicity, permeability and reactivity as per the requirement[26].

Paper has emerged as a promising platform for preparing sensing in the analytical, clinical, food and environmental chemistry because of its simplicity, low cost, versatility and high abundance[1, 27-29]. These analytical devices can be integrated in a manner that is flexible, portable, disposable, easy to operate, point-of-care (POC) diagnostics. The first paper-based device was demonstrated in 1956, device for the semi-quantitative detection of glucose in urine[30]. These devices were further developed as immune chromatographic paper test strips (lateral flow or dipstick tests), with a well-known example of pregnancy test kit[31].

The most common techniques reported for paper based quantitative analysis are chemiluminescence[2], electrochemical[1,13], colorimetric[3,5,6,32,33,34], electrochemiluminescence[35,36] and electrical conductivity[37,38].Colorimetric detection has been most common for paper-based sensors because it provides strong contrast with a coloured substrate and can be disposed of easily and safely after use. These analytical devices are efficient but produce semi-

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quantitative results. Martinez et al.[3] were the first to demonstrate paper-based devices. The method is less precise as compared to UV-visible and atomic absorption spectroscopy as visual interpretation of colour and intensity is different for different individuals, condition of light and the type or condition of paper substrate. Martinez et al.[39] modified the accuracy by measuring the visible colour changes with use of camera phone or scanner and using digital imaging.

Most commonly used paper-based diagnostic devices are urinalysis dipsticks and litmus paper. Paper is also used as a support for qualitative spot tests for analytes with applications covering clinical diagnostics, organic and inorganic chemical analysis, environmental and geochemical analysis, and pharmaceutical and food chemistry[40-42]. According to the World Health Organization, "diagnostic devices for developing countries should be ASSURED: affordable, sensitive, specific, user-friendly, rapid and robust, equipment free and deliverable to end-users"[43].

Owing to such important and diverse applications of paper-based sensing devices, our group also developed paper-based sensing devices with improved accuracy by inclusion of digital image processing tool of MATLAB[44-48]. More advancement in this area are needed towards development of such analytical devices as these are need of an hour and can contribute towards cheap on-site and in-time monitoring of toxic entities. Present paper is an effort to determine lead concentration at point and in real samples using MATLAB assisted paper-based sensor and thereby minimizing its ill effects. The sensor comprises of a Whatman filter paper no 1 impregnated with potassium iodide and ninhydrin.

Materials and Methods

Apparatus and software

The scanner (HP-laser Jet M1005MFP) was used for scanning the Whatman No.1 strips. The scanner was set at resolution of 300 dpi. For analysing colour values in the RGB (red, green and blue) system, MATLAB 2011 software written in Visual Basic Media was used. For injection of sample aliquot onto the sensing strip micropipette (Eonpipette-A07088) was used.

Chemicals and reagents

All reagents used in making sensor were of analytical grade chemicals. The water used during the experiment was doubled distilled and de-ionised water. Stock solution of 74 ppm of Pb was prepared by dissolving1g of $Pb(Cl)_2$ in 100 mL of distilled water. Required dilutions were prepared daily. potassium iodide (KI) solution (1% KI) was prepared by dissolving 1 g KI in 100 mL of doubled distilled water. 1% Ninhydrin solution was prepared by dissolving 1 g ninhydrin in 100 mL doubled distilled water.

Procedure

Lead produces yellow coloured complex product when reacted with KI and ninhydrin. The sensor strips for lead was constructed by immersing the Whatman strip of a particular dimension in known concentration of KI for few seconds followed by drying in oven at temperature 60 °C then again immersing in known concentration of ninhydrin and followed by drying in oven at same temperature. Injection of aliquots of 6 μL lead solution on sensing strip was done. A spot of yellow colour was produced instantly, sensor was scanned and then image of spots were transferred and analysed by MATLAB software to deduce the value of R, G and B. The RGB colour model is an additive colour model in which red, green and blue light are added in various ways to produce a broad array of colours. Wide range of colour can be analysed to get their corresponding R, G and B value. The effective intensity of colour spot of any colour value can be calculated by following formula.

$$A_r = -\log (R_s/R_b) \quad \ldots(1)$$

$$A_g = -\log (G_s/G_b) \quad \ldots(2)$$

$$A_b = -\log (B_s/B_b) \quad \ldots(3)$$

Where, A_r, A_g, A_b are effective intensities of the red, green and blue colour, respectively, R_s, G_s, B_s and R_b, G_b, B_b refer to R, G and B values of the sample and blank, respectively. For getting calibration curve, effective intensities of R, G and B values were plotted against analyte concentration.

Results and Discussion

Lead(II) react with KI to liberate iodine which then binds with ninhydrin to form a coordination complex species of yellow colour (exact composition of complex is under investigation). Yellow colour is produced only on introduction of sample containing Pb(II) on to the sensor. The sensor so developed will efficiently diagnose the presence of lead in various aqueous samples and serve as potent device to prevent the toxicity. Sensing strips should be stored in air tight

packaging with minimal exposure to sunlight because this on exposure to light and air gets coloured due to oxidation which in turn affects effective intensity.

Optimization of conditions

Injection volume

The volume of analyte which is injected onto sensing strip was studied. It was observed that optimum volume of sample is 6 µL which produces maximum intensity on the strip. It was seen that when we increased the volume of injected sample it resulted in dispersion of spot at periphery thus decreasing the intensity of colour.

Effect of reagent concentration

To optimize the concentration of KI and ninhydrin, sensors with different concentrations of reagents were made by immobilizing the Whatman filter paper. Each time 6 µL of stock solution (74 ppm) of Pb(II) was introduced to each strip with the help of micropipette followed by drying. Scanning of the coloured spots and RGB analysis with help of MATLAB software was done using IP tool. The effective intensities obtained for R, G and B values were plotted against concentration of KI/ninhydrin to know the optimum volume. Figs 1 and 2 depict the impact of KI and ninhydrin concentration on intensities of spots, respectively and the maximum colour intensity was observed for both 1% of KI and 1% of ninhydrin. For this reason, 1% KI and 1% ninhydrin were set as the optimum concentration of the reagents.

Drying methods

After introducing the reagent onto the sensing strip, it is needed to be dried. It was observed that no considerable change in intensity of signal has been noticed when dried through different methods like drying naturally at room temperature, in hot air oven or in normal oven. But it is recommended to dry the strip under oven to increase the rate of analysis. Heating the sensing strip at high temperature should not be done for longer period of time as it will result in turning of colour of strip which will raise blank spot intensity.

Calibration curves

The calibration curve was found to be linear for 0.09–0.5ppm (Fig. 3; Photo 1). Figure 3 can be used for determination of Lead concentration. It is clear from Fig. 3 that the B-values are most sensitive of all. The graph is perfectly linear as indicated by the trend line for B-values and nearly linear for G-values, but R-values are nearly ineffective. Thus, B-values are recommended for determination. For lower concentrations B-values can be used as they are more sensitive.

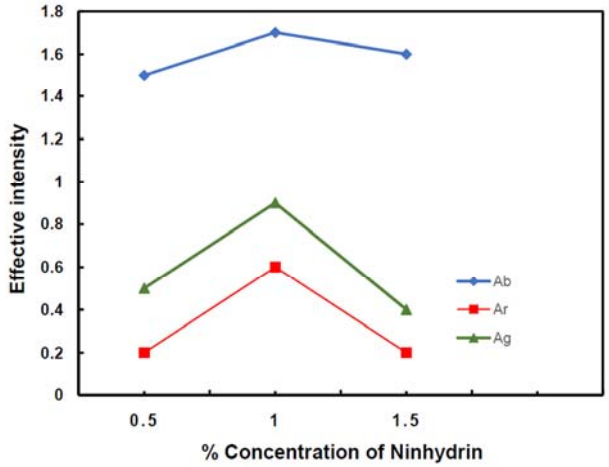

Fig. 1 — Variation of KI concentration

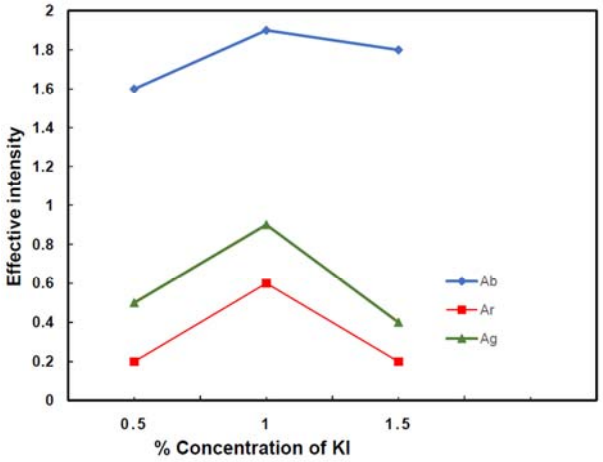

Fig. 2 — Variation of NEDA concentration against

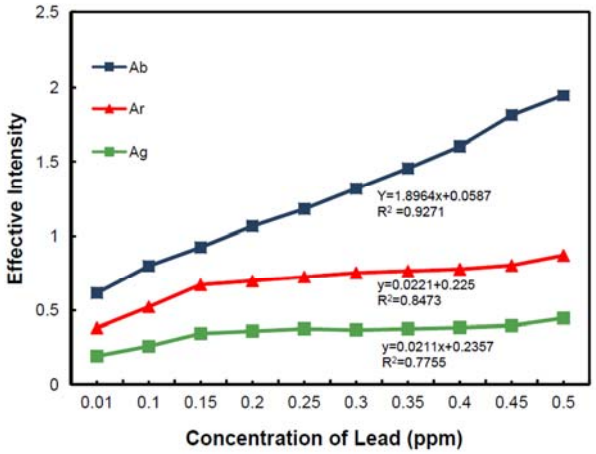

Fig. 3 — Calibration graph for 0.01–0.5 ppm of lead

Reproducibility, response time, stability and detection limit of the system

The sensor was tested for reproducibility and it was found that intensity of spot remains same when tested for several times for particular concentration of Lead. It was tested for different concentration of Lead, the conclusion was drawn from SD and RSD deviation values for A_g and A_b, that the method is reproducible. To test for the response time, the sensor was injected with sample containing Lead and time required to get 90% of steady colour intensity was calculated. The spot was generated instantly as the sample was introduced to the strip. No change in intensity was observed on heating.

Selectivity

The effect of other metallic species for studying selectivity of proposed reagent system on developed paper-based sensor for determination of lead was tested under optimum conditions without any masking (Table 1).

Stability

The intensity of the spots which were obtained on injecting Lead solution on sensor was calculated with help of MATLAB and it was seen that the intensity after 30, 60, 90,120,150, 180, 210, 240, 300 and 360 min and then after 24 h remains nearly stable and constant. After 24 h it was observed that spots diffuse thus it was concluded that the spot remains stable only for 24 h after injection of sample containing lead.

To test the stability of sensor and effectiveness of sensor from its date of production, the sample containing lead was tested from sensor on each day for 10 days. It was found that the sensor worked with the same efficiency. After the 10th day it was found that the no spots were obtained on injecting the sample to TLC strip. This leads us to conclude that the sensor will be effective for minimum 10 days. For each RGB factor there is only one DL. DL of the method practically found was 0.01 ppm. The practical DL is the lowest concentration, which gives colour on the strip.

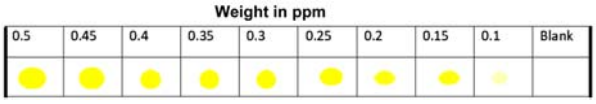

Photo 1 — Colour variation on sensor with concentration

Analysis of real samples

With the help of calibration curve, the amount of lead present in different brands sindoor and lipsticks were tested. The data that were inferred is given in Table 2. It was concluded that the quantitative determination of Lead can be performed under region assessed. Table 3 depicts its wider linear range in

Table 1 — Tolerance limit of various interfering species on 100 ppm determination of Pb(II)

S. No.	Interfering Metallic Species	Tolerance Limit (ppm)[a]
1	Mn^{2+}	10
2	Cr^{3+}, K^{+}	15
3	As^{3+}, Se^{3+}	8
4	Fe^{2+}, NH_4^{+}, SO_4^{2-}	4
5	Cu^{2+}, Cd^{2+}, Zn^{2+}	12
6	Hg^{2+}, Ba^{2+}, Cl^{-}	10
7	Na^{+}, HCO_3^{-}, CO_3^{2-}	5

[a]The limit of tolerance with ±5% error

Table 2 — Analysis of lead in real samples by the proposed sensors

Sample	lead originally present (ppm)	lead added (ppm)	lead found[a] (ppm)	% Recovery
Sindoor brand 1	0.4	22	25	113.6
Sindoor brand 2	0.3	30	29	96.6
Lipstick brand 1	0.2	22	26	118.1
Lipstick brand 2	Not found	30	29	96.6

[a] Total lead found after addition

Table 3 — Comparison of proposed method with other methods

S. No.	Reagent / reference	Technique	Range of determination	Remark	Detection limit	Ref.
1	5'-Cy5.5-SH-3(CH2CH20) 6-GAAGGTGTGGAAGG-3' Linked to Au NPs	Fluorescence	1×10^{-11} to 1×10^{-6} M	Costly and time consuming	2.7×10^{-13} M	49
2	Flavonoid moiety incorporated carbon	Quantum dots	1×10^{-10} to 2×10^{-8} M	Not user friendly	5.5×10^{-11} M	50
3	Rhodamine 6g derivative	Fluorescence	1×10^{-8} to1×10^{-5} M	Limited sensitivity	2.7×10^{-9} M	51
4	Coumarin	Fluorescence	6×10^{-6} to 2×10^{-5} M	HEPES Aqua medium required.	3.36×10^{-11} M	52
5	Mizo Tetra(N methyl -4Pyridyl) porphine tetrachloride	Spectrophotometry	5×10^{-6} M	Cd^{2+},Fe^{3+},Hg^{2+},Cu^{2+}	0.5×10^{-6} M	53
6	Silica coated ZnS QDs(ZnSOSiO2 QDs)	Fluorescent	1×10^{-9} to 2.6×10^{-4} M	Cd^{2+} interference	-	54
7	KI + Ninhydrin	MATLAB assisted spectroscopy	0.08×10^{-5} to 0.05×10^{-5} M	Remark	0.44×10^{-5} M	Proposed Method

Table 4 — Advantages of proposed MATLAB assisted paper-based sensing device with other sensing devices

S. No.	Substrate	Coating	Type of method	Detect	Detection limit (ppm)	Remark	Ref.
1	Grapheme oxide sheet	Aptamer	Fluorescence microscopy	Pb^{2+}	0.5	Sophisticated instrument	55
2	Nitrocellulose paper	Ag/AgCl	Electrochemical	$Cd^{2+,}$ $Pb^{2+,}$ $Bi^{3+,}$ Zn^{2+}	2×10^{-3}	Costly	56
3	Whatman Filter paper	Screen printed carbon electrodes	Electrochemical	$Pb^{2+,}$ Cd^{2+}	2×10^{-3}	Not layman friendly	57
4	Whatmann filter paper 4	ZI, ALS, Cyclohexane, Cyanide, DPC, CPZ, PAN	Colour picker application	Pb, Cr, Mn, Cu, Zn	0.2	Require many chemicals	58
5	Whatmann filter paper 1	KI + Ninhydrin	MATLAB assisted	Pb, Ni, As, Cd	0.01	Cheap, Robust, Layman friendly	Present method

comparison to others methods and found that our paper-based sensing method has various advantages over other methods[49-54] This paper-based sensor was used as potent diagnostic tools and are found to have advantageous over other such devices in terms of robustness, user-friendliness, cost effectiveness, simplicity, rapid on-site detection or home-care testing[54-58] (Table 4).

Conclusions

In conclusion, our paper-based sensing method is a simple and efficient, robust, sensitive and user-friendly method for point of diagnosis, even by a layman. It is cost-effective and hence can easily viable by all rapid on-site detection or home-care testing. Moreover, the fibrous network of paper stores reagents in active form which increases the surface area with respect to volume, providing easy and efficient detection and the use of MATLAB software accurately determines the concentration of metal. Paper being porous substrate, the test result may be easily obtained within short range of time. Its novelty lies in the fact that it is not restricted to detection of transparent sample we can thus elaborate use of this sensor in testing of various sample in case of emergency.

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