Development of Microfluidic Paper Based Analytical Device for Detection of Phosphate in Water

Bhavishya Waghwani, Suresh Balpande, Jayu kalambe

Abstract**:** ***In this paper, the researchers have developed a microfluidic paper-based analytical device (µ-PAD). In order to quantify the precise concentration of phosphate in water, ImageJ tool has been used. The ImageJ is used to calibrate the color range and intensity for phosphate concentration detection. For creating a hydrophobic barrier on filter paper poly-dimethylsiloxane (PDMS) and rubber stamp with desired pattern of µ-PAD was used. The microfluidic device developed is a low cost solution which is simple to use in rural and urban areas for detection of contaminants in water. The excess amount of phosphate in water causes eutrophication which is harmful for aquatic animals likes fishes. The microfluidic based platform is developed and experiments are performed for detecting the concentration of phosphate ranging from 1 mg to 20 mg/ml. For the spot test, the user just needs to drops 1 ml of water and the results are obtained instantly.***

Index Terms**: *Microfluidics, Phosphate, Water***

I. INTRODUCTION

The groundwater contamination occurs due to either natural causes or anthropogenic activities which need regular monitoring of water quality to devise ways and means to look after it. Contaminants concentrations in surface and groundwater bodies in India are increasing due to rapid urban development because of increasing economic-growth rates. High concentration of contaminants in drinking water poses warning to human, plants/crops and creature's health. It is reported that the most impacting contaminants are nitrogen and phosphate parameters occurring due to overuse of chemical fertilizers and pesticides in agriculture. Phosphate has significant values in both agriculture and biomedical applications. To address this problem, we proposed to develop a low cost microfluidics paper based sensors for monitoring of water quality with a special emphasis on detection of contaminants like phosphate and nitrogen contents [1]. Phosphate is the most essential content for the nutrient for plant and animal species in natural waters. Phosphate is a limiting factor in the growth of most aquatic ecosystems. Phosphates are basically the salts of various phosphoric acids [2]. Phosphates are used in animal fertilizers, water treatment, fertilizers, domestic and industrial reagents, metal treatment to inhibit corrosion and ceramic production. Increase in level of phosphate in waters includes input from agriculture (fertilizers and animal wastes), municipal and industrial sources (treated and untreated). Elevated nutrient levels lead to excessive of growth of algae and aquatic fauna. An Algal boom can direct to water becoming contaminated to animals and human and impairing the use of water. Excessive nutrient also causes eutrophication and it is one of the major causes for death of aquatic animals. An acceptable limit of phosphate varies accordingly [3]. Eutrophication decreases the amount of sunlight and increase the concentration in water bodies. Increase in carbon dioxide deprives aquatic organisms of oxygen which results the death of fishes [4]. There are several methods reported for qualitative detection of phosphate in water with chemical reactions. As reported in [5] the microelectrode is integrated with the microfluidics systems for the phosphate detection. This process uses the gold nanoparticles along with the molybdophosphate complex for total phosphate determinations in water. The microelectrode fabricated using microelectromechanical systems (MEMS) techniques and porous, branching gold nanoparticles (AuNPs) were electrodeposited on the microelectrode to improve its sensitivity. In [6] the system is based on microsystem technology for analysis of phosphate detection in water. The microfluidic system has been developed for the analysis. For determining the concentration of phosphate, apparatus used is the spectrometer, due to which the cost of system becomes high. The electrochemical phosphate sensor was using the ammonium molybdate tetrahydrate/silver nanowires by modified screen printed electrodes for phosphate detection. In [7] silver nanowire is used which is quite costly which increases the overall budget of the system and also the set up is too complicated to understand. The rural and underdeveloped areas cannot use these methods due to high cost, large instrumentation size, bulky reactions and large quantities of required samples. However, in current field testing the user need to wait for hours to get the result. Expensive testing facilities make it difficult to monitor only the quality of phosphate in the water [8]. Inexpensive field testing helps in recognizing the problem and in improving the quality of water.

To conquer these problems microfluidics platform provides an excellent solution because of its various advantages like it requires a low volume of fluid, high specificity of chemical results in uniform reaction condition and higher grade products in single or multistep reaction [9]-[10]. A paper-based microfluidics has involved

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the attention as a new point of care (POC) diagnostic platform due to their inexpensive, usable and designed especially for the use in developing countries [11].This allows the fabrication of simple sensor that can be prepared very easily and maintain the advantage of polymer-based microfluidic devices [12]-[13]. Microfluidic paper-based analytical devices (µPAD) made up of patterned paper enable fluid handling and quantitative chemical analysis when applying it in the field of medicine, healthcare and environmental monitoring [14]. Developing countries (DCs) and in developed countries demands development of relatable assays for water and food safety as they are of greater importance. Appropriate practical detection and quantifying analytic methodology must be robust, lightweight, easy to handle, low-cost and it should also be quantitative. The attempt has been made to detect the adulteration in milk using this methodology has been reported in our previous work [15].

This paper proposes a combination of the paper-based assay and a portable detector offering a low cost and effective solution for phosphate detection. We have developed a POC system comprised of µPAD that analyses the concentration of phosphate in water. The µPAD run colorimetric assay and the ImageJ quantifies the concentration of phosphates based on the RGB value of color produced in the assays. A droplet-based µPAD, stores the reagent used for detecting phosphate in water. The µPAD is printed with the assist of rubber stamp using PDMS material and parched in an oven to create a hydrophobic barrier on paper that forms a region for testing and storing the chemical reagent. Chemical reagents are applied on paper and they are stored for use. A laboratory test of detecting phosphates on droplet-based µPAD is done. The developed system has a variety of characteristic relevant to POC applications that are appropriate for the low-income countries like utilizing the low volume of fluid for testing phosphates. The database is prepared for 1mg to 20 mg in 1ml of water is prepared with experimental results. The developed detection methodology is discussed in this paper.

The paper is organized is as follows, in section 2 Experimental procedure is discussed, Results and discussions are presented in section 3 followed by conclusion in section 4.

II. EXPERIMENTS

A. Material and Chemicals

All the chemicals used were of analytical reagent graded quality and were employed as received. The following materials were used to prepare spot tests Ammonium Molybdate, Ammonium Metavandate and Potassium Dihydrogen OrthoPhosphate. Whatman® grade 1 filter paper and Sylgard 184 were acquired and hexane (Sigma) respectively for µPAD.

B. Fabrication of test spot and µPAD

An economical methodology is used for fabricating µPADs via PDMS as a hydrophobic barrier. PDMS was used for creating a barrier as it performed fine with organic solvents [16]-[18]. Using contact printing methodology, an elastomeric stamp was developed for the fabrication of µPADs. Firstly, the elastomeric stamp with custom design surface containing the pattern of µPAD was applied to a solution of PDMS (10:1 mixture of Sylgard 184 prepolymer and curing agent) and hexane 10:1.5 (w/w) and lightly pressed against the surface of the filter paper. A mixture having a lower concentration of hexane requires a long time to go through and with high concentration gives high deterioration in the resolution. The total thickness of the filter paper requires 30 seconds to blend and then PDMS was cured for 30 minutes at 80º C. The stamp which was used here is shown in fig. 1. The most attractive aspect of this rubber stamp is that it is of low cost and it can be used for longer extent without any potential degradation.

Fig. 1: Rubber Stamp

The numerous arrays of 0.75 mm diameter circle were printed on one side of the filter paper using elastomeric stamp and for creating the hydrophobic barrier the back area of the test spot was enclosed. For chemicals not to get evaporated from the filter paper, the transparent plastic layer was used to cover the front side of the test spot. The fabricated µPAD was kept in a zipped tight Mylar bag at room temperature. After packaging, stable result for the detection of phosphate were obtained till 5 days.

C. Spot test imaging

The images of µPAD were taken inside the packed box which was made from a normal shoe box. The box was covered completely with a black paper for avoiding the external lights and a hole was created on the top to accommodate camera lens. The light emitting diode light series was kept on both the sides and it was enclosed with numerous layers of printing paper so that the light diffuse within the box. The experimental setup is shown in the figure 2.

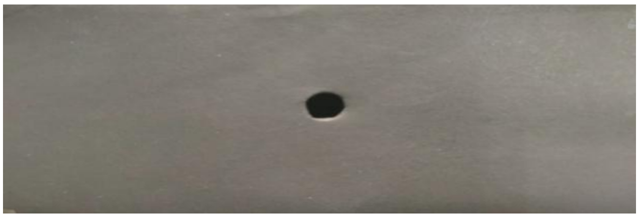

Fig. 2 a

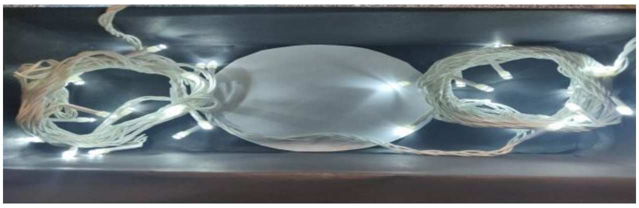

Fig. 2 b

Fig 2 shows the experimental set up (a) for accommodating the camera lens on top of the box (b) for placing the filter paper along with LED light inside the box.

III. Results & Discussions

For sample preparation, the phosphate was added manually ranging from 1 mg to 20 mg in 1 ml of water sample. Some of the experimental results are shown here. Each experiment was performed in three sets for checking the consistency in the results. The same method used for the detection of phosphate in water can also be used for the detection of nitrogen, heavy metals and other contaminants dissolved in water.

A. Detection of phosphate in water

The analyzer is based on the molybdenum yellow method. This method involves mixing the sample with an equivalent volume of a reagent containing 0.357 gm of ammonium metavanadate and 7.143 gm of ammonium molybdate in an acidic standard. Vanadomolybdophosphoric acid is formed, which is yellow in color [2]. The standard deviation and relative standard error were calculated as 0.90 and ± 0.011. The results of phosphate detection are shown in fig. 2.

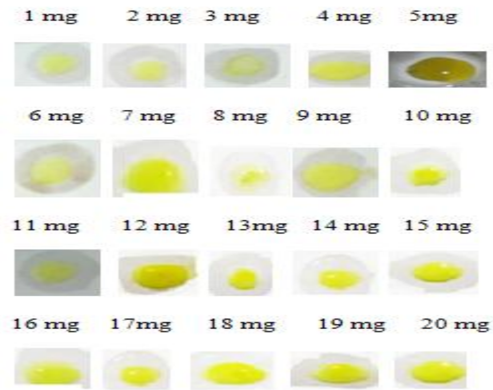

Fig. 3: Spot test results

B. Colour Intensity

Image J software is used for finding the color intensity for the detection of phosphate and the results were obtained instantly. To enumerate the mean color intensity of each spot the Image J software was used. The mean intensity was resolute by drawing a circle using an oval tool over the image and selecting measurement from image toolbar to calculate average intensity in that area. The error bar graph is plotted with the average of standard error and is shown in fig 3.

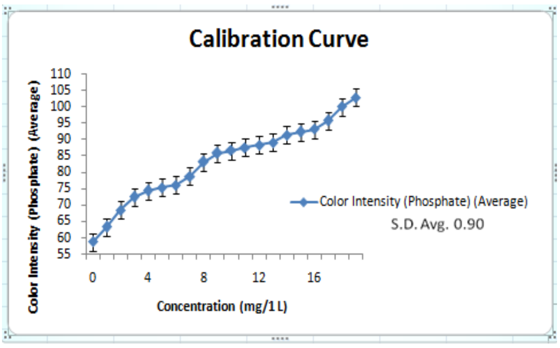

Fig. 4: Calibration Curve for phosphate detection

IV. Conclusion

An inexpensive µPAD is developed for detection of phosphate in water. All the chemicals used for the detection were stored in µPAD. The user just need to apply a single drop of water to the spot test and the presence of phosphate in the sample will be detected instantly. The results obtained are more specific and precise with ImageJ software. ImageJ gives the result of the presence of phosphate in the form of intensity. The low-cost µPAD solution is developed and making it easily available for users and water testing centers. In future, the studies will be focused on enhancing the stability of spot test for a longer period of time and developing spot test for detection of phosphate in soil, seawater, wastewater and domestic water and for the detection of other water contaminants like nitrogen, cadmium and lead. The µPAD developed is easy to use, handy inexpensive and could be distributed to low-income countries where water contamination is an immense issue.

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