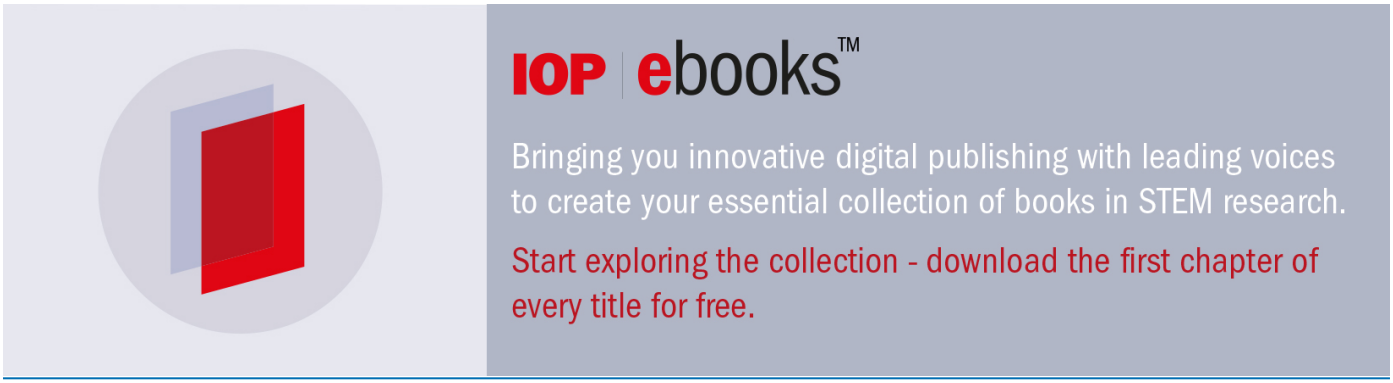
Microfluidic Paper-based Analytical Devices (μPADs) For Analysis Lead Using Naked Eye and Colorimetric Detections



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Microfluidic Paper-based Analytical Devices (µPADs) For Analysis Lead Using Naked Eye and Colorimetric Detections

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Abstract. This work describes comparation two designs of a microfluidic paper-based analytical devices (µPADs) using a wax printing for fabrication of hydrophobic zones on the chromatographic paper and their application to the detection of lead in waste samples. Two different designs used in this research were distance-based method and analysis image method. Sodium rhodizonate in tartrate buffer solution (pH 2.8) solution are used as the colorimetric reagents for the direct naked eyed detection of lead(II) on µPADs. The detection of lead(II) concentration is conducted by measuring the distanceof a colored reaction product which propagate in the detection zone (design 1) and the change in color intensity on the µPADs is also used for the lead detection using ImageJ software to determine the RGB value (design 2). Related to both methods can be used to determine the concentration of lead(II) with a comparable coefficient of determination, but based on the analysis method, design one is a design that is very simple, easy, fast, inexpensive and only uses the naked eye for detection.

1. Introduction

Lead (Pb) is a heavy metal with high toxicity, whose contamination in the enviromental has received much attention for its negative effect on human's health, causing anemia, IQ degradation, and organ malfunctions such as liver and kidney[1]. Its main sources of contamination are industrial waste and fossil fuel residue from transportations [2], mostly accumulated in bodies of water [3].

The analyses on lead vary from simple tests to complicated tests using sophisticated instruments such as atomic absorption spectroscopy (AAS), inductively couple plasma-optical emission spectroscopy (ICP-OES), inductively couple plasma-mass spectroscopy (ICP-MS), X-ray fluorescence (XRF), and scanning electron microscopy (SEM)[4]. In regards to sensitivity and accuracy, the techniques using sophisticated instruments are more promising.However, the procedure is quite complicated, consumption of reagents is large[5], then the limited costs and availability of instruments, especially for developing countries, have not been adequate[6]. For this reason, it is necessary to develop new devices with a method that is simple, inexpensive, easy to use, does not use instruments, and sample analysis can be done on a micro scale [5,6].

µPADis one of the analytic devices first developed by Whiteside's group in 2007[7], in these devices, hydrophilic cellulose fibers form a network of capillaries where in hydrophilic liquids can penetrate and be transported using capillary force only, without requiring an external driving force. In

order to confine and control liquid flow with in the hydrophilic paper, microfluidic channels limited by hydrophobic barriers were patterned [7].

The most common detection method in µPADs analysis is colorimetry because this method uses specific reagents that states analyte concentration marked by color changes [8].Lead metal can be analyzed using µPAD, color changes occur when lead(II) reacts with sodium rhodizonate as a complexion[9]. The color changes that occur can be calculated by analyzing the intensity of the color using computer or smart phone software. Conversely, naked eye is a potentially excellent detector. The first attempt at using the naked eye was in distance based detection, which was developed by Henry's group[10]. The principle of this method is that the analyte concentration is determined by the length of the color band in the µPAD channel, which will be long if the analyte concentration increases [6,10].

Therefore, the purpose of this research is to develop µPADs by comparing two different designs and analysis processes. Design 1 uses a distance-based method with the naked eye as a detector whereas the design 2 is a general design of µPAD, which consists of one sample zone and four detection zones. Design two uses computer software as a detector to measure the concentration of analytes. Some factors, such as fabrication hydrophobic barriers, sample volume and reagents, the length and width of the channel and the length of reaction time are optimized to improve the precision and accuracy of the results obtained.

2. Experimental

2.1. Design and fabrication of the µPAD

A design µPAD was created using drawing software CorelDRAW X7 and was then printed onto filter paper (200× 200 mm, Chromatography paper 1CHR, Whatman, GE Healthcare Lifesciences, United Kingdom) using a Xerox ColorQube 8580DN, CT, USA wax printer. The printed devices were placed on a hot plate, which caused the wax to permeate through the paper, forming hydrophobic barriers that control liquid flow.

Two different types of designs are printed with their respective objectives. Design 1 used distance-based type and design 2 is general design µPAD consist of four reaction zones, which are connected through small channels to a sample zone located at the center of theµPAD as shown in figure 1.

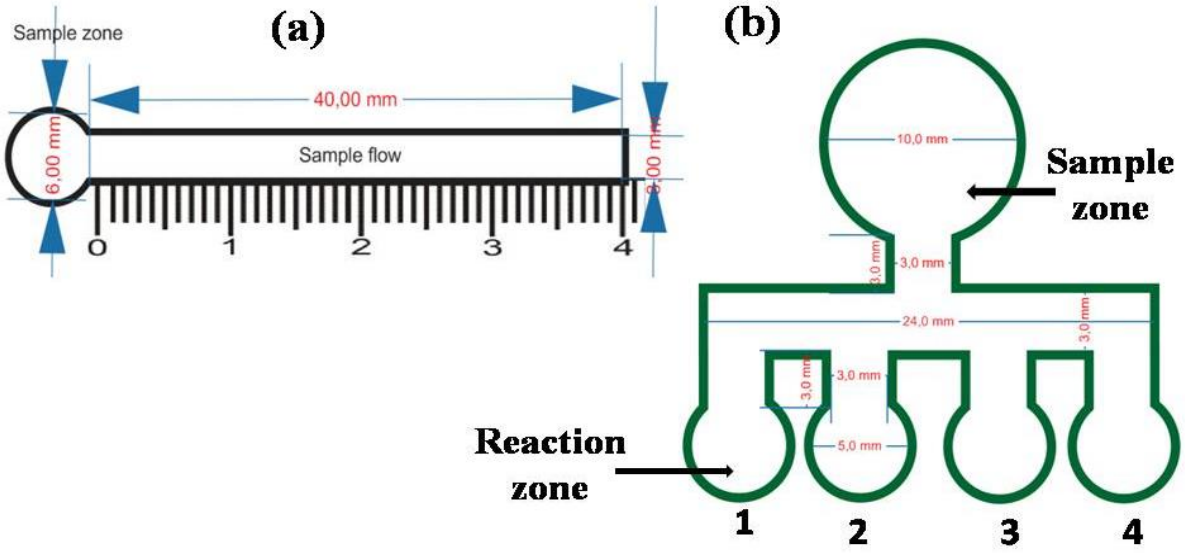


Figure 1. The design of the µPADs (a) distance-based µPAD (b) general design µPAD

2.2. Apparatus and reagents

Some glassware and micropipettes commonly used for analysis are used in this work, chomatography paper, printer Xerox ColorQube 8580 DN, hot plate and scanner (hpDeskJet Ink Advantage 2135 (300dpi).

The material used in this study was distilled water, lead(II) nitrate from Sigma-Aldrich (USA). A 0.16 g lead(II) nitrate is dissolved in 100mL distilled water consisting of nitric acid 0.5 $molL^{-1}$ to produce a stock solution of 1000 mgL^{-1}, then a standard solution of lead(II) is made by diluting lead(II) stock solution with water distilled to get the desired concentration. A 10 mM of tartrate buffer solution (pH 2.8) was prepared by dissolving 1.13 g of tartaric acid (Sigma-Aldrich, US) and 0.58 g of sodium tartrate in dihydrate (Sigma-Aldrich, Spain) in deionized water to produce 500 mL. The reagent concentration for design 2 varied from 0.1-0.4% (b/v) and 0.1% for design 1 sodium rhodizonate (NaR), which was made always fresh when used by dissolving 0.01 g of dibasic sodium rhodizonate 97 % (NaR) (Sigma-Aldrich, Austria) for 0.1% in 10 mL of deionized water

Additional metal salts were used to test potential interferences: barium chloride (Sigma-Aldrich, USA), calcium chloride (Sigma-Aldrich, USA), zinc chloride (Sigma-Aldrich, USA), iron(II) chloride tetrahydrate (Sigma-Aldrich, Germany). All solution for analysis interference study were prepared at 1000 mgL^{-1}in 0.5 $molL^{-1}$nitric acid. The solution were added to the test solution containing 6 mg L^{-1}lead(II) with the lead(II)/interference ions ratio of 1/1, 1/5 and 1/10.

2.3. The procedure of detection

The procedure to detection lead(II) shown in figure 2. A 1µL volume of Sodium rhodizonate reagent (NaR) 0.1% added in detection zone in design 1 and 0.25µL NaR0.1-0.4% respectively, added in detection zone in design 2. After 5 minutes of drying, the device is ready to use. A 10µL stock solution added to the sample zone design 1 and 20µL in design 2. The solution flow in the channel and reacts with NaR, resulting in a color change in the detection zone. The lead(II) ion reacts with NaR to give a complex color of Pb-rhodizonate. After reaction, lead(II) can be analyzed by measuring the color band length in the design 1 and analysis the RGB value in design 2.

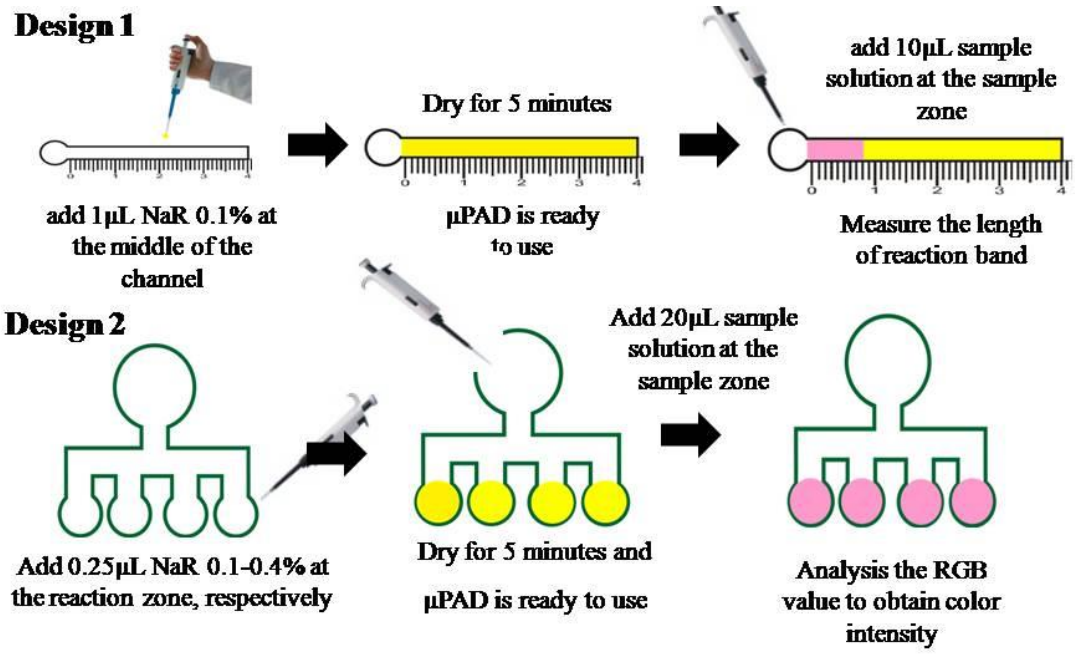


Figure 2. The procedure to detection lead(II) in waste sample

2.4. Artificial waste

Artificial waste was comprised of barium chloride (3 mgL^{-1}, Aldrich), calcium chloride (1 mgL^{-1}, Aldrich), zinc chloride (1 mgL^{-1}, Aldrich), iron(II) chloride tetrahydrate(1 mgL^{-1}, Aldrich) per 100 mL of water. The variation in lead(II) concentration starts at 3, 5, 7 and 10 mgL^{-1}.

3. Results and discussion

3.1. fabrication hydrophobic barrier

The aim of making a hydrophobic barrier is to find out that the penetrated wax maximally forms a hydrophobic pattern on both sides of the paper. To determine the maximum penetration of wax, variations in temperature and penetration time were carried out from 120-180℃ for 30-180 seconds. The best results occur at a temperature of 150℃/120 seconds (Fig. 3), this can be analyzed in two simple steps: 1. measures the width of the spread of wax on the front and back of the paper surface, 2. If the width of the spread of wax is the same on both sides of the paper, then the sample to be tested does not experience overflow from its hydrophobic pattern.

The ink is made of a mixture of hydrophobic carbamates, hydrocarbons, and dyes that melts around 120℃ and is then suitable for piezoelectric printing [11].The viscosity of the wax is a function of the temperature, and a uniform and well-controlled heat source is required for reproducible, wax melts quickly at temperatures above 120℃, but the wax penetration process does not work optimally because some parts of the paper are not penetrated. temperatures above 150℃ speed up the melting process of wax, but it results in the paper substrate being used. Cellulose fibers on paper are easily damaged by too high temperatures.

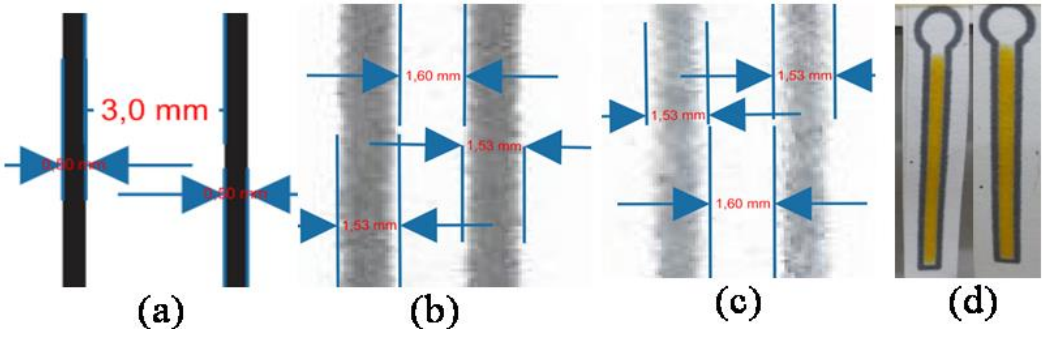


Figure 3. Patterning hydrophobic barriers in paper by wax printing. (a) after wax printing, (b) the front surface of the device after being heated, (c) the back surface of the device after being heated, (d) addition of reagents on the device

3.2. Optimization of reagent concentration

The concentration of rhodizonate (NaR) has an effect on sensitivity in detecting lead(II). The analyte concentration is determined by the length of the color band in the µPAD channel, which will be long if the analyte concentration increases. Fig.4 show the sensitivity level of the variation in NaR concentration 0.1-0.4%. As a result, 0.2% NaR has a better level of sensitivity compared to 0.1%; 0.3% and 0.4% NaR, cause the sample zone distance to the reaction zone 2 is closer than the sample zone distance to the reaction zones 1, 3, and 4, so the reaction time between sample and NaR in the reaction zone 2 is longer. The results in figure 4, a 0.2% NaR concentration was chosen for the next lead(II) analysis. The red intensity in design 2 has a higher value compared to green and blue, so the next measurement taken is the value of red intensity.

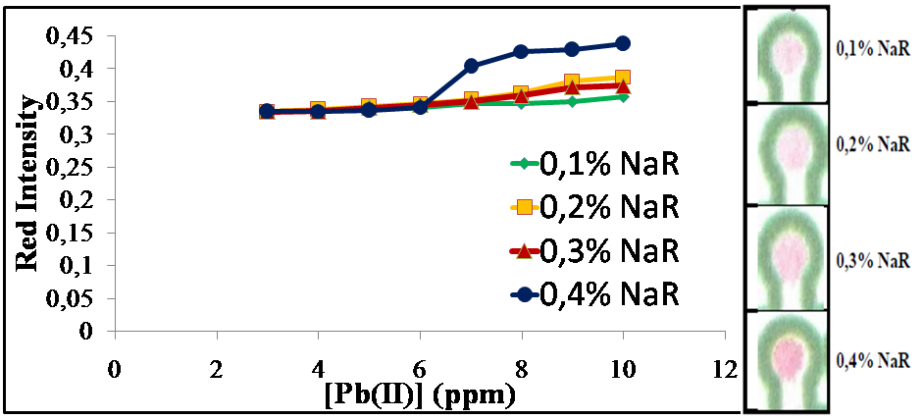
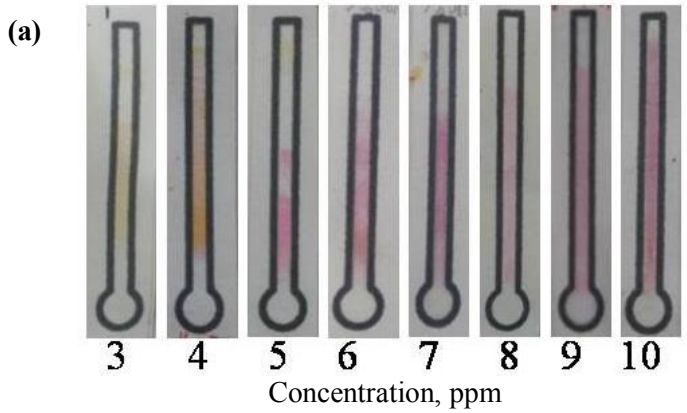


Figure 4. The relationship between reagent concentration to sensitivity for detection of lead (II) (0,1%; y= 0.003x + 0.323, R^2 = 0.966); (0.2%; y= 0.007x + 0.305, R^2 = 0.928); (0.3%; y= 0.006x +0.310, R^2 = 0.946); (0.4%; y= 0.018x + 0.262, R^2 = 0.866).

3.3. Detectors and analysis of images

A variety of detectors can be applied for optical sensing from the common devices such as scanners or camera phones to more specialized devices. The simplest and least expensive method that does not require any additional equipment is naked eye detection. Another common device is an office scanner, which provides high resolution and ensures focus of the digitalized image; furthermore, image intensity is not affected by external lighting conditions[12].

The naked eye is used as a detector in analyzing the length of color bands in design 1. The intensity of the color was recorded by a scanner (hpDeskJet Ink Advantage 2135 (300dpi) and imageJ software, used to determine the RGB intensity in design 2. The calibration curve obtained can be seen in Figure 5 with a concentration range of 3-10 mgL^{-1}. The optimized conditions for NaR concentration are 0.1% and 0.2% for design 1 and design 2, respectively, which resulted ingood linearity of calibration curve as can be seen in Fig 4 (b and c). In design 1 (Fig.4a) the length of the pink-colored band increased with increasing concentration of lead(II).



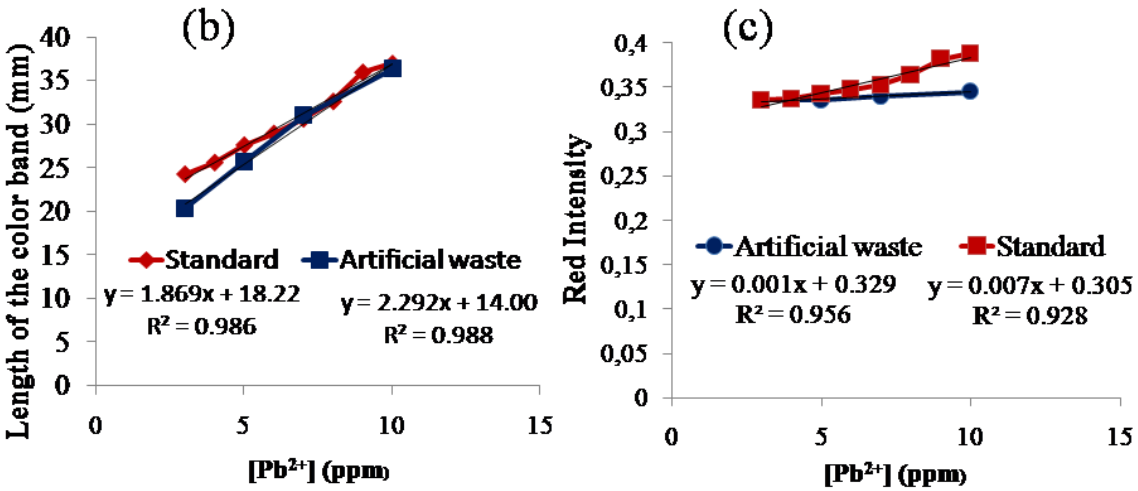


Fig 5. Signals of the µPADs with different concentration. (a) Images of the distance-based µPAD, (b) Effect the concentration and the length of the color band (design 1), (c) the relationship between the concentration and the red intensity (design 2).

The methods can be applied to determine the concentration of lead(II) with a comparable coefficient ($R^2 \cong 1$). However, from the practical point of view of the analytical method, design 1 is better than design 2 in term of simplicity, easiness, analysis time, cost (inexpensive) and only uses the naked eye for detection.

3.4. Validation Methods

The validation methods consist of linearity, limit of detection and precision (Fig.5). The repetition was carried out 10 measurements to analyze the concentration of lead(II) with a relative standard deviation (RSD) of 2,839 and 0,230 % for µPADs design 1 and design 2, respectively. In this work, the obtained % recovery was 83,56% and 99,91% for design 1 and design 2, respectively. The quantitation limit (LOQ) 2,519 mg L^{-1} and limit of detection (LOD) 0,756 mgL^{-1} for design 1.

3.5. Interference ions

Several variations of metal ions commonly found in lead(II) wastes such as Ba(II), Zn(II), Ca(II) and Fe(II) are reacted with NaR, thus giving interference effect on the analysis of lead (II) in sample. The tolerance limit for disturbing ions can be seen in table 1.

Ba (II) provides positive storage because it produces pink deposits with rhodizonate reagents under acidic conditions[13]. The results of Fe (II) analysis have a tolerance limit of 30 mgL^{-1}, if the concentration of Fe(II) is increased, it will affect the distance of the color band produced (the distance will decrease).

Table 1. Tolerance limits of interference ions

Interference ions	Tolerance limits (mg/L)
Ba^{2+}	60
Fe^{2+}	30
Ca^{2+}, Zn^{2+}	> 60

Ca (II) and Zn (II) cannot provide a tolerance effect on acid buffer conditions, these ions will react with NaR if the conditions are neutral or in other words the tolerance limit is greater than 60 mg/L[13].

4. Conclusion

This research presents comparation two design of μPAD suitable for identifying a lead(II) in wastesampel. In order to detect lead(II) can analyze the complex reactions of lead and NaR reagents. Two designs with different forms and methods of analysis were developed in this research. The first design is based on a distance-based method with the naked eyes as a detector, while the second design use analysis image to determine the intensity of the color. The designs can be used to determine the concentration of lead(II) with a comparable coefficient of determination, but based on the analysis method, design 1 is a design that is very simple, easy, fast, inexpensive and only uses the naked eye for detection.

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