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Diagnostics Devices for Improving the World: µPADs Integrated with Smartphone for Colorimetric Detection of Dopamine

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ABSTRACT

In recent years, microfluidic paper-based analytical devices (µPADs) were used; becuase of their low cost, ease of use, low sample consumption and reagent, and portability, specially in developing countries. In this research, colorimetric detection of dopamine (DA) was proposed on fast, simple, sensitive and low-cost µPADs, which is fabricated by using laser cutting technique. In paper-based microfluidic systems, wax printing is commonly used to create a hydrophobic barrier, but in this study, labels were used for the first time to create hydrophobic barriers due to their cost-effectiveness and easy access. Also in this study, the effect of various parameters on the performance improvement of developed µPADs such as DA volume, reaction time, drying time and volume ratio of ferric ion to DA was investigated. The results showed that the presence of DA made the red color bolder and a quantitative amount of DA was obtained by taking pictures of colored areas with a smartphone. Finally, after drawing the calibration curve, the limit of detection value 0.1 µM was defined.

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1. INTRODUCTION

µPADs offer lower fabricating cost than traditional microfluidic devices, do not need microstructure equipment, and they are using for Point-of-care (POC) diagnostics in limited resource environments [1-3]. So far, several detection methods have been used for µPADs, for example: fluorescence, colorimetry, electrochemistry, and chemiluminescence [4-11]. Between these techniques, colorimetric detection is the most widely used for integrating with µPADs because it is easy to perform, does not require complex equipment and suitable for integrating with partly cheap devices, like scanners and smartphones [12-14].

DA related to a group of catecholamine that play a very important role in the mammalians central nervous, cardiovascular and endocrine systems. Most importantly, an imbalance of DA levels can lead to neurological disorders including Huntington's disease, immunosuppressive diseases and addiction problems, schizophrenia, depression, and Parkinson's [15-18]. Hence, detection of DA in biological fluids including serum and blood plasma is very important for clinical objects [13].

In recent years, various researches have been done in this field. For example, Liu et al. [19], in 2020, Provided an engineering insight that focuses on practical strategies for enhancing the analytical performance of ePADs while maintaining the desired simplicity and performance. Teepoo et al. [20], in 2019, reported a one-step approach for fabricating screened printed microfluidic paper-based analytical devices (µPADs) using polylactic acid as a new hydrophobic material.

In 2019, Liu et al. [21], reported the colorimetric detection of DA in µPADs using the redox method. In the present study, similar to the method proposed by Liu et

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al. [21], colorimetric measurements of DA were developed in simple and low-cost µPADs, except that the label because of its cheapness and availability was used for the first time as a hydrophobic barrier. In addition, for the first time in recent studies, the influence of different geometries and the presence of microfluidic channels were discussed.

In this study, first, a suitable hydrophobic material was selected from the publicly available materials and then the effect of various factors on the detection of DA was investigated. In the next step, these factors were optimized to create the best paper-based microfluidic device. Finally, the effect of microfluidic channel on improving the results was discussed and analyzed.

2. MATERIALS AND METHODS

2. 1. Chemicals and Materials Dopamine hydrochloride powder (Sigma-Aldrich, USA) was obtained from Safir Azma Company (Tehran, Iran). Iron (III) chloride, Phenantroline, K_2HPO_4 and KH_2PO_4 were purchased from Merck (Germany). Whatman® Filter paper No.1 was obtained from Azmiran Company (Tehran, Iran). DA solution and ferric chloride solution were prepared with a certain amount of deionized water. Na_2HPO_4 and NaH_2PO_4 solutions were used to construct phosphate buffer solution with different pH. Phenantroline solution was obtained by adding a certain amount of phosphate buffer.

2. 2. Construction of µPADs µPADs Structure were designed by Microsoft PowerPoint software 2013. Then Whatman filter paper No.1 was cut according to the desired design by a laser cutting machine. At the first step, we were trying to use wax printing for hydrophobic barriers, but unfortunately we could not find 3-D wax printer. Because of that we were looked for some materials that had the same function as wax to manually create hydrophobic barriers.

Various materials and compounds available were used. However, each of the materials and compounds have been used had disadvantages that were not suitable for selection as a hydrophobic barrier. Finally, we got the label using trial and error. After assembly, a colorimetric test was performed and obtain the best result among the other options. Because the label cutting was done with a laser cutting machine, it had a very high accuracy and delicacy compared to other methods. At the same time, it was very economical. At the end, the results showed the suitability of using label as a hydrophobic barrier and then the potential of our proposed method as a simple and economical method.

2. 3. Analysis Technique A similar analysis technique was performed by Liu et al. [21] (Figure 1).

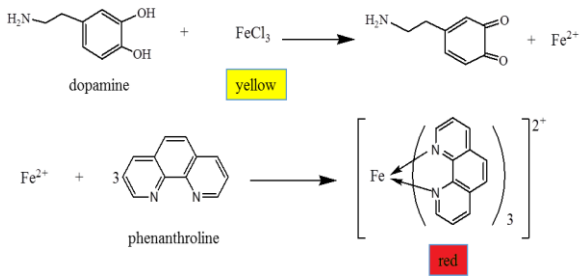

Figure 1. Schematic of DA assay reaction [21]

According to these reactions, ferric ions oxidize DA is reduced to ferrous ion. In the next step, the ferrous ion reacts with phenanthroline to form the red iron (II) tris complex (1 -10-phenanthroline).

2. 4. Sample Preparation 4.2 µl of DA solution and 8.4 µl of ferric chloride solution were reacted for 6 minutes, then the mixture was poured into uptake zone 1 and 12.6 µl phenanthroline was poured into uptake zone 2. After the solutions flowed through the capillary adsorption process, a red complex was formed in the detection area. After drying for 10 minutes, photos of the colored areas were taken using a smartphone camera in a light box. The images were then transferred to Photoshop software and the average red color intensity generated on the device was obtained (all the above steps were performed according to the instructions proposed by Liu et al. [21]).

3. RESULT AND DISCUSSION

3. 1. Construction of µPADs To use the label in the structure of the paper-based microfluidic systems, we designed a label border for the paper substrate and then distributed the filter paper in the middle of layer and fixed it completely (Figure 2).

3. 2. The Effect of Different Parameters on Color Intensity Many parameters can affect the color intensity created in the colorimetric assay. For example, in the study conducted by Gash et al. [22], Paper

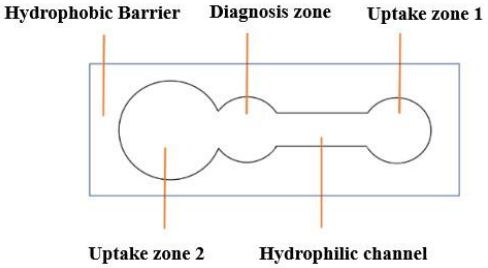

Figure 2. Schematic of the fabricated µPADs

Topography, μPAD capability in liquid confinement, channel resolution and minimum barrier width, as well as stability against surfactants and solvents were investigated. In the study of Lin et al. [23], UV exposure time, resolution of hydrophobic-hydrophilic patterns, concentration of polyurethane acrylate (PUA) and chemical resistance of PUA-based barriers were evaluated. Aksoren et al. [24], Studied the effects of reagent volume in the detection area, the concentration of reagents and solutions, and the reaction time of the analytes. Based on the researches, in the present study, the parameters of DA volume, reaction time, drying time, and volume ratio of ferric ion/DA, etc. have been investigated. In the following sections, we examined these parameters in more details.

3. 2. 1. Volume of DA To evaluate the effect of sample volume on color intensity, first different volumes of DA, ferric chloride and phenanthroline solutions were prepared. Then, for DA detection test, other parameters were fixed. After that, the colorimetric detection test was performed according to the mentioned steps. Finally, using the obtained data, a graph of sample volume changes in terms of average color intensity was drawn. According to Figure 3, as the sample size increases, the color intensity increases. But when the sample size exceeds a certain limit, a little solution leaks from the detection area, which is not desirable for us. So that, optimizing the sample volume, in addition to affecting the color intensity, can also reduce the sample consumption and thus reduce the cost.

3. 2. 2. Ferric Chloride/DA Reaction Time The reaction times varied from 2 - 10 minutes with an interval of 2 minutes and other parameters were fixed. Ferric chloride / DA reaction time affected the color intensity as shown in Figure 4. As you can see, as the reaction time is greater, the average color intensity gets higher and then its value is fixed. We can see the highest color intensity during the reaction time of 4 minutes. Therefore, it can be said that the time required to perform the oxidation-reduction reaction in this study is 4 minutes.

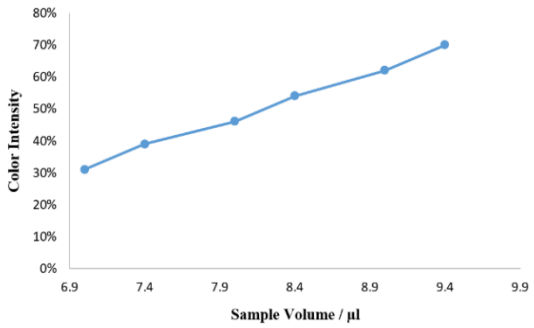

Figure 3. Effect of DA volume on the average color intensity

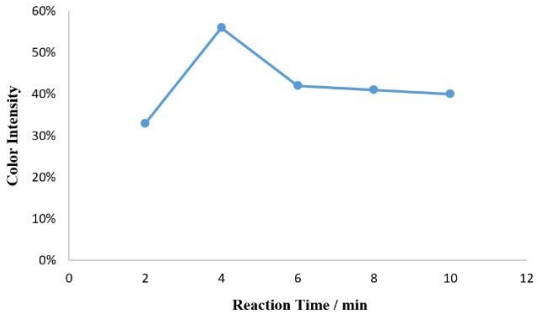

Figure 4. Effects of ferric chloride / DA reaction time on color intensity

3. 2. 3. Drying Time Drying time varied between 6-14 minutes and ferric chloride / DA reaction time, sample volume, and DA/ferric ratio were fixed. The graph shows that over time the color intensity goes up and for a longer time, the color intensity had decrease. The reason for the upward trend can be attributed to the slow drying of the paper substrate, followed by a gradual increase in color intensity (Figure 5).

3. 2. 4. Volume Ratio of Ferric Ion / DA The results showed that with increasing the ratio of Ferric ion / DA, the color intensity increases and reaches a peak value in 2 /1 and a little reduced in ratios higher than that (Figure 6). According to the obtained results, since the highest color intensity was obtained in the ratio of 2 / 1 for the volume ratio of ferric chloride to dopamine, then the volume ratio of 2 / 1 will be considered as the optimal volume ratio.

3. 3. μPAD Dimension Optimization To achieve optimal geometry and also to prove the superiority of the presence of microfluidic channel in the system structure, 4 different geometries were designed and fabricated (Figure 7). Designs 1 and 2 were with microfluidic channel, designs 3 and 4 were without microfluidic channel. Then, the effect of DA / ferric chloride reaction time was performed for each geometries and the results were obtained.

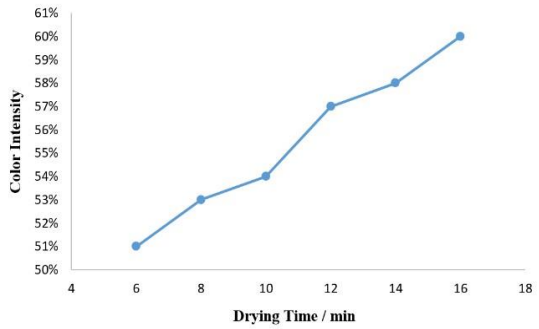

Figure 5. Effect of drying time on the average color intensity

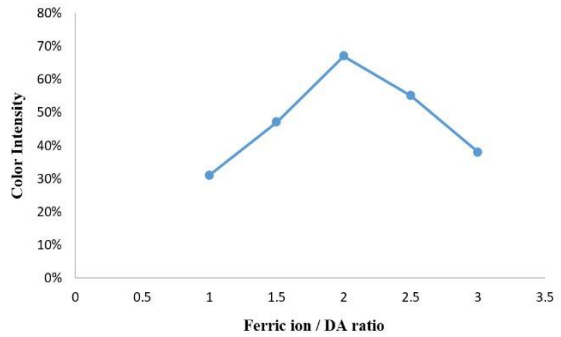

Figure 6. Effect of Ferric ion / DA ratio on the average color intensity

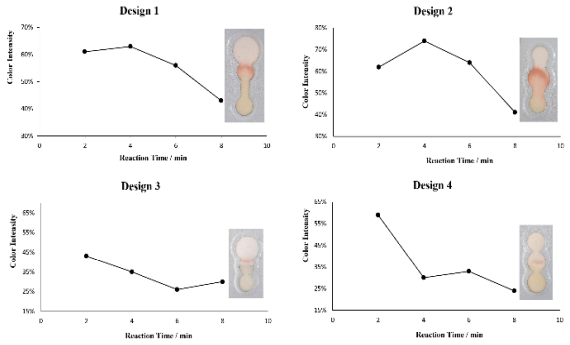

Figure 7. Effect of reaction time for 4 deferent geometries

The results show that, in geometries with microfluidic channels, it was observed that as the reaction progressed, the intensity of the color obtained increased and reached its peak in 4 minutes, and after that declining. For geometries without microfluidic channels, the trend of color intensity changes did not follow a specific rule and there was no peak in the diagrams. The cause of this phenomenon can be attributed to the random movements of the fluid along the paper because the presence of a microfluidic channel determines the path of movement and prevents such a problem. Therefore, it can be concluded that the presence of microfluidic channel causes proper guidance of the solution in the system and prevents additional movements of the solutions. At the end, among the two geometries with microfluidic channels, the geometry with the highest color intensity (design 2) was selected as the optimal geometry.

3. 4. Calibration Curve In order to determine the concentration of DA in the samples, the calibration curve was obtained. To reach the calibration curve, different solutions of DA with different concentrations in the range of 1-6 µM were prepared. As a result, a linear relationship was obtained between the color intensity and the concentration of DA (Figure 8). The mathemtical relation is expressed as y = 23.4x + 189.38 and the linear

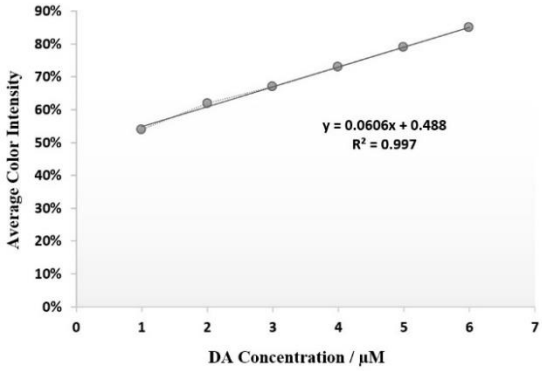

Figure 8. Analytical calibration curve for different concentration of DA from $1 \times 10^{-6} - 6 \times 10^{-6}$ M

correlation coefficient were $R^2 = 0.997$. This calibration curve allowed determining an experimental limit of detection of 0.1µM.

4. CONCLUSIONS

A simple, inexpensive, portable, and environmentally friendly colorimetric method was developed to determine DA concentrations. The analytical platform is made of cellulosic material that is compatible with many chemicals. The developed method is easy to use and can be easily stored. Therefore, these devices can be used for other chemical reactions that include analytical methods. In this study, the LOD value was 0.1 µM, which is an acceptable number compared to the LOD obtained and reported in literature (0.37µM).

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Persian Abstract

چکیده

در سال‌های اخیر، دستگاه‌های تحلیلی مبتنی بر کاغذ میکروسیال (μPADs) به دلیل هزینه کم، سهولت استفاده، مصرف نمونه و معرف کم و قابلیت حمل، به‌ویژه در کشورهای در حال توسعه مورد استفاده قرار گرفته‌اند. در این تحقیق، تشخیص رنگ سنجی دوپامین بر روی μPADهای سریع، ساده، حساس و کم‌هزینه پیشنهاد شد که با استفاده از تکنیک برش لیزر ساخته شده است. در سیستم‌های میکروسیالی مبتنی بر کاغذ، معمولاً از چاپ موم برای ایجاد مانع آبگریز استفاده می شود، اما در این مطالعه برای اولین‌بار از برچسب‌ها به دلیل مقرون به صرفه بودن و دسترسی آسان استفاده شد. همچنین تأثیر پارامترهای مختلف بر بهبود عملکرد μPADهای توسعه‌یافته مانند حجم دوپامین، زمان واکنش، زمان خشک شدن و نسبت حجمی یون آهن به دوپامین مورد بررسی قرار گرفت. نتایج نشان داد که وجود دوپامین سبب پررنگ تر شدن رنگ قرمز شده و مقدار کمّی آن با گرفتن عکس از مناطق رنگی توسط تلفن هوشمند به‌دست آمد. در نهایت پس از رسم منحنی کالیبراسیون، مقدار LOD ۰/۱ میکرومولار محاسبه شد.