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Proceeding Paper

Smartphone-Addressable Paper-Based Devices for the Colorimetric Detection of Ampicillin Based on Salt-Induced Aggregation of Gold Nanoparticles †

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Abstract: In this work, we describe the fabrication of paper-based aptasensing devices for ampicillin determination that rely on the salt-induced aggregation of gold nanoparticles (AuNPs) in the presence of the target. Circular paper-based devices were created on paper via pen-plotting (using water-repellent ink to create hydrophobic barriers) and modified with NaCl. The sample was incubated with an ampicillin aptamer and AuNPs and was added to the assay zones of the paper-based devices. In the absence of ampicillin, the aptamer prevented the aggregation of the AuNPs, and the assay zones remained red. When ampicillin was present, it selectively bound with the aptamer and the AuNP aggregate, producing a purple color. The color of the assay zones was monitored via a smartphone, and the color graduation was related to the ampicillin concentration in the sample. Different experimental parameters (type of paper, concentration of reagents) were investigated, and the analytical features of the method for the determination of ampicillin were established.

Keywords: paper-based devices; ampicillin; gold nanoparticles; smartphone; colorimetric detection; pen-plotting

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1. Introduction

Antibiotics are being widely used for the prevention and treatment of bacterial infections in farming [1]. The extensive use, abuse or misuse of antibiotics in food-producing animals may lead to residues finding their way into animal-derived foods (such as meat, milk and dairy products) and the natural environment. As a result of this process, long-term exposure to antibiotic residues can increase antibiotic resistance and potentially cause health problems to human consumers [2–4].

Therefore, it is important to develop low-cost, simple, fast, selective and sensitive detection technologies for the determination of antibiotics in different matrices. The "golden standard" for the identification and determination of antibiotics are liquid chromatography approaches, often hyphenated to mass spectrometry (LC-MS), which offer unambiguous confirmation, high sensitivity and multi-analyte capabilities [5,6]. However, these methodologies require expensive and bulky equipment, well-trained staff and extensive sample pretreatment. On the other hand, immunoassays (based on the use of antibodies for target recognition) [7] and biosensors [8,9] offer distinct advantages over LC-MS in terms of portability, rapidity, cost and, more importantly, scope for on-site and field assays.

Aptamers are gaining increasing popularity for antibiotic detection, serving as bioreceptors in biosensors and bioassays [10–13]. Aptamers, also named "artificial enzymes", are short oligonucleotide sequences that exhibit binding affinity towards selected target analytes and have some distinct important advantages over antibodies. Paper-based analytical devices (PADs) have attracted increased attention in the last fifteen years, as they are

inexpensive, portable sensing platforms for different analytical applications, using cellulose paper as a functional support [14–19].

In this work, we describe a new type of paper-based aptasensing devices for ampicillin determination that rely on the salt-induced aggregation of gold nanoparticles (AuNPs) in the presence of the target [20]. To the best of our knowledge, this is the first report of a simple paper-based device for ampicillin detection. Although lateral flow assays on functional nitrocellulose strips have been reported for ampicillin detection [21], these devices are complex to fabricate and require modified capture probes. The principle of the assay proposed in this work is illustrated in Figure 1. Initially, paper-based devices are patterned on paper via pen-plotting using hydrophobic ink and modified with NaCl. The sample is incubated with an ampicillin aptamer and AuNPs and added to the paper-based device. In the absence of ampicillin, the aptamer prevents the aggregation of the AuNPs, and the devices are colored red. When ampicillin is present, it selectively binds with the aptamer and the unprotected AuNP aggregate, producing a purple color. The color of the paper-based devices is monitored via a smartphone, and the color graduation is related to the ampicillin concentration in the sample.

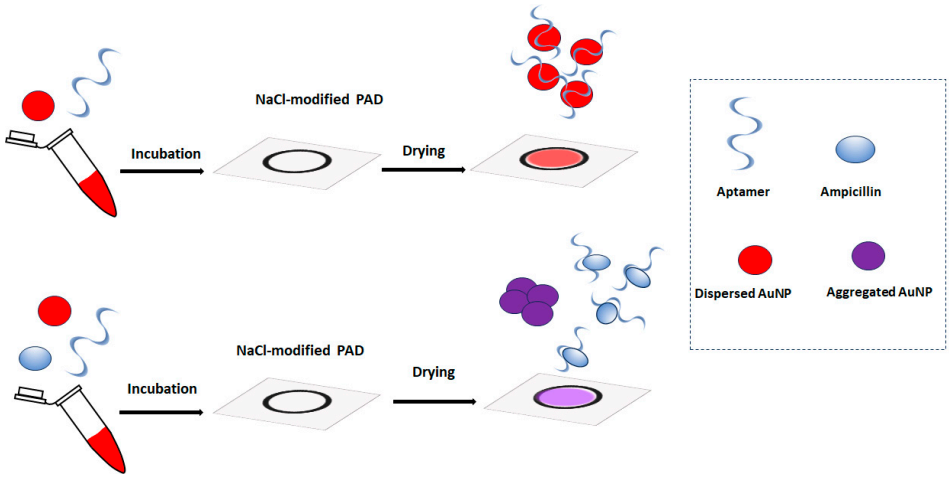

Figure 1. The principle of the paper-based colorimetric aptamer assay for ampicillin with salt-induced aggregation of AuNPs.

2. Experimental

2.1. Reagents and Materials

All chemicals used for the preparation of stock and standard solutions were of analytical reagent grade and purchased from Sigma-Aldrich. The ampicillin aptamer was purchased from Integrated DNA Technology (IDT) (USA), and its sequence was 5′-TGG GGG TTG AGG CTA AGC CGA C-3′.

2.2. Experimental Protocol

The experimental protocol is schematically illustrated in Figure 2.

The paper-based devices were plotted using an AxiDraw desktop x-y plotter (Evil Mad Science LLC, Sunnyvale, CA, USA). The control software was the AxiDraw extension for Inkscape operated via the open-access software Inkscape (Inkscape Project, https://inkscape.org/about/, accessed on 24 October 2023). The paper support was Whatman grade 42 filter paper, and a hydrophobic marker pen (Edding 780 0.8 mm tip thickness (black)) was used for plotting.

The paper based-devices were modified with salt by adding 8 µL of a 1.0 M NaCl solution, and the devices were left to dry.

A 13.3 mM AuNPs solution was incubated with 5 µM of aptamer solution for 1 h. Then, an ampicillin standard in the range 50–750 µg L^{-1} was added to the aptamer/AuNPs

solution and further incubated for 30 min. Finally, 8 µL of the aptamer/AuNPs/ampicillin solution was added to the salt-modified paper substrate and left to dry at room temperature.

Upon drying, the image of the paper-based devices was captured using a smartphone (Samsung A12), and the image file was transferred to InkScape. The scanned image was filtered using the fluorescent preset filter, making the red color more vibrant. The "color picker" tool was implemented to measure the H-value, using the HSV (hue, saturation, value) color space. The H-value for each measurement was subtracted from the H-value of the blank experiment; higher blank-subtracted H-values corresponded to stronger "purple" color intensity. Data plotting and reporting were performed in Excel.

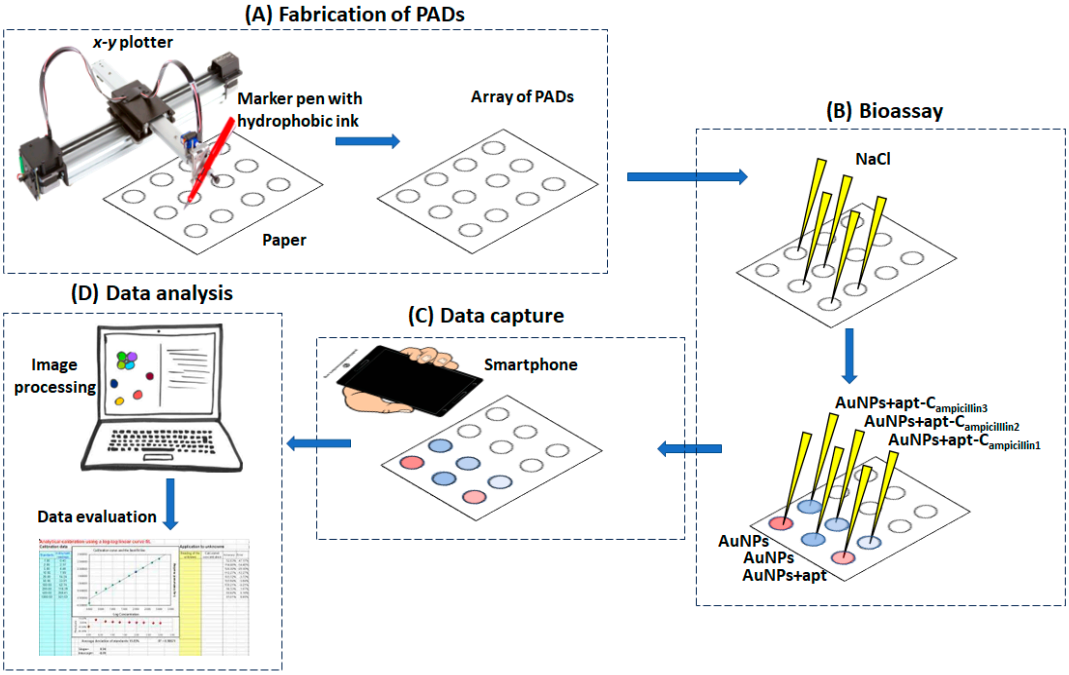

Figure 2. The experimental protocol for the fabrication of the PADs, the bioassay and data capture, analysis and evaluation.

3. Results and Discussion

The method optimization involved study of the type of the paper support, the NaCl concentration used to modify the devices and the aptamer concentration. Four types of paper support were studied (namely Mackerey Nagel MN261 chromatography paper, Whatman grade 1 chromatography paper, Whatman grade 42 filter paper and Whatman grade 1 filter paper) in terms of the aggregation capacity of AuNPs in the presence of NaCl (expressed in terms of the H-value). As illustrated in Figure 3a, the strongest aggregation was obtained with the Whatman grade 42 filter paper, which was used for further experiments. Next, the NaCl concentration that induced the most efficient aggregation of AuNPs was investigated. As shown in Figure 3b, NaCl concentrations $\geq$ 1 M were sufficient to induce the maximum aggregation of AuNPs, and 1 M NaCl was selected. Finally, the aptamer concentration that was required to protect the AuNPs from the salt-induced aggregation was selected. Figure 3c indicates that the protection of AuNPs from aggregation increased as the aptamer concentration increased (reflected in the decreasing H-values); 5 µM of aptamer was selected for the rest of this work.

Then, the analytical features of the assay were evaluated. Calibration for ampicillin was carried out in the concentration range 0–1000 µg L^{-1}. The calibration plot is shown in Figure 4, while photographs of the respective paper-based devices with different target concentrations and the linear-log calibration plot are shown as inserts. Each calibration point is the mean of three assays, and the error bars in Figure 4 represent the standard deviation of the three assays. The limit of detection was calculated as 10 µg L^{-1} using

the formula LOD = 3.3 × s_b/S (where s_b is the standard deviation of the intercept of the calibration plot, and S is the slope of the linear part of the calibration plot). The mean relative standard deviation across the calibration range (including six calibration points) was 16.9%.

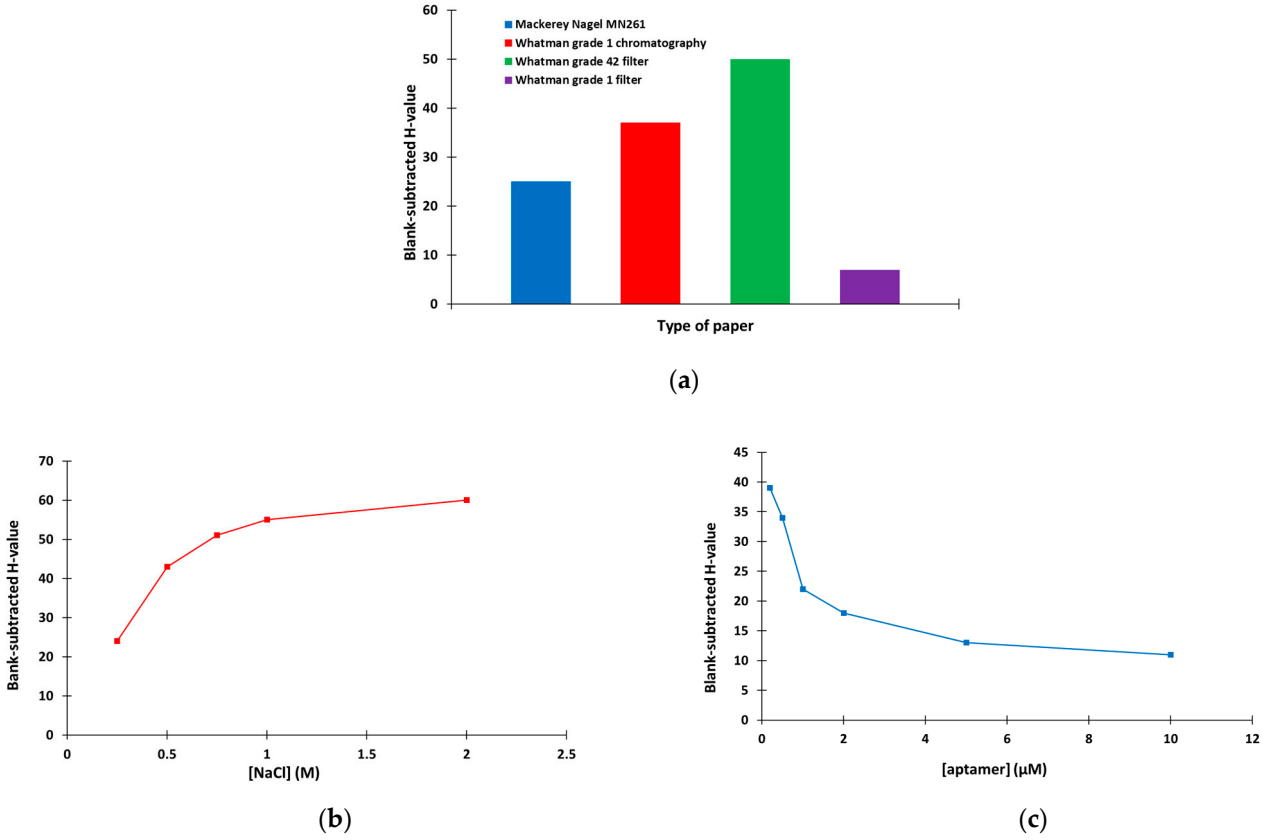

Figure 3. Selection of (**a**) the type of paper (8 μL of 13.3 mM AuNPs + 8 μL of 1 M NaCl), (**b**) the concentration of NaCl (8 μL of 13.3 mM AuNPs + 8 μL of NaCl at Whatman grade 42 filter paper) and (**c**) the concentration of aptamer (different concentrations of aptamer diluted in 10 mM of phosphate buffer (pH 7.4) containing 2 mM of $MgCl_2$ was incubated with 13.3 mM AuNPs for 1 h and applied to the paper-based device modified with 1 M NaCl).

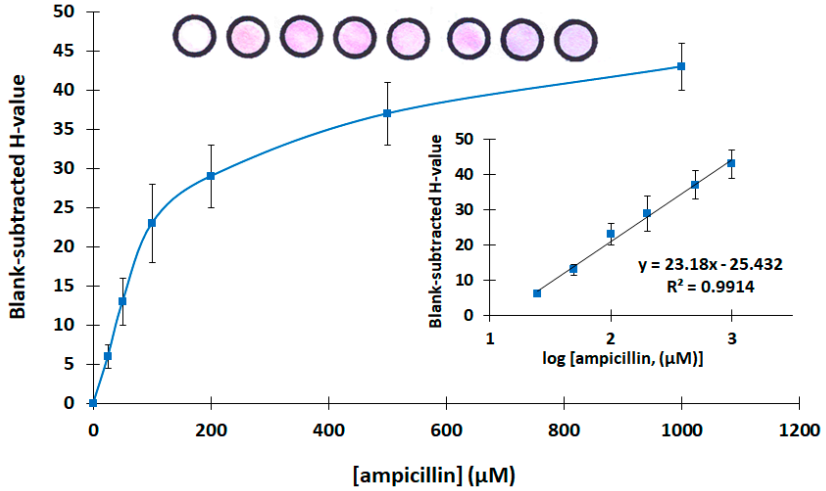

Figure 4. Calibration of ampicillin (the linear-log transformation of the calibration plot and photographs of the paper-based sensors at the different ampicillin concentrations are shown as inserts. The photograph at the left is a device with buffer only).

4. Conclusions and Prospects

In this work, a colorimetric paper-based aptasensing approach for the assay of ampicillin was developed. The method for the fabrication of the paper-based devices (pen-plotting with hydrophobic ink) is fast, low-cost and convenient, and the protocol of the aptamer-based assay is simple, without the requirement for labels or other probes. Instrument-free quantitative analysis can be performed using only a smartphone as a recording device. Work is in progress to improve the limit of detection and implement the assay using real samples.

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