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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 by pen-plotting (using water-repellent ink to create hydrophobic barriers) and were 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 aggregation of the AuNPs and the assay zones remained red. When ampicillin was present, it selectively bound with the aptamer and the AuNPs 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 which find their way into animal-derived foods (such as meat, milk and dairy products) and into 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 the determination of antibiotics are liquid chromatography approaches, often hyphenated to mass spectrometry (LC-MS), which offers 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 coined "artificial enzymes", are short oligonucleotide sequences which exhibit binding affinity towards selected target analytes and have some distinct important advantages over antibodies. Paper-based analytical devices (PADs) have attracted increase attention in the last fifteen years as 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 is illustrated in Figure 1. Initially, paper-based devices are patterned on paper by pen-plotting using hydrophobic ink and are modified with NaCl. The sample is incubated with an ampicillin aptamer and AuNPs and is added to the paper-based device. In the absence of ampicillin, the aptamer prevents aggregation of the AuNPs and the devices are colored red. When ampicillin is present, it selectively binds with the aptamer and the unprotected AuNPs 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.

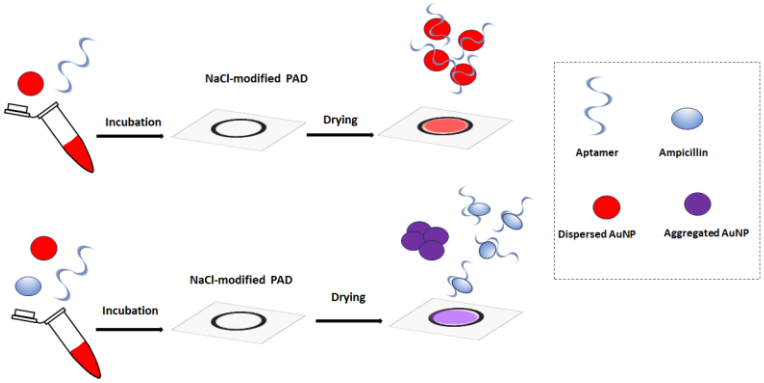

Figure 1. The principle of the paper-based colorimetric aptamer assay for ampicillin with salt-induced aggregation of AuNPs.

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 potted using an AxiDraw desktop x-y plotter (Evil Mad Science LLC, Sunnyvale, CA). The control software was the AxiDraw extension for Inkscape operated *via* the open-access software Inkscape (Inkscape Project, https://inkscape.org/about/). 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 and the devices were left to dry.

A 13.3 mM AuNPs solution was incubated with 5μM aptamer solution for 1 hour. Then, an ampicillin standard in the range 50-750 μg L^{-1} was added in 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 correspond to stronger "purple" color intensity. Data plotting and reporting were performed in Excel.

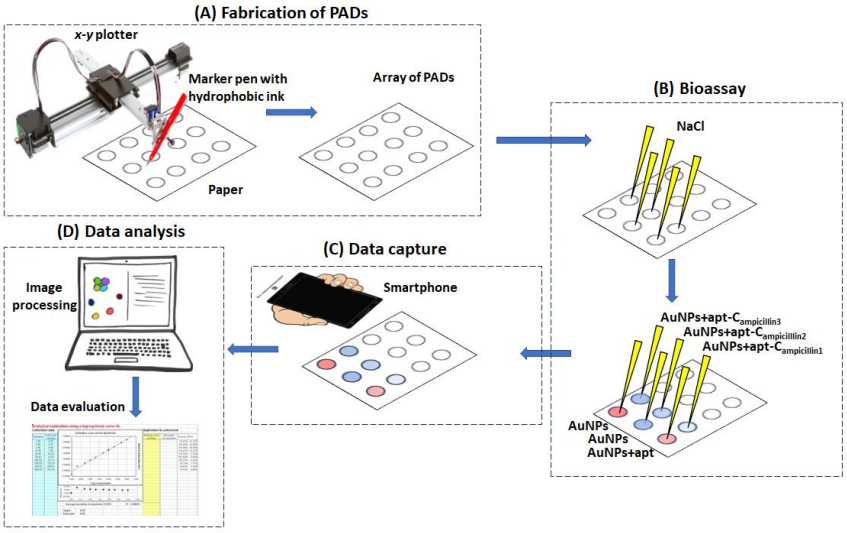

Figure 2. The experimental protocol for the fabrication of the PADs, the bioassay and the data capture, analysis and evaluation.

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, the Mackerey Nagel MN261 chromatography paper, the Whatman grade 1 chromatography paper, the Whatman grade 42 filter paper and the 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 Fig. 3b, NaCl concentrations ≥ 1 M were sufficient to induce 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 AuNPs protection from aggregation increased as the aptamer concentration increased (reflected in the decreasing H-values); 5 μM of aptamer was elected for the rest of this work.

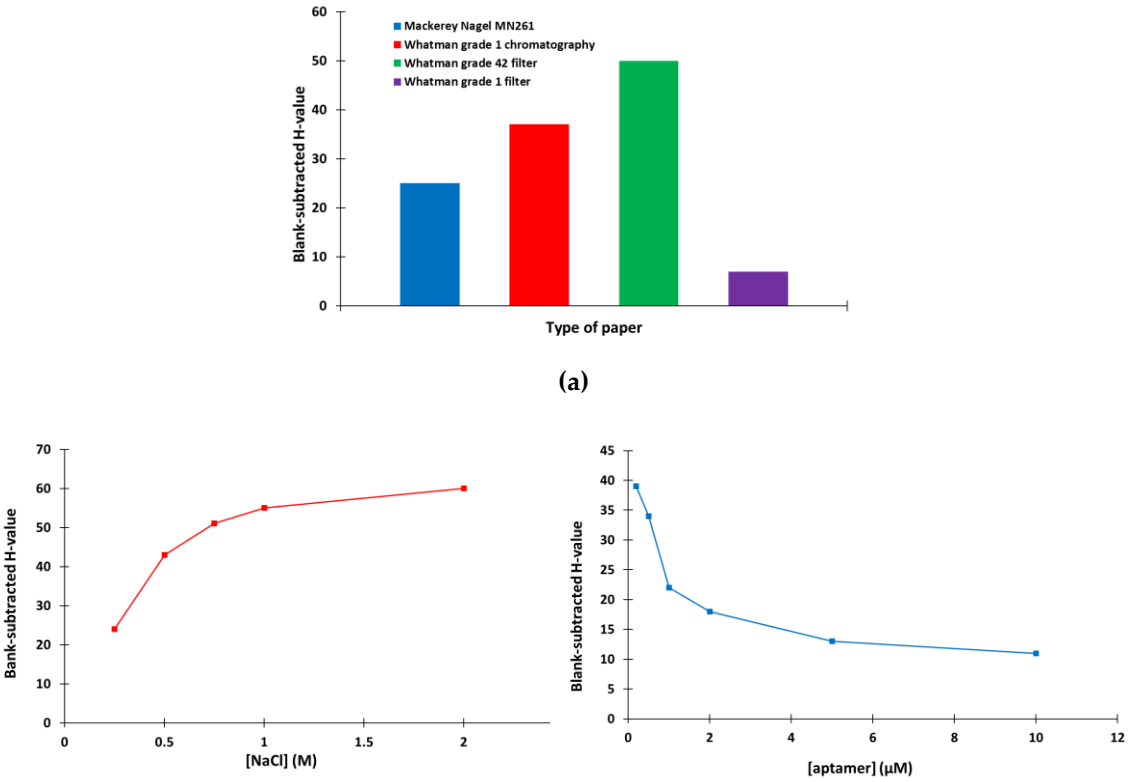

Figure 3. Selection of (a) the type of paper (8 μL of 13.3 mM AuNPs + 8 μL 1 M NaCl), (b) concentration of NaCl (8 μL of 13.3 mM AuNPs + 8 μL of NaCl at Whatman grade 42 filter paper), (c) concentration of aptamer (different concentrations of aptamer diluted in 10 mM phosphate buffer (pH 7.4) containing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

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 photos 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 3 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 $LOD=3.3\times s_b/S$ (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 6 calibration points) was 16.9 %.

3. 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 to implement the assay to real samples.

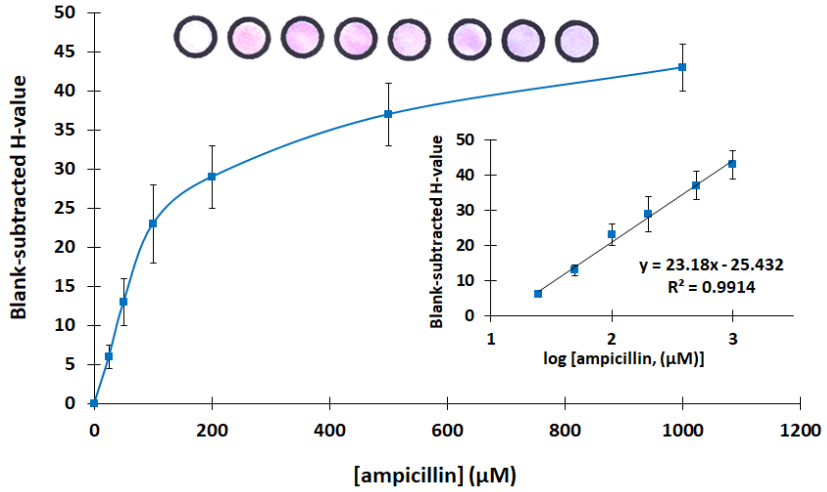

Figure 4. Calibration of ampicillin (the linear-log transformation of the calibration plot and photos of the paper-based sensors at the different ampicillin concentrations are shown as inserts. The photo at the left is a device with buffer only).

Author Contributions: Conceptualization, AE., C.K.; methodology, A.E., C.K and D.S.; validation, D.S.; investigation, D.S.; data curation, A.E., D.S.; writing—original draft preparation, A.E., D.S.; writing—review and editing, A.E., D.S.; supervision, A.E.; project administration, A.E.; funding acquisition, A.E. All authors have read and agreed to the published version of the manuscript.

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References

1. Landers, T.F.; Cohen, B.; Wittum, T.E., Larson, E.L. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. *Public Health Rep.* **2012**, *127*(1), 4–22. doi: http://www.jstor.org/stable/41639470.
2. Ben, Y.; Fu, C.; Hu, M.; Liu, L.; Wong, M.H.; Zheng C. Human health risk assessment of antibiotic resistance associated with antibiotic residues in the environment: A review. *Environ Res.* **2019**, *169*, 483-493. doi: 10.1016/j.envres.2018.11.040.
3. Larsson, D.G.J.; Flach, CF. Antibiotic resistance in the environment. *Nat. Rev. Microbiol.* **2022**, *20*, 257–269. https://doi.org/10.1038/s41579-021-00649-x
4. Patangia, D.V.; Anthony Ryan C.; Dempsey, E.; Paul Ross, R.; Stanton C. Impact of antibiotics on the human microbiome and consequences for host health. *Microbiologyopen* **2022**, *11*, e1260. doi: 10.1002/mbo3.1260..
5. Peris-Vicente, J.; Peris-García, E.; Albiol-Chiva, J.; Durgbanshi, A.; Ochoa-Aranda, E.; Carda-Broch, S.; Bose, D.; Esteve-Romero, J. Liquid chromatography, a valuable tool in the determination of antibiotics in biological, food and environmental samples. *Microchem. J.* **2022**, *177*, 107309. doi: https://doi.org/10.1016/j.microc.2022.107309.
6. Li, F.; Luo, J.; Zhu, B.; Liu, Z. Pretreatment Methods for the Determination of Antibiotics Residues in Food Samples and Detected by Liquid Chromatography Coupled with Mass Spectrometry Detectors: A Review. *J. Chromatogr. Sci.* **2022**, *60(10)*, 991-1003. doi: https://doi.org/10.1093/chromsci/bmac021.
7. Ahmed, S.; Ning, J.; Peng, D.; Chen, T.; Ahmad, I.; Ali, A.; Lei, Z.; Abu Bakr Shabbir, M.; Cheng, G.; Yuan Z. Current advances in immunoassays for the detection of antibiotics residues: a review. *Food Agric. Immunol.* **2020**, *31(1)*, 268-290. doi: 10.1080/09540105.2019.1707171.
8. Zhou, C.; Zou, H.; Sun, C.; Li. Y. Recent advances in biosensors for antibiotic detection: Selectivity and signal amplification with nanomaterials. *Food Chem.* **2021**, *361*, 130109. doi: https://doi.org/10.1016/j.foodchem.2021.130109.
9. Hong, J.; Su, M.; Zhao, K.; Zhou, Y.; Wang, J.; Zhou, S.-F.; Lin, X. A Minireview for Recent Development of Nanomaterial-Based Detection of Antibiotics. *Biosensors* **2023**, *13*, 327. doi: https://doi.org/10.3390/bios13030327
10. Sharma, A.; Singh, A.C.; Bacher, G.; Bhand, S. Recent Advances in Aptamer- Based Biosensors for Detection of Antibiotic Residues. *Aptamers Synth. Antibodies* **2013**, *2*, 43–54.

11. Mehlhorn, A.; Rahimi, P.; Joseph, Y. Aptamer-Based Biosensors for Antibiotic Detection: A Review. *Biosensors*. **2018**, *8(2)*, 54. doi: 10.3390/bios8020054.
12. Evtugyn, G.; Porfireva, A.; Tsekenis, G.; Oravczova, V.; Hianik, T. Electrochemical Aptasensors for Antibiotics Detection: Recent Achievements and Applications for Monitoring Food Safety. *Sensors* **2022**, *22*, 3684. doi: https://doi.org/10.3390/s22103684.
13. Liang, G.; Song, L.; Gao, Y.; Wu, K.; Guo, R.; Chen, R.; Zhen, J.; Pan, L. Aptamer Sensors for the Detection of Antibiotic Residues— A Mini-Review. *Toxics* **2023**, *11*, 513. doi: https://doi.org/10.3390/toxics11060513.
14. Tribhuwan Singh, A.; Lantigua, D.; Meka, A.; Taing, S.; Pandher, M.; Camci-Unal, G. Paper-Based Sensors: Emerging Themes and Applications. *Sensors* **2018**, *18*, 2838.
15. Kung, C.T; Hou, C.Y.; Wang, Y.N.; Fu, L.M. Microfluidic paper-based analytical devices for environmental analysis of soil, air, ecology and river water. *Sens. Actuat. B. Chemical* **2019**, *301*, 126855.
16. Fu, L.M.; Wang, Y.N. Detection methods and applications of microfluidic paper-based analytical devices. *Trends Anal. Chem. (TrAC*) **2018**, *107*, 196-211.
17. Lim, H.; Turab Jafry, A.; Lee, J. Fabrication, Flow Control, and Applications of Microfluidic Paper-Based Analytical Devices. *Molecules* **2019**, *24*, 2869.
18. Ozer, T.; McMahon, C.; Henry, C.S. Advances in Paper-Based Analytical Devices. *Annu. Rev. Anal. Chem.* **2020**, 13, 85-109.
19. Noviana, E.; Carrão, D.B.; Pratiwi. R.; Henry C.S. Emerging applications of paper-based analytical devices for drug analysis: A review. *Anal. Chim. Acta* **2020,** *1116*, 70-90. doi: 10.1016/j.aca.2020.03.013.
20. Nooranian, S.; Mohammadinejad, A.; Mohajeri, T.; Aleyaghoob, G.; Kazemi Oskuee, R. Biosensors based on aptamer-conjugated gold nanoparticles: A review. *Biotechnol. Appl. Biochem.* **2022**, *69*, 1517–1534. doi: https://doi.org/10.1002/bab.2224.
21. Lin, H.; Fang, F.; Zang, J.; Su, J.; Tian, Q.; Kumar Kankala, R.; Lin, X. A Fluorescent Sensor-Assisted Paper-Based Competitive Lateral Flow Immunoassay for the Rapid and Sensitive Detection of Ampicillin in Hospital Wastewater. *Micromachines* **2020**, *11*, 431. https://doi.org/10.3390/mi11040431