Paper-based colorimetric sensor for potassium ion detection in urine by crown ether modified gold nanoparticles

Khwanrudee Chitbankluai¹,², Chittanon Buranachai¹,²,³, Warakorn Limbut¹,⁴,⁵ and Panote Thavarungkul¹,²,³,⁵*

¹Center of Excellence for Trace Analysis and Biosensor, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
²Department of Physics, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
³Thailand Center of Excellence in Physics, Commission on Higher Education, 328 Si Ayutthaya Road, Bangkok, Thailand, 10400
⁴Department of Applied Science, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla 90112, Thailand
⁵Center of Excellence for Innovation in Chemistry, Faculty of Science, Prince of Songkla University, Hat Yai, Songkhla, Thailand, 90112

*Email: panote.t@psu.ac.th

Abstract. Detection and identification of potassium ion (K⁺) level in urine is essential for diagnosis of several diseases or for guiding treatments. We have developed a simple, rapid, low cost and naked-eye quantitative method for K⁺ analysis in urine using a paper-based colorimetric array test strip. The array comprised three sensing elements printed using a wax-printing technique and filled with different amounts of crown ether (4-aminodibenzo-18-crown-6) modified gold nanoparticles (AuNPs). The detection is achieved by measuring the color change (from red to violet) when AuNPs aggregated following crown ether-K⁺ complexation. Furthermore, the detection sensitivity could be controlled by changing the amount of the crown ether. The strip sensor array based on crown ether modified AuNPs successfully detected K⁺ level in the ranges of 5-1000 µM by naked eye. Thus, the developed sensor is very simple yet has a potential to be of real use in detecting K⁺ in real urine samples.

1. Introduction

Potassium ion (K⁺) is an essential electrolyte in biological systems. In urine, normal potassium ion concentration ranges from 25 to 125 mM and abnormal values may be an indicator of several diseases such as renal diseases, alkalosis, diabetes or cirrhosis [1-3]. Therefore, the detection of K⁺ in urine is important for health monitoring and medical diagnoses. There have been various analytical methods developed and applied to detect K⁺ including flame photometry [4], spectrophotometry [5] and electrochemical method [6]. Although these methods show good resolution, high sensitivity and speed, they are costly and mostly requires complicated instruments. In addition, they require professional technicians and laboratories [6, 7]. This limits the access to healthcare for general public, especially the patients in remote areas. Therefore, it is interesting and necessary to find a portable system based on a simple and sensitive K⁺ detection, which can be easily read out by naked eye.

Accordingly, Qiu and co-workers [7] developed a colorimetric detection method based on crown ether (4′-aminodibenzo-18-crown-6) modified gold nanoparticles (AuNPs) for K⁺ detection. Their
method was later successfully applied to detect K⁺ in real urine samples. However, there is still room for improvement, especially on the issue of high sample-reagent usage and high detection limit (40 μM) by naked eye. In spired by their work, herein we develop a paper-based colorimetric array test strip with crown ether modified AuNPs to obtain a simpler and more sensitive method for K⁺ detection.

2. Materials and methods

2.1. Chemicals and reagents
All chemicals used in this study were of analytical grade. Ultrapure water was from a maximum ultrapure water system (18.2 MΩ Milli-Q, Germany), it was used for preparation and dilution of all solution. Gold(III) chloride (HAuCl₄) and 4′-aminodibenzo-18-crown-6 (C₂₀H₂₅NO₆) were from Sigma-Aldrich (St. Louis, MO, USA). Trisodium citrate (Na₃C₆H₅O₇) and potassium chloride (KCl) were from Ajax Finechem (Australia). All the glassware containers were cleaned by using aqua regia solution (HCl–HNO₃=3:1 v/v).

2.2. Preparation of citrate-stabilized AuNPs
AuNPs were synthesized according to a method reported in a prior literature [7]. Briefly, AuNPs prepared by rapidly adding a sodium citrate solution (1wt%, 4 mL) into a boiling aqueous solution of HAuCl₄ (96 mL, 0.26 mM) with continuous heating and stirring. During the synthesis, the color of the solution changed from colorless to dark blue and finally wine red. After heating for 20 min, the solution was cooled to room temperature.

2.3. Sensing mechanism
The crown ether was functionalized onto the surface of AuNPs through gold-nitrogen bonds and the presence of K⁺ caused AuNPs aggregation via a sandwich complex of crown ether and K⁺ (figure 1A). The characteristic color changes of AuNPs from red to violet with increasing concentrations of K⁺ was then observed due to the change in surface plasmon resonance (SPR) frequency [8] as previously described in the work of Qiu and co-workers [7]. The crown ether is specific and suitable for K⁺ detection because the pore size of the crown ether matches with the size of K⁺. To further increase the sensitivity and ease of use, we coated the crown ether modified AuNPs on filter paper to fabricate a test strip (figure 1B). It was expected that by doing so, the modified AuNPs are highly concentrated on the filter paper and the sensitivity should increase dramatically. In addition, the test strip should be easy to use to detect K⁺ in urine samples with naked eyes or with a regular table top scanner.

Figure 1. (A) Schematic illustration of the sensing mechanism and (B) the developed paper-based colorimetric array test strip for the detection of K⁺.
2.4. Fabrication of a paper-based colorimetric array test strip

A paper-based colorimetric array test strip was fabricated by a wax printing method. The strip contains an array of circular-shaped test zones (6 mm in diameter) (figure 1B). The fabrication process consisted of two steps. First, the wax pattern was printed on the surface of filter paper by a wax printer (Xerox Color Qube 8570, Japan). Second, the wax patterned paper was heated on a hot plate at 150 °C for 15 minutes causing the wax to penetrate through the paper to form hydrophilic region surrounded by hydrophobic barrier within the paper.

2.5. Analytical procedure and image processing

For colorimetric detection of K⁺, the three sensing elements were prepared using different concentrations of crown ether, as presented in figure 1B 65 µL of citrate-stabilized AuNPs solution were incubated with 15 µL of the crown ether (final concentration 3.5, 4.0, 4.5 µM) for 3 minutes with mild shaking. The crown ether-AuNPs mixture (12 µL) was added to the test zones and dried at room temperature. After drying completely, 12 µL of K⁺ solutions were added to monitor the color change. After that, the image of the array was recorded with a table top scanner (Epson Perfection V33, Netherlands) and imported to the ImageJ program to determine the mean color intensity. In this colorimetric reaction, the red color obviously changed to violet. Thus, the value of the red intensity was used for the quantitative analysis.

3. Results and discussion

Five concentrations of crown ether on AuNPs were used in order to compare color changing behaviors of a paper-based colorimetric array test strip. As shown in figure 2A, at each concentration of the crown ether, the color of the test zones gradually shifts from red to violet with increasing concentration of K⁺ as hypothesized. In addition, at each concentration of K⁺, the color profiles exhibit gradual changes from red to violet in the direction of increasing crown ether concentration. Quantitatively, the mean red intensities are inversely proportional to the concentrations of K⁺ with linear ranges of 250-1000 µM, 250-1000 µM, 50-500 µM, 5-50 µM and 5-50 µM for the case of AuNPs coated with 3.0, 3.5, 4.0, 4.5 and 5.0 µM crown ether, respectively (figure 2B). Accordingly, AuNPs modified with 3.0 and 5.0 µM crown ether were excluded from the test strip due to their low detection sensitivities in the range 250-1000 and 5-50 µM K⁺.

![Figure 2](image-url) (A) The optical image of test zones coated with AuNPs modified with five different concentrations of crown ether in the presence of various concentrations of K⁺. (B) The variations of red intensity for five concentrations of crown ether in the presence of K⁺ concentration ranging from 0 to 1000 µM. The inset plot shows that in the range of 0 to 50 µM.
Next, a colorimetric array test strip was fabricated by coating the test zones with AuNPs modified with 3.5, 4.0, 4.5 µM of crown ether (number 1, 2 and 3 in figure 3A). The results indicate that at least 5 µM could be roughly quantified by the naked eye detection of coloration. The proposed method is very simple, rapid, inexpensive and can be used in conjunction with a common smartphone camera or scanner as a detector for quantitative determination of K⁺. To further simplify the analysis, in figure 3B we construct a color chart from the mean RGB values calculated from the RGB values over the test zones in figure 3A. It is clear that this sensor improves the limit of detection almost 8 times compared with the previous work [7].

![Figure 3](image_url)

**Figure 3.** (A) an optical image of the test strip after adding K⁺ at various concentrations and (B) a processed image of the test strip (A). The color in a given square in (B) is from the mean RGB value calculated from the RGB values over the test zone at the same position in (A).

### 4. Conclusion

A paper-based colorimetric array test strip consisting of three test zones coated with AuNPs modified with different concentrations of crown ether was successfully developed for the detection of K⁺ concentrations. It is able to quantify the concentrations of K⁺ in the range 5-1000 µM by naked eye visualization. This sensor offers the advantages of a simplicity, rapidity, low sample consumption, and low cost for determination of K⁺ in equipment-free fields.

### Acknowledgements

This work was financially supported by the Thailand Center of Excellence in Physics (ThEP) (grant no. ThEP-61-PHM-PSU2), the Graduate School Dissertation Funding for Thesis Fiscal Year 2019 and the Research Assistantship, Faculty of Science, Prince of Songkla University (contract no. 1-2560-02-007). The partial supports from the Center of Excellence for Trace Analysis and Biosensor (TAB-CoE), Department of Physics, Prince of Songkla University, Hat Yai, Songkhla, Thailand are also gratefully acknowledged.

### References

[1] Xu L, Sun N, Zhou L, Chen X, Wang J, Wang Q, Wang K, Zhang J and Pei R 2015 Analyst 140 3352–5
[2] Yang L, Qing Z, Liu C, Tang Q, Li J, Yang S, Zheng J, Yang R and Tan W 2016 Anal. Chem. 88 9285–92
[3] Naderi M, Hosseini M and Ganjali M R 2018 Spectrochim. Acta A 195 75–83
[4] Overman R R and Davis A K 1947 J. Biol. Chem. 168 641–9
[5] David D J 1960 Analyst 85 495–503
[6] Zhu B, Booth M A, Woo H Y, Hodgkiss J M and Travas-Sejdic J 2015 Chem.: Asian J. 10 2169–75
[7] Qiu J, Zhang Y, Dong C, Huang Y, Sun L, Ruan H, Wang H, Li X and Wu A 2019 Sens. Actuators B Chem. 281 783–8
[8] Chen Z, Guo J, Ma H, Zhou T and Li X 2014 Anal. Methods 6 8018–21