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Short communication

One-step signal amplified lateral flow strip biosensor for ultrasensitive and on-site detection of bisphenol A (BPA) in aqueous samples

Zhanlong Mei [a,1], Wei Qu [a,1], Yi Deng [a], Huaqin Chu [a,c], Jinxuan Cao [b,*], Feng Xue [a], Lei Zheng [a], Hani Said El-Nezamic [d], Yucheng Wu [a], Wei Chen [a,*]

[a] School of Biotechnology and Food Engineering, Anhui Provincial Key Lab of Advanced Functional Materials & Devices, Hefei University of Technology, Hefei 230009, PR China
[b] Department of Food Science & Technology, Ningbo University, Ningbo 315211, PR China
[c] School of Food Science & Technology, Jiangnan University, Wuxi 214122, PR China
[d] School of Biological Science, The University of Hong Kong, Hong Kong, PR China

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ABSTRACT

A one-step signal amplified lateral flow strip (LFS) biosensor has been developed for ultrasensitive and on-site visual detection of bisphenol A (BPA). This signal amplified LFS was based on the dual labeling using different-sized gold nanoparticles (**Duo-LFS**). This Duo-LFS could achieve BPA detection with 0.5 ng/mL as the visual sensitivity by naked eye observation and with 0.076 ng/mL as the limit of detection (LOD) for semi-quantitative detection by software analysis, which is at least 10-fold improvement of the sensitivity of traditional LFS based methods. This one-step signal amplified lateral flow strip biosensor and related signal enhancement method could be adopted as a potential generous technique for all LFS-based detection methods.

1. Introduction

Bisphenol A (BPA) is one kind of common chemical engineering materials and has been widely used in the production of epoxy resins and polycarbonate plastic used in food packaging (Jo et al., 2011; Mei et al., 2013a, 2013b). However, it has been proven that BPA could mimic the effect of endogenous hormones, estrogens and androgens by binding to the estrogen receptor and proliferation (Diamanti-Kandarakis et al., 2009). Even worse, BPA is postulated to cause reproductive disorders, various kinds of cancers and has diverse pleiotropic actions in the brain and cardiovascular systems. Even now, there are still many commercial and professional controversies about the exact adverse effects of BPA at a certain amount (Siva, 2012; Brody, 2012). For effective risk assessment of BPA to human health at the low level, the quantitative measurement of BPA in different biological and aqueous samples is of key importance. However, one of the existing obstacles of BPA detection for risk assessment is that the sensitivity of the adopted method could not achieve the successful determination at low levels. Therefore, developing an easy and sensitive method for on-site detection of BPA is of critical significance for food safety and human public health.

Current methods adopted for BPA detections are mainly relied on the instruments based methods. For example, gas chromatography–mass spectrometry (GC–MS), liquid chromatography–mass spectrometry (LC–MS) were all used for BPA detections (Becerra and Odermatt, 2012; Nam et al., 2010; Brenn-Struckhofova and Cichna-Markl, 2006) while the solid-phase extraction coupled with isotope dilution-HPLC (high performance liquid chromatography) is used as the “gold standard” for BPA detection in urine samples adopted by CDC (Centers for Diseases Control and Prevention) in USA (Aveyard et al., 2007). However, the high price of various instruments, time-consuming sample pre-treatments and expert personnel for operations limit the practical applications of these methods for on-site detections. Comparatively, antibody-based surface plasmon resonance (SPR) (Abera and Choi, 2010; Hegnerová et al., 2010) and enzyme linked immunosorbent assay (ELISA) methods have been adopted in the field of on-site and high throughput screening. These methods avoid the drawbacks of the instrumental-based methods effectively. However, for non-specialized laboratories and for in-field and on-site detections in urban areas in developed countries as well as rural areas in developing countries, it can still be difficult to perform labor intensive operations including repeated incubation and washing steps, and enzyme reactions for the final signal generation in ELISA.

* Corresponding authors.
E-mail addresses: chenweishnu@163.com, chenweishnu@hfut.edu.cn (W. Chen).
[1] These two authors contributed equally to this paper.

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Considering the urgent demand for overall speed and simplicity of detection, the lateral flow strip (LFS) could be a more suitable alternative for on-site screening with the advantages of user-friendly format, short assay time, and cost-effectiveness (He et al., 2010, 2011; Mabey et al., 2004). In this field, the most successful and famous product of lateral flow strip should be the home pregnancy strip and glucose testing strip, which have been widely commercialized and used. Recently, our group also has done the related research of BPA detection using traditional lateral flow strip method (Mei et al., 2013a, 2013b). However, due to the intrinsic properties of traditional lateral flow strip methods and the of the titer of the used antibody, it is noted that the sensitivity of the traditional lateral flow strip method is not high enough to meet the detection requirements of the special target analyte at low or trace concentrations including BPA, which inhibit the further applications of the lateral flow strip in many important fields. For example, several bio-monitoring studies have reported the occurrence of BPA at ng/mL level in human tissues and fluids, which could not be effectively monitored by current rapid detection methods (Liao and Kannan, 2011; Vandenberg et al., 2010). Unfortunately, current LFS-based rapid detection methods also could not meet the above mentioned requirements for BPA detection due to the existing high limit of detection (LOD) of traditional LFS.

Recently, so many researches about signal enhancement in lateral flow strip biosensors have been extensively reported such as using silver enhancement technology (Shyu et al., 2002; Yang et al., 2011), fluorescent quantum dots as new tags (Wang et al., 2011), and core-shell structured Ag/Au nanoparticles as the reporter (Liao and Li, 2010), and optimization of antibody conjugated to the surface of gold nanoparticles on the conjugation pad (Zhang et al., 2011a,b). Besides, the pre-enrichment treatment of the detection samples by magnetic nanoparticles and gold nanoparticles before using LFS has just been reported for signal amplification (Nash et al., 2012). All these researches are of great significance for the further wide applications of LFS-based detection methods. However, among all these researches, complicated instrumentations and multi-step operations are still needed, which prevents the wide use for in-field detections in many basal laboratories and un-developed districts. And to the best of our knowledge, there is still no reporting about using one-step signal amplified lateral flow strip for small molecule detections including BPA while there was only one reporting about signal amplified lateral flow strip for macromolecule (Choi et al., 2010). Of note, the LFS based methods for detection of small and macro molecules are based on the different recognition and strip design model. So, it is of great importance and interests to develop the novel LFS based method for rapid and ultrasensitive detection with low molecular weight in the comparable simple way. In this study, a new lateral flow strip method was developed using two kinds of gold nanoparticles for dual labeling and signal amplification (Duo-LFS). The small molecule BPA could be successfully detected qualitatively and quantitatively with the excellent sensitivity in the one-step model. More importantly, the sensitivity of this Duo-LFS method is even comparable to some instrumental based methods, which paves a new way for ultrasensitive and on-site screening of BPA and other lateral flow strip based methods.

2. Materials and methods

2.1. Chemicals

$HAuCl_4$, sucrose, Tween-20, bovine serum albumin (BSA), trisodium citrate, were all purchased from Sangon Biotech (Shanghai) Co. Ltd. Conjugate pads, cellulose fiber sample pads, laminated cards, and nitrocellulose membranes were purchased from Millipore. All the solutions used in this article were prepared by ultrapure water (>18 MΩ). All the antibodies were obtained from Sangon Biotech (Shanghai) Co. Ltd.

2.2. Methods

Gold nanoparticles with average diameter of 15 and 40 nm were both prepared using the trisodium citrate reduction method with slight modification. The difference is in the dosage of the added trisodium as the reduce reagent. 100 mL of 0.01% $HAuCl_4$ in a 250 mL beaker were brought to through boil with vigorous stirring. Then, 2 mL of 1% trisodium citrate were added and the color of the solution changed from dark then to purple and finally to wine-red. The solution was kept heating and stirring for another 10 min at room temperature. The 40 nm gold nanoparticles were prepared in the same way except 1 mL 1% trisodium citrate added.

The synthesized gold nanoparticles were stored at 4 °C before use.

The BPA–BSA conjugates were prepared according to the reported methods with minor modifications (Zhao et al., 2002). Briefly, the 4,4-bis(4-hydroxy-phenyl) valeric acid (BVA) was used as the antigen of BPA for preparation of complete antigen. First, BVA was dissolved in DMSO and activated with NHS and EDC with stirring for 2 h. The reaction mixture was treated as solution A. Second, BAS solution was prepared by dissolving in 0.1 mol/L $NaHCO_3$ (pH 7.0). Following, solution A was added into BAS solution slowly with continuous stirring for 2 h. The final reaction mixture was purified by dialysis against 0.01 mol/L PBS at 4 for 24 h. The solution was further dialyzed against pure water for 48 h. Afterwards, the solution was lyophilized and the product was treated as BPA–BSA conjugates for further research.

The gold nanoparticle-antibody conjugates were prepared as reported (Mei et al., 2013a,2013b). Before the conjugation, the minimum amount of antibody for stabilizing the gold nanoparticles was determined. Typically, the pH of the colloidal gold solution was adjusted to 8.0 by 0.1 M Na_2CO_3. Antibody at different concentration was added into the pH adjusted colloidal gold solution and incubated for 1 h respectively. Following, 10% NaCl was added, and the minimum amount of antibody stabilizing the colloid gold solution was treated as the optimum amount to conjugate with the colloidal gold nanoparticles. After optimization, antibody at defined concentration was added into 1 mL pH adjusted colloidal gold and agitated for 1 h, after that 100 μL 10% BSA was added and agitated for another 30 min. The mixture was centrifuged at 9.5*g* for 10 min, and the pellet was resuspended by 0.5 mL resuspend buffer (10% sucrose, 2% trehalose, 0.05% PEG, 0.5% Tween-20 in 10 mM Tris-HCl) as the gold nanoparticle-antibody conjugate solution. The conjugate solution was stored at 4 °C before use.

The Duo-LFS was constituted by sample pad, the recognition conjugation pad, the signal amplification conjugation pad, nitrocellulose membrane, and absorbent pad. 15 nm GNPs conjugated with anti-BPA MAb were blocked by the BSA and were dispersed on the first recognition conjugation pad; 40 nm GNPs modified with anti-BSA mAb were blocked by the HSA and were dispersed on the second signal amplification conjugation pad. BPA–BSA conjugates and goat-anti-mouse antibody were dispersed on the nitrocellose membrane by the Biojet BJQ 3000 dispenser to form the test and control line, respectively. The five parts were assembled on a plastic adhesive backing in order, and each part overlapped 2 mm to ensure the sample could migrate on the strips. The strips were cut into 3 mm in width and stored at 4 °C in dry till use.

Sample solutions containing different concentrations of BPA was prepared by spiking BPA in 10 mM PBS (pH 7.4). In a typical test, 100 μL sample solution was dropped on the sample pad, after

10 min, the intensities of the test and control line were determined using ImageJ.

3. Results and discussions

In this study, we present the one-step signal enhancement lateral flow strip (**Duo-LFS**) for ultrasensitive detection of BPA without any further post-treatments in less than 15 min. The construction and the basic detection principle of the Duo-LFS are depicted in Fig. 1. Fig. 1 shows a schematic illustration of the detection principle of the signal enhanced Duo-lateral flow strip. It is easy to find that there are two conjugation pads on the designed lateral flow strip. The first pad is the same as the conventional one for antibody labeling while the second one is for signal amplifications. It is also easy to understand the conventional technique using 15-nm gold nanoparticles for labeling of antibody against BPA on the first conjugation pad. How to use the second conjugation pad (the enhancement pad) for signal amplification? It can be described in the following two aspects: (1) it is well known that BSA is widely used in the first conjugation pad for blocking the non-covered sites of gold nanoparticles and inhibiting the non-specific adsorptions. Skillfully, we used the second gold nanoparticles coated with the antibody against BSA to further label the first gold nanoparticles on the test line through immunoreaction. (2) The property of high surface to volume effect of nanoparticles was applied here, which is beneficial for the biomolecule immobilization and signal amplification.

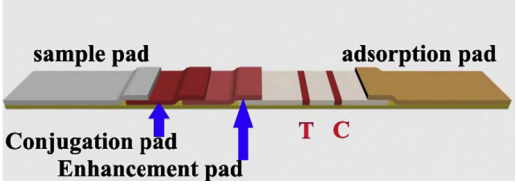

Fig. 1. The schematic diagram of the detection principle of the signal amplified lateral flow strip.

Before the real detection of BPA in water samples using this Duo-LFS, several key factors, which could influence the performance of the lateral flow strip including the antibody concentration and the amount of gold nanoparticle labeled conjugates on the pad, were optimized firstly (see details in the Supporting information). Specifically, the diameter of the second gold nanoparticles was also optimized for getting the best signal amplification performance in BPA sensing. Under the optimal conditions, the sensitivity of the assembled signal enhanced Duo-LFS was examined by using the standard stock BPA solutions at different concentrations. The detection results of different BPA standard solutions by both the Duo-LFS and the traditional strips were shown and compared in Fig. 2. As shown in Fig. 2a, it is easy to find that the optical density on the test line was decreased with the increase of BPA concentrations due to the adopted competitive immunoreaction model. According to the rule of empirical experience, the limit of detection observed by naked eyes could be determined as the minimum target concentration producing the color on the test line significantly different to that on the test line

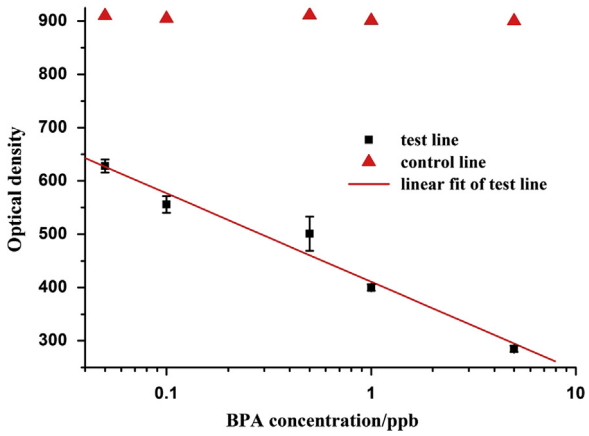

Fig. 3. The calibration curve for BPA detection using the signal amplified lateral flow strip.

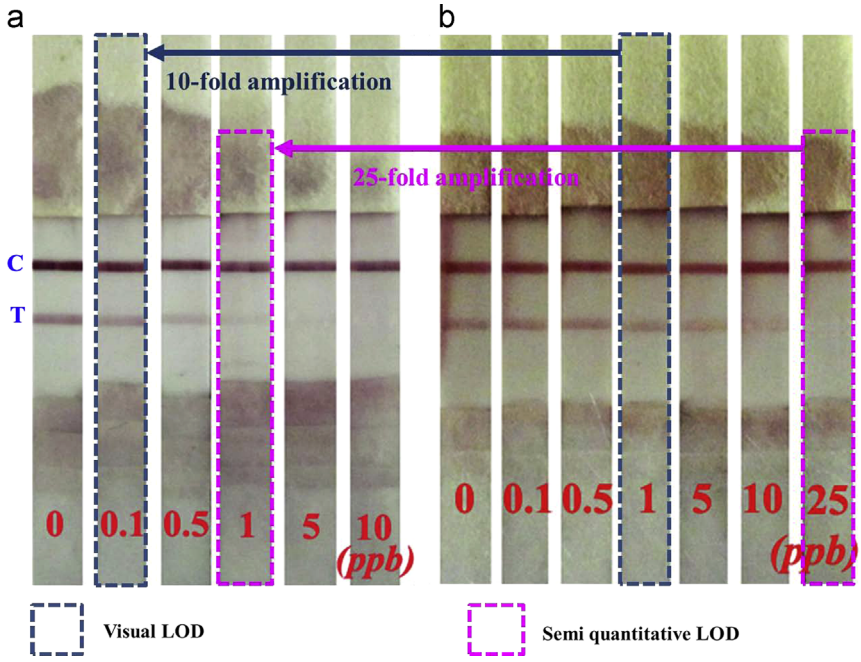

Fig. 2. The BPA sensing results by signal amplified (a) and traditional lateral flow strips (b).

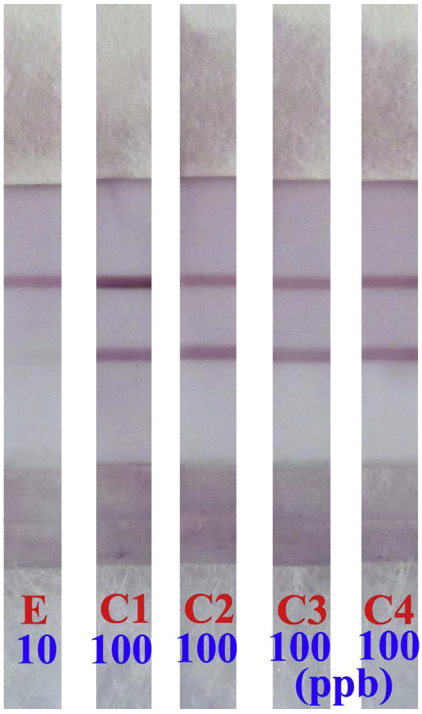

Fig. 4. The selectivity of the developed signal amplified lateral flow strips. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

of the negative control strip without the target analyte (Wang et al., 2011; Xie et al., 2009). Therefore, the visual limit of detection (LOD) of the Duo-LFS could be defined as 0.5 ng/mL, at which the color of the test line was almost disappeared on the strip. Of great significance, this sensitivity of the developed Duo-lateral flow strip is even comparable to some reported instrumental based methods for BPA detections (Marchesini et al., 2005). Meanwhile, it is also noticed that the color of the control lines on the strips is almost the same, which indicates the effectiveness of the developed Duo-LFS. In contrast, BPA detection results of the traditional strips at the same conditions were also shown in Fig. 2b. And similarly, only 5 ng/mL of BPA in the sample could induce almost the same disappearing of the test line as Duo-LFS, which could be attributed as the visual LOD of traditional LFS. For quantitative analysis, the optical density of test lines of both strips was analyzed by the ImageJ Analysis Software (the detailed analysis shown in Supporting information) and average value of three parallel samples at the same concentration was adopted for calibration curve construction. According to the final quantitative result (Fig. 3), the useful range of the quantitative detection of the Duo-LFS is from 0.05 to 10 ng/mL. More exactly, the good linear relationship from 0.05 to 5 ng/mL was achieved based on the analyzed results with R square of 0.9931 and the limit of detection is 0.076 ng/mL (The LOD was defined as the concentration of BPA in the samples that caused a 10% decrease of the optical density of the test line on the strip compared with that of the blank group) (Xie et al., 2009; Zhang et al., 2006).

Besides, another important factor of the Duo-LFS was interrogated by using the developed Duo-LFS to measure other analogues of BPA. As it is shown in Fig. 4, 10 ng/mL of BPA (the first strip in Fig. 4) has induced the complete disappearance of the red color on the test line indicating the strong positive signal. However, it is also observed that the analogues at an even ten-fold concentration of BPA (100 ng/mL) could not induce the intensity decrease of the test line. All these results demonstrate the excellent specificity of the new signal enhanced Duo-LFS.

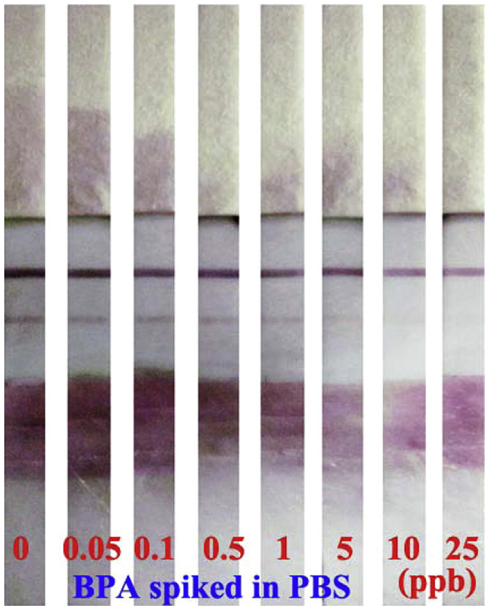

Fig. 5. The BPA detection results in the real BPA spiked samples.

Finally, the real applications of the developed signal enhanced Duo-LFS were carried out by detecting BPA spiked samples in PBS and tap water samples, respectively. Both spiked samples in PBS and tap water samples could be successfully determined with satisfied results. The results of spiked BPA samples in PBS were almost the same as the standard solution (Fig. 5). As to that of the spiked BPA in tap water samples shown in Fig. 5, the visual LOD could be determined as low as 1 ng/mL, which is still very sensitive comparable to the traditional LFS and meets the requirement of on-site screening. The detection results of Duo-LFS were also further confirmed by HPLC (see details in Table S1), which also indicated the reliability of the developed Duo-LFS.

4. Conclusion

A one-step signal enhanced lateral flow strip (Duo-LFS) was developed for sensitive and on-site detection of BPA in less than 15 min. The signal amplification was based on the dual labeling on the test line of the strip using gold nanoparticles with different size. More importantly, the detection and amplification operations are both completed in one step without any extra treatments, which is of great importance for rapid and on-site high throughput routine screening. And the visual LOD of the Duo-LFS could be as low as 0.5 ng/mL without using any further instruments, which could meet the requirements of the on-site BPA screen and provide a 10-fold improvement of sensitivity of traditional LFS based methods. Besides, this adopted signal amplification treatment could be applied to any other strip-based research for sensing target with low molecular weight. Further research on sensing of other targets and using nucleic acid probes as the signal enhancement recognition system is on-going in our lab. And we believe that this signal amplification technique would further speed up the commercial of lateral flow strip products and greatly broaden the application of lateral flow strip based methods in different fields including on-site BPA detection.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.06.006.

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