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SERS based immunochromatographic assay for rapid and quantitative determination of bisphenol A

Lei Zhang [a,*], Yingshan Chen [b], Qin Zhu [a], Wenjin Ji [a], Suqing Zhao [b,*]

[a] Department of Analytical and Testing Center, School of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou, 510006, PR China
[b] Department of Pharmaceutical Engineering, School of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou, 510006, PR China

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ABSTRACT

Bisphenol A (BPA) is a typical endocrine-disrupting chemical (EDC), which has been banned for food packing and baby - bottle material due to its potential endocrine harm to human body. In this paper, an easy and rapid immunochromatographic assay (ICA) combined with the technology of surface-enhanced Raman spectroscopy (SERS) was fabricated to improve the sensitivity and rapidity for quantitative determination of BPA. After 10 min of a quick chromatographic process, the specific Raman signal on T line of the test strip was measured. Compared with the traditional ICA, the SERS-ICA realized quantitative test and showed the limit of detection (LOD) was 0.1 ng/mL, getting approximately 300-fold lower. The method displayed high specificity and precision with RSD of 4.1%, and the test strip indicated favorable stability for two weeks without loss of activity. For river, tap and commercial drinking water sample, the SERS-ICA results were confirmed by HPLC, and the recoveries of spiked BPA were from 90.80-117.80%. It was shown that the proposed method takes advantages both of SERS and ICA, exhibiting high sensitivity, precision, stability, quantification and rapidity for field test, expecting a widely useful method for various small molecules in food safety, medical diagnosis and environmental samples.

1. Introduction

Bisphenol A (BPA) is a kind of typical endocrine-disrupting chemicals (EDCs), which have attracted the attentions of researchers for its reproductive toxicity to liver damage, disrupted pancreatic β-cell function, thyroid hormone disruption, obesity-promoting effects, *etc.* [1–3]. The in-vitro studies have found that low doses of BPA disrupted the cell function by genomic and nongenomic estrogen-response mechanisms even as low as 1 pM or 0.23 ppt [4,5]. Unfortunately, it was detected that BPA was presented in 93% of tested urine samples with detectable levels in the United States, and was found in blood as high as 1–18 nM [6], which was potentially harmful to the human body. The commonly used techniques for BPA detection include high performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC–MS), electrochemical sensors and immunoassays [7–10]. These methods have a long detection cycle, high cost, and require miscellaneous and toilsome procedures, which restrict their applications in rapid detection and field test. Hence, it is necessary to develop a sensitive, specific, portable and rapid method for BPA detection.

The immunochromatography assay (ICA) is a powerful method for rapid screening and field test because of its advantages of simplicity, flexibility, rapidity and low costs [11]. However, ICA strips are of low sensitivity and can only provide qualitative or semi-quantitative results of analyte concentration. Therefore, a new quantitative analysis method were introduced to supplement.

Surface enhanced Raman spectroscopy (SERS) is a high sensitive spectral technique based on the adsorption of molecules on rough metal surfaces, and we know it can detect analyte accurately by its fingerprint characteristics [12]. There is no doubt that SERS has been widely applied in various analytical applications. It has been reported SERS is used to detect BPA in some papers [13,14]. However, the molecule construction of BPA is unfit for adsorption on rough metal surfaces, so that indirect or coupled methods were applied for BPA determination by SERS [15,16]. In our previous paper, a SERS coupled Enzyme-linked immunoassay (SERS-ELISA) method was prepared for sensitive detection of BPA, which was established on the ELISA theory [17], however which involved complicated procedures and spent long time in incubation and washing steps. ICA combined with SERS method can quickly give the qualitative result within 15 min on one hand, and on the other hand the quantitative result can be obtained by SERS sensitively, which is appropriate for rapid and field test [18–20]. In this paper, as illustrated in Fig.1, a new analysis method SERS-ICA was proposed for

* Corresponding authors.
E-mail addresses: zhangleishg@163.com (L. Zhang), sqzhao@gdut.edu.cn (S. Zhao).

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qualitative and quantitative detection for BPA. The SERS tags 4MBA labeled monoclonal antibody against BPA on gold nanoparticles (4MBA@AuNPs@mAb) was fabricated firstly, then the ICA process based on the indirect competitive immunoassay principle (ic antigen antibody binding) was finished within 10 min. After the capillarity migration process, the antigen-SERS tags complexes were aggregated and concentrated at the T line. On the one hand, it could be detected of BPA qualitatively by the color change. On the other hand, it could be analyzed quantitatively by the SERS tags signal because of the "hot spots" formed between gold nanoparticles. The sensitivity, specificity, precision and stability of the SERS-ICA system were evaluated. In the end, the concentrations of BPA in real water samples were measured simultaneously by SERS-ICA and HPLC. It is shown that SERS-ICA can be used for the field analysis and quantitative test for food safety, environmental pollution and medical diagnosis in the future.

2. Materials and methods

2.1. Reagents and chemicals

Hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), 4-mercaptobenzoic acid (4MBA), bovine serum albumin (BSA), sodium Citrate ($Na_3C_6H_5O_7 \cdot 2H_2O$) were purchased from Sinopharm Chemical Reagent Co., Ltd, China. bisphenol A (BPA), benzene, hydroquinone, phenol, o-hydroxyaniline and o-cresol were purchased from Aladdin China Ltd. Monoclonal antibody against BPA and coating antigen were prepared according to our previous work [10,21]. The goat anti mouse IgG was obtained from BOSTER biological company (Wuhan, China). Phosphate buffer saline (PBS) and phosphate buffer saline with tween (PBST) were prepared according to the conventional method. All chemical reagents were of analytical grade and the ultrapure water (18.2 MΩ cm) was used throughout the experiment. Nitrocellulose (NC) membranes, PVC sheets, adsorption pads and sample pads were purchased from Jieyi Biotechnology Co. Ltd. (Shanghai, China).

2.2. Instruments

The UV Laser Raman Spectroscopy (LabRAM HR 800, HORIBA Jobin Yvon) was used to measure the Raman spectra. An air-cooled He-Ne laser with 633 nm excitation for an accumulation time of 20 s was used. The ultraviolet visible spectrophotometer (T9) from Beijing Purkinje General Instrument Co. Ltd (Beijing, China) was used to record the absorption spectrum of gold nanoparticles. Scanning electron microscope (SEM) image was obtained using a Nova NanoSEM 430 scanning electron microscopy. Sample homogenizing was carried out using vortex mixer (KH3200B) from Qilin Medical Instrument Factory (Haimen, Jiangsu province, China). The coating antigen was carried out on a XYZ three-dimensional spraying machine (HM3030) from Shanghai Kinbio Tech. Co., Ltd (Shanghai, China). High performance liquid chromatography (HPLC) analysis was performed by LC-20A (Shimadzu, Japan) with a C18 column.

3. Experimental

3.1. Production of mAb against BPA and synthesis of coating antigen

The 4, 4-bis(4-hydroxyphenol) valeric acid (BVA) was selected as the hapten [10,21], and the BVA-BSA conjugate (bovine serum albumin as the carrier protein) was used as the immunogen to prepare monoclonal antibody (mAb) against BPA, which exhibited high specificity and binding affinity for BPA. The BVA-PLL (poly-L-lysine) conjugate was prepared as the coating antigen, showing a high sensitivity of indirect competitive ELISA (ic-ELISA) for BPA detection.

3.2. Preparation of SERS labeled immunoprobe of 4MBA-AuNPs-mAb

Firstly, the sodium citrate capping colloidal gold nanoparticles (AuNPs, 20 nm in diameter) was prepared according to our previous work [22] with small modifications. 200 mL of 1×10^{-4} g/mL $HAuCl_4 \cdot 3H_2O$ solution was heated to boiling and added rapidly with 1.8 mL of 1×10^{-2} g/mL $Na_3C_6H_5O_7 \cdot 2H_2O$ solution. The mixture was

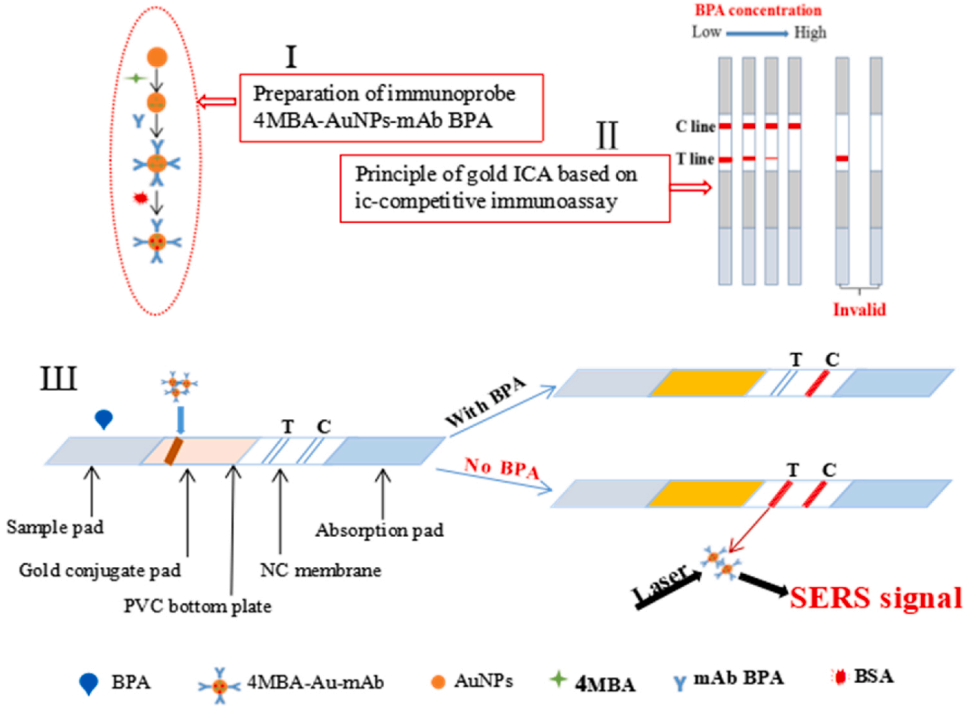

Fig. 1. Schematic diagram and principle of SERS-ICA, including (I) the preparation of SERS tagged immunoprobe of 4MBA-Au-mAb, (II) the ic-immunoassay chromatography process, (III) the structure of gold ICA strip and the principle of SERS-ICA.

vigorously stirred and kept boiling for 15 min. After the colloidal gold solution was cooled, 10 μL of the 4MBA (in ethanol) in 1 mM was added to 10 mL AuNPs and reacted for 1 h at room temperature. Then 0.1 mol L^{-1} K_2CO_3 solution was subsequently added to adjust pH value to 9, and 10 μL of mAb against BPA was labeled to 1 mL of the 4MBA-tagged AuNPs. After mixing for 1 h, 100 μL of 3% BSA/PBS was added for blocking the extra active sites on the immunoprobe and continuously incubated overnight. Then, the SERS labeled immunoprobe was washed by centrifugation at 7000 rpm for 10 min to remove the free mAb and 4MBA, the sedimented immunoprobe of 4MBA-AuNPs-mAb was re-diluted with 2 mL PBS and stored at 4 °C.

The prepared spherical AuNPs with the size about 20 nm was characterized in Fig. S1, a dominant UV absorption peak at 520 nm was observed, which is suitable for gold ICA (20 nm diameter) [23,24] and can form more "hot spot" between nanoparticles, resulting in strong signal enhancement, thus improving the sensitivity of SERS [25].

3.3. Assembly of SERS-ICA strip

Before assembling the SERS-ICA strip, the coating antigen (T line) and second antibody (C line) on NC membrane were immobilized. It was done by XYZ three-dimensional spraying machine. 10 μL of 1.0 mg/mL BVA-PLL and 0.1 mg/mL goat anti mouse IgG were sprayed at T line and C line respectively. Then NC membrane was placed at room temperature for 0.5 h, and dried at 37 °C for 1 h.

Fig. 1(III) showed the strip structure consisted of absorbent pad, NC membrane, sample pad, and a PVC substrate. The NC membrane was pasted at the center of the PVC substrate. The sample pad and absorbent pad were pasted on left and right sites of the NC membrane with a 2 mm overlapping. Then the assembled plate was cut into single test strips of 6 mm in width, being sealed into a clean box and stored at 4 °C for use.

3.4. Analysis of traditional ICA and SERS signal measurement

A traditional ICA for BPA detection was carried out as follows. 2 μL of 4MBA-AuNPs-mAb tags was dropped onto sample pad about 1 cm near the NC membrane, then 100 μL of BPA standard solution (a series of concentration of 100, 50, 30, 10 and 5 ng/mL) was pipetted onto the sample pad at 0.5 cm from the end of the strip. Driven by the capillary action, the BPA standard solution mingled with the immunoprobes would flow toward the direction of absorbent pad. The colloidal gold color would appear on the T line and C line, which could be observed by the naked eyes in 10−15 min for a rapid qualitative analysis (the result as shown in Fig. 3 (a)). Last, the strips at T line were measured by UV Laser Raman Spectroscopy for a SERS-ICA quantitative analysis.

3.5. SERS-ICA for BPA determination in actual water samples

In order to demonstrate a practical-application feasibility, three types of water samples were selected as the solution for BPA, including the tributary of Zhujiang river (denoted as sample 1#, after centrifuged at 6000 rpm for 10 min, and the sediment removal), tap water (denoted as sample $2^{\#}$) and the commercial barreled water (denoted as sample $3^{\#}$). Each sample was filtrated through 0.45 μm microporous filter and spiked with BPA standard solution at the final concentrations of 0, 5, 10 and 50 ng/mL. Then 100 μL of these water samples were dropped onto the assembled strips. The recovery of BPA from the spiked samples was calculated as:

Recovery (%) = [(BPA concentration measured -Blank) / BPA concentration spiked] ×100%.

4. Results and discussion

4.1. Principle of SERS-ICA

The SERS-ICA test is as shown in Fig.1. Firstly, the sample solution containing BPA is applied on the sample pad and gradually migrates in the longitudinal direction due to capillary action. When the sample solution migrates through the conjugate pad, the antigen BPA can specifically bind to the antibody of SERS immunoprobes (4MBA-AuNPs-mAb) immobilized on the conjugate pad. In BPA negative samples, the SERS immunoprobes conjugate with the coating antigen and accumulate on the T line, resulting in purple lines on the NC membrane. Due to competition between BPA and BVA-PLL for binding to SERS immunoprobes, the color of the T line gets weaker with the increasing of BPA concentration. Therefore, the color green of the T line was inversely proportional to the BPA concentration. The results showed that BPA could be qualitatively detected by the naked eyes as well as quantitatively detected by measuring the SERS peaks of 4MBA on the T line.

4.2. Feasibility of SERS-ICA for BPA

Four ICA test strips were assembled as follows: a. 4MBA-Au-mAb as immunoprobe, BVA-PLL coated on the T line; b. 4MBA-Au-PBS as probe (without antibody), BVA-PLL coated on the T line; c. 4MBA-Au-mAb as immunoprobe, BSA-PBS sprayed on the T line (no coating antigen); d. Au-mAb as immunoprobe (without Raman reporter), BVA-PLL coated on the T line. The solution of 0 ng/mL BPA was tested with results shown in Fig. 2. The Raman spectrum of (a) displayed an obvious SERS signal of 4MBA especially at peaks of 1077 cm^{-1} and 1580 cm^{-1}, which was absence in (b), (c) and (d). It indicated that the antibody labeled on the immunoprobe was specifically bound with the antigen coated on T line without nonspecific adsorption. In the case of condition d, there was no Raman signal because 4MBA had not been tagged on the immunoprobe, illustrating that the quantitative determination signal was generated from the Raman reporter. Therefore, there was a specific binding between antigen and antibody, and the SERS-ICA was feasible for quantitative determination of BPA.

4.3. Optimization of the SERS-ICA

In order to improve the sensitivity and accuracy of SERS-ICA for BPA detection, a series of parameters were optimized elaborately. (i) 10 μL of

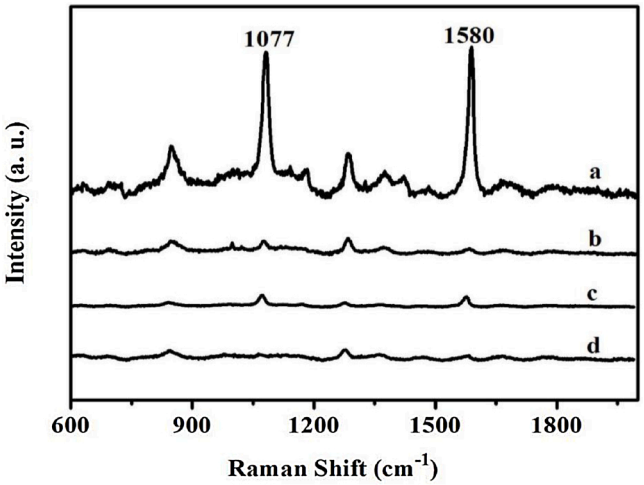

Fig. 2. Feasibility test of (a) 4MBA-Au-mAb as immunoprobe, BVA-PLL coated on T line; (b) 4MBA-Au-PBS as probe, BVA-PLL coated on T line; (c) 4MBA-Au-mAb as immunoprobe, BSA-PBS sprayed on T line; (d) Au-mAb as immunoprobe, BVA-PLL coated on T line.

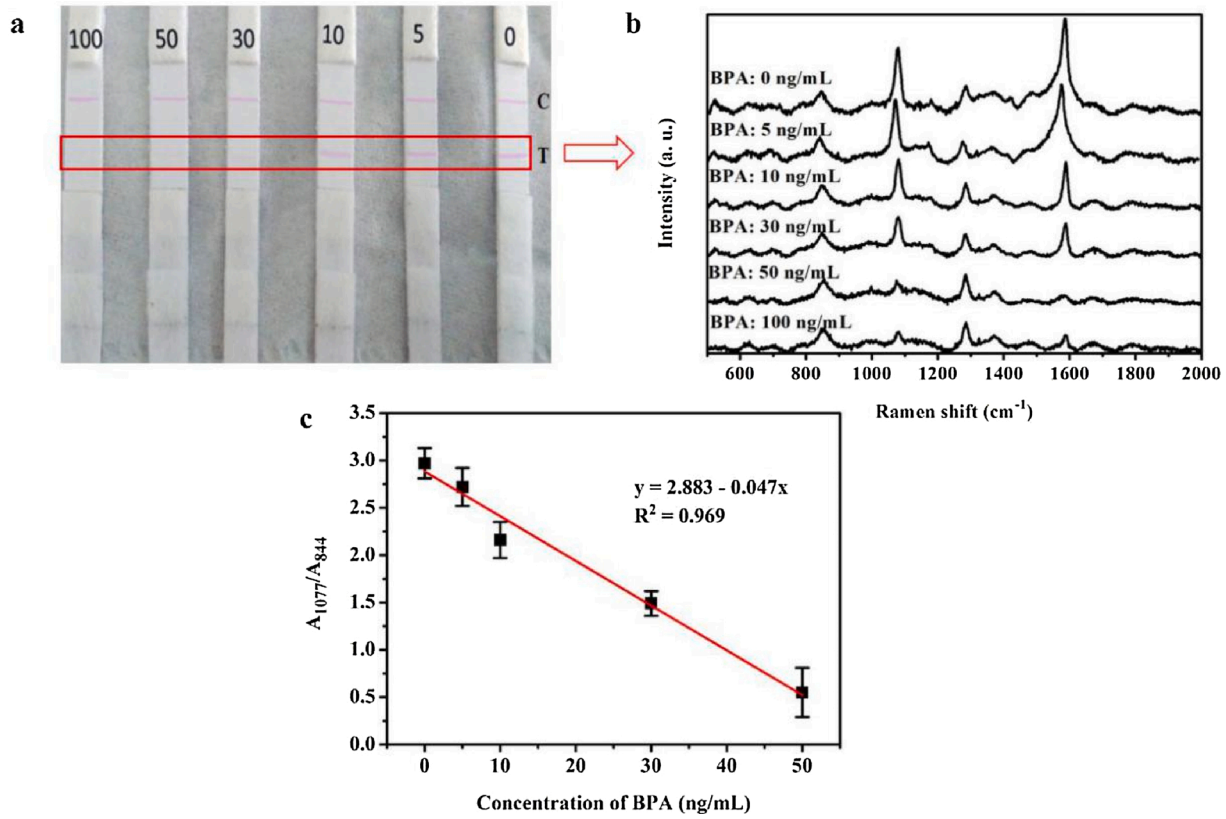

Fig. 3. Specificity of the SERS-ICA for BPA. (a) Visual observation of the ICA strips for BPA standard solutions. (b) SERS spectra of strips on T line for different BPA concentrations. (c) Standard curve of SERS-ICA for BPA, the error bars represent the SD of A1077/A844 measured from 6 different sites in the middle parts on T line. The BPA standard concentrations were 100, 50, 30, 10, 5 and 0 ng/mL, and prepared by dilution with methanol aqueous solution.

different dilutions of mAb against BPA (1:100, 1:200, 1:500, 1:1000 and 1:2000, diluted with PBS) were labeled to 1 mL of 4MBA-tagged AuNPs respectively. The surface plasma resonance (SPR) band of AuNPs varied in intensity and location after labeling. AuNPs showed the SPR peak at 520 nm, which was used to evaluate the optimal antibody dilution. As shown in Fig. S2 (a), when mAb against BPA of 1:1000 was labeled to 4MBA-AuNPs, the SPR peak intensity reached a maximum. (ii) The optimal concentration of coating antigen and the volume of immunoprobe were evaluated by the inhibition ratio (I_0/I_{10}) of SERS intensity of 4MBA at 1077 cm^{-1} (I_0 and I_{10} were the SERS intensity measured on T line when BPA concentration was 0 and 10 ng/mL respectively). The greater ratio value implies the stronger immune competition reactivity. As shown in Fig. S2 (b), 10 μL of different concentrations (0.2, 0.5, 0.7, 1.0, 1.2 and 1.5 mg/mL of BVA-PLL) were coated on T line, the value of the inhibition ratio was the biggest when coating 1.0 mg/mL. (iii) As shown in Fig. S2 (c), it was found when 0.5, 1.0, 1.5, 2.0 and 2.5 μL of the SERS immunoprobe were pipetted onto the strips, the biggest inhibition ratio value was obtained at 2.0 μL. Therefore, the optimal experimental conditions of SERS-ICA for BPA were: dilution of 1:1000 mAb used for SERS immunoprobe, 1.0 mg/mL of BVA-PLL coated on T line, and 2.0 μL of the 4MBA-Au-mAb pipetted onto the strips.

4.4. Sensitivity of the SERS-ICA for BPA

Under the optimal conditions, series of BPA standard (0, 5, 10, 30, 50 and 100 ng/mL) in PBS were tested. As shown in Fig. 3 (a), purple band appeared on C line in all test strips, but the color of the T line turns shallow as the BPA concentration increased from 0 to 30 ng/mL. And when the BPA standard concentration was 30 ng mL^{-1}, the T line became colorless, indicating a LOD by the naked eye observation. Fig. 3 (b) showed the SRES spectra of the strips on T line after the above naked eye determination. It was noted that with BPA concentration increasing, the peak intensity at 1077 cm^{-1} was gradually decreased, but all the spectra had the similar intensity at 844 cm^{-1}, which was the background signal of NC membrane (as shown in figure S3). Therefore, the standard curve of SERS-ICA for BPA was plotted by the peak area ratio of A_{1077}/A_{844} *vs.* BPA concentration (Fig. 3 (c)). The result at 0−50 ng/mL exhibited a good linear correlation with the equation of $y = 2.883-0.047x$, $R^2 = 0.969$, with the LOD of 0.1 ng/mL (3 times the standard deviation above the blank), approximately 300-fold lower than that of the traditional ICA, and 50-fold lower than that obtained by the fluorescence polarization immunoassay (FPIA) with the same mAb [21].

4.5. Specificity of the SERS-ICA for BPA

The specificity of this method was tested as follow: 100 μL of six benzene homogenous analytes (Benzene, Hydroquinone, Phenol, o-hydroxyaniline, O-Cresol and BPA) with concentration of 100 ng/mL were tested by SERS-ICA, with water as the blank control. As shown in Fig. 4, the peak intensity on T line at 1077 cm^{-1} of these analytes showed a medium reduction when compared with the blank solution, but appeared a sharply reduction when testing BPA, which suggested that there was little cross-reactivity with Benzene, Hydroquinone, Phenol, o-hydroxyaniline and O-Cresol, showing a high specificity of SERS-ICA for BPA.

4.6. Precision and stability of the SERS-ICA test strips

The precision of the intra-assay was investigated by one test strip. The BPA standard solution of 100 ng/mL concentration was detected, then 20 points on the T line from different positions along the middle parts were measured. In Fig. 5 (a), it showed the 20 points value of the peak area ratio (A_{1077}/A_{844}) and the RSD value was worked out 4.1%, showing good precision of the method. For stability test, the strips were

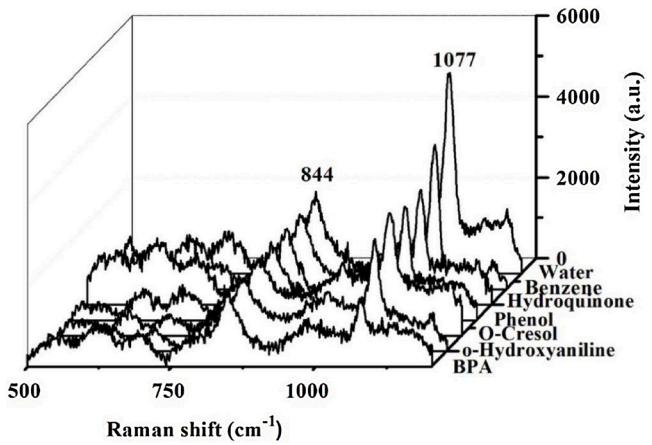

Fig. 4. SERS spectra measured on T line for water, Benzene, Hydroquinone, Phenol, o-hydroxyaniline, O-Cresol and BPA.

assembled under the optimal conditions and preserved in 4 ℃ box for 2 weeks. Consequently, 0 ng/mL and 100 ng/mL BPA standard solution were detected respectively. As shown in Fig. 5 (b), there was no obvious changes in both of the SERS spectral peak and shape after a period of storage, which indicated that there was a high stability of the SERS-ICA assay.

4.7. Quantitative determination of BPA in water samples

The quantitative test results were listed in Table 1. There were three actual samples with 4 levels of BPA concentrations were measured by SERS-ICA. 0 ng/mL was the original sample without BPA spiked, but 5 ng/mL, 10 ng/mL, and 50 ng/mL were spiked samples, which were applied to detect the BPA spiked recovery. The result showed that sample 1 was detected of BPA at 8.12 ng/mL, while the other 2 samples were not checked out. Each test was measured 3 times and the average value was adopted to calculate the BPA content. The results indicated that the recoveries of BPA from spiked samples were 90.80–117.80 % with standard deviation (SD) in the range of 2.1–6.9 % (n = 3).

To demonstrate the practicability of SERS-ICA for BPA, three water samples were analyzed by both SERS-ICA and HPLC methods. It was shown in Fig. 6 that the correlation analysis of BPA in water samples by HPLC and SERS-ICA were linear, with the correlation coefficient of 0.991, indicating the good possibility and reliability of SERS-ICA for BPA.

5. Conclusions

In this study, a highly rapid and sensitive immunochromatographic assay combined with SERS was developed for quantitative detection of BPA. Its principle was similar to the traditional ICA, but with the conjugate of 4MBA-AuNPs-mAb as the immunoprobe, the detection sensitivity was greatly improved due to SERS intensity. The results showed that the ICA procedure was completed within 10 min, and the LOD was 30 ng/mL observed by the naked eyes. Combining with the SERS signal measurement, the LOD of SERS-ICA was improved to 0.1 ng/mL, approximately 300-fold lower than that of the traditional ICA. In addition, the actual water samples were analyzed by SERS-ICA and HPLC, which showed a good correlation and demonstrated that this proposed method of SERS-ICA was reliable for rapid, sensitive and precise determination of BPA. In the future, SERS-ICA could be used as an effective means of detection for analytes in food safety, pesticide and environmental monitoring.

CRediT authorship contribution statement

Lei Zhang: Conceptualization, Investigation, Writing - original draft. **Yingshan Chen:** Data curation, Writing - review & editing. **Qin Zhu:** Data curation, Formal analysis, Writing - review & editing, Methodology. **Wenjin Ji:** Software, Formal analysis, Validation. **Suqing Zhao:** Conceptualization, Project administration.

Table 1
Results of BPA determination in water samples.

Sample [a]	BPA spiked (ng/mL)	Measured by SERS-ICA (ng/mL)	Recovery (%)	SD (n = 3)
1#	0	8.12		4.2
	5	14.01	117.80	5.1
	10	19.04	109.20	3.5
	50	58.85	101.46	4.3
2#	0	–		
	5	4.54	90.80	2.1
	10	10.93	109.30	3.3
	50	51.29	102.58	4.0
3#	0	–		
	5	5.09	101.80	6.9
	10	9.23	92.30	3.2
	50	52.06	104.12	6.7

1#: Zhujiang river water near the Nanting wharf in Guangzhou Higher Education Mega Center, Guangzhou, China;
2#: tap water in the laboratory.
3#: commercially available barreled water.
- : No checked out.

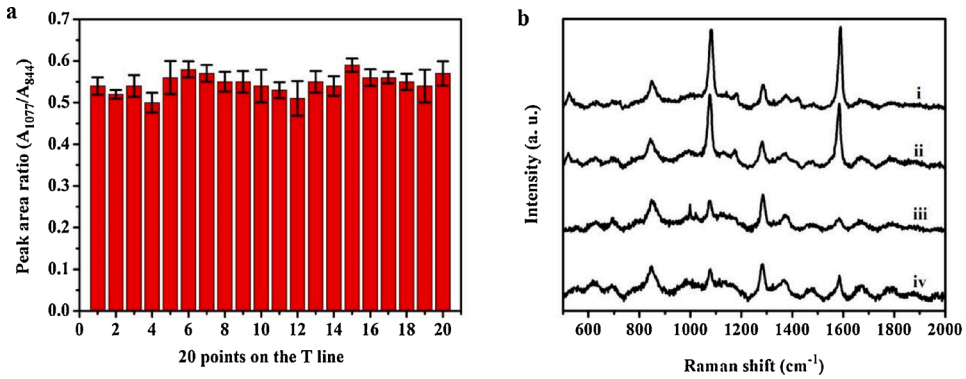

Fig. 5. (a) SERS peak area ratio of 4MBA at 1077 cm^{-1} and 844 cm^{-1} from 20 different points on the T line, with the RSD value of 4.1 %. (b) SERS spectra of (i) 0 ng/mL BPA tested on the freshly assembled strip; (ii) 0 ng/mL BPA tested on the stored strip for 2 weeks; (iii) 100 ng/mL BPA tested on the freshly assembled strip; (iv) 100 ng/mL BPA tested on the stored strip for 2 weeks.

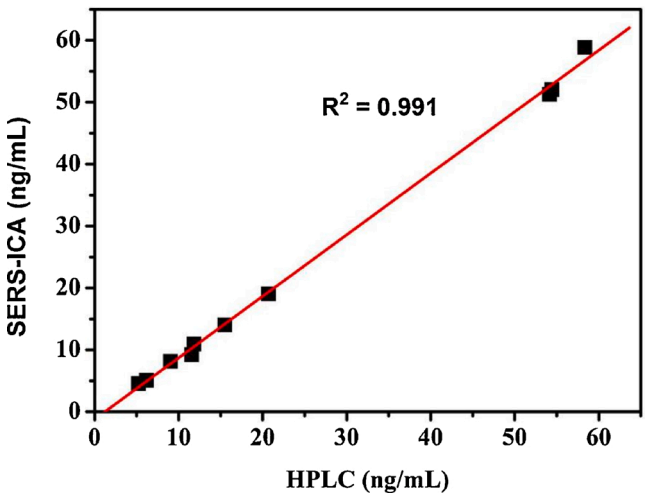

Fig. 6. Correlation analysis of BPA in water samples by HPLC and SERS-ICA.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vibspec.2021.103225.

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