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Design of elution strategy for simultaneous detection of chloramphenicol and gentamicin in complex samples using surface plasmon resonance

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ABSTRACT

For the analysis of massive samples containing multiple analytes, the enhancement of detection efficiency is crucial. In this study, a facile method was developed for sequential detection of chloramphenicol (CAP) and gentamicin (GEN) in complex samples, e.g. milk, using a surface plasmon resonance (SPR)-based biosensor. Based on the immune inhibition format, two conjugates of antigen and bovine serum albumin (BSA)—denoted as CAP–BSA and GEN–BSA—were grafted on the same channel of the SPR sensor chip. Two standard curves for CAP and GEN were separately obtained by first mixing a single antibody with different concentrations of the relevant antigen. Moreover, different regeneration solutions were screened for sequential analysis. An alkaline solution was found to completely remove the antibody against GEN (AbGEN) from the chip, but it exhibited limited ability to dissociate the antibody against CAP (AbCAP). Therefore, alkaline solution and Gly-HCl solutions are successively applied to elute AbGEN and AbCAP, respectively. By gradual elutions, CAP and GEN concentrations were simultaneously calculated with limit of detection values of 5.28 ng/mL and 2.26 ng/mL, respectively. Furthermore, the spiking milk samples with CAP and GEN validated the assay with recoveries of 77.6–101.1%. Therefore, this method is expected to improve the detection efficiency of SPR biosensors.

1. Introduction

Surface plasmon resonance (SPR) is an important optical physical phenomenon involving the resonant transfer of pumping light energy to a surface-plasmon mode in the form of collective oscillations of electrons at the metal surface (Homola, 2003). Based on this phenomenon, the SPR sensor had been developed to measure the changes in the refractive index of the metal surface, as well as the molecular interaction (Shi et al., 2015). Generally, a biorecognition element is immobilized on the metal surface to capture analytes, giving a local change in refractive index that will be translated into output signal by the SPR sensor (Homola, 2008). The change on the chip surface can be calculated for the precise measurement of the interactions between a ligand and an analyte (Malmqvist, 1993). The SPR analysis exhibits several advantages including less time consumption (Zhang, 2016), non-labelling, high sensitivity and high efficiency (Gao et al., 2016). Therefore, it has been widely applied in numerous fields, such as clinical diagnosis (Soler et al., 2015), drug screening (Olaru et al., 2015; Wang et al., 2015), environmental monitoring (Jang et al., 2015) and food safety (Vaisocherova-Lisalova et al., 2016).

In recent years, food safety has attracted immense interest, and most of samples require urgent monitoring (Hanning et al., 2012). However, the detection efficiency is severely hampered because of the complex matrix in actual samples and wide varieties of toxic residues (Kagan, 2016). A lot of conventional analytical techniques, such as gas/liquid chromatography–mass spectrometry (Steinborn et al., 2016), enzyme-linked immunosorbent assay (Zhu et al., 2016), lateral flow immunoassay strips (Song et al., 2016), fluorescence (Gao et al., 2014), gated resonance energy transfer (Stobiecka, 2014; Stobiecka and Chalupa, 2015) and electrochemical biosensor (Rotariu et al., 2016) have been utilised to determine various targets in complex samples. Nevertheless, these techniques exhibit some disadvantages, such as

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operation inconvenience, complicated sample preparation, lengthy analysis time and poor reproducibility (Chiou et al., 2015). Therefore, a more effective method is always required to solve these issues.

In this regard, the use of SPR biosensors, as a new technique, has solved some aforementioned issues (Singh, 2016). Nonetheless, multi-detection is regarded to be very crucial for practical applications. However, it is still difficult to be realised by SPR biosensors which are usually equipped with single channel or two channels (a control and a test) in the labs. To overcome the challenge of multi-detection, some studies have been reported, e.g., by the increase of the number of test channels and the addition of imaging systems (Puiu and Bala, 2016; Abadian et al., 2015). Fernández et al. (2010) have integrated a gold diffraction grating surface into a six-channel microfluidic device to achieve on-site label-free analysis of multiplexed antibiotics in milk samples. Liu et al. (2016) have reported a sensitive method with SPR imaging biosensor for small saccharides (glucose) and large glycoconjugates (carcinoembryonic antigen). However, in a majority of SPR sensors, only a single test channel is typically equipped; hence, the detection of multiple analytes is still difficult. For realizing the multi-detection, a new method of detection using the single-channel SPR instrument is required.

During the SPR detection, removing the analytes (antibodies, antigens and aptamers etc.) from the sensor chip called “regeneration” is a crucial process (Morton and Myszka, 1998). For reusing sensor chips, specific mild acidic or basic solutions are used to break the non-covalent bonds between the ligand and analyte without degrading the ligand on the chip for subsequent binding (Homola et al., 1999). To achieve optimum results, the sensor chip should be regenerated without a shift in the baseline and a change in the surface capacity. Hence, the strict optimisation of the elution solutions in an SPR assay is mandatory (Myszka, 1999). However, some analytes bound to the sensor chip may not be completely dissociated using specific elution solutions (Andersson et al., 1999). Different elution solutions exhibit different capabilities for dissociating the binding between the analyte and ligand. As a result, different analytes can be possibly removed successively from the chip surface under different regeneration conditions (van Oss, 2000).

In this study, a facile method was developed for the sequential detection of chloramphenicol (CAP) and gentamicin (GEN) using gradient elution solutions. Two conjugates of antigen and bovine serum albumin (BSA), e.g. CAP–BSA and GEN–BSA, were covalently immobilised on the sensor chip. For gradient elution, various regeneration conditions were applied for the elution of CAP and GEN. Based on the immune inhibition format, CAP and GEN were simultaneously detected using a single injection. Furthermore, the analytical performance was demonstrated by the recovery results obtained by spiking milk samples with CAP and GEN.

2. Materials and methods

2.1. Reagents and materials

CAP, GEN and glycine (Gly) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Hydrochloric acid (HCl) and sodium hydroxide (NaOH) were purchased from Tianjin Chemical Reagent Factory. Acetate buffers at different pH values (4.0, 4.5, 5.0, 5.5), 2-(4-(2-Hydroxyethyl)-1-piperazinyl)ethanesulfonic acid-buffered saline (HBS) buffer, 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-Hydroxysuccinimide (NHS) and ethanolamine (EA) were purchased from General Electric Co. (Uppsala, Sweden). CAP and GEN (1.0 mg/mL each) were prepared using ultrapure water and stored at 4 °C. The CAP–BSA conjugate (CAP–BSA), GEN–BSA conjugate (GEN–BSA), and the antibodies against CAP and GEN (AbCAP, AbGEN, respectively) were obtained from Huaan Magnech Bio-Tech Co. (Beijing, China). Ultrapure water was obtained from a Millipore Direct-Q ultrapure water system (Boston, MA, USA). Milk samples purchased from a local market and they were processed by centrifugation (Sigma, 3–30 K) using ultrafine filters (Sartorius, Germany).

2.2. Modification of sensor chips

CM5 sensor chips obtained from GE Healthcare (Uppsala, Sweden) contained carboxymethylated dextran matrices on gold surfaces. The EDC/NHS method was employed to immobilise the target conjugates on the chips for further application. Briefly, 0.4 mol/L EDC and 0.1 mol/L NHS were mixed to reach a final volume of 170 μL in a 1:1 ratio, and the mixture was injected at a flow rate of 30 μL/min into channel 2 for activating the carboxyl groups of the sensor chip. Next, CAP–BSA and GEN–BSA (2.0×10^4 ng/mL each) were successively injected at a rate of 5 μL/min for approximately 300 s for immobilisation. Finally, 126 μL of an EA solution (pH 8.5) at a rate of 10 μL/min was used to deactivate the unreacted sites.

2.3. Screening of regeneration conditions

A majority of the typically used elution solutions were screened in detail during regeneration. Gly-HCl at different pH values (1.2, 1.5, 2.0, 2.5), NaOH at different concentrations (15, 25, 30, 35, 50 mM), 0.1 M HCl and 5 M $MgCl_2$ were tested for the regeneration of the sensor chips. Briefly, 1.0×10^4 ng/mL AbCAP was injected at a rate of 10 μL/min for 60 s to bind with CAP–BSA on the sensor chip, followed by successive regeneration using the aforementioned elution solutions for approximately 30 s. The similar processes were performed to optimize the regeneration conditions when AbGEN bound to the sensor chip.

2.4. Assay procedure

SPR experiments were performed on a Biacore X100 system, a commercial SPR device from Biacore (GE Healthcare). The temperature of the Biacore X100 system was maintained at 25 °C during the experiment. This system contains two channels, a test channel and a control channel, respectively. The SPR biosensor combined with the immune inhibition format has typically been utilised for the detection of small molecules (Lu et al., 2014). By this method, CAP–BSA and GEN–BSA were immobilised on a sensor chip, and separate detections were first performed. The AbCAP was mixed with different concentrations of CAP standard. In the mixtures, the final concentration of AbCAP was fixed to 1.0×10^4 ng/mL, while the final concentrations of CAP standards were varied (0, 5, 10, 20, 30 ng/mL). 100 μL AbCAP/CAP mixtures were incubated for approximately 5 min and then injected so that the free AbCAP in the mixtures bound to the CAP–BSA on the sensor chip. Before the next cycle of detection, the sensor chip was regenerated with Gly-HCl (pH 1.2) for approximately 30 s to elute AbCAP and rinsed with HBS buffer. Subsequently, the results were collected and fitted to a standard curve of CAP. Similarly, a standard curve was obtained for GEN. The final concentration of AbGEN was adjusted to 1.0×10^3 ng/mL after mixing with GEN standards with the final concentrations of 0, 1.0, 2.5, 5.0, 7.5, 10 ng/mL. Furthermore, NaOH solution (25 mM) was used to elute AbGEN after the detection. For ensuring the validity of test results, each cycle was performed in triplicate.

During the sequential detection of CAP and GEN, regeneration was performed in two steps, in contrast to the separate detection. Fig. 1 shows the schematic for the simultaneous detection of CAP and GEN. First, the weakly binding target was eluted using one regeneration solution. Then the response shift was subsequently calculated and correlated to the amount of the remaining antibody. The remaining antibody was eluted using the second regeneration solution. Based on the common immune inhibition format, the first step involved the incubation of the two antibodies and antigens. The AbCAP and AbGEN concentrations (similar to those utilised for the separate detection

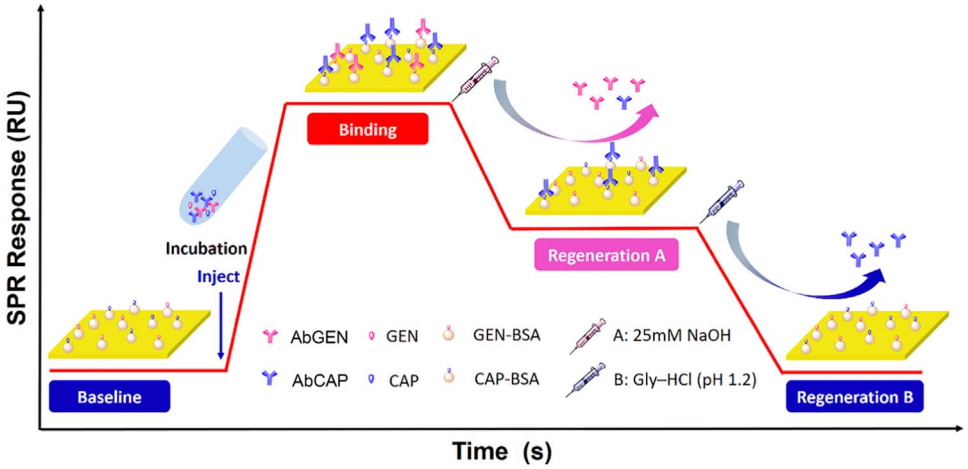

Fig. 1. Schematic for the simultaneous detection of chloramphenicol and gentamicin using SPR by gradual elution. The SPR response of the chip immobilized with CAP-BSA and GEN-BSA was set as the baseline at first. The mixtures of antibodies (AbCAP and AbGEN) and antigens (CAP and GEN) were injected after incubation. The free antibodies were binding on the surface with the response increase. Then the regeneration A (25 mM NaOH) were injected to elute the weak binding target (AbGEN), which may also carry little AbCAP away. The response value was declined after regeneration, but more than the first baseline, which means the remaining antibody (AbCAP) was still binding on the chip. Then the response shift could be used to obtain the CAP concentrations directly. Finally, the regeneration B (Gly-HCl pH 1.2) was used to remove the remaining antibody to bring the response to the baseline for the next detection.

mentioned above) were maintained constant, and mixed with different concentrations of CAP and GEN. Specially, the final concentrations of AbCAP and AbGEN were was fixed to 1.0×10^4 ng/mL and 1.0×10^3 ng/mL in the mixtures, while the final concentrations of CAP (0, 5, 10, 20, 30 ng/mL) and GEN (1.0, 2.5, 5.0, 7.5, 10 ng/mL) were as the same as separate detections. Each mixture was incubated for approximately 5 min. Subsequently, the mixtures were injected at a rate of 10 μL/min for 60 s. And then different solvents were used to elute AbCAP and AbGEN, i.e. 25 mM NaOH was used to regenerate AbGEN for 30 s firstly, and then Gly-HCl (pH 1.2) was used to remove AbCAP for the next detection.

2.5. Spiking tests of milk

For the spiking tests, 5 mL of a milk sample was spiked with CAP and GEN. Then, HBS buffer was added in the milk sample to reach the final volume of 10 mL for ultrafiltration–centrifugation. The ultrafiltrate was collected and diluted 10 times with HBS buffer for detection. In the spiking samples, the final concentrations of CAP and GEN were 10, 20, 30 ng/mL and 2.5, 5.0, 7.5 ng/mL. All the tests were performed at least three times for the precise results.

3. Results and discussion

3.1. Immobilisation of BSA on the chip surface

For ensuring successful immobilisation on the sensor chip, acetate buffer (pH 4.5) was used to dilute the solutions of CAP–BSA and GEN–BSA. At pH 4.5, the chip surface coated with carboxymethylated dextran (pK_a 3.5) exhibited negative charges, while BSA with the pI value of 4.7 carried positive charges. Due to electrostatic interactions, BSA can be well combined with the chip surface. Furthermore, the EDC/NHS method was utilised to activate the carboxyl groups on the surface for covalent, stable binding with BSA.

3.2. Regeneration

In this work, regeneration is a crucial process, not only for reusing chips, but also for simultaneous detection. During the regeneration, analytes were dissociated from the functional surface of chips. If the analytes are well dissociated from undestroyed functional surface of chips during the regeneration, the latter detection of the samples with the same analytes can be fulfilled according to the response shift (Drake and Klakamp, 2011). Therefore, the optimisation of regeneration conditions is elaborately executed herein to enhance the stability of the sensor response and increase the repeatability of sensor chips (Andersson et al., 1999).

Fig. 2A shows the comparison of the baseline shift and sample response for the elution of AbGEN using four regeneration solutions including 0.1 M HCl, Gly-HCl (pH 1.2), 25 mM NaOH and 5 M $MgCl_2$. Using 25 mM NaOH, the baseline and sample response were stable without significant fluctuations. In contrast, the baseline gradually increased after two reuse cycles using 5 M $MgCl_2$, indicating that AbGEN was not completely eluted from the chip. Moreover, using 0.1 M HCl and Gly-HCl (pH 1.2), marginal difference was observed between the response and baseline, also demonstrating that some AbGEN is still bound to the chip. Hence, NaOH solution was confirmed to be suitable for the elution of AbGEN.

For the optimised regeneration, NaOH solutions at the concentrations of 20, 25, 30 and 35 mM were further compared to elute AbGEN. As observed in Fig. S1 (Supporting information), 25 mM NaOH was more stable as compared to other concentrations, with little change observed in the baseline and sample response. Therefore, 25 mM NaOH was the optimum concentration for the regeneration of AbGEN. Furthermore, different concentrations of AbGEN were injected and then eluted by 25 mM NaOH for testing the regeneration performance. Fig. 2C shows good regeneration obtained using 25 mM NaOH, as confirmed by the return of the baseline to the origin value with high repeatability.

Similar to the regeneration of AbGEN described above, the regeneration of AbCAP was performed using four elution solutions including 0.1 M HCl, pH 1.2 Gly-HCl, 50 mM NaOH and 5 M $MgCl_2$. As shown in Fig. 2B, using Gly-HCl, the baseline and sample response were stable. Using 0.1 M HCl, the sample response was stable after two cycles, but the baseline significantly increased, indicating that HCl is not the most suitable regeneration solution for completely removing AbCAP. In contrast, using the $MgCl_2$ and NaOH solutions, it was both difficult to regenerate AbCAP: the baselines increased above 200 RU, whereas sample responses decreased. Hence, the Gly-HCl solution was selected for regeneration of AbCAP. In addition, the Gly-HCl solution at different pH values (1.2, 1.5, 2.0, 2.5) was further optimised (Fig. S2), and the regeneration deteriorated with the increasing pH value. As shown in Fig. 2D, using Gly-HCl at a pH of 1.2, highly stable regeneration of AbCAP was observed; these versatile results were

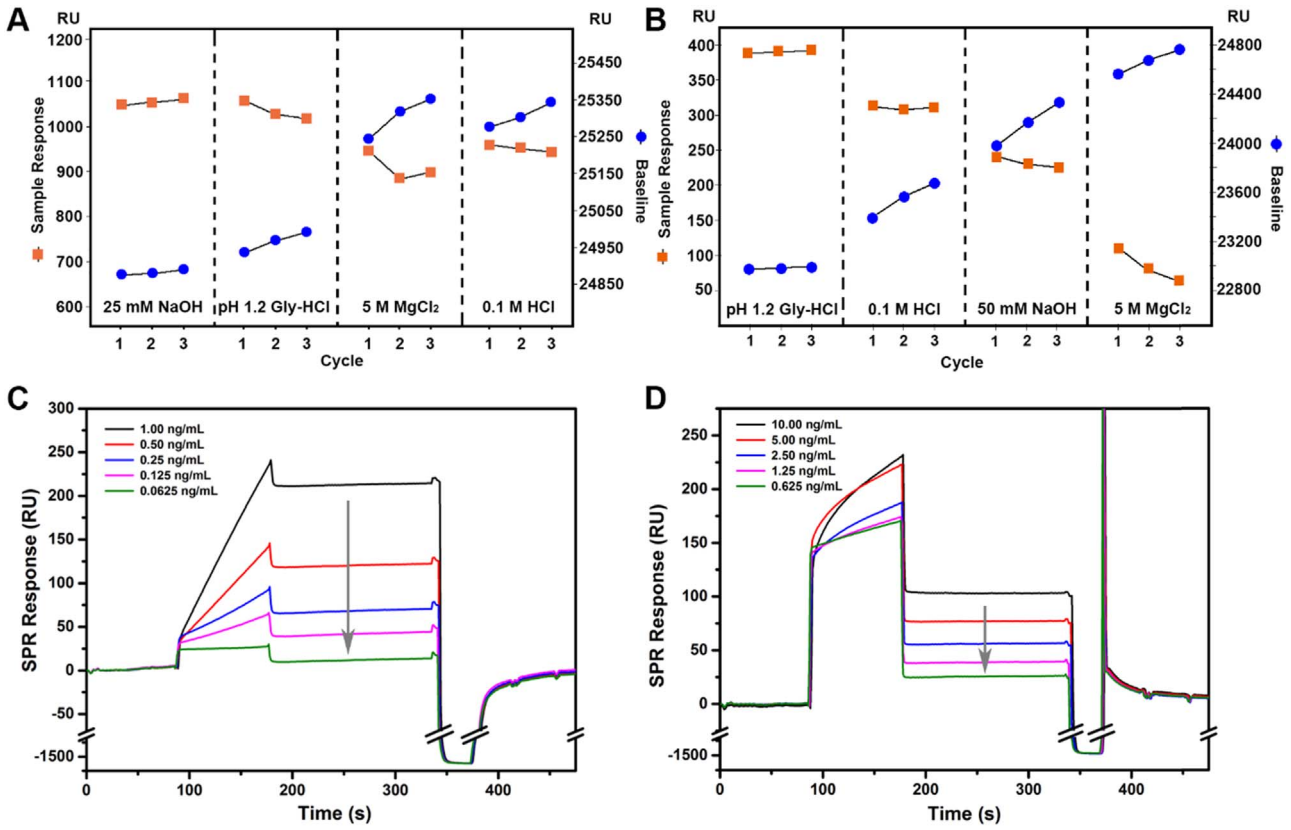

Fig. 2. Comparison of the baseline shift and sample response using the four regeneration solutions for AbGEN (A) and AbCAP (B). The square (orange) and the circle (blue) represent the sample response and baseline, respectively. Each regeneration solution was tested for three cycles and separated with imaginary line, which was used to compare the stability after regenerating with these solutions. Regeneration for eluting different concentrations of AbGEN using 25 mM NaOH (C) and AbCAP using pH 1.2 Gly-HCl (D). The gray arrows in (C) and (D) indicate the trend of response shift with the decreasing AbGEN and AbCAP concentration, respectively. The baseline was also back after regeneration.

attributed to the binding characteristics of different antibodies and antigens (Karlsson and Fält, 1997).

3.3. Application of SPR sensors for simultaneous detection

SPR sensors demonstrate an outstanding ability to determine molecular interactions with high sensitivity (Liu et al., 2014). However, it is difficult to detect small molecules, caused by the weak signals obtained as a result of the change in their mass on the chip surface (Zeng et al., 2014). Furthermore, in an immune competitive format, antibodies should be immobilized on the chip, which need to consider issues of the correct Ab orientation (Stobiecka and Chalupa, 2016). Hence, for a simple detection of small molecules (e.g., CAP and GEN), an immune inhibition format is applied in this study.

First, CAP and GEN were separately detected. To ensure the effective binding between the antibody and antigen, an adequate amount of a AbCAP solution with a fixed concentration was incubated with a CAP sample with different concentrations for 5 min, followed by the injection of the AbCAP/CAP mixtures for detection. Fig. 3A shows the results obtained from the sample response shifts. With the increasing CAP concentrations, the sample response shifts decreased. The response shift depended on the amount of AbCAP bound to CAP–BSA on the chip surface. Since the AbCAP concentration was constant in the mixture, the free AbCAP was reciprocally related to the CAP concentrations. As shown in Fig. 3C, an S-logistic curve was fitted to the response shift dependence on CAP concentration. Furthermore, a similar process was performed for the detection of GEN. As shown in Fig. 3B, with the increasing concentrations of GEN in the mixture, SPR response shifts also decreased, and a similar calibration curve was obtained (Fig. 3D).

In the process of simultaneous detection, CAP and GEN were mixed together. Different regeneration conditions were used to elute AbCAP and AbGEN, caused by the difference of their bond strength on the chip surface. In other words, a strongly bound antibody was possibly retained on the sensor chip after weak regeneration, and the amount of the remaining antibody resulted in a response shift. The amount of such remaining antibody (strongly bound) on the chip was found to be directly correlated to that of the injected free antibody after incubation with the corresponding antigen. Thus, the response shift values were reciprocally related to the antigen concentrations. According to the regeneration results (Fig. 2), the NaOH solution was capable of easily breaking the bonds between AbGEN and GEN–BSA, but it was unable to completely dissociate AbCAP from the sensor chip. Hence, 25 mM NaOH is selected as the first elution solution. During regeneration A (Fig. 1), AbGEN was eluted by NaOH with the simultaneous dissociation of some amounts of AbCAP. The response shift observed after the first regeneration can be regarded as the amount of AbCAP remaining on the chip. Thus, the concentrations of CAP in the mixture (CAP/AbCAP) can be determined.

After the elution using NaOH solution, the response shift was inversely correlated to the concentration of CAP in the mixture (Fig. 4A). Next, using Gly-HCl as the second elution solution to remove the remaining antibody, the baseline returned back to the origin value. As shown in Fig. 4B, a binomial standard curve was obtained between the concentrations of CAP and the response shift due to the remaining AbCAP.

According to the curve equation in Fig. 4B, the CAP concentration in the test sample can be calculated based on the response shift during Gly-HCl elution (Regeneration B in Fig. 1). Using the separate curve of CAP (Fig. 3C), we can obtain the response shift resulted from CAP during the first binding step (Binding in Fig. 1). Then the response shift for GEN in the binding step was determined by subtracting the

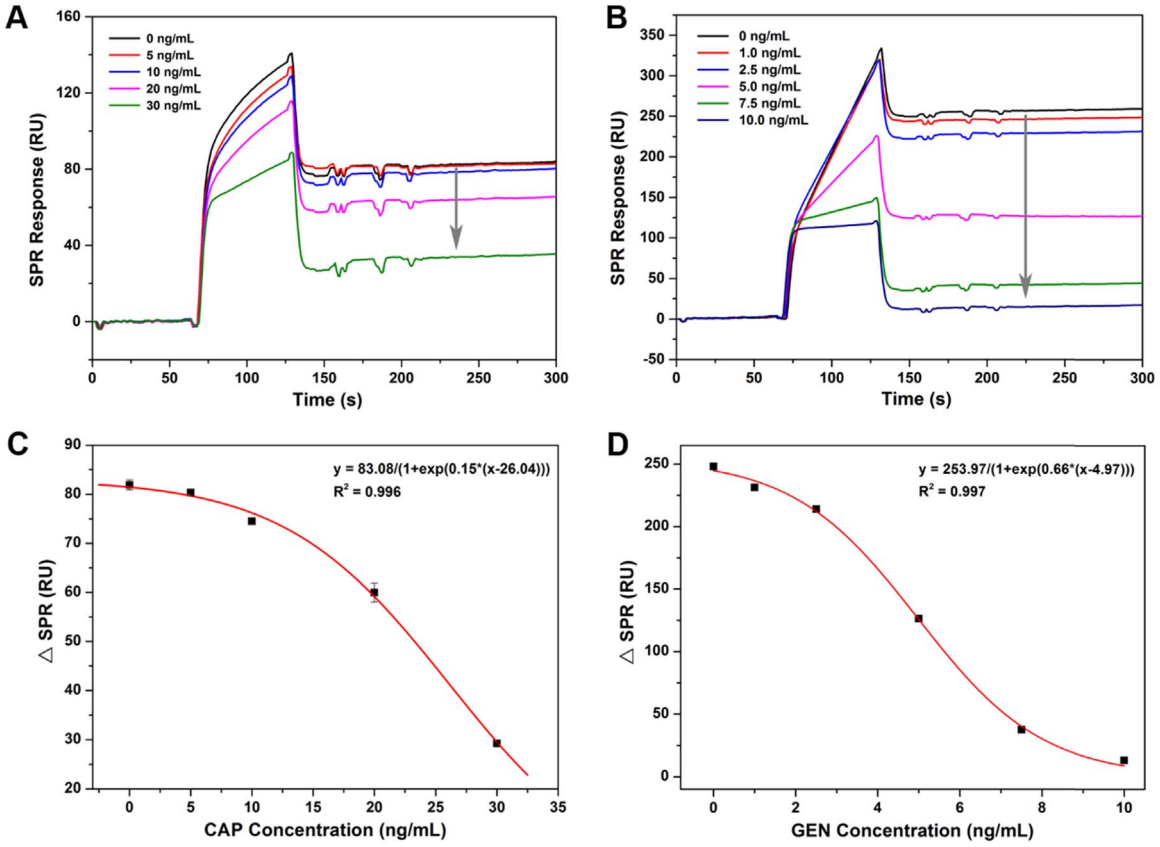

Fig. 3. (A) Results obtained from response shifts corresponding to the mixing of AbCAP with different CAP concentrations. (B) Results obtained from response shift corresponding to the mixing of AbGEN with different GEN concentrations. Standard curves of SPR for CAP (C) based on the results of response shift of CAP concentrations and for GEN (D) based on the results of response shift of GEN concentrations. The gray arrows in (A) and (B) indicate the trend of response shift with the increasing CAP or GEN concentrations, respectively.

calculated response shift for CAP from the total response shift (CAP/GEN, in Fig. 4A). Therefore, the GEN concentration can be calculated by the curve equation in Fig. 3D. Based on these three curve equations, the CAP and GEN concentrations were simultaneously determined with the limit of detection (LOD) values of 5.28 ng/mL and 2.26 ng/mL, respectively. For confirming the reliability of the detection results, different concentrations of GEN and CAP were mixed with AbCAP and AbGEN, and then the mixtures were evaluated. As shown in Table S1, the detection results were in agreement with the actual values. Some errors existed, which may be attributed to the following reasons. Firstly, the instrument error can lead to fluctuation of responses during sample injection, as well as manual operation. Secondly, the calculation process can decrease the accuracy. Finally, three standard curves were applied, and a few errors were observed during the fitting of curves. Although the results are slightly affected by errors in one curve, the errors can be amplified with the successive calculation of three curve equations. Thus, the measurement accuracy of GEN concentration (based on three curves) was less than that of CAP concentration (based on one curve). On the other hand, the deviation observed for CAP concentration was greater than that observed for GEN concentration (Table S1), attributed to the lower response shift and characteristics of the curve. In other words, a few changes in the ΔSPR (Fig. 4B) possibly

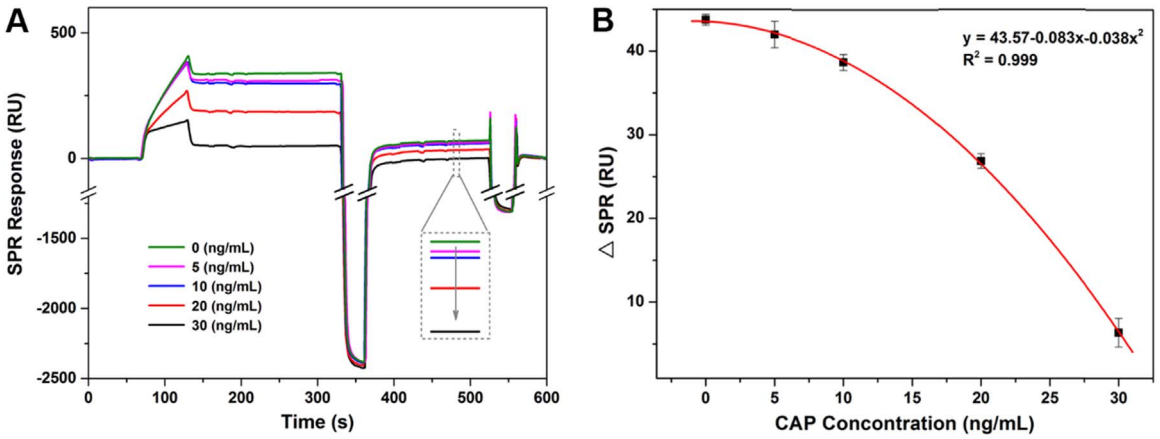

Fig. 4. (A) Results obtained from real-time monitoring of response shifts observed with the addition of different concentrations of CAP into the AbCAP/AbGEN mixture. The process contained two regenerations, 25 mM NaOH and pH 1.2 Gly-HCl, successively. The dotted area shows the amplification of a part of curve after the first regeneration. The gray arrow indicates the trend of response shift with the increasing CAP concentrations. (B) Standard curve of the shifts normalised with different CAP concentrations. The ΔSPR values in the curve were correspond with five SPR responses in the Fig. 4A.

affected the CAP concentration. However, the same changes in ΔSPR (Fig. 3D) resulted in no significant fluctuation for GEN concentration because GEN had a higher response shift in the curve. Thus, the GEN concentration exhibits better precision. To decrease these errors, the results were repeatedly tested to obtain the average values.

Compared with separate detection, simultaneous detection exhibits advantages of time savings and detection efficiency. Conventionally, a sensor chip can only be used to detect one target in biosensors (single test channel). If there are more than one targets, multiple chips should be applied. The process of switching chips and priming the channel surfaces are quite time-consuming. In this study, two targets in the samples were simultaneously injected and detected using one chip by performing two regeneration steps (Regeneration A and Regeneration B in Fig. 1). Besides time savings, the sensor chips can be efficiently used for multi-detection, contributing to a certain degree of cost reduction.

3.4. Application of the SPR immunosensor for the detection of CAP and GEN in milk

A recovery study was performed to detect CAP and GEN in milk using this simultaneous detection method. The complex matrix in the milk should always be considered, which may not only disturb the interaction between the antibody and antigen but also increase the probability of nonspecific adsorption (Ye et al., 2016). Hence, ultrafiltration–centrifugation and dilution were employed to reduce the interference from the protein and fat before injection (Lu et al., 2012). As shown in Fig. 5, the matrix curves overlapped with the HBS standard curves, indicating that no significant effect of the sample matrix on the detection.

The milk was then spiked with different concentrations of CAP and GEN. Sample treatment was conducted according to the above-mentioned steps, and the results are listed in Table 1. The recoveries of CAP and GEN were 93.0–101.1% and 77.6–97.1%, respectively. Although the recoveries were affected by the matrix to some degree, the method was found to be suitable for the simultaneous detection of CAP and GEN in milk.

Table 1
Recovery studies for milk with three concentrations of CAP and GEN by the multi-detection method.

Target sample	Spiked concentration (ng/mL)	Average ± SD (ng/mL)	Recovery (%)
CAP	10	9.30 ± 1.27	93.0
	20	18.80 ± 0.76	94.0
	30	30.33 ± 0.52	101.1
GEN	2.5	1.94 ± 0.08	77.6
	5.0	4.66 ± 0.06	93.2
	7.5	7.28 ± 0.04	97.1

4. Conclusions

In summary, an innovative method was developed using SPR biosensor for the simultaneous detection of CAP and GEN. Under optimum conditions, this assay exhibited high efficiency and sensitivity. Two samples were detected in a process without switching sensor chips, which demonstrates a high-efficiency usage of chips and cost reduction to some extent. The LOD values of CAP and GEN were 5.28 ng/mL and 2.26 ng/mL, respectively. For verifying the feasibility for milk, the recovery studies of CAP and GEN were implemented with

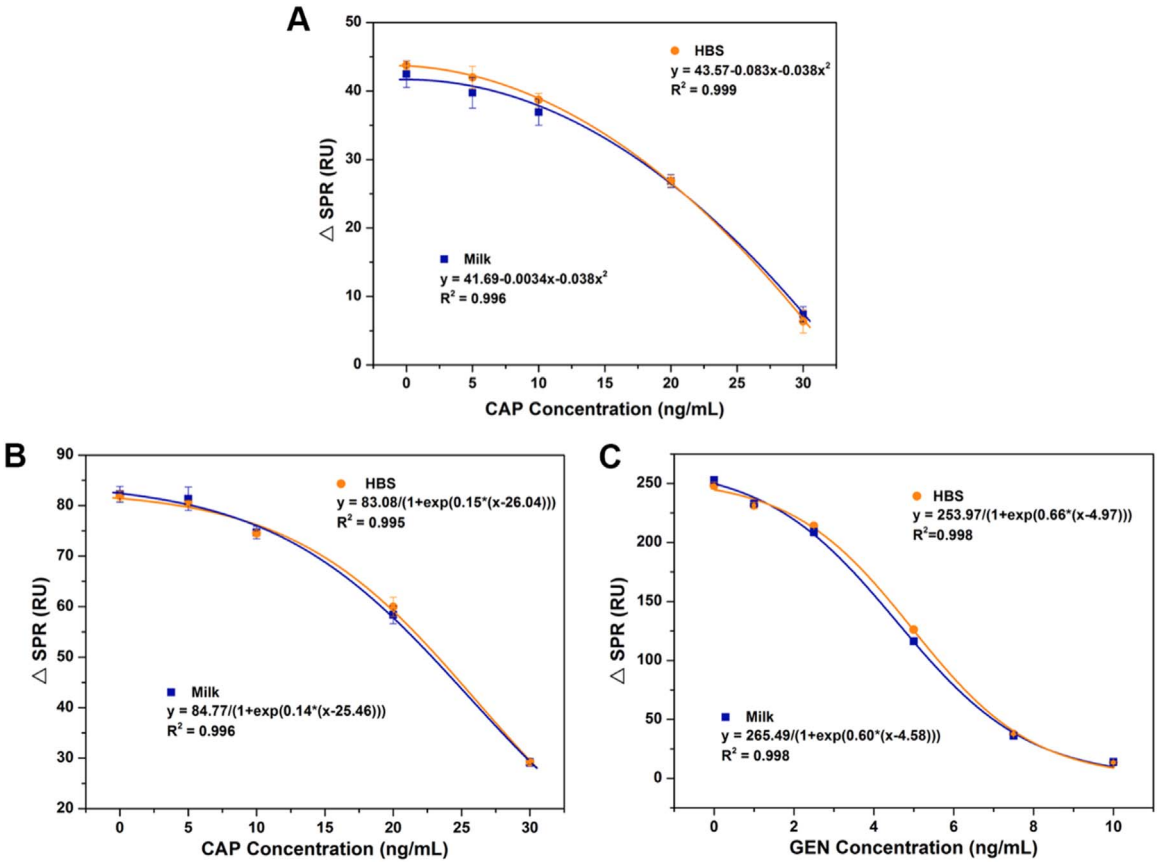

Fig. 5. Standard curves for the detection of CAP and GEN in milk compared to those in HBS. The square (blue) curve and the circle (orange) represented detections in milk and HBS, respectively. (A) The standard curves were obtained in the process of simultaneous detection after two regenerations. (B) The standard curves were obtained in the separate detection of CAP. (C) The standard curves were obtained in the separate detection of GEN.

satisfactory results (77.6–101.1% recoveries). All the results obtained demonstrate good applications for multi-detection on one chip. Moreover, this method can also be utilised in the instrument with multiple channels, which may enhance the ability for simultaneous detection of several samples. Hence, the SPR assay proposed herein demonstrates potential and significance for rapid multi-detection applications.

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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.2017.02.022.

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