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Original article

Development of a surface plasmon resonance biosensor for accurate and sensitive quantitation of small molecules in blood samples

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ABSTRACT

Therapeutic drug monitoring (TDM) has played an important role in clinical medicine for precise dosing. Currently, chromatographic technology and immunoassay detection are widely used in TDM and have met most of the needs of clinical drug therapy. However, some problems still exist in practical applications, such as complicated operation and the influence of endogenous substances. Surface plasmon resonance (SPR) has been applied to detect the concentrations of small molecules, including pesticide residues in crops and antibiotics in milk, which indicates its potential for in vivo drug detection. In this study, a new SPR-based biosensor for detecting chloramphenicol (CAP) in blood samples was developed and validated using methodological verification, including precision, accuracy, matrix effect, and extraction recovery rate, and compared with the classic ultra-performance liquid chromatography-ultraviolet (UPLC-UV) method. The detection range of SPR was 0.1–50 ng/mL and the limit of detection was 0.099 ± 0.023 ng/mL, which was lower than that of UPLC-UV. The intra-day and inter-day accuracies of SPR were 98%–114% and 110%–122%, which met the analysis requirement. The results show that the SPR biosensor is identical to UPLC-UV in the detection of CAP in rat blood samples; moreover, the SPR biosensor has better sensitivity. Therefore, the present study shows that SPR technology can be used for the detection of small molecules in the blood samples and has the potential to become a method for therapeutic drug monitoring.

1. Introduction

With the development of personalized medicine, clinicians require therapeutic drug monitoring (TDM) to maintain dosage within an effective range [1]. TDM is mainly used for drugs with narrow therapeutic windows or concentration-dependent adverse drug reactions and is mostly used in immunosuppressive agents, antiepileptics, antibiotics, and antipsychotics [2]. Currently, there are two main detection methods for therapeutic drug monitoring: traditional chromatography and emerging immunoassays. Chromatography has been implemented in clinical studies for an earlier start to trials, and it is the most widely used method in clinical practice; however, its clear disadvantages include low throughput, complicated operation, expensive instrument cost, and time-consuming optimization requirements. The immunoassay has the advantages of simple operation, high affinity, and a short detection cycle, but the measurement results may be affected by metabolites and endogenous substances in the sample [3]. These two methods cover most of the drugs in the TDM demand. However, there is still a lack of convenient methods to perform TDM for more drugs to provide personalized medication data for precision medicine. Based on results from surveys on the situation of TDM in hospitals and laboratories [4–6], the actual effects of TDM are not satisfactory in most hospitals and have been affected by the high cost of instruments, long turnaround time, and incorrect sample collection and pretreatment, which suggests that the existing methods are not sufficiently friendly for routine clinical tests. In addition, considering convenience, cost, and demand, there is little

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implementation for the monitoring of relatively safe drugs, which have wide target ranges and minor adverse drug reactions. However, TDM of these drugs can reduce the occurrence of drug resistance and improve the effectiveness of drug treatments [7]. Thus, it is important to develop a new TDM technology that provides rapid results and is accurate, easy to use, and inexpensive.

Surface plasmon resonance (SPR) is a biosensor based on physical optics technology used to detect the interaction between two molecules. It has the characteristics of label-free detection, real-time dynamic monitoring, high specificity, and high sensitivity [8]. It can provide information such as kinetic parameters and affinity constants during the interaction of biomolecules and is suitable for detecting almost all substances that can produce molecular interactions, including proteins, peptides, DNA, and small molecule compounds. SPR is widely used in food analysis, clinical diagnosis, environmental monitoring, pathogenic microorganism detection, and new drug research and development [9–11].

Owing to its high selectivity and sensitivity, SPR has received attention for application in small-molecule concentration detection in recent years. Guo et al. [12] proved that a direct SPR biosensor with an anti-triazophos monoclonal antibody could be used for the trace detection of triazophos. The SPR biosensor showed high specificity and a low detection limit for triazophos and could be reused. Srivastava et al. [13] developed an SPR sensor chip with a prism-based Kretschmann configuration for the detection of glucose and improved its sensitivity and stability compared to previous reports. Bereli et al. [14] prepared SPR and quartz crystal microbalance sensors using molecular imprinting technology to detect amoxicillin in commercial and local eggs. The sensor showed high selectivity for amoxicillin and was highly precise and reusable. In addition, the detection results were verified using liquid chromatography-tandem mass spectrometry. Çimen et al. [15] developed an SPR biosensor based on a molecular-imprinted polymeric film for the detection of L-phenylalanine, which proved that the L-phenylalanine-imprinted SPR sensor had good recognition ability for L-phenylalanine, and the method was faster, more convenient, and more reproducible and sensitive.

It has been shown that SPR is feasible for detecting the concentration of small molecule compounds. However, few quantitative studies have systematically used SPR biosensors for the detection of drug concentrations in blood. The existing small molecule concentration detection method is mainly used for samples with relatively simple components, such as food and water, whereas the components of in vivo samples, such as blood and urine, are complex. Endogenous substances may combine with drugs or antibodies, which affects the detection process and increases the difficulty of quantification. The existing detection method based on SPR sensors is mainly qualitative or semi-quantitative. Whether it can be used for accurate quantification of complex biological samples still requires systematic methodological research. This study aimed to develop an SPR biosensor that utilizes chloramphenicol (CAP) antibodies as recognition molecules immobilized on a CM5 chip to detect CAP in rat blood samples (Fig. 1). The feasibility of SPR quantitation was systematically evaluated in vitro and in vivo in terms of precision, accuracy, matrix effect, and extraction recovery rate, and was then used in the practical application of rat blood after administration of CAP. The results showed that the performance of the SPR biosensor is identical to that of the classical ultra-performance liquid chromatography-ultraviolet (UPLC-UV) method in detecting small molecules in blood samples and that the sensitivity of the SPR biosensor is even higher. Therefore, this study provides credible evidence for the application of SPR biosensors to the quantitative analysis of biological samples and shows that SPR technology has great potential in therapeutic drug monitoring.

2. Materials and methods

2.1. Drugs and reagents

Standard compounds including CAP, ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR), azithromycin (AZM), cefuroxime (CXM), and cefoperazone (CFP) were purchased from the National Institute for Pharmaceutical and Biological Products of China (Beijing, China). The purity of all standard chemicals was >99.8%. The CAP antibody was purchased from GeneTex (Irvine, CA, USA). CAP succinate sodium was purchased from Efebio (Shanghai, China). High performance liquid chromatography-grade methanol was purchased from Merck KGaA (Darmstadt, Germany). CM5 chips, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride, *N*-hydroxysuccinimide (NHS), ethanolamine, phosphate-buffered saline (PBS), and HBS-EP buffer were provided by GE Healthcare (Chicago, IL, USA). Dimethyl sulfoxide (DMSO) with a purity of >99.5% was purchased from Sigma-Aldrich (St. Louis, MO, USA). Purified water was obtained using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. In vitro sample preparation

CAP, CIP, LEV, NOR, AZM, CXM, and CFP were first dissolved to 10 mmol/L in DMSO as stock solutions. Later in the study, they were

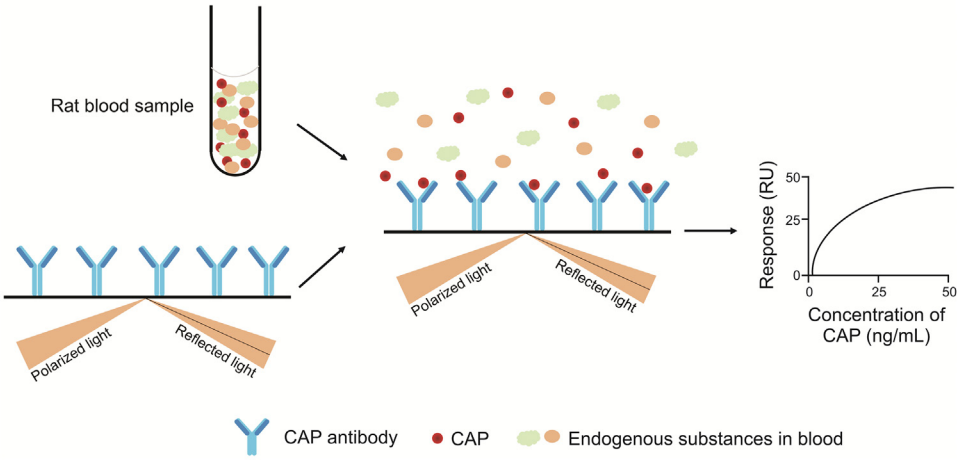

Fig. 1. Principle of the surface plasmon resonance (SPR) biosensor for the process of chloramphenicol (CAP) detection in rat blood samples.

diluted with PBS with 5% DMSO for SPR analysis and with methanol for UPLC-UV analysis.

2.3. Immobilization of CAP antibody on the SPR sensor

All SPR-based detections were performed on a Biacore T200 system (GE Healthcare, Chicago, IL, USA). The system temperature was 25 °C and the flow rate of the EP buffer was 30 μL/min. Flow cell (FC) 1 was set as the reference cell, and FC 2 as the detection cell. Suitable immobilization conditions were determined through the physical absorption progress using four 10 mM sodium acetate buffers (pH 4.0, 4.5, 5.0, and 5.5) to dilute the CAP antibody to two different concentrations (50 and 100 μg/mL). The antibody was then immobilized on the detection cell using an amino coupling reaction.

2.4. Activity of the SPR biosensor

The 10 mmol/L CAP sample was diluted in a 100 ng/mL PBS solution containing 5% DMSO, and then 5% DMSO PBS was used to sequentially dilute the solution to 50, 20, 10, 5, 2, 1, 0.5, 0.2, and 0.1 ng/mL for injection. All samples in the SPR analysis were injected over the FC 1 and 2 on the sensor surface for 120 s at 30 μL/min and dissociated for 300 s.

2.5. Specificity of SPR analysis

Stock solutions of CAP, CIP, LEV, NOR, AZM, CXM, and CFP were diluted in 256 nmol/L of PBS solution containing 5% DMSO and injected over the FC 1 and 2 on the sensor surface for 120 s at 30 μL/min and dissociated for 300 s in the proper order, with CAP processed last.

2.6. Stability of SPR analysis

Starting from the day after the CAP antibody was coupled to the chip, 10 ng/mL of CAP samples were tested for 10 consecutive days, and the stability of the chip was obtained by observing the change in the response value of the sample at this concentration.

2.7. Detection range and limit of detection (LOD) of SPR analysis

The CAP stock solution was diluted into a series of 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, and 0.1 ng/mL with PBS solution containing 5% DMSO for injection. All samples in the SPR analysis were injected over the FC 1 and 2 on the sensor surface for 120 s at 30 μL/min and dissociated for 300 s.

Three curves were randomly selected from the results of three experiments and a smooth straight line was intercepted to determine the noise value of the instrument. The LOD was the corresponding concentration when the response was three times greater than the noise value.

2.8. Intra-day and inter-day precision and accuracy of SPR analysis

Intra-day precision and accuracy were evaluated using three repetitive tests of samples at three different concentration levels (0.3, 3, and 30 ng/mL). Inter-day precision and accuracy were defined using data from three analytical runs performed on three consecutive days. Precision was defined as the variance between replicate samples and expressed as the coefficient of variation (CV %). When evaluating the intra- and inter-day precision, the CV should not exceed 15%. The accuracy was calculated to express the difference between the measured and nominal concentrations of the samples. To estimate intra- and inter-day accuracy, the concentration of samples should be within 85%–115% of the nominal concentrations.

2.9. Experimental conditions of UPLC-UV analysis

Gradient separation chromatography was performed on an Agilent 1290 Infinity UPLC system (Agilent Technologies, Waldbronn, Germany) using an ACQUITY UPLCTM BEH C_{18} column (2.1 mm × 100 mm, 2.5 μm; Waters, Milford, MA, USA) at 40 °C with a 0.4 mL/min mobile phase consisting of a gradient mixture of 0.1% (*V*/*V*) aqueous formic acid (A) and acetonitrile 0.1% (*V*/*V*) aqueous formic acid (B) ranging during elution 5% B over 0–2 min, 5%–80% B over 2–6 min, and kept 80% B for 2 min. The injection volume was 2 μL, and the UV wavelength was set at 277 nm.

2.10. Standard linearity, intra-day and inter-day precision, and accuracy of UPLC-UV analysis

The 10 mmol/L CAP sample was diluted with methanol into three sets of solutions with concentration gradients of 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, and 0.05 μg/mL for injection. Each sample was injected three times. The peak area of the analyte was fitted to the concentration of each standard analyte by using a linear regression equation. The intra- and inter-day precision and accuracy were the same as those of SPR, but the sample concentrations were 0.2, 1, and 5 μg/mL.

2.11. Animal experiment

Five male SD rats with a body weight of 225 ± 14 g were purchased from regular animal suppliers to our department. The rats were randomly divided and placed into plastic cages with filter bonnets and sawdust bedding for 2 days after arrival. Five rats were housed per cage with food and water provided ad libitum. The animal room was maintained at a temperature of 20–25 °C, 50%–70% humidity, and a 12-h light/dark cycle. Before dosing, rats were fasted overnight for 12 h with free access to water. Animal care and experimental procedures were approved by the Laboratory Animal Experimental Committee in Naval Medical University.

2.12. Preparation of in vivo samples

Rats were injected 30 mg/mL CAP succinate sodium PBS solution through the tail vein at a dose of 180 mg/kg. Before administration and at 0.75, 1, 2, and 4 h after administration, 0.4 mL of blood was collected from the retro-orbital venous plexus at each point and allowed to stand at 25 °C for 2 h. Then, centrifugation was conducted at 3,000 r/min for 10 min and serum was collected. The serum was centrifuged again at 12,000 r/min for 10 min at 4 °C. The supernatant was promptly frozen at −80 °C until analysis.

2.13. Serum sample pretreatment

Protein precipitation with methanol was used to prepare all in vivo samples. A 150 μL aliquot of serum was mixed with 450 μL of methanol, which was set at −80 °C for 30 min. After the vortex for 3 min, the samples were centrifuged at 13,000 r/min for 5 min at 4 °C, and the supernatant (containing 75% methanol) was separated for subsequent tests.

2.14. Effect of methanol on SPR analysis

Two sets of 5 and 20 ng/mL CAP samples with the same gradient concentrations of 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 1%, and 2% (*V*/*V*) methanol without serum were configured, and the other two groups of 5 and 20 ng/mL of CAP samples containing 0.025% (*V*/*V*) of

serum and the same gradient concentrations of 0.075%, 0.125%, 0.25%, 0.5%, 1%, and 2% (*V*/*V*) methanol were used for injection. The effect of methanol on SPR was determined using linear regression analysis of the response and methanol concentration.

2.15. Matrix effect and extraction recovery rate of in vivo samples

Low, medium, and high concentrations (0.2, 1, and 5 μg/mL) of the UPLC samples were prepared first, and the low-, medium-, and high-concentration samples (0.3, 3, 30 ng/mL) of SPR were diluted from those UPLC samples. Among both sets of samples, group A was a standard solution without serum, group B was a blank serum sample with a standard solution added before protein precipitation, and group C was a blank serum sample with a standard solution added after protein precipitation. The measured chromatographic peak areas of the samples of group A at low, medium, and high concentrations were A1 and the SPR response value was A2; similarly, the results of group B were B1 and B2, and the results of group C were C1 and C2. The matrix effects of UPLC and SPR were the ratio of C1 to A1 and C2 to A2, respectively, and the extraction recovery rate was the ratio of B1 to A1 and B2 to A2, respectively.

2.16. Determination of CAP concentrations in blood

Rat-medicated serum samples were directly injected into the UPLC system after protein precipitation. For SPR injection, the samples were diluted 1000 times with SPR running buffer to reduce the concentration of methanol to 0.075%. The differences between the results of the two analysis methods were compared.

3. Results and discussion

3.1. Characterization of the surface plasmon resonance biosensor

The CAP antibody was diluted to 100 μg/mL with sodium acetate buffer (pH 5.0) and immobilized on the FC 2 of a CM5 sensor chip. The immobilization level was 15977.7 resonance unit (RU), which met the requirements for accurate determination. The activity of the chip was first verified by injection of the serial concentration (0.2–50 ng/mL) of the positive drug CAP. As shown in Figs. 2A and B, CAP bound to the sensor surface in a concentration-dependent manner, and the equilibrium dissociation constant (K_D) was determined as 18.14 nM.

To ensure that other small molecules do not affect the detection of CAP, the specificity of the sensor chip was evaluated using several compounds. CAP and six other antibacterial drugs (256 nM each) were injected into the SPR system and the response values were recorded (Fig. 2C). The response value of CAP was higher than that of the other compounds, indicating that the biosensor had a high affinity for CAP, whereas the responses of the biosensor to the other six antibacterial drugs were negligible. These results show that the CAP antibody on the sensor surface had good activity and specificity for CAP, and the SPR system could be used to detect CAP in our further studies.

3.2. Stability

To confirm whether the chip could maintain a stable state within a certain period, a standard CAP sample (10 ng/mL) was monitored daily for 10 consecutive days under normal conditions. As shown in Fig. 2D, the response values of the standard sample were nearly the same over 10 days (27.31 ± 0.56 RU). This result shows that the chip remained stable for at least 10 days. Therefore, the credibility of the experiment could be ensured within 10 days after the sensor chip was prepared, and all SPR assays in this study were performed during this period to obtain reliable results.

3.3. Detection range and LOD of SPR and UPLC-UV analyses

The UPLC-UV technique is extensively used in medical research and is considered as a classic method for TDM in clinical practice

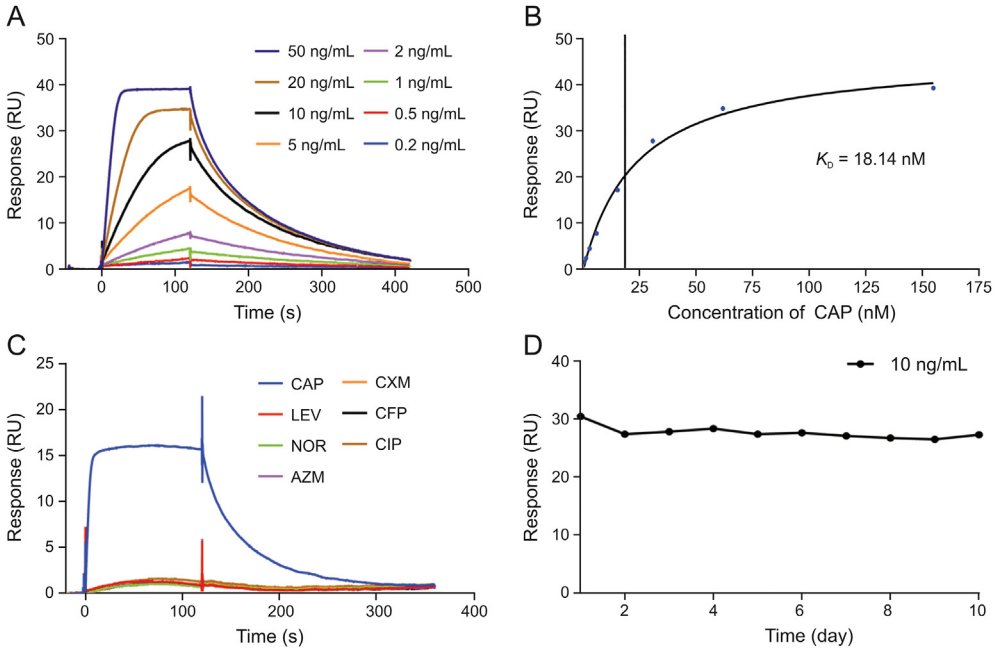

Fig. 2. Characterization of the surface plasmon resonance (SPR) sensor chip. (A) Sensorgrams of chloramphenicol (CAP) at different concentrations. (B) Fitting curves of CAP. (C) Response of CAP, ciprofloxacin (CIP), levofloxacin (LEV), norfloxacin (NOR), azithromycin (AZM), cefuroxime (CXM), and cefoperazone (CFP) with CAP antibody on CM5 chip. (D) Response of 10 ng/mL CAP sample for 10 consecutive days.

[3]. Therefore, the feasibility of SPR analysis in the area of drug quantitation was evaluated using the same analytical method validation index as the classic UPLC-UV analysis, including the detection range, LOD, precision, accuracy, matrix effect, and extraction recovery rate; the results of the two methods were compared.

For SPR analysis, the detection range was determined by calculating LOD and the saturation concentration of the chip. The LOD was found to be 0.099 ± 0.023 ng/mL by calculating instrument noise (3 × means of instrument noise), which also confirmed that the lower LOD was 0.1 ng/mL (Fig. 3A). Because the binding of CAP to the sensor surface reached saturation at 50–100 ng/mL (Fig. 3B), the upper LOD was determined as 50 ng/mL. Thus, the reliable detection range of CAP through SPR analysis was determined as 0.1–50 ng/mL. In previous SPR quantification studies, only the first several points with a good linear relationship were selected as the concentration detection range [16], but the findings of this study indicated that the detection range could be expanded to the whole concentration range before the sensor surface reached saturation, and the following methodological experiments were designed to prove this hypothesis.

Experiments showed that UPLC-UV analysis can achieve a good linear regression in the concentration range of 0.1–20 μg/mL (Figs. 3C and D). The correlation coefficient was 0.9997 and the regression equation was $y = 4.640x - 0.4381$, where x is the concentration of the CAP samples and y is the area of the CAP peak. LOD was found to be 1.56 ± 0.71 μg/mL by calculating instrument noise (3 × means of instrument noise).

From the above results, it can be concluded that the LOD and detection range of SPR were lower than those of UPLC-UV in the analysis of CAP, which was ng/mL grade compared to μg/mL grade. A lower detection range of SPR indicates better sensitivity and is more suitable for detecting low-concentration components of samples.

3.4. Intra- and inter-day precision and accuracy of SPR and UPLC-UV analyses

For SPR analysis, to prove the reliability of the expanded detection range, three concentrations of 0.3, 3.0, and 30.0 ng/mL, which represent low, medium, and high concentrations, respectively, according to the detection range of 0.1–50 ng/mL, were selected for precision and accuracy detection. The precision (shown as CV%) of 3.0 and 30.0 ng/mL samples was under 15% both intra- and inter-day, and the intra-day accuracy ranged from 98% to 114% (Table 1).

The precision and accuracy of UPLC-UV for CAP were also evaluated using low, medium, and high concentrations of CAP (0.2, 1.0, and 5.0 μg/mL, respectively), according to the UPLC-UV detection range. Table 1 shows that the precision of medium- and high-concentration samples, both intra- and inter-day, was less than 17%. The intra- and inter-day data indicate that the accuracy of medium- and high-concentration samples was in the range of 97%–104%.

Therefore, in terms of precision and accuracy, the SPR and UPLC-UV methods were not significantly different. Although the SPR response and CAP concentration showed a non-linear relationship in medium- and high-concentration samples, the precision and accuracy could meet the analysis requirements. Thus, the expanded detection range of SPR analysis is feasible, and this can render SPR detection promising for various samples with a wider concentration range.

3.5. Effect of methanol on SPR analysis

Since the in vitro experimental results have proven the feasibility of SPR in quantitation analysis, methodological studies were performed to demonstrate the feasibility of SPR analysis for blood

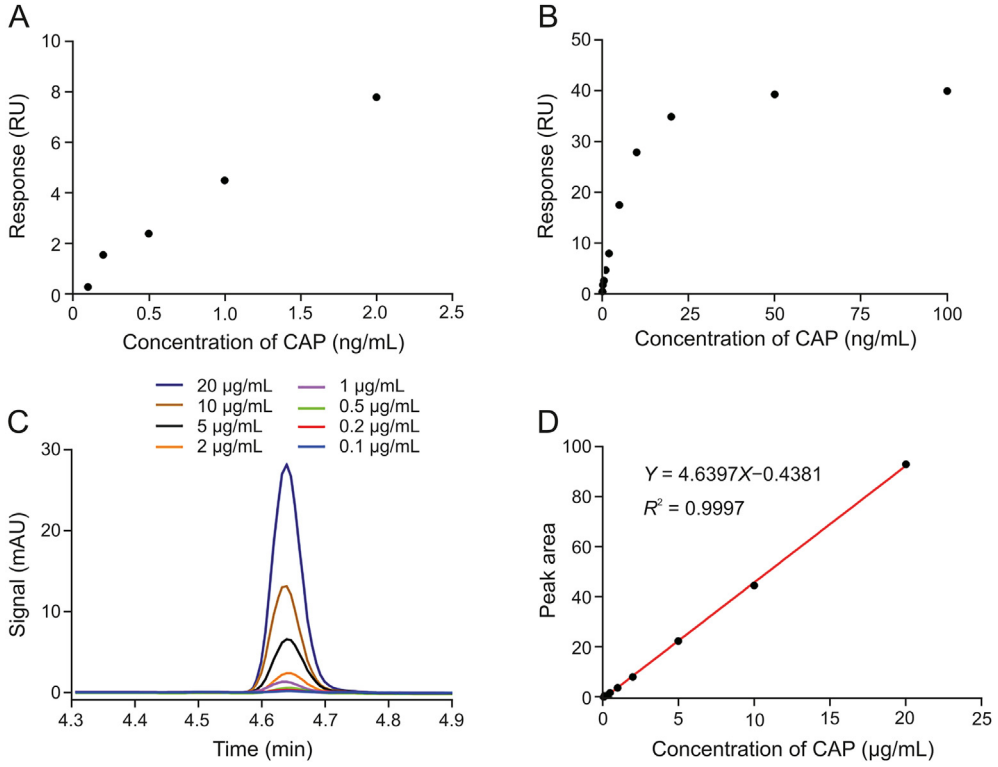

Fig. 3. Detection range of surface plasmon resonance (SPR) and ultra-performance liquid chromatography-ultraviolet (UPLC-UV) analyses. (A) Response of chloramphenicol (CAP) (0.1–2.0 ng/mL) in SPR analysis. (B) Response of CAP (0.1–100 ng/mL) in SPR analysis. (C) UPLC-UV spectrum of CAP (0.1–20 μg/mL). (D) Good linear regression of CAP (0.1–20 μg/mL) for UPLC analysis.

Table 1
Intra-day and inter-day precision and accuracy of surface plasmon resonance (SPR) and ultra-performance liquid chromatography-ultraviolet (UPLC-UV) analyses ($n = 3$).

Methods	Nominal concentration	Intra-day			Inter-day		
		Calculated concentration (mean ± SD)	Precision (CV, %)	Accuracy (%)	Calculated concentration (mean ± SD)	Precision (CV, %)	Accuracy (%)
SPR	0.3 ng/mL	0.29 ± 0.11 ng/mL	36	98	0.36 ± 0.13 ng/mL	35	118
	3.0 ng/mL	3.19 ± 0.04 ng/mL	1	106	3.30 ± 0.21 ng/mL	6	110
	30.0 ng/mL	34.25 ± 1.11 ng/mL	3	114	36.68 ± 3.14 ng/mL	8	122
UPLC-UV	0.2 μg/mL	0.11 ± 0.04 μg/mL	38	55	0.16 ± 0.06 μg/mL	42	78
	1.0 μg/mL	0.97 ± 0.16 μg/mL	17	97	1.04 ± 0.16 μg/mL	15	104
	5.0 μg/mL	4.96 ± 0.20 μg/mL	4	99	5.06 ± 0.20 μg/mL	4	101

CV: coefficient of variation.

samples. The methanol precipitation method was used in the processing of blood samples; therefore, all blood samples contained methanol. However, whether methanol interferes with CAP detection through SPR analysis is still unknown. Therefore, we determined the extent of the influence of methanol on the surface of the SPR sensor.

First, different concentrations of methanol (0%, 0.03125%, 0.0625%, 0.125%, 0.25%, 0.5%, 1%, and 2% (*V*/*V*)) were added to medium or high concentrations of in vitro CAP samples (5 and 20 ng/mL) without serum. The results are shown in Figs. 4A and B. The response signal increased with increasing concentrations of methanol in a concentration-dependent manner in both medium- and high-concentration samples. Then, blank serum was used to prepare methanol and CAP-containing blood samples, in which the concentration of serum was fixed at 0.025% (*V*/*V*), the concentrations of methanol were adjusted to 0.075%, 0.125%, 0.25%, 0.5%, 1%, and 2% (*V*/*V*), and the CAP concentration was medium or high (5 or 20 ng/mL). The response value was also positively correlated with methanol concentration (Figs. 4C and D). However, the correlation coefficients were not as positive as those of the serum-free samples, mainly because of the influence of the complex serum matrix. From these results, it can be concluded that methanol affects the response value of the sample regardless of the presence or absence of serum. Therefore, to eliminate the influence of methanol on the detection of CAP, the methanol content of all blood samples and the accompanying curve were fixed at 0.075% (*V*/*V*) in subsequent SPR analyses.

3.6. Matrix effect and extraction recovery rate

Matrix effect and extraction recovery rate experiments were conducted to determine the influence of endogenous substances in the serum and the protein precipitation process, respectively. CAP was added to the blank serum to prepare low-, medium-, and high-concentration samples. The samples were then diluted to their respective concentration ranges and subjected to SPR and UPLC-UV analyses. All results for the two methods, as shown in Table 2, were nearly within 68%–108%, of which the matrix effect of the low concentration was clearer, and the other results of the matrix effect and extraction recovery rate were acceptable. It can be concluded

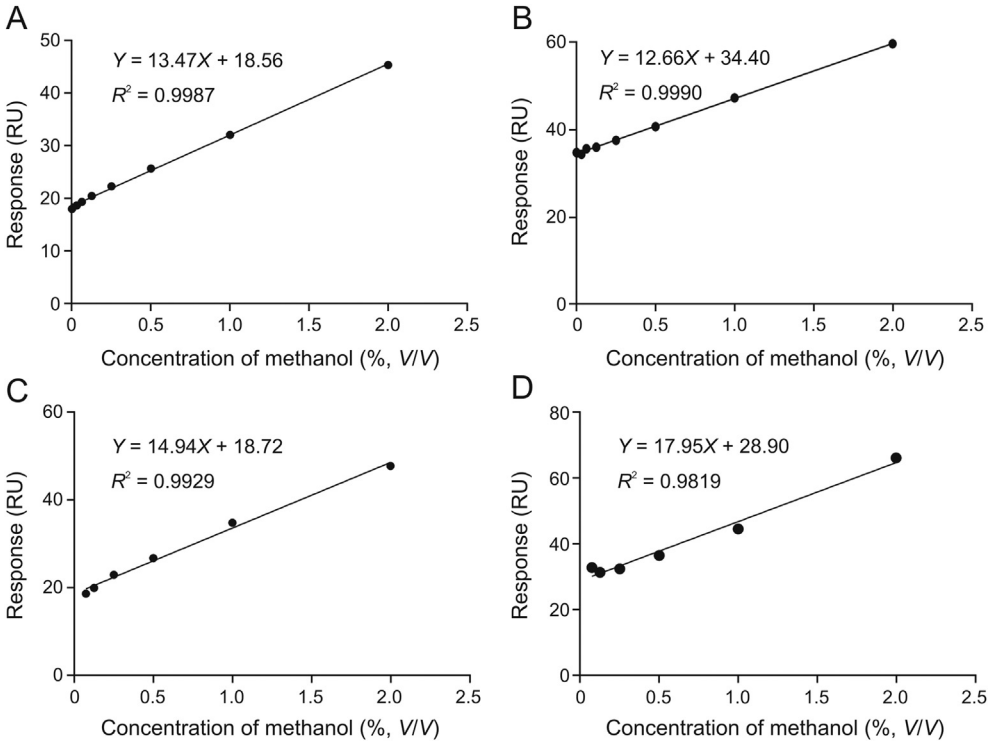

Fig. 4. Effect of methanol on detecting chloramphenicol (CAP) using surface plasmon resonance (SPR). (A) Response of 5 ng/mL CAP in the presence of 0%–2% (*V*/*V*) methanol and the absence of serum. (B) Response of 20 ng/mL CAP in the presence of 0%–2% (*V*/*V*) methanol and the absence of serum. (C) Response of 5 ng/mL CAP in the presence of 0.075%–2% (*V*/*V*) methanol and 0.025% (*V*/*V*) serum. (D) Response of 20 ng/mL CAP in the presence of 0.075%–2% (*V*/*V*) methanol and 0.025% (*V*/*V*) serum.

Table 2
Matrix effects and extraction recovery rate of surface plasmon resonance (SPR) and ultra-performance liquid chromatography-ultraviolet (UPLC-UV) analyses ($n = 3$).

Methods	Nominal concentration	Mean response (RU)/Mean peak area [a]			Matrix effect (%)	Extraction recovery rate (%)
		A1 (A2)	B1 (B2)	C1 (C2)		
SPR	0.3 ng/mL	2.33	1.57	1.59	68	99
	3.0 ng/mL	13.78	11.76	14.83	108	79
	30.0 ng/mL	47.36	44.49	44.64	94	100
UPLC-UV	0.2 μg/mL	1.08	0.76	0.83	76	92
	1.0 μg/mL	4.19	3.55	3.92	94	90
	5.0 μg/mL	22.24	17.91	20.57	92	87

[a] Groups A1, B1, and C1 are analyzed by SPR method and groups A2, B2, and C2 are analyzed by UPLC-UV method. Groups A1 and A2 were the mean response and mean peak area of the standard solution without serum, respectively; groups B1 and B2 were the mean response and mean peak area of the blank serum sample with standard solution added before protein precipitation, respectively; and groups C1 and C2 were the mean response and mean peak area of the blank serum sample with standard solution added after protein precipitation, respectively.

Table 3
Determination of chloramphenicol (CAP) concentrations in blood samples obtained from each rat at 0.75, 1, 2, and 4 h after intravenous administration of CAP by surface plasmon resonance (SPR) and ultra-performance liquid chromatography-ultraviolet (UPLC-UV) (mean ± SD, $n = 5$).

Methods	CAP concentrations (μg/mL)			
	0.75 h	1 h	2 h	4 h
SPR	25.51 ± 2.04	23.50 ± 6.12	5.99 ± 2.78	1.67 ± 0.84
UPLC-UV	35.41 ± 4.41	27.96 ± 1.45	7.37 ± 2.21	–

–: not detected.

that the two methods exhibit limited differences in matrix effects and extraction recovery rates, and the concentration of CAP in medicated rat serum can be measured more extensively.

3.7. Determination of CAP concentrations in blood

When collectively considering all the methodological indices of this study, SPR quantitation analysis is comparable to UPLC-UV. Finally, this method was applied to determine CAP in samples from rat blood after intravenous administration of CAP. Similarly, the results of SPR and UPLC-UV analyses for in vivo samples were compared to demonstrate the feasibility of SPR in practical applications. Blood samples from five rats were obtained before intravenous administration and 0.75, 1, 2, and 4 h after intravenous administration of CAP. The medicated serum samples were directly analyzed by the UPLC after protein precipitation and then diluted 1000 times before SPR analysis, because the detection range of SPR was 1000 times lower than that of UPLC-UV. The concentration detected by SPR was slightly lower than that detected by UPLC (Table 3), which was probably caused by the dilution procedure that might affect the SPR response or different detection principles between different methods. However, because the above methodological indices for both methods are acceptable, the quantification results of SPR are credible. It also showed that SPR can detect samples with lower concentrations than UPLC-UV, which proves that SPR analysis is more sensitive to the detection of serum samples. Therefore, the SPR sensor can detect samples with a concentration of ng/mL grade in practical applications. Collectively considering these findings as well as other advantages of SPR analysis, such as being rapid, convenient, and cost-effective, shows that it will have wider application fields in the quantitation of small molecules in blood.

4. Conclusions

In the present study, a comparison between SPR and UPLC-UV analyses for the quantitation of CAP was explored. The SPR biosensor was comparable to the UPLC-UV in terms of precision, accuracy, matrix effect, and extraction recovery rate. In addition, the detection range and LOD of SPR were lower than those of UPLC-UV, indicating better sensitivity, and SPR could detect lower concentrations of medicated serum. The results indicated that the SPR biosensor could be used for the quantitation of blood samples and was not significantly different from the classic UPLC-UV analysis through methodological verification. Therefore, SPR biosensors have potential for detecting small molecules in vivo, such as blood, urine, and other complex biological samples.

Although SPR technology has achieved success in the analysis of biomolecular interactions, SPR sensors are rarely used in the quantitative study of small molecules in blood samples. In this study, a new SPR method was developed to quantitively detect small molecules in complex blood samples, and its feasibility was preliminarily proven through methodological evaluation. However, further details regarding the application of this method need to be verified. For example, methanol and acetonitrile are commonly used in the pretreatment of blood samples; however, the effect of these solvents on the detection of target molecules is unknown. These issues warrant further studies to develop an optimized SPR-based quantitation method.

In addition, this study showed that the precision and accuracy of low-concentration samples detected by SPR were not satisfactory. The main reason is that the SPR sensor was constructed using an immobilized antibody and was thus a biological activity-based detector; therefore, its stability and lifespan may not be as high as those of the chemical detector. The development of new SPR sensors with better stability and longer lifespan is important for facilitating the development of SPR in quantitative analysis. Some recognition molecules, such as molecularly imprinted polymers and nucleic acid aptamers, are promising options, and SPR sensors with these recognition molecules will be the focus of future research.

This study has proven the feasibility of SPR for the detection of small molecules in blood samples by developing an SPR-based CAP biosensor, evaluating the methodological index, and comparing it with the classic UPLC-UV method. SPR analysis instruments have some intrinsic advantages; for example, they are simple to operate, do not require specialized technical training, and the SPR instrument has the potential to become miniaturized and portable. If it can further overcome the limitations in terms of stability and repeatability, the SPR biosensor can supplement the UPLC-UV method or become a mainstream method in blood sample analysis and play an important role in providing a new technology for drug development and clinical pharmacy.

CRediT author statement

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and Editing; **Diya Lv:** Methodology, Resources, Data curation, Writing - Reviewing and Editing; **Ying Zhang:** Visualization; **Dongyao Wang:** Validation; **Xiaofei Chen:** Resources; **Zhenyu Zhu:** Resources; **Zhanying Hong:** Resources, Investigation; **Yifeng Chai:** Supervision, Conceptualization; **Hai Zhang:** Conceptualization, Resources, Writing - Reviewing and Editing; **Yan Cao:** Supervision, Conceptualization, Methodology, Validation, Writing - Original draft preparation, Reviewing and Editing, Funding acquisition.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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