Analytical Methods

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *Anal. Methods*, 2021, **13**, 1919

Enhanced performance of a surface plasmon resonance-based immunosensor for the detection of glycocholic acid

Qiyi He,[ab] Yanxiong Fang,[b] Huiyi Yang,[a] Ding Shen,[a] Yingshan Chen,[a] Yingying Zhong,[a] Xiangguang Li,[a] Sergei A. Eremin,[c] Xiping Cui*[a] and Suqing Zhao*[a]

The concentration of glycocholic acid (GCA) in urine and blood is an important biomarker for liver cancer. Monitoring of GCA depends to a large extent on the availability of appropriate analytical techniques. In this work, based on the immobilization of GCA-OVA onto the sensor chip surface, a label-free competitive inhibition immunoassay for the determination of GCA with the surface plasmon resonance (SPR) technique was developed. The proposed SPR immunosensor is simple to prepare, recyclable and exhibits excellent sensitivity to GCA (a linear range of 13.3–119.4 ng mL^{-1} and a limit of detection (LOD) of 2.5 ng mL^{-1}), which was 14 times lower than that of the traditional immunoassay. Excellent recoveries and correlation between these two methods were observed (R^2 = 0.995). Hence, it can be proved that the SPR immunosensor could be used to achieve rapid and sensitive quantitative detection of GCA in real urine samples and meet clinical needs.

Received 2nd March 2021
Accepted 4th March 2021

DOI: 10.1039/d1ay00357g

rsc.li/methods

1. Introduction

Liver disease is one of the common human diseases.[1] The incidence of primary liver cancer is concealed, and the degree of malignancy is developing rapidly.[2] Glycocholic acid (GCA) is a compound of cholic acid and glycine in the liver.[3,4] Under normal circumstances, the amount of GCA that is infused into the system is less than 1% of the total amount, and the content of GCA in peripheral blood is extremely small.[4,5] When liver function is impaired, GCA in the portal vein is not efficiently absorbed by liver cells, resulting in an increased concentration of GCA in the blood.[6] Previous studies have shown that the content of GCA in blood and urine was significantly increased in patients with intrahepatic cholestasis during pregnancy, liver fibrosis, cirrhosis, and liver cancer, suggesting that GCA could be used as a biomarker for early diagnosis of liver disease.[2,7] In general, the concentration of GCA is less than 3 μg mL^{-1} in blood (http://www.hmdb.ca/) and less than 4 μg mL^{-1} in urine.[8]

At present, a variety of instruments and methods have been developed to detect GCA in biological samples such as plasma and urine. The limits of detection (LOD) for these methods are 5.60 μg mL^{-1} for high-performance liquid chromatography (HPLC),[9] 10 ng mL^{-1} for liquid chromatography-tandem mass spectrometry (LC-MS/MS),[10] 0.50 ng mL^{-1} for ultraperformance liquid chromatography-mass spectrometry (UPLC-MS),[11] 4.20 μg mL^{-1} for matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF-MS),[12] and 1.0 ng mL^{-1} for ultra-high performance liquid chromatography coupled with triple quadrupole linear ion trap mass spectrometry (HPLC-QqQ-MS/MS).[13] These instrumental analysis methods have the advantages of high sensitivity and accuracy, but they are time-consuming, expensive and require special operations.[14] Therefore, it is necessary to develop inexpensive, simple and efficient approaches with high throughput. Immunoassay is considered as a simple, economical and high-throughput assay.[15,16]

Immunoassays for GCA have also been reported. For instance, polyclonal antibody (pAb)-based radioimmunoassays (RIA) have a half-maximum signal inhibition concentration (IC_{50}) of 2.20 μg mL^{-1} in GCA determination.[17] In addition to its advantage of high sensitivity, RIA inevitably requires the use of radioactive reagents, which means radiation damage to technicians, as well as dangerous and expensive waste management. In addition to RIA, other immunoassay methods have also been reported for the determination of GCA, such as single-chain variable fragment antibody (scFv)-based enzyme linked immunosorbent assay (ELISA) (IC_{50} = 0.06 μg mL^{-1})[18] and pAb-based fluorescence polarization immunoassay (FPIA) (IC_{50} = 0.31 μg mL^{-1}).[19] It is essential to label antibodies or antigens in

[a]*Department of Pharmaceutical Engineering, School of Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou 510006, People's Republic of China. E-mail: xipcui@gdut.edu.cn; sqzhao@gdut.edu.cn*

[b]*Department of Chemical Engineering and Technology, School of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou 510006, People's Republic of China*

[c]*Faculty of Chemistry, M. V. Lomonosov Moscow State University, Moscow, 119991, Russia*

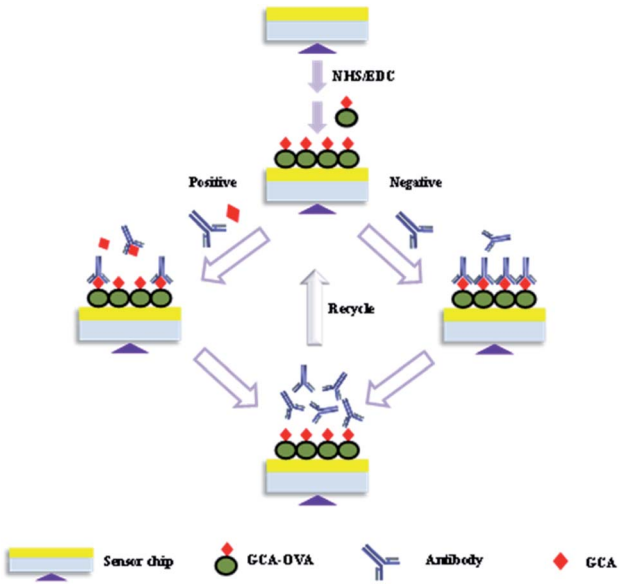

Fig. 1 Basic principle of the SPR immunosensor.

traditional immunoassays, however, chemical or physical methods are generally used for labeling, which might result in reagent consumption and the incapability of antibody recognition.[20] Different from conventional immunoassays, surface plasmon resonance (SPR) is a real-time, highly sensitive, label-free technique that directly monitors the interaction of specific antibodies and antigens in one step.[21,22] The detector is like an analytical weighing balance, sensing the change of the refractive index (RI) caused by fixed ligands and flowing analyte and generating quality-dependent signals and achieving real-time detection.[23,24]

In this work, based on the anti-GCA monoclonal antibody fixed on the surface of the CM5 sensor chip, a highly sensitive and label-free SPR immunosensor was established. The main principle of the established SPR immunosensor is depicted in Fig. 1. Under optimized conditions, a new SPR immunosensor was successfully constructed for the detection of GCA in human urine samples, which was verified by ELISA. Therefore, the SPR immunosensor proposed in this paper can be designed to determine GCA in a fast, high-throughput, competitive and indirect manner.

2. Materials and methods

2.1 Materials and reagents

Taurodeoxycholate, glycylursodeoxycholic acid, ursodeoxycholic acid, chenodeoxycholic acid, deoxycholic acid, sodium taurocholate, hyodeoxycholic acid, GCA, *N*-hydroxysuccinimide (NHS), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC·HCl) were obtained from Aladdin Co. Ltd. (Shanghai, China). Sensor chip CM5 research-grade (BR-1000-14) was purchased from GE Healthcare (Little Chalfont, U.K.). Ethanolamine was obtained from the Damao Chemical Reagent Factory (Tianjin, China). Coating antigen GCA-OVA and the monoclonal antibody (mAb) against GCA were synthesized and obtained according to our previous study.[18] Ultrapure water was used throughout the experiments and all reagents were of analytical grade unless otherwise specified.

The SPR-based Au film-chip system (BIAcore T200) was obtained from GE Healthcare (Pittsburgh, USA). The microplate reader (Infinite F200) was purchased from Tecan Trading AG (Switzerland).

2.2 Fabrication of the SPR immunosensor

The coating antigen GCA-OVA was fixed on the surface of the CM5 sensor chip by the active ester method.[25] Firstly, EDC buffer (0.4 M) and NHS buffer (0.1 M) were mixed and injected into the flow cell (gold film modified by dextran) at a steady rate of 30 μL min^{-1} for 15 min. Secondly, the coating antigen GCA-OVA (100 μg mL^{-1}) was injected into the flow cell for 15 min in acetate buffer (pH = 4.0). Finally, ethanolamine buffer (1 M, pH = 8.5) was injected into the flow cell to block the unreacted carboxyl group of the dextran at a rate of 30 μL min^{-1} for 10 min. PBST (PBS containing 0.5% Tween-20) was used as the running buffer during the whole experiment.

2.3 SPR-based indirect competitive immunoassay

In a conventional indirect competitive immunoassay, it is quite important to investigate the amount of antibodies and coating antigens separately. Firstly, the dilution of monoclonal

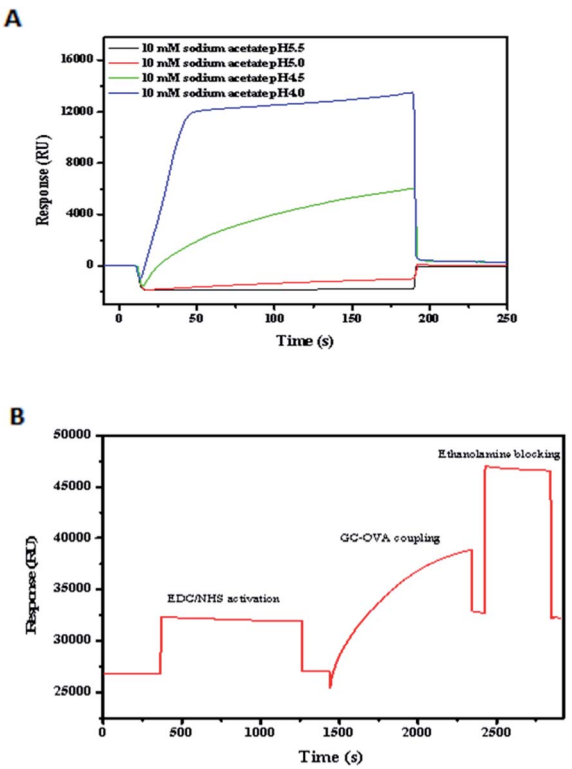

Fig. 2 (A) Effect of different pH in the pre-immobilization process of the GCA-OVA conjugate on the SPR chip surface. (B) The immobilization process of the GCA-OVA conjugate on the SPR chip surface. (a) EDC/NHS activation process; (b) GCA-OVA immobilization; (c) ethanolamine blocking process.

antibodies against GCA was optimized by a direct immunoassay with 0 and 50 ng mL^{-1} GCA. Under the optimal antibody concentration, the suitable dilution of the mAb was pre-mixed with the GCA standard solution in PBS buffer (0, 1, 10, 10^2, 10^3, 10^4, 10^5 ng mL^{-1}) for 30 min at 37 °C. After lifting the chip surface to a stable baseline of 30 μL min^{-1} with the running buffer, the mixture was injected into the chip surface (filled with GCA-OVA) at 10 μL min^{-1}. In this study, the GCA standard solution in the PBS buffer competed with the GCA-OVA conjugation on the chip surface, and the remaining antibodies were anti-GCA mAbs. Subsequently, the regeneration buffer Gly–HCl (0.01 M, pH 1.5) was injected into the flow cell at a rate of 30 μL min^{-1} for 2 min, and then the sensor chip was cleaned with running buffer for the next measurement.

Origin 8.5 was used to determine the assay sensitivity (IC_{50}), limit of detection (LOD, IC_{10}) and linear detection range (IC_{20}–IC_{80}) of the SPR immunosensor from the four-parameter logistic calibration diagram. The RUs-RU_0/RU_0 values were plotted against the GCA concentration, and a four-parameter logistic equation defined below was used to fit the immunoassay data,

$$Y = (A - D)/[1 + (X/C)B] + D$$

where A = response at high asymptote, B = slope factor, C = concentration corresponding to 50% specific binding (IC_{50}), D = response at low asymptote, and X = calibration concentration.

2.4 Indirect competitive ELISA

Under optimized conditions, an indirect competitive ELISA (icELISA) was developed. Briefly speaking, the 96 well microplates were coated with GCA-OVA (100 μL per well) in a carbonate buffer (pH = 9.5) at 37 °C for 2 hours. After microplates were washed five times with PBST, non-specific bindings were blocked by using 3% skim milk powder (300 μL per well) at 37 °C for 1 hour. After secondary washing, anti-GCA mAbs (50 μL per well) and a series of concentrations (0, 1, 10, 10^2, 10^3, 10^4, and 10^5 ng mL^{-1}) of GCA standard product or its analogs (50 μL per well) were mixed and incubated in the microplates for 1 hour at 37 °C. After the third washing, the anti-mouse IgG-HRP (100 μL per well) was added into the microplates and incubated at 37 °C for 1 hour. After washing again with PBST, the colorimetric substrate TMB (100 μL per well) was added into the microplates and the plates were incubated for 15 min. The reaction was terminated with sulfuric acid (50 μL per well, 0.5 M), and measured by using a microplate reader at OD_{450} nm. The calculation of IC_{50}, LOD and the linear range was based on a four-parameter logistic equation, where the Abs/Abs_0 values were plotted against the GCA concentration.

2.5 Selectivity of the SPR immunosensor

The cross-reactivity (CR) of the SPR immunosensor was investigated by using other bile acids with structural similarity to GCA, *i.e.*, taurodeoxycholate, glycylursodeoxycholic acid, ursodeoxycholic acid, chenodeoxycholic acid, deoxycholic acid, sodium taurocholate, and hyodeoxycholic acid. All the selected analogs were individually tested and CR was calculated according to the following equation:[26]

$$CR\% = [IC_{50}\ (GCA)/IC_{50}\ (analogue)] \times 100\%$$

2.6 Spiked samples analysis

The urine samples from volunteers in our laboratory were collected and centrifuged at 13 000 rpm for 10 minutes at 4 °C to remove any solid waste. The syringe filter (pore size 0.22 μm) was used for physical filtration. The supernatant was collected and stored at −80 °C for further use. The standard GCA product in PBS buffer was spiked into the above samples to respectively obtain the final concentrations of 50 ng mL^{-1} and 100 ng mL^{-1} and to validate the accuracy and precision of the developed SPR immunosensor.

3. Results and discussion

3.1 Immobilization of the GCA-OVA conjugate

It is well known that in conventional ELISA, coated antigens are fixed on polystyrene microplates by physical adsorption. But in the SPR immunosensor, the competitive antigen (so-called coating antigen) was fixed to the gold film by chemical coupling. After activation by the active ester method, the CM5 sensor chip provided covalent bonding sites for the GCA-OVA conjugate *via* a crosslinking reaction between the carboxyl of the dextran and amino groups of protein. In this process, the pH of the acetate buffer is the key factor affecting the formation of amide bonds. In order to coat more GCA-OVA on the CM5 sensor chip surface and generate a sufficiently strong signal, GCA-OVA (100 μg mL^{-1}) was prepared with 10 mM acetate buffer of different pH values (4.0, 4.5, 5.0, and 5.5) to optimize the immobilization on the chip surface, and 50 mM NaOH acted as regeneration buffer. The effect of different pH on the sensor chip surface during the pre-immobilization of the GCA-OVA conjugate is shown in Fig. 2A. It is clear that there was little increase in the signal when the pH of the acetate buffer was 5 or 5.5, indicating that the GCA-OVA conjugate was barely bound to the chip

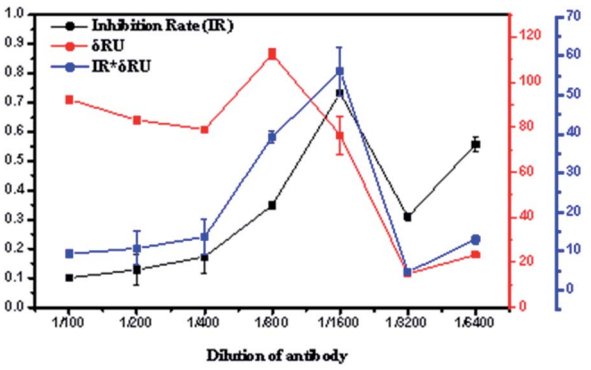

Fig. 3 Results of optimization of antibody concentration in the detection process.

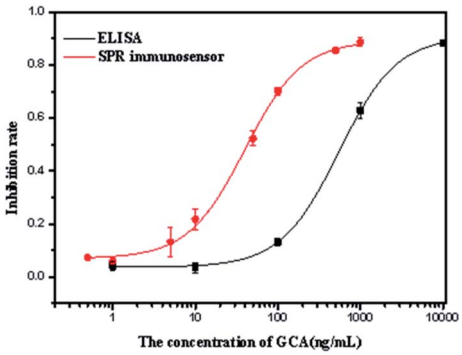

Fig. 4 Comparison between icELISA and the SPR immunosensor.

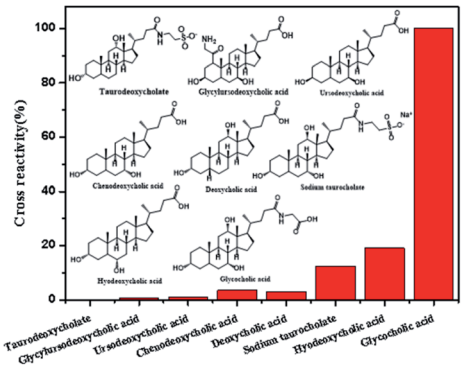

Fig. 5 Cross-reactivity of the antibody to GCA and related compounds by the SPR immunosensor.

surface. The signal increased over time when the pH of acetate buffer was 4 or 4.5, suggesting that the GCA-OVA conjugate was more likely to bind to the activated carboxyl groups on the surface of the sensor chip under these conditions. In order to generate a stronger signal, the acetate buffer of a pH of 4.0 was used for subsequent immobilization.

GCA-OVA prepared with acetate buffer (100 μg mL^{-1}, pH 4.0) was activated by the active ester method on the sensor chip surface for 15 min. GCA-OVA should be attached to the surface of the sensor chip surface whenever possible. Fig. 2B shows that the GCA-OVA immobilized signal could reach the 5135 RU value after optimization after blocking with ethanolamine, which was suitable for further immunoassay.

3.2 Optimization of the anti-GCA mAb concentration

In the indirect competitive immunoassay, the interaction between the anti-GCA mAb and the GCA-OVA conjugate coated on the surface of sensor chip was impeded when GCA existed in the samples. Prior to the immunoassay, the anti-GCA mAb at a fixed concentration was mixed with GCA samples and incubated to achieve a balance. A decrease in GCA concentration increased the number of free anti-GCA monoclonal antibodies, which in turn increased the response signal of their binding to the GCA-OVA conjugate. Therefore, it is very important to optimize the concentration of the anti-GCA monoclonal antibody in the above determination method, and then optimize the antibody dilution (1 : 6400, 1 : 32, 1 : 16, 1 : 8, 1 : 400, 1 : 200, 1 : 100) with or without 50 ng mL^{-1} free GCA.

When there was no GCA in the reaction system, the dilution of the anti-GCA mAb decreased from 6400 to 100 and the signal on the SPR chip increased from 42.1 to 914.0 RU, proving that the anti-GCA mAb concentration would affect the binding amount of GCA-OVA fixed on the surface of the sensor chip, thus affecting the strength of the SPR signal. As shown in Fig. 3, when there was 50 ng mL^{-1} free GCA in the reaction system, the SPR signal increased from 18.6 to 821.5 RU compared with the reaction system without GCA. The signal changed because the remaining anti-GCA mAb bound to the free GCA interacted with the GCA-OVA on the surface of the sensor chip. As the anti-GCA mAb was diluted from 1 : 100 to 1 : 6400, the inhibition rate increased from 0.10 to 0.73. RU–RU_0 (δRU) and δRU × IR were

Table 1 Comparison between different methods used for the determination of free GCA in biological samples[a]

Method	LOD (μg mL^{-1})	Linear range (μg mL^{-1})	Analysis time (min)	Reusability	Reference
HPLC	5.6	N.S.	>30 min	N.S.	9
UPLC-MS	5.0×10^{-4}	N.S.	>30 min	N.S.	11
HPLC-MS/MS	1.0×10^{-2}	N.S.	>30 min	N.S.	10
MALDI-TOF-MS	4.2	8.0–171.0	>30 min	N.S.	12
UHPLC-QqQ-MS/MS	1.0×10^{-3}	0.1–0.8	>30 min	N.S.	13
FPIA	9.0×10^{-3}	4.0×10^{-2}–2.6	10 min	No	19
icELIA-ScFv	1.0×10^{-2}	2.0×10^{-2}–0.2	>120 min	No	18
icELISA-mAb	7.0×10^{-2}	0.2–1.7	>120 min	No	This work
SPR immunosensor	2.5×10^{-3}	1.0×10^{-2}–0.1	7 min	Yes	This work

[a] N.S. – not stated.

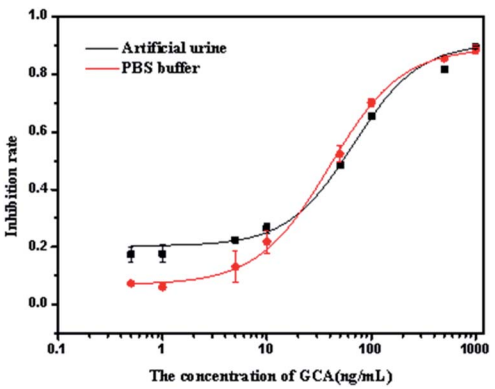

Fig. 6 Calibration curves in PBS buffer and artificial urine with the SPR immunosensor.

Table 2 Recovery of GCA from spiked human urine samples by ELISA and the SPR immunosensor

		ELISA ($n = 3$)			SPR immunosensor ($n = 3$)		
Samples	Spiked level (ng mL^{-1})	Found (ng mL^{-1})	Recovery (%)	CV (%)	Found (ng mL^{-1})	Recovery (%)	CV (%)
1	50	58.5 ± 3.1	117.0	5.2	53.3 ± 0.9	106.6	1.7
	100	88.8 ± 10.3	88.8	11.6	101.3 ± 2.9	101.3	2.8
2	50	57.6 ± 0.3	115.2	0.6	43.9 ± 8.0	87.7	18.2
	100	79.7 ± 1.8	79.7	2.3	95.6 ± 1.9	95.6	2.0
3	50	46.1 ± 9.3	92.3	20.1	59.8 ± 1.4	119.7	2.4
	100	117.2 ± 2.9	117.2	2.5	90.0 ± 2.3	90.0	2.5

also calculated. The higher the δRU × IR was, the higher the IR and δRU were. Therefore, from the perspective of the SPR reaction and cost, the anti-GCA monoclonal antibody diluted to 1 : 1600 was selected as the optimal antibody concentration for further immunoassay.

3.3 Comparison between indirect competitive ELISA and the SPR immunosensor

ic-ELISA and the SPR immunosensor were used in this study. Under optimized conditions, the anti-GCA monoclonal antibody was diluted at 1 : 16 000, and goat anti-mouse IGG-HRP was diluted as a secondary antibody at 1 : 5000. As shown in Fig. 4, the four-parameter logistic equations were $y = -0.87/[1 + (x/547.1)^{1.24}] + 0.91$ while the value of R^2 was 0.9999 for ELISA, and $y = -0.89/[1 + (x/39.8)^{1.26}] + 0.89$ while the value of R^2 was 0.9993 for the SPR immunosensor. With regard to ELISA, the value of IC_{50} was 547.1 ng mL^{-1}, LOD was 70.3 ng mL^{-1} and the working range was 178.1–1680.1 ng mL^{-1}. And the linear range of the SPR immunosensor was 13.3–119.4 ng mL^{-1}, the IC_{50} value was 39.8 ng mL^{-1} and LOD was 2.5 ng mL^{-1}. Compared with conventional ELISA, the IC_{50} and LOD of the SPR immunosensor were 14 times and 28 times lower, respectively. Conventional ELISA is limited by the coloration of the label signal (HRP). In many cases, sensitivity can be enhanced by amplifying the labeled signal such as mimic enzymes,[27] streptavidin–biotin[28] or multi-HRP nanocomposites,[29] *etc.*

The SPR immunosensor also has significant advantages in terms of increased sensitivity and the absence of markers and reagent savings. Compared with most of the previously reported methods (Table 1), the SPR immunosensor developed is more sensitive and efficient than other immunoassay and chromatography methods. It is worth mentioning that this newly established method can realize fully automated detection, and the immunosensor chip can be reused nearly 300 times, which can save the usage of coating antigens and labeling reagents.

3.4 Specificity of the SPR immunosensor

To evaluate the specificity of the SPR immunosensor for determining GCA, CR was measured by comparing the IC_{50} (concentration corresponding to $\delta RU/RU_0 = 50\%$) of seven structurally and functionally similar bile acids with that of GCA. As shown in Fig. 5, hyodeoxycholic acid (19.1%) and sodium taurocholate (12.6%) had the same CRs as that of GCA, which might be due to their similar structure in the nucleus of steroid. The recognition between the antibody and molecules was mainly based on the production of the antibody. In these cases, the immunogen was prepared by using the carboxyl group of the D ring and the farthest part of the molecule might be the primary unit for identification. Though there were CRs in liver hyodeoxycholic acid and sodium taurocholate, the content of analogs is positively correlated with liver diseases, which is still of significance for the diagnosis of liver diseases.[19]

3.5 Recovery tests of clinical samples

The matrix effect is quite important in immunoassays. If the matrix seriously affects the accuracy of experiments, it is quite important to pre-treat complex samples. Thus, in order to investigate the influence of matrix effect on the SPR immunosensor, artificial urine was used to simulate human urine.[30] The artificial urine samples were diluted to prepare a series of GCA standard solutions. As shown in Fig. 6, the IC_{50} of the PBS buffer was 39.8 ng mL^{-1} while that of artificial urine was 67.0 ng mL^{-1}. The sensitivity of artificial urine decreased slightly, indicating that the SPR immunosensor was partially resistant to the matrix effect. Considering the amount of GCA in human urine or serum, the linear range of the proposed method was 25 times lower than actual and clinical use. The dilution of samples might be beneficial to reduce the matrix effect. Puncture and recovery analyses were performed on urine to check the accuracy of the proposed SPR immunosensor and ic-ELISA.

As shown in Table 2, the recovery of GCA in human urine was 87.7–119.7% by using the SPR immunosensor and 79.7–117.2% by ELISA. It should be noted that the sensitivity of the two methods is different. To fit the linear range, the dilution of urine was adjusted according to the method. As can be seen from the results of the spiked experiments, both methods exhibited good accuracy, high recovery rate, and good correlation with each other $R^2 = 0.995$ (data not shown), which proved that the SPR immunosensor was an effective method for detecting GCA in human urine.

4. Conclusions

Conventional immunoassays are inseparable from antibody labeling. In this study, as a newly developed sensor technique, SPR plays a more direct role in antigen–antibody recognition. It

has been reported to combine immunoassay and SPR techniques for determining GCA. Although the two are based on similar competitive principles, the SPR immunosensor is 14 times more sensitive than indirect competitive ELISA, suggesting that the SPR technology has the potential to enhance sensitivity. In addition, the SPR immunosensor has advantages in reagent consumption, detection time, sensitivity and automatic operation. In summary, the newly developed SPR immunosensor can be used as a potential method for monitoring clinical samples in a real-time manner and quantifying GCA residues.

Conflicts of interest

The authors declare that there is no conflict of interests to publish this paper.

Acknowledgements

This work was financially supported by the Guangzhou Science and Technology Foundation (2016201604030025 and 201902010430100011) and Guangdong Science and Technology Foundation (2016B090934002, 2017A050501034, and 2018A030313926). The expert technical assistance of Miss Lu Huang and Jing Li is also greatly appreciated. We wish to acknowledge the Analysis and Test Center of the Guangdong University of Technology for supplying the instrumental support.

References

1 P. Luo, P. Yin, R. Hua, Y. Tan, Z. Li, G. Qiu, Z. Yin, X. Xie, X. Wang, W. Chen, L. Zhou, X. Wang, Y. Li, H. Chen, L. Gao, X. Lu, T. Wu, H. Wang, J. Niu and G. Xu, *Hepatology*, 2018, **67**, 662–675.
2 T. Chen, G. Xie, X. Wang, J. Fan, Y. Qiu, X. Zheng, X. Qi, Y. Cao, M. Su, X. Wang, L. X. Xu, Y. Yen, P. Liu and W. Jia, *Mol. Cell. Proteomics*, 2011, **10**(7), M110.004945.
3 T. Li and J. Y. Chiang, *J. Lipids*, 2012, **2012**, 754067.
4 A. F. Hofmann, *Hepatology*, 2009, **49**, 1403–1418.
5 G. A. Kullak-ublick, S. Bruno and P. J. Meier, *Gastroenterology*, 2004, **126**, 322–342.
6 J. Han, W. X. Qin, Z. L. Li, A. J. Xu, H. Xing, H. Wu, H. Zhang, M. D. Wang, C. Li, L. Liang, B. Quan, W. T. Yan, F. Shen, M. C. Wu and T. Yang, *Clin. Chim. Acta*, 2019, **488**, 68–75.
7 C. Manzotti, G. Casazza, T. Stimac, D. Nikolova and C. Gluud, *Cochrane Database Syst. Rev.*, 2017, (2), CD012546.
8 Q. F. Li, Y. M. Zhan, Y. G. Zhong, B. Zhang and C. Q. Ge, *Biomed. Chromatogr.*, 2016, **30**, 1706–1713.
9 E. Persson, L. Lofgren, G. Hansson, B. Abrahamsson, H. Lennernas and R. Nilsson, *J. Lipid Res.*, 2007, **48**, 242–251.
10 A. Sanchez-Guijo, C. Blaschka, M. F. Hartmann, C. Wrenzycki and S. A. Wudy, *J. Steroid Biochem. Mol. Biol.*, 2016, **162**, 117–125.
11 L. Yang, A. Xiong, Y. He, Z. Wang, C. Wang, Z. Wang, W. Li, L. Yang and Z. Hu, *Chem. Res. Toxicol.*, 2008, **21**, 2280–2288.
12 D. Mims and D. Hercules, *Anal. Bioanal. Chem.*, 2003, **375**, 609–616.
13 J. Zhang, Y. Fan, Y. Gong, X. Chen, L. Wan, C. Zhou, J. Zhou, S. Ma, F. Wei, J. Chen and J. Nie, *J. Chromatogr. B: Anal. Technol. Biomed. Life Sci.*, 2017, **1068–1069**, 245–252.
14 A. Kolosova, K. Maximova, S. A. Eremin, A. V. Zherdev, J. V. Mercader, A. Abad-Fuentes and B. B. Dzantiev, *Talanta*, 2017, **162**, 495–504.
15 Y. Zhou, X. Huang, S. Xiong, X. Li, S. Zhan, L. Zeng and Y. Xiong, *Anal. Chim. Acta*, 2018, **1038**, 112–119.
16 N. Zhu, Y. Zhu, J. Wang, E. Gyimah, X. Hu and Z. Zhang, *Talanta*, 2019, **199**, 72–79.
17 P. Miller, S. Weiss, M. Cornell and J. Dockery, *Clin. Chem.*, 1981, **27**(10), 1698–1703.
18 X. Cui, N. Vasylieva, P. Wu, B. Barnych, J. Yang, D. Shen, Q. He, S. J. Gee, S. Zhao and B. D. Hammock, *Anal. Chem.*, 2017, **89**, 11091–11097.
19 Q. He, X. Cui, D. Shen, Y. Chen, Z. Jiang, R. Lv, S. A. Eremin and S. Zhao, *J. Pharm. Biomed. Anal.*, 2018, **158**, 431–437.
20 H. Zhang and B. L. Miller, *Biosens. Bioelectron.*, 2019, **141**, 111476.
21 Q. He, Y. Chen, D. Shen, X. Cui, C. Zhang, H. Yang, W. Zhong, S. A. Eremin, Y. Fang and S. Zhao, *Talanta*, 2019, **195**, 655–661.
22 M. Khodami and P. Berini, *Sens. Actuators, B*, 2018, **273**, 1156–1161.
23 H. Yu, R. Han, J. Su, H. Chen and D. Li, *Clin. Chim. Acta*, 2020, **502**, 9–14.
24 N. Mudgal, A. Saharia, A. Agarwal, J. Ali, P. Yupapin and G. Singh, *Opt. Quantum Electron.*, 2020, **52**, 307.
25 M. Pan, X. Wang, J. Wang, Y. Lu, K. Qian and S. Wang, *Food Anal. Methods*, 2016, **10**, 2027–2035.
26 X. Cui, P. Wu, D. Lai, S. Zheng, Y. Chen, S. A. Eremin, W. Peng and S. Zhao, *J. Agric. Food Chem.*, 2015, **63**, 9372–9378.
27 L. Wu, G. Li, X. Xu, L. Zhu, R. Huang and X. Chen, *TrAC, Trends Anal. Chem.*, 2019, **113**, 140–156.
28 R. Nie, X. Xu, X. Cui, Y. Chen and L. Yang, *ACS Omega*, 2019, **4**, 6210–6217.
29 Y. J. Chen, M. Chen, T. L. Cheng, S. R. Roffler, S. Y. Lin, H. L. Hsu, C. H. Wang, C. Y. Chen, A. P. Kao, J. J. Cheng and K. H. Chuang, *Anal. Chem.*, 2019, **91**, 8310–8317.
30 D. C. Kabiraz, K. Morita, K. Sakamoto, M. Takahashi and T. Kawaguchi, *Talanta*, 2018, **186**, 521–526.

