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# Enhanced optical immuosensor based on surface plasmon resonance for determination of transferrin

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**Abstract**

Wavelength modulation surface plasmon resonance biosensors (SPR) using colloidal Au nanoparticles and double-linker sensing membrane enhancement are reported for determination of transferrin. The 2-mercaptoethylamine (MEA) was immobilized on the biosensor surface with traditional amine coupling method. The interaction between colloidal Au nanoparticles and MEA was investigated. The anti-transferrin was immobilized on the biosensor surface prepared with staphylococcal protein A (SPA). The interaction of the antibody and antigen was monitored in real time. The good response was obtained in the concentration range 1–20, 0.1–20 and 0.05–20 μg/mL for directly immune assay, double-linker assay and colloidal Au-amplified assay. The result clearly demonstrates that these methods may obtain significantly enhancement of sensitivity for the wavelength modulation SPR biosensor.


*Keywords:* SPR; Wavelength modulation; Transferrin; Double-linker assay; Colloidal Au-amplified assay

## 1. Introduction

Transferrin, the major serum iron transport glycoprotein in human, is essential for normal cell growth and maintenance. The transferrin molecule consists of a single peptide chain of 679 amino acids and is arranged in two globular domains, the N-terminal (AA 1–336) and the C-terminal (AA 337–679) domain. Each domain contains a metal binding site, but only the C-terminal domain contains two N-glycosylation sites located at the positions 413 and 611 [1]. So the transferrin is able to bind two iron atoms per molecule and therefore is half or fully saturated at N- and C-terminal binding site. Serum transferrin is synthesized mainly in hepatocytes, from which it is secreted in blood, but also in other cell types including oligodendrocytes and its major function is to transport iron from sites of absorption to tissues throughout the body [2,3]. Its iron-binding capacity is of importance, because free iron is presented in the alveolar system of the neonatal lung. Free iron ions are able to promote highly toxic hydroxyl radical-formation. Therefore, the transferrin may play a regulatory role in iron metabolism, protect against the toxic effects of free iron [2].

Many diseases are associated with the change of the transferrin content in serum. People with acute hepatitis, anemia, and pregnancy are associated with the symptoms of the increased levels of transferrin, while nephritic syndrome, rheumatism, cirrhosis, malignant tumor, acute leukemia are characterized by the decrease of this carrier [4–7]. Thus, the determination of transferrin gains attention from both medical experts and clinical chemists. Some conventional methods for diagnostic determinations of the transferrin are enzyme-linked immunosorbent assay (ELISA) [8], immunochemical nephelometry [9], capillary zone electrophoresis (CZE) [10], piezoelectric immunoassay [11], immunological turbidimetric assay (ITA) [12], radioimmunoassay (RIA) [13], as well as electrochemical immunoassay [14]. However, most of these methodologies have not sufficiently low detection limit to

*Abbreviations:* SPR, surface plasmon resonance; SPA, staphylococcal protein A; PBS, phosphate-buffered saline; BSA, bovine serum albumin; MPA, 3-mercaptopropionic acid; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, *N*-hydroxysuccinimide; MEA, 2-mercaptoethylamine

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meet the need of clinical diagnosis. Despite ELISA and RIA have high sensitivity, they usually need to label with enzyme, radioactive isotopes or fluorescein, which may be complicated and time-consuming operations. Recently, Li et al. [15] developed a renewable fluorometric enzyme immunosensing system using prochlorperazine (PCP) as the substrate for horseradish peroxidase (HRP) and Lei et al. [16] proposed a new electrochemical immunoassay strategy based on electrostatic interaction of natural polymers for the determination of transferrin in serum samples.

Surface plasmon resonance (SPR) is a well-known reliable tool for investigating interaction between biomolecular, especially antibody–antigen complexes owing to its features of simplicity, low cost, no label, high selectivity, and real-time monitoring [17,18]. In the last two decades, there is remarkable progress in the development of the SPR biosensor. Various SPR biosensors have been developed and applied to detection and identification of specific ananlytes in the areas of environmental protection, biotechnology, medical diagnostis, drug screening, food safety, and security [19]. As a quantitative tool, SPR biosensors can be used to determine reaction kinetic and affinity constants for molecular interactions, as well as the active concentration of biomolecules in solution. As a qualitative tool, its application range from orphan ligand and small-molecule screening to epitope mapping and complex assembly studies. In general, direct detection is preferred in application where direct binding of analyte of interest produces a sufficient response. If necessary, the lowest detection limits of the direct SPR biosensors can be improved by using sandwich strategy. The antibodies or antigens may also be coupled to large particles such as liposomes [20] and Au nanoparticles [21] as labels to further enhance the SPR biosensor signal. Recently, there is a growing concern about the use of Au nanoparticles for enhancing SPR biosensor sensitivity. The possibility that synthesizing metal nanoparticles of controlled size and surface capping allows the generation of low molecular weight- or biomaterial-Au nanopartical conjugates. Specifically, the electronic coupling between the localized surface plasmon of the Au nanoparticals and the plasmon wave associated with the surface leads to enhanced shifts of the resonance angles [22].

The objective of this study is to develop an enhanced immumosensor for the detection of the transferrin based on double-linker assay and colloidal Au-amplified assay using a self-assembled wavelength modulation SPR apparatus in our laboratory [23,24]. An efficient immobilization of transferrin antibody using double-linker sensing membrane is developed. By use of a double-linker system to immobilize transferrin antibody on the biosensor surface, transferrin binding gave increased SPR signal. The real time monitoring of transferrin binding to its antibody, and the enhancement effect provided by the Au nanoparticles are elucidated. Previously, we developed an enhanced wavelength modulation SPR immunosensor based on the sandwich assay and colloidal-Au-enhanced assay for determining the human complement factor 4 [23]. Colloidal Au was used as label by antigen (antibody) conjugating to enhance sensitivity of SPR biosensor. However, in this paper, the colloidal Au is not as labels but as active components for the amplified SPR analysis of bimolecular interaction. Thus, colloidal Au is not easy to be removed owing to immobilization of colloidal Au on the biosensor surface. On the other hand, it is not necessary to consider if protein activity is influenced because colloidal Au-protein conjugation is elided. The interaction between colloidal Au nanoparticles and SPR-active Au films was studied. The study shows that covalent attachment of submonolayers of colloidal Au nanoparticles (10 nm) leads to very large shift in the resonance wavelength. Such large changes can be coupled to biomolecular recognition events so as to further enhance the SPR sensor sensitivity. These assay methods demonstrate superior detection limits and signal strengths over the direct assay.

## 2. Materials and methods

### 2.1. Equipment

The details of the self-assembled wavelength modulation SPR apparatus have been described previously [25,26]. Briefly, the SPR instrument consists of a halogen tungsten lamp, collimating device, optical system, and Fullwave Spectrophotometer. Halogen tungsten lamp and collimating device were purchased from Changchun Fifth Optics Precision Instrument (PR China). The lenses, prisms and various optical adjusting frames were purchased from the Precision Optical Instrument Factory of Changchun (PR China). Fullwave Spectrophotometer was obtained from Ocean Optics Inc., USA. The resolution of the CCD used here is 0.3 nm. All bioassays were performed entirely in solution to facilitate real-time monitoring of surface reactions.

### 2.2. Au film preparation

Thin Au films (50–60 nm) were prepared by vacuum-deposited. To deposit the gold film, the prism was mounted in an electron-beam evaporator system in an arrangement so that the flux of evaporated metal was perpendicular to the bottom of the right prism. The deposition process was monitored using a quartz crystal detector.

### 2.3. Reagents

Commercially obtained reagents were used without further purification. Bovine serum albumin (BSA), staphylococcal protein A (SPA), anti-transferrin, and transferrin were obtained from Shanghai Biology Product Research Institute. Colloidal Au (10 nm), 3-mercaptopropionic acid (MPA) and 2-mercaptoethylamine (MEA) were purchased from Sigma (St. Louis, USA). 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and *N*-hydroxysuccinimide (NHS) were obtained from Shanghai

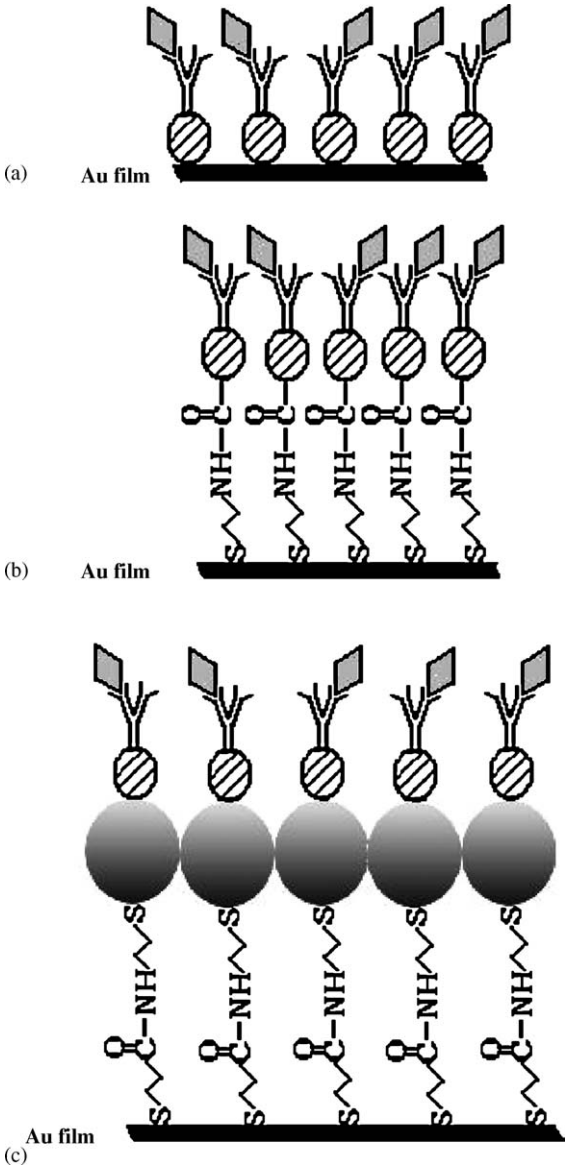


Fig. 1. The scheme of the presented immunoassays. (a) Directly immune reaction; (b) double-linker assay; (c) colloidal Au-amplified assay. ( ) Antigen, ( ) antibody, ( ) Au colloid, ( ) SPA.

Lizhu Dongfeng Biotechnology Co. All other chemicals were of analytical-reagent grade. PBS buffer solution (pH 7.4, 0.01 mol/L phosphate-buffered saline solution, 0.2 g KCl, 8.0 g NaCl, 0.24 g $KH_2PO_4$ and 1.44 g $Na_2HPO_4$ dissolved in 1000 mL water) was used as running buffer in wavelength modulation SPR biosensor.

### 2.4. Immunoassay procedure

The schematic representation of three immunoassays is showed in Fig. 1.

#### 2.4.1. Directly immune reaction

Anti-transferrin at 1:10 (v/v) was flowed over the biosensor surface prepared with SPA, and the assembling of the antibody was carried out for 12 h to organize the processing anti-transferrin on the SPA surface and thus the anti-transferrin membrane was stable. The biosensor surface was blocked with BSA for at least 20 min after the biosensor surface was rinsed for washing off non-covalently bound anti-transferrin to stabilize the surface baseline. Then, the transferrin was diluted with PBS buffer and flowed over the biosensor surface, and the response due to antibody–antigen reaction was recorded as time prolonged.

#### 2.4.2. Double-linker assay

The biosensor surface was rinsed with ultrapure water, and then was exposed to PBS buffer until resonance wavelength kept constant. The solution containing 10 mmol/L MEA was injected into flow cell for 1 h to give an SPR response of 11.58 nm. The 0.1 mg/mL SPA solution was then injected into flow cell. The immobilization of antibody on the SPA surface was performed by injection anti-tranerrin solution (v/v 1:10). After self-assembling of anti-transferrin was carried out for 12 h and unreacted sites on the biosensor surface was blocked with BSA solution, antigen binding test was performed by injecting transferrin for 30 min.

#### 2.4.3. Colloidal Au-amplified assay

The biosensor surface was rinsed with ultrapure water, and then was exposed to PBS buffer until resonance wavelength kept constant. The MPA solution (10 mmol/L) was flowed over biosensor surface for 1 h. The surface was then activated by injection of a mixture of NHS (40 mg/mL) and EDC (40 mg/mL) for 7 min. After biosensor surface was rinsed with PBS buffer, the solution containing 0.4 mg/mL MEA was injected into flow cell. Immobilization of colloidal Au (10 nm) onto modified (MEA-coated) surface was performed for 1 h from aqueous at room temperature. The flow cell was rinsed with PBS buffer and was incubated with 0.1 mg/mL SPA solution for 1 h. The solution containing (v/v 1:10) anti-transferrin was injected into flow cell and allowed to react for 12 h. Before the determination of the transferrin, the surface was blocked with BSA for 20 min.

## 3. Results and discussion

### 3.1. Directly immune reaction

The use of SPA as a membrane to connect antibody and Au is the best strategy because SPA binds specifically to the Fc region of immunoglobulin molecule without interacting at the antigen binding sites. SPA is a polypeptide of the staphylococcus and has four binding sites with antibody. In the directly immune reaction, the biosensor surface was modified with SPA. The solution containing 0.1 mg/mL SPA was flowed over biosensor surface, and the self-assembling of SPA on the Au film was monitored in real time. The biosensor surface of exposure to the SPA solution leads to a 8.13 nm shift in the resonance wavelength within 35 min, and the reso-

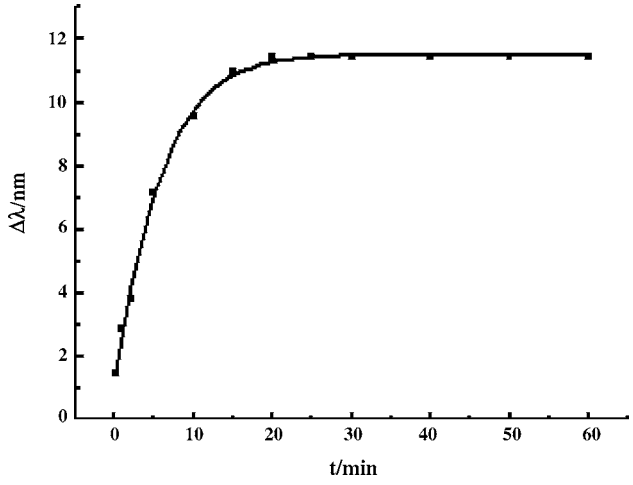


Fig. 2. The kinetic adsorption curve of anti-transferrin on the SPA monolayer in the directly immune assays.

nance wavelength remains almost constant as time prolongs. This suggests that the self-assembling monolayer has been completed.

To wipe off non-specific adsorption on the gold surface, the biosensor surface was rinsed with PBS buffer until resonance wavelength kept constant. Then anti-transferrin solution was injected into the flow cell to monitor its assembling on the SPA surface. Fig. 2 shows the kinetic adsorption curve of anti-transferrin on the SPA monolayer in 1 h. It can be seen from Fig. 2 that association between antibody and SPA is very fast. The process was carried out for 12 h in order to form densely packed layers on the SPA surface. Prior to determine of transferrin, biosensor surface was blocked with BSA. The transferrin-binding test was performed in PBS buffer for 30 min at the room temperature. The relationship between transferrin concentration and the shift of the resonance wavelength was obtained. The transferrin is determined in the concentration range from 1 to 20 μg/mL.

### 3.2. Double-linker assay

The MEA-SPA double sensing membrane was developed because the sensitivity of the double sensing membrane is higher than that obtained with the monolayer sensing membrane. The first linker (MEA) can be attached strongly to the gold film surface by its sulfide bonds, and the amines terminal group is an active group that can bind to protein. The second linker (SPA) can bind the Fc region of the antibody at near neutral pH, leaving the antigen-binding free sites. Meanwhile, the −COOH of SPA can react with amines group of MEA.

The biosensor surface was rinsed with PBS buffer until the resonance wavelength remained constant. That is, the baseline had been stabilized. Then, the biosensor surface was exposed to the MEA solution and was incubated for 1 h. The association of the MEA and gold film was monitored in real time according to the shift of the resonance wavelength.

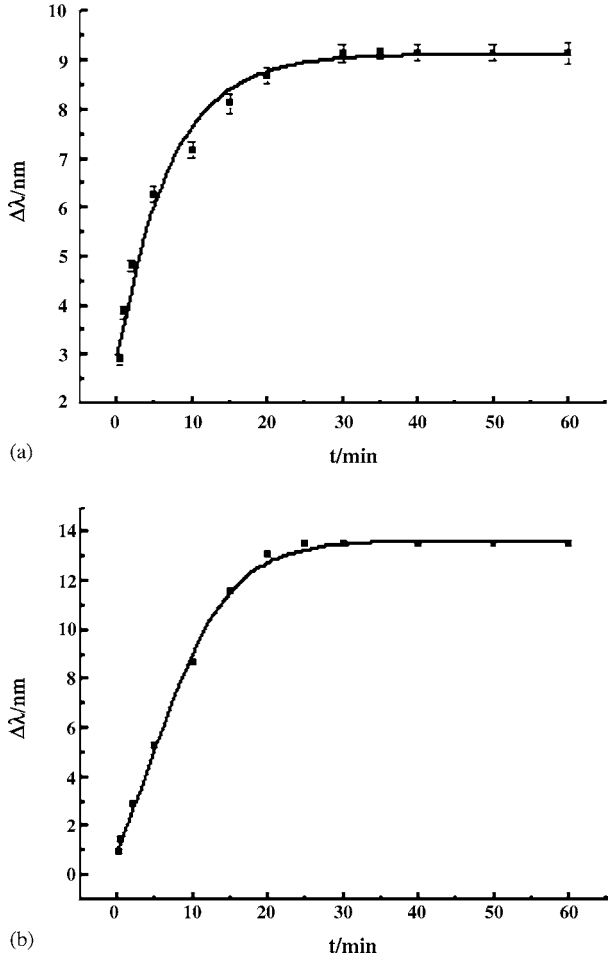


Fig. 3. (a) The kinetic adsorption curve of SPA on the MEA surface. The error bars represent the S.D. of the values determined from three the same assays. (b) The adsorption curve of the anti-transferrin on the double-linker sensing membrane.

About 30 min later, the reaction of the MEA and gold film was completed. The maximum shift of resonance wavelength is 11.58 nm. Following, after the biosensor surface was rinsed with PBS buffer, the SPA solution (the second linker) was flowed over MEA surface. The sensorgram of the interaction of the SPA and MEA is showed in Fig. 3a. As seen from Fig. 3a, the association of the SPA and MEA is very fast, and the shift of the resonance wavelength reaches about 90% of total shift in 15 min. After 30 min later, the reaction between the SPA and MEA almost completed. Then anti-transferrin was self-assembled on the double-linker (MEA-SPA) sensing membrane. The adsorption curve of the anti-transferrin on the double-linker sensing membrane is showed in Fig. 3b. If the binding sites of SPA are in a plane, the binding rate is fast. However, if the same binding sites of SPA are not in a plane, the binding rate will be lowered because of the steric exclusion effect. In this experiment, the binding of SPA for the monolayer sensing membrane is easier to be in a plane than that for the double-linker sensing membrane. Therefore, it is seen from Fig. 3b and Fig. 2, the binding rate of the

anti-transferrin to SPA on double-linker sensing membrane is slower than that on monolayer sensing membrane in a 10 min. The different concentrations of the transferrin were separately flowed over the anti-transferrin surface after biosensor surface was blocked with BSA. The transferrin is determined in the concentration range from 0.1 to 20 μg/mL. Theoretical analysis shows that the sensitivity of the spectral prism-based SPR sensor is higher at long wavelength than that at short wavelength, and the sensitivity of the spectra prism-based SPR sensor increases rapidly with increasing wavelength towards long wavelength [27]. As mentioned aboved, the shift of the resonance wavelength towards long wavelength caused by double-linker sensing membrane immobilized on the sensor surface is larger than that caused by monolayer sensing membrane immobilized on the sensor surface. As expected, for the double-linker assay, the lowest determination concentration is 10-fold lower than that obtained by directly immune reaction. At 0.1 μg/mL of the concentration, binding reaction of transerrin can not be detected directly by SPR, but double-linker sensing membrane method still leads to 0.47 nm shift of the resonance wavelength. The primary response due to specific binding of 20 μg/mL transferrin to anti-transferrin immobilized is about 2.30 nm shift of the resonance wavelength, however, the specific binding of 20 μg/mL transferrin to anti-transferrin immobilized is about 3.79 nm shift of the resonance wavelength in the double-linker assay.

### 3.3. Colloidal Au-amplified assay

The SPR response signal is enhanced using the colloidal Au method. The antigen-colloidal Au conjugate results in enhancement of the SPR sensitivity to protein–protein interaction, and this aspect has been extensive investigated [21,23,28]. In the presented study, colloidal Au is adsorbed onto the self-assembled monolayer (SAM) formed on the SPR substrate. The adsorbed colloidal Au could collectively or selectively alter the three signature SPR features of the substrate/SAM system, thereby enhancing the detection sensitivity of the SPR device [29]. The interaction between colloidal Au nanopartical and SPR-active Au film was studied in detail by Lyon et al. In addition, they further illustrated how small Au nanoparticles could be selectively enlarged SPR signal [30].

The MEA was immobilized on the MPA surface with traditional amine coupling method in this study. Because the sulfide bond of MPA can stick firmly to the gold film and the –COOH group of MPA can react with NHS by using EDC as catalyzer to form *N*-hydroxysuccinimide esters, these esters can then react spontaneously with amine group of the MEA. Thus, the sulfide of the MEA may free bind to colloidal Au surface introduced into solution. The covalent attachment of colloidal Au nanoparticles leads to large shift of the resonance wavelength. The maximum shift of the wavelength resonance is 36.36 nm. The first possible mechanism is the coupling of the localized surface plasmon of the colloidal Au with the propagating plasmon in the Au film. The second possible mechanism is that the large bulk of the colloidal Au nanoparticle leads to large change of the refractive index. After the colloidal Au nanoparticles was immobilized on the MEA-coated surface, the surface was rinsed with PBS buffer until resonance wavelength kept constant. Alike, colloidal Au surface was modified using 0.1 mg/mL SPA. Then anti-transferrin was flowed over SPA surface and was incubated for 12 h. Following, BSA was injected into flow cell in order to block unreacted sites on the colloidal Au surface. Fig. 4 depicts the relationship between concentration of analyte and resonance wavelength shift for three immnoassays. The SPR biosensor has good response to transferrin in the concentration range 0.05–20 μg/mL. The effect of the Au nanoparticles on the SPR signal enhancement depends on the particle diameter and concentration [28]. On the one hand, with the addition of the Au nanoparticles, the resonance wavelength will shift towards long wavelength. As mentioned above, the sensitivity of the sensor increases with the shift of the resonance wavelength towards long wavelength. On the other hand, SPR dip will be broaden with the shift of the resonance wavelength towards long wavelength and this will deteriorate the detection limit. So, use of the Au nanoparticles is limited at concentration of the Au nanoparticles. This aspect has been studied [28]. Therefore, for the colloidal Au-amplified assay, the lowest determination concentration is two-fold lower than that obtained by the double-linker assays, and 20-fold lower than that obtained by the directly immune reaction. At 0.05 μg/mL of the concentration, binding of transerrin can not be determined directly by SPR, but resonance wavelength still occurred change by using colloidal Au nanoparticles. When the colloidal Au-amplified assay is applied, exposure to a solution of 20 μg/mL transferrin leads to a 5.35 nm shift of the resonance wavelength. However, the specific binding of 20 μg/mL transferrin to anti-transferrin immobilized is about 2.30 and 3.79 nm shift of the resonance wavelength in the direcly immune assay and double-linker assay.

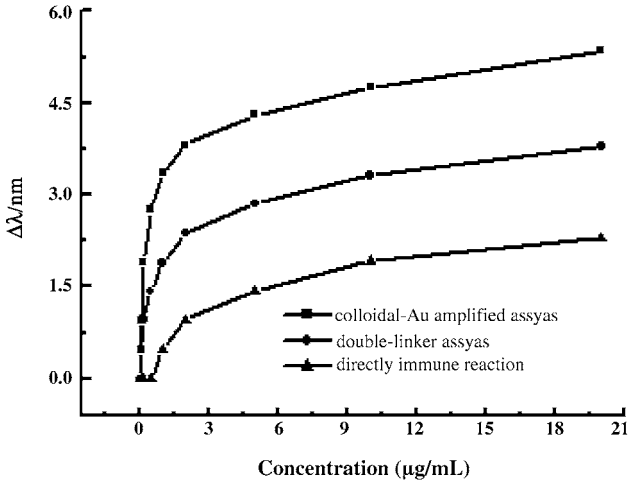


Fig. 4. Biosensor response of three assays in dependence on the applied transferrin concentration.

## 4. Conclusion

The use of colloidal Au nanoparticles has been shown to enhance the sensitivity of a wavelength modulation SPR biosensor. In previous studies the colloidal Au nanoparticles were linked to surface on analyte as labels for amplifying SPR biosensor signals. In this paper, the colloidal Au nanoparticles were used as active components to detect transferrin. The immobilization of colloidal Au nanoparticles on the MEA-coated surface was described. The binding of the colloidal Au nanoparticles leads to large changes in the resonance wavelength and transferrin can be determined in the concentration range of 0.05–20 μg/mL. A double-linker assay measurement for transferrin was demonstrated. This double sensing membrane method may also amplify the SPR signals and transferrin can be determined in the concentration range of 0.1–20 μg/mL. However, transferrin can only be determined in the concentration range of 1–20 μg/mL by directly immune reaction. The results of the experiments indicate that colloidal Au amplified and double-linker assay have extraordinary potential in wavelength modulation SPR application.

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## References

[1] J. Peter, C. Unverzagt, W.-D. Engle, D. Renauer, C. Seidel, W. Hosel, Biochim. Biophys. Acta 1380 (1998) 93.
[2] S. Goldwurm, C. Casati, N. Venturi, et al., Hematol. J. 1 (2000) 390.
[3] A.-R. Zhou, R.-B. Huang, Medical Biochemistry, The United Press of Beijing Medical University and Xiehe Medical University of China, Beijing, 1997, p. 239.
[4] A.G. Das, A. Abbi, Am. J. Hematol. 72 (2003) 158.
[5] S.A. Franziska, A. Pierre, D. Lena, D.C. James, F.H. Richard, Am. J. Clin. Nutr. 74 (2001) 776.
[6] A.M. Spiekerman, Anal. Chem. 67 (1995) 429.
[7] H. Li, H. Sun, Z.M. Qian, Trends Pharmacol. Sci. 23 (2002) 206.
[8] C.E. Gerber, G. Bruchelt, B. Gotze-Speer, C.P. Speer, J. Immunol. Methods 233 (2000) 41.
[9] R. Gottschalk, R. Wigand, C.F. Dietrich, G. Oremek, et al., Clin. Chim. Acta 93 (2002) 127.
[10] Q. Dong, D.Q. Yu, X.Y. Ye, W.R. Jin, Electrophoresis 22 (2001) 128.
[11] Z.Y. Wu, G.L. Shen, L.J. Xie, R.Q. Yu, Sens. Actuators B 71 (2000) 99.
[12] H.J.H. Kreutzer, J. Clin. Chem. Clin. Biochem. 14 (1976) 401.
[13] G.J. Anderson, A. Mackerras, U. Mack, L.W. Powell, J.W. Halliday, J. Lab. Clin. Med. 107 (1986) 59.
[14] G.D. Liu, K.S. Hu, G.L. Shen, R.Q. Yu, Analyst 125 (2000) 1595.
[15] Z.Z. Li, F.C. Gong, Sens. Actuators B 99 (2004) 562.
[16] C.X. Lei, J. Wu, H. Wang, G.L. Shen, R.Q. Yu, Talanta 63 (2004) 469.
[17] R.J. Green, R.A. Frazier, K.M. Shakeshe, M.C. Davies, C.J. Roberts, S.J.B. Tendler, Biomaterials 21 (2000) 1823.
[18] B.K. Oh, Y.K. Kim, W. Lee, Y.M. Bae, W.H. Lee, J.W. Choi, Biosens. Bioelectron. 18 (2003) 605.
[19] R. Karlsson, J. Mol. Recognit. 17 (2004) 151.
[20] T. Wink, S.J. Van Zuilen, A. Bult, W.P. Van Bennekom, Anal. Chem. 70 (1998) 827.
[21] L.A. Lyon, M.D. Musick, M.J. Natan, Anal. Chem. 70 (1998) 5177.
[22] M. Zayats, S.P. Pogorelova, A.B. Kharitonov, O. Lioubashevski, E. Katz, I. Willner, Chem. Eur. J. 9 (2003) 6108.
[23] X. Liu, Y. Sun, D.Q. Song, Q.L. Zhang, Y. Tian, S.Y. Bi, H.Q. Zhang, Anal. Biochem. 333 (2004) 99.
[24] D.Q. Song, Y. Mu, X.J. Zhao, H.Q. Zhang, J. Sun, F. Liang, Y.B. Cao, Q.H. Jin, Chem. J. Chin. Univ. 21 (2000) 686.
[25] X. Liu, D.Q. Song, Q.L. Zhang, Y. Tian, H.Q. Zhang, Talanta 62 (2004) 773.
[26] X. Liu, J.Y. Wei, D.Q. Song, Z.W. Zhang, H.Q. Zhang, G. Luo, Anal. Biochem. 314 (2003) 301.
[27] J. Holmola, I. Koudla, S.S. Yee, Sens. Actuators B 54 (1999) 16.
[28] S. Kubitschko, J. Spinke, T. Bruckner, S. Pohl, N. Oranth, Anal. Biochem. 253 (1997) 112.
[29] E. Hutter, J.H. Fendler, D. Roy, J. Phys. Chem. B 105 (2001) 11159.
[30] L.A. Lyon, M.D. Musick, P.C. Smith, B.D. Reiss, D.J. Pena, M.J. Natan, Sens. Actuators B 54 (1999) 118.