



The development of lateral flow immunoassay strip tests based on surface enhanced Raman spectroscopy coupled with gold nanoparticles for the rapid detection of soybean allergen β -conglycinin

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ABSTRACT

β -Conglycinin is an important storage protein in soybean, which can potentially cause food allergies in human. In this study, a sensitive mouse monoclonal antibody (3D11 mAb) with a high affinity was prepared, and sandwich lateral flow immunochromatographic detection strips were developed for the rapid detections of the soybean allergen β -conglycinin. The 3D11 mAb was combined with a rabbit polyclonal antibody in order to establish strips. The titer of 3D11 mAb was $1:2.56 \times 10^5$. The affinity constant of the 3D11 mAb was 9.6×10^9 . The lowest detection limit with the naked eye of the double antibody sandwich strips was $1 \mu\text{g/mL}$. In addition, chemical molecules p-aminothiophenol with colloidal gold were used as Raman enhancement signals in order to achieve quantitative detections of the β -conglycinin. It was determined in this study that the practical working range of the β -conglycinin concentrations was between 160 ng/mL and $100 \mu\text{g/mL}$ with the developed assay.

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1. Introduction

β -Conglycinin exists in the form of a soluble heterotrimeric glycoprotein. β -Conglycinin generally accounts for approximately 30% of the storage protein in soybean. There have been found to be so many advantages of β -conglycinin associated with human health. Previously, hundreds of studies have focused their attention on those benefits. For example, soybeans contain abundant essential amino acid and less saturated fat [1]. And soybeans exert their protective effects against heart disease, cancer, and osteoporosis [2]. It has been determined that β -conglycinin has the ability to increase satiety and reduce hunger in cholecystokinin secretions. Since β -conglycinin peptone in the lumen stimulating endogenous cholecystokinin release with direct acceptance to the intestinal cells, thus it suppresses food intake [3]. Furthermore, previous studies have revealed that a certain percentage of β -conglycinin included in daily protein intake can promote cholesterol reduction and reduce the incidence of atherosclerosis [4]. Kewabeta found that β -conglycinin had mediated or influenced the hepatic lipid metabolism by increasing plasma adiponectin to increase insulin sensitivity [5]. Since β -conglycinin peptides of enzyme-mediated production stimulate the growth of bifidobacterial, the protein may be beneficial to improve gastrointestinal health [6].

However, along with the benefits, it has been found β -conglycinin can result in food allergy reaction which may potentially harm human

health. It was determined that between 1% and 6% of babies are affected by soy allergies. Also, the incidence of adult soy allergies has continuously increased due to the escalation in soy product consumption in recent years. Symptoms of allergies to soy may include allergic reactions to the skin, digestive tract, or respiratory tract, similar to yak diarrhea [7]. Chen found that $7 \mu\text{g}$ of crude soybean extract caused redness and inflammation in piglets within 5 min, and more than $200 \mu\text{g}$ of β -conglycinin caused the inflammation areas to enlarge [8]. Therefore, for human dietary safety, the detection of allergens has suddenly become particularly important. As shown in Table 1, the current detection methods for β -conglycinin or soy protein involve chromatography [9,10], enzyme-linked immunosorbents [11–15], polymerase chain reactions [16,17], and so on. Previously, Geng summarized and discussed the current methods available for the identification and quantification of soybean allergens when completing the broader safety assessments of genetically modified crops [18]. At the present time, detection methods for soybean allergenic proteins, such as Gly m Bd 28K [19], Gly m 4 [20], Gly m Bd 30K [21], glycinin [22], Gly m 8 [23] have been developed. Although these detection methods are diverse, each has its own features and advantages. Due to the characteristics of high accuracy, sensitivity and convenience, gold immunoassay strips assays are often chosen to achieve the rapid analysis of β -conglycinin.

Meanwhile, a Raman spectrum is the plot of light intensity expressed as arbitrary units, or counts, versus the frequency of scattered light in frequency units. This surface-enhanced Raman scattering technique has been found to have high sensitivity, high affinity, and fingerprint resolution, which can effectively enhance the signal strength by 6

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Table 1Comparison of the different analytical methods for the detection of β -conglycinin.

Analytical method	Aimed compound	Linearity range	Detection limit	Ref.
HPLC	β -Conglycinin			[9]
HPLC/MS	β -Conglycinin α subunit's synthetic peptide	1.6–500 ng/mL	0.14 ng/mL	[10]
ELISA	β -Conglycinin α' subunit's epitope peptide		2 ng/mL	[11]
ELISA (indirect competition method with rabbit pAb)	β -Conglycinin	20–320 ng/mL		[12]
ELISA (double antibodies sandwich method)	β -Conglycinin	3–100 ng/mL	1.63 ng/mL	[13]
Colloidal gold immuno-chromatography	β -Conglycinin	100–6400 ng/mL	110.5 ng/mL	[15]
PCR	Soybean PCR product Le1	1–70 ng/ μ L		[16]
PCR	Soy protein isolate	5.75 pg DNA	0.1 g/kg	[17]

to 10 orders of magnitude [24]. Therefore, Raman spectroscopy was used in this study to detect p-aminothiophenol (4-PATP), which was modified on the gold particle surfaces to amplify the signals [25]. The development of lateral flow immunoassay strip tests based on surface enhanced Raman spectroscopy (SERS-LFA) was aimed at achieving a higher prediction accuracy. Based on the preparation of β -conglycinin antibodies, a double antibody sandwich method for the rapid detection of β -conglycinin and a Raman enhanced immunoassay with colloidal gold as active substrate was established. The detection method of soy allergens presented in this study will provide a convenient and quick detection tool for consumer foods with allergic potential.

2. Material and methods

2.1. Material and apparatus

In this study, SPF BALB/c female mice (8 to 10 weeks of age) were provided by the Zhengzhou University Laboratory Animal Co. Ltd. (Zhengzhou, China). The soybean isolate protein used in this study was purchased from a local supermarket. The Freund's complete adjuvant (FCA), Freund's incomplete adjuvant (FIA), 50% PEG1500, and HAT/HT medium supplement were purchased from Sigma-Aldrich (St. Louis, MO, USA). The horseradish peroxidase-labeled goat anti-mouse immunoglobulin (GaMlgG-HRP), horseradish peroxidase-labeled goat anti-rabbit immunoglobulin (GaRlgG-HRP), SDS-PAGE Gel Kit, and TMB single-component substrate solution were supplied by the Solarbio Science and Technology Co. Ltd. (Beijing, China). The chemiluminescent HRP Substrate (ECL) was purchased from Millipore (USA). The fetal bovine serum was supplied by the Zhejiang Tianhang Biotechnology Co. Ltd., and the RPMI-1640 medium was obtained from Hyclone. This study purchased chloroauric acid ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$, 99.9%) and citrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$, 99.9%) from the Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China), and the p-aminothiophenol (4-PATP, 98%) were obtained from the Aladdin-Reagent Co., Ltd. (Shanghai, China). In addition, various gels and rotator systems (Tanon), along with such equipment as a Raman spectroscope (LabRAM HR-Evolution), Multiskan FC Microplate reader, magnetic stirrer, Nanodrop 2000/2000C spectrophotometer (Thermo), inverted microscope (BM), water jackets and series II CO2 Incubator (Thermo Scientific) were used in the present study's experimental processes.

2.2. Production and characterization of monoclonal antibodies

The β -conglycinin was separated and extracted via an isoelectric precipitation method, and purified by an ammonium sulfate precipitation method, according to procedures presented by He et al. [26] After several primary immunization processes, this study selected a few mice which had been determined to have high-titer and high sensitivity characteristics for the next experimental process. The mouse spleen cells and myeloma cells were fused into hybridoma cells using a cell fusion technique, and the selective medium (HAT or HT) allowed the fusion cells to survive. Then a natural β -conglycinin protein was used to

screen the major positive clones by indirect enzyme-linked immunosorbent assay (iELISA). Cell lines were obtained by a limited dilution of the positive clones. A large number of specific monoclonal antibodies were prepared by injecting cells into the intraperitoneal cavities in order to induce ascites [27]. The specificity and immunological properties of the monoclonal antibodies were identified by Western Blot and sodium dodecyl sulfate- polyacrylamide gel electrophoresis (SDS-PAGE) procedures.

2.3. Preparation of the immunological probes

This study referred to the method of Ni et al. [28], in which 1.0 μL or 2.5 μL of 1 mmol/L 4-aminothiophenol ethanol solution was added to 1 mL colloidal gold (approximately 30 nm, 0.2 M K_2CO_3 adjusted to a pH of 7.5) and magnetically stirred for 12 h. After centrifugation at 10,000 r/min for 10 min, the supernatant was removed and the precipitate was resuspended in a certain volume of ultrapure water (pH of 7.5). This procedure was repeated twice in order to wash away the free PATP and resuspended. The gold nanoparticles-PATP conjugates (referred to as AuNPs-PATP) were prepared.

The AuNPs-PATP solution was adjusted to a pH of 8.0 according to the optimal pH adjustment of the labeled antibody in order to avoid the aggregation of the gold particles. 1 mg/mL of β -conglycinin rabbit antiserum were added to 1 mL of pH-adjusted colloidal gold solution with an optimum concentration prior to being constantly stirred overnight. The β -conglycinin rabbit polyclonal antibody was supplied via laboratory preservation. Following the addition of 100 μL of 10% (w/v) bovine serum albumin (BSA) solution as a blocking agent, the solution mixture was left standing undisturbed for 3 h. Because of the good blocking effect of BSA, it effectively eliminated the adsorption between IgG and the surface of gold particles due to the hydrophobic effect or the formation of Au—S bonds. Then, the solution was centrifuged at 12,000 r/min for 30 min. The precipitation was suspended in a 20 mmol/L sodium borate buffer (PBS). After washing three times, the colloidal gold conjugate was suspended in an appropriate amount of PBS with 1% BSA, after which the colloidal gold could be either seen or detected on the test strips. The Raman immunoprobe (abbreviated in this study as Au-PATP-pAb) was obtained and then stored under refrigerated condition at 4 °C [25].

2.4. Fabrication of the colloidal gold immunoassay strips

In the current study, double antibody sandwich mode colloidal gold immunochromatography test strips were developed for the rapid detection of β -conglycinin based on the of rabbit-derived pAb and murine mAb. This study prepared the colloidal gold nanoparticle-labeled rabbit polyclonal antibody as a signal for the immunochromatography. Then by reference to the direct sandwich ELISA method for pecan detection presented by Liu et al. [29], this study designed a novel method for fabricating two types of strips and then provided the experimental assessments results. One type of strip was based on a combination of antigen and monoclonal antibody, in which the antigen was dispensed at the

site of the test line (T-line). Another type was based on the reaction of two antibodies and antigen, referred to as a “sandwich strip”, in which the monoclonal antibody was coated at the test line. The strips assemblage contained a sample pad, conjugate pad, nitrocellulose membrane and an absorbent pad. Cutting and spraying materials included automated instruments, such as a Bio-Dot CM 4000 Cutter, XYZ 3000 Dispensing Platform and so on. On the nitrocellulose membrane, the antigen or monoclonal antibody at a concentration of 1 mg/mL were dispensed as the test line. Then the control line (C-line) was dispensed with both goat anti-rabbit IgG. Then, 2 μ L of immunoprobe was dripped at the position of the conjugate pad. After drying, the finished strip products were sealed and stored at 4 °C with desiccant until used. When the products were put into use, the sample pads of the assembled test paper were immersed in 100 μ L of the β -conglycinin standard solution, water, or samples, respectively, and then placed horizontally for 2 min to observe the results.

2.5. Testing procedures and principles

For the “sandwich strip”, it was observed that when the sample solution reached the conjugate pad, the gold-labeled antibody or Raman immunoprobe had bound to the β -conglycinin from the sample solution, and the conjugates flowed to the water-absorbing side due to the capillary action. When the conjugates crossed the nitrocellulose membrane, they were caught by the mAb at the T-line. The complexes developed coloration and Raman intensity which were detected using Raman spectroscopy. When the intensity of the Raman signal was proportional to the antigen concentration, the sample was said to be positive; However, if there was no Raman signal at T-line, the sample was believed to be negative. The C-line coated goat anti-rabbit IgG was used to judge the validity of the test strips and the probes. For example, if coloration had developed at the C-line, then the test strip system was considered to be normal. Otherwise, the test strip was considered to be invalid.

2.6. SERS measurements

A Raman microscope couples a Raman spectrometer to a standard optical microscope, allowing high magnification visualization of a sample and Raman analysis with a microscopic laser spot. When a high intensity laser light source shines and focuses on the sample surface, molecule scatters incident light. Among them, a small amount of light (typically 0.0000001%) is scattered at different wavelengths (or colors) and Raman Scatter is made.

In the present research study, with the labeled polyclonal antibody-monoclonal interception mode, a Raman spectrometer was used to establish a standard curve of the Raman signal intensity and antigen concentration. Molecule 4-aminothiophenol as a Raman signal was modified on the colloidal gold in order to bind to the antibody protein for preparing a Raman immunoprobe. The samples of β -conglycinin were diluted five-fold with 0.015 M of PBS for the purpose of testing the suitable concentration of antigen for the immunoprobe with the first strip (for example, 8.5, 1.7, 0.34, and 0.07 mg/mL). The β -conglycinin samples were then diluted 10-fold with 0.015 M of PBS in order to test for sensitivity with the “sandwich strip” (for example, 100, 10, and 1 μ g/mL). The specificity of the “sandwich strip” was evaluated using Albumin from bovine serum (BSA) and other edible protein (for example, peanut protein, wheat germ albumin, and wheat germ globulin) as test antigens. Meanwhile, a blank control was set.

The Raman spectrometer had an excitation wavelength of 632.8 nm, laser power of 10 mW, and an integration time of 10 s. The immunoprobe and PATP were dropped on a coin, wafer or strip as substrate and measured under Raman spectrometer. Different with coin and wafer, the nitrocellulose membrane was rough and porous, the immunoprobe are randomly distributed over and inside the membrane. Khlebtsov et al. obtained SEM images for GERT immunocomplexes in the LFIA area by using a backscattered-electron detector [30]. It is

helpful for Raman spectrometer to focus the “hot-spot” with immunoprobe. The optimal detection way was selected at first. After that, the signal on the T-line of “sandwich strips” was tested. Each measurement collected three points evenly on the T-line, and their average value was taken as valid data by which to ensure the authenticity of the data [31]. This “sandwich strip” with a Raman signal PATP aiming at quantitative detection of β -conglycinin was called as surface-enhanced Raman scattering lateral flow immunochromatographic assay (SERS-LFA). In addition, in order to estimate the accuracy of the obtained data, milk samples containing 0.5, 5, and 50 μ g/mL of β -conglycinin, respectively, were tested by SERS-LFA using the same batch of strips, and the accuracy rates were indicated as recovery rates. The precision was indicated as relative standard deviations (RSD).

2.7. Verification of the double antibody sandwich ELISA

This study's double antibody sandwich ELISA method was built for the purpose of verifying the effects of the SERS-LFA. 3D11 mAb was used as the capture antibody, and rabbit-derived pAb was used as the detection antibody. In addition, goat anti-rabbit IgG antibody was used as the enzyme-labeled secondary antibody. The basic operational steps for the double antibody sandwich ELISA were as follows: 1. The capture antibody was first diluted by 2000-fold with a 0.1 M carbonate buffer (CBS, pH 9.6). Then, 100 μ L of capture antibody solution (mAb) were placed in the wells of a 96-well plate and coated overnight at 4 °C. The wells were washed three times with PBST, after which the water removed with paper but moisture in the wells was maintained (simply referred to in this study as washed plates); 2. 200 μ L of blocking solution (skimmed milk) was added to each well, and the specimens were incubated at 37 °C for 1 h. Similarly, the aforementioned washed plate procedures were carried out; 3. the β -conglycinin protein was diluted a phosphate buffer (PBS, pH 7.2) and 100 μ L were added to the microplate. Also, a phosphate buffer was only set as a blank control. The specimens were incubated for 1 h at 37 °C, and washed plate procedures were again carried out; 4. the detection antibody (pAb) was diluted 3000-fold with a blocking solution and 100 μ L were added into each well. The specimens were incubated for 30 min at 37 °C, prior to the washed plate procedure being carried out; 5. the enzyme-labeled secondary antibody was diluted 5000-fold (corresponding to the detection antibody), and 100 μ L were added to each well. The specimens were then incubated for 30 min at 37 °C; 6. during the final steps, 100 μ L of TMB solution were added to each well in order to observe any color reactions within a 10 min time frame. Finally, 50 μ L per well of 2 mol/L H_2SO_4 were used to terminate the reaction, and the $\text{OD}_{450\text{ nm}}$ values were obtained using a microplate reader.

In the present study, the logarithm of the concentration of the protein solution with different concentrations was the abscissa and the $\text{OD}_{450\text{ nm}}$ was the ordinate. The standard curve of the double antibody sandwich ELISA was drawn in order to determine the linear range of the ELISA method [32].

3. Results

3.1. Preparation of the β -conglycinin and mAbs

The purity levels of the soy protein isolate and the 5 μ g of β -conglycinin antigen were identified using gel electrophoresis (Fig. 1b to c). During the primary immunization process, the serum titer and fusion effects of the No. 5 mouse were observed to be better than those of the other mice. Then, following the screening with ELISA, and the cloning and ascites preparation in vivo, the mAb was obtained. The biological activities of the 3D11 mAb were tested using a Western Blot method (Fig. 1d), and a 1:15,000-fold dilution of the 3D11 mAb was reacted with β -conglycinin. Also, a 1:20,000 dilution of enzyme-labeled sheep anti-mouse secondary antibody was used for chemiluminescence. As can be seen in Fig. 1d, bands of β -conglycinin α' subunit and α subunit

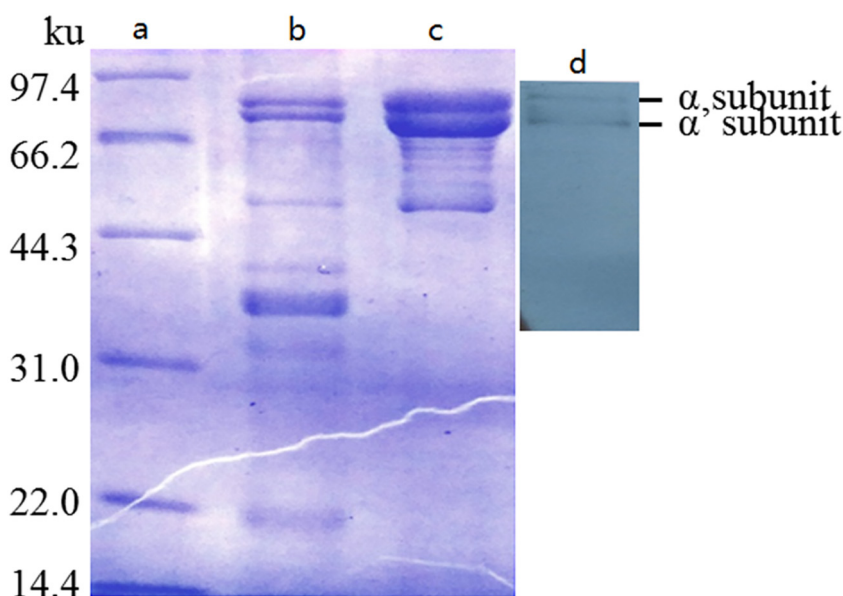


Fig. 1. Identification of the β -conglycinin and 3D11 mAb specificity using SDS-PAGE and Western Blot methods: a) Marker; b) SPI isolate soybean protein; c) β -conglycinin; d) 3D11 mAb react with β -conglycinin.

were visible on the NC membrane, which indicated that the β -conglycinin α' subunit and α subunit may have been specific antibody epitopes for 3D11 mAb [33]. However, due to the high homology of the two subunits, this mAb may potentially have been a specific antibody against common epitope for the two subunits.

3.2. Titers, inhibitive titers and affinity constant curves of the 3D11 and 3C9 mAb detected by ELISA

The supernatant titers of the cells were selected to be the maximum dilution factors of the antibodies which were 2.1 times larger than the negative control [34]. The monoclonal antibody titers were evaluated by the dilution factor in which the OD value was equal to 0.1. The titers of 3D11 and 3C9 were $1:2.56 \times 10^5$ and $1:1.28 \times 10^5$, respectively; the affinity constants (K_a) of the 3D11 and 3C9 mAb were 9.6×10^9 and 1.42×10^{11} L/mol [35], respectively, which represented high affinity levels [36]. The affinity constant curves are shown in Fig. 2a. In addition, as detailed in Fig. 2b, the logarithm of B/B_0 and competitive protein concentration in the range of 93.75 to 3000 ng/mL of the 3D11 mAb were linear, and its IC_{50} value was 911 ng/mL [37]. The IC_{50} value of 3C9 mAb was determined to be 2.91 μ g/mL. It was observed that the 3D11

mAb (with its antibody subtype belonging to IgG1) had a better IC_{50} value of 911 ng/mL. Therefore, it was selected to be used in the development process of the LFA.

3.3. Characterization and optimization of the binding conditions of immunological probes

As shown in Fig. 3A, a pH level of 8.5 was chosen as the optimal pH for the colloidal gold of the rabbit polyclonal antibody using a Mey stability test. Due to the visual difference related to different observes in the pH precision test paper, this study chose a pH of 8.5 of 9 mL of colloidal gold with 130 μ L of 0.2 mol/L K_2CO_3 in the experimental tests. The optimum labeling concentration for the rabbit polyclonal antibody was determined in order to maintain a stable red color and was diluted 400-fold (Fig. 3B). The actual labeling amounts were increased by 20% according to the experimental results. In other words, 1 mL of colloidal gold solution was labeled with 0.5 μ L of rabbit polyclonal antibody. As previously presented by Arielle, the colloidal gold particle size will affect Raman intensity [38,39]. Therefore, this study utilized three types of gold in the experimental tests in order to determine which achieved the best results. The particle size is about 23 nm, 30 nm, 49 nm,

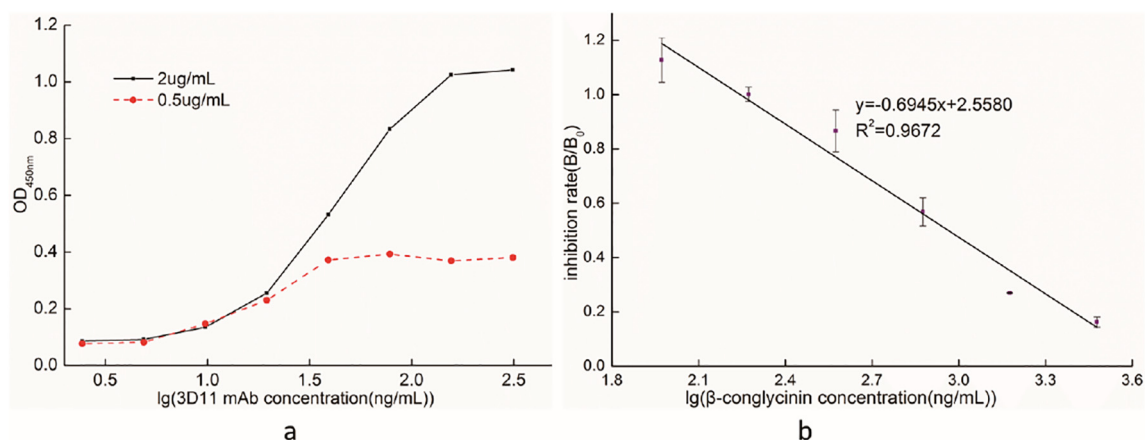


Fig. 2. Affinity constant and inhibitive titers curves of the 3D11 mAb against β -conglycinin by indirect competitive ELISA.

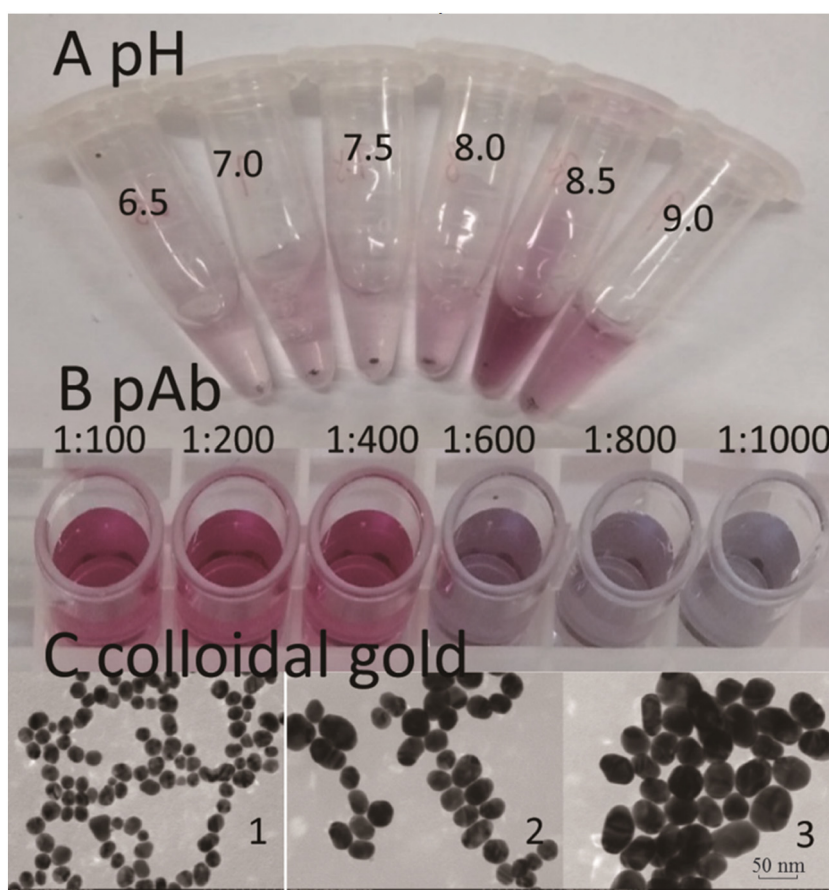


Fig. 3. Effects on the colloidal gold-labeled rabbit polyclonal antibody of different pH condition and pAb concentration.

respectively. Then, in accordance with the results, we choose the No. 2 colloidal gold (about 30 nm) was selected for the subsequent experiments, as shown in Fig. 3C.

This study obtained the PATP-AuNPs-pAb immunoprobes, with Raman spectroscopy similar to that shown in Fig. 4. There existed two distinct spikes, at 1080 and 1589 cm^{-1} , respectively. The SERS peak intensities at 1589 cm^{-1} were used to construct calibration curves. When the carriers like coin, wafer or strips were placed on the microscope stage, the infrared light source illuminates immunoprobe surface to

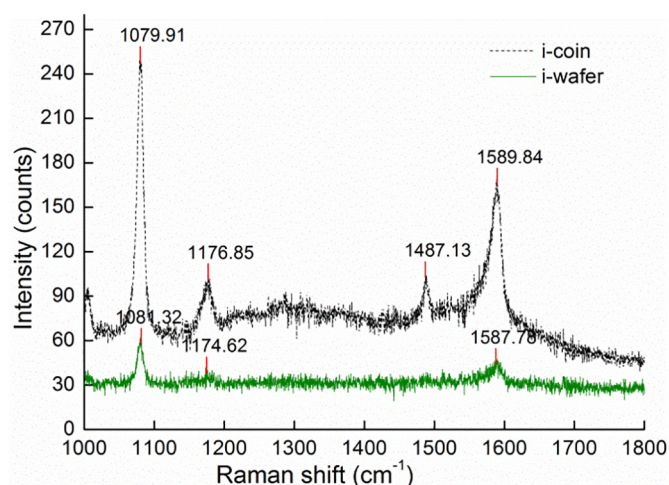


Fig. 4. PATP modified immunoprobe Raman spectroscopy on different bases.

generate signal. It was found that when a coin was chosen as the carrier, the immunoprobe had stronger spectrum signals when compared to that achieved using a wafer. Compared with Liu's [40], the surface Raman spectra of PATP-capped Au NPs was similar, which appeared the Raman intensities of peaks at ca. 1076 ($\nu_{\text{C-C}} + \nu_{\text{C-S}}$, a_1), 1176, 1487, and 1585 ($\nu_{\text{C-C}}$, a_g) cm^{-1} [41]. And his explanation was intriguing. He found when the particle size became larger from 15 nm to 70 nm, the Raman intensities of peaks raised. And after laser illuminated for 1 min than 1 s, the peaks had a red shift and an obvious increase in the Raman intensities of peaks at ca. 1137, 1387, 1429, and 1573 cm^{-1} was found. The size and laser illumination time may be the important factors. Zhang believed that O_2 promotes the formation of 4,4'-dimercaptoazobenzene, a new substance containing -N=N- double bonds, which have been found to lead to the appearance of surface-enhanced Raman signals [42]. However, some researchers believe that only the rough surface of the gold colloid can obtain high sensitivity Raman signal [43]. The SERS enhancement mechanism mainly focuses on two modes: Electromagnetic field enhancement and chemical enhancement. However, with the results obtained in the current research experiments, this study has provided valuable data support for future research in this field of study.

3.4. Sensitivity and specificity of the colloidal gold lateral flow test strips

Fig. 5 demonstrated the quantitative assay results using LFA strips for β -conglycinin detection. As detailed in Fig. 5A, the first strips used in the detection of the concentration levels of β -conglycinin were diluted to 8.5 mg/mL, 1.7 mg/mL, 340 $\mu\text{g/mL}$, 68 $\mu\text{g/mL}$, 13.6 $\mu\text{g/mL}$, respectively, as sample solutions dispensed on the test line. Then, a "sandwich strip" was used (Fig. 5B and C), in which 0.6 μL of a 1:10

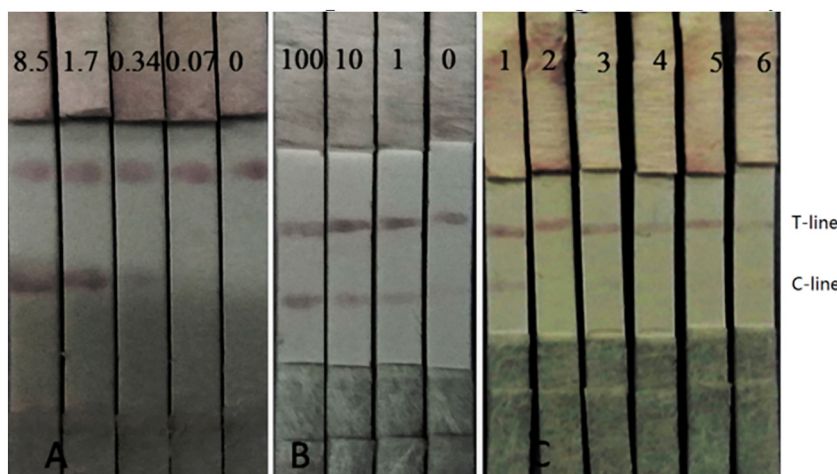


Fig. 5. Digital photograph of the test line area in the NC membranes: A. Different concentration of β -conglycinin on the T-line (0.07 to 8.5 mg/mL); B. β -conglycinin with different concentration as samples (1 to 100 μ g/mL); C. 5 mg/mL of the different proteins as samples on the T-line: 1. Glycine; 2. BSA; 3. peanut protein; 4. wheat germ albumin; 5. wheat germ globulin; 6. physiological saline or ultrapure water.

dilution of monoclonal antibody was dripped at the test-line position, and 0.6 μ L of a 1:10 dilution GaRIgG-HRP was administered at both ends the control line. The membrane was then blow dried. First of all, the assays were performed in the 0 to 8.5 mg/mL range. It was observed that with the increasing β -conglycinin concentrations, the coloration of the T-line became weaker. As shown in Fig. 5A, 2 μ L of gold-labeled rabbit antibody probes were able to detect the 340 μ g/mL, and the 68 μ g/mL β -conglycinin had not been successfully detected. In addition, the sensitivity of the double antibody sandwich strips was evaluated with 0 to 100 μ g/mL of β -conglycinin in 10 mM of PBS. The lowest detection limit with the naked eye was 1 μ g/mL, as illustrated in Fig. 5B. The strips were determined to be quite specific without cross reactivity with the soy protein and its analogues in the food products (Fig. 5C). However, there was some cross reactivity observed with the glycine sample. It should be noted that that sample may have contained some β -conglycinin. In the present study's experiments, no cross-reactivity was observed with peanut protein, wheat germ albumin, wheat germ globulin, or BSA.

3.5. Antibodies applied in the Raman detection processes

While observing the color changes and Raman signals of T- and C-lines, the curve relationships were established according to the Raman

peak intensities at 1589 cm^{-1} and concentration data. Then, in accordance with the logarithm of the Raman intensity and β -conglycinin concentration, it was confirmed that the SERS-LFA method satisfied $y = 65.14x + 113.92$ in the range of 160 ng/mL to 100 μ g/mL (Fig. 6a). The error bars indicate the RSD from three independent measurements. In addition, according to the 3σ principle of method detection limits, the concentration corresponding to three times the blank noise value was selected as the detection limit. The detection limit of this method was 32 ng/mL. This study's results of the addition and recovery of the β -conglycinin from the SERS-LFA test paper are shown in Table 2. The rates were considered to be within the normal range, indicating that the SERS-LFA test strips had achieved good accuracy results.

3.6. Verification of the double antibody sandwich ELISA method

In the current study, a standard curve was established in order to arrive at a linear range. As shown in Fig. 6b, it was found that the combination of pAb and mAb had assisted in the detection of the soy protein concentrations ranging between 78.125 ng/mL and 10 μ g/mL. In addition, this study found that the corresponding enzyme-linked immunosorbent assay had similar linear concentration ranges relative to the SERS-LFA test strips. It was determined that the SERS-LFA test strips

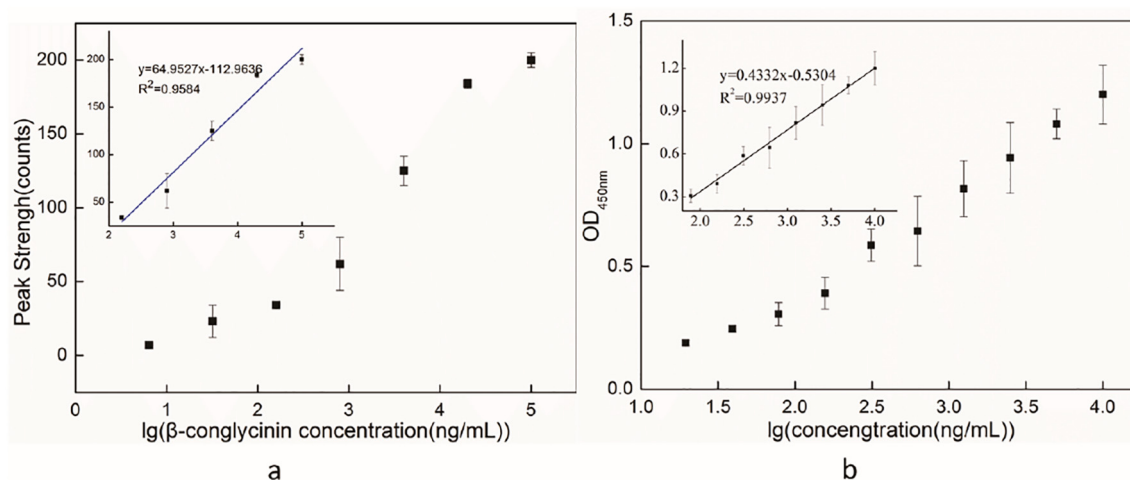


Fig. 6. Calibration curves of the SERS-LFA strips for β -conglycinin between the relative Raman intensity and the β -conglycinin concentrations; Curves of the double antibody sandwich ELISA for the β -conglycinin.

Table 2Recovery rate and relative standard deviations of milk added with β -conglycinin by SERS-LFA.

Sample	Original concentration of β -conglycinin ($\mu\text{g/mL}$)	Recovered concentration of β -conglycinin ($\mu\text{g/mL}$)	Recovery rate (%) (n = 3)	RSD (%)
Skimmed milk	0.5	0.58 ± 0.05	116 ± 9.2	8
	5.0	5.2 ± 0.3	103 ± 6.4	6
	50	48.7 ± 2.3	97.5 ± 4.6	5

could potentially have a wider detection range, and hopefully achieve super sensitive β -conglycinin detection results.

4. Discussion

In the present study, dual-antibody sandwich immunochromatographic test strips were prepared using rabbit anti- β -conglycinin polyclonal antibody as the detection antibody and 3D11 mAb as the immobilized capture antibody. A Raman immunoprobe was prepared by modifying colloidal gold with 4-aminothiophenol and then binding it to the antibody for the purpose of establishing surface-enhanced Raman lateral immunochromatographic test strips for rapid protein detection. It was found that the SERS-LFA method had the ability to accurately detect the β -conglycinin content in samples ranging from 160 ng/mL to 100 $\mu\text{g/mL}$, which satisfied $y = 65.14x + 113.92$. The SERS-LFA detection limit was confirmed to be 32 ng/mL.

The Raman test strips for the β -conglycinin detection designed in this study were a combination of a surface enhanced Raman technique and immunochromatography technology. The immunochromatographic test strips themselves had displayed the advantages of fast detection results, low cost, and high-throughput detection in the field. Therefore, it was found that the surface-enhanced Raman scattering technology had significantly enhanced the sensitivity of the test strips, and the detection ranges no longer were limited to just visual color distinctions. In regard to the results achieved in this study's experimental processes, it was found that improvements in the experimental conditions would be required in order to achieve lower detection limits, which would subsequently lead to better advantages in the surface-enhanced Raman scattering techniques. However, due to the promotion of portable Raman instruments, the surface-enhanced Raman immunological detection method has been found to be more convenient in practical applications. The method presented in this study could potentially realize that the rapid and accurate identifications of soy allergen ingredients on food labels, and the technology could provide improved convenience for food export enterprises in the future. Also, the method may provide an effective monitoring tool for the development of desensitized foods. Therefore, this study successfully laid a foundation for the development and research of new immunochromatography technology.

Recently, portable SERS readers for lateral flow assays have been introduced with line illumination abilities using custom-made fiber optic probes. These types of equipment are able to achieve ultrasensitive point-of-care testing in clinical chemistry, similar to the common gold test strips [44]. It is believed that there will be extensive research applied using new material and techniques for immunochromatographic test strips, and traditional confirmation methods for food allergens will be replaced. Such advancements will result in significant contributions to the innovation of detection technology and food safety in the future. This study showed that the proposed SERS-based LFA technique exhibits major potential to be a valuable tool in the early detection of specific allergens due to its excellent analytical sensitivity performance.

CRedit authorship contribution statement

Jun Xi: Conceptualization, Methodology, Writing - review & editing, Supervision, Funding acquisition. **Qiurong Yu:** Data curation, Formal analysis, Visualization, Writing - original draft.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.saa.2020.118640>.

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