

Immunocolorimetric assay based on amplified gold nanoparticles and magnetic separation beads for detection of sesame allergens in food

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Research Article

Keywords: Sesame allergen, Immunocolorimetric assay, Magnetic separation, Monoclonal antibody, seed growth liquid

Posted Date: May 20th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1635298/v1>

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Abstract

We had developed a magnetic separation immunocolorimetric assay to detect sesame allergens. Sesame monoclonal antibody (Ab) was modified to gold nanoparticles (AuNPs) as signal probes (AuNPs-Ab). Sesame allergens (SA) were attached to the carboxyl-functional magnetic polystyrene microspheres (MPMs) as capture probes (MPMs-SA). Based on the competition format, the capture probes competed with the sesame allergens in the sample to bind the corresponding signal probes. When sesame allergens were in the sample, two immune complexes (AuNPs-Ab@MPMs-SA and AuNPs-Ab@SA) were formed. The immune complex AuNPs-Ab@SA was used to quantify the sesame allergens in the sample. This immunoassay had a detection linear from 50 to 800 $\mu\text{g L}^{-1}$ with a limit of detection (LOD) of 45.53 $\mu\text{g L}^{-1}$. Based on the optimized conditions, the recovery results of sesame allergens in bread, biscuits, almond beverage and energy bars were between 82.50% and 116.67%. The LODs for the bread, biscuit, almond beverage and energy bar samples were 0.36, 0.36, 0.27 and 0.55 mg kg^{-1} respectively.

1. Introduction

Sesame, also known as flax, is one of the main oil crops in China. It has a broad range of applications, and owing to its high nutritional content, it is widely used as a condiment to add to baked goods and dishes. However, sesame has emerged as a common source of food allergens. Sesame allergic reactions, which occur in people of all ages, are immune reactions mediated mainly by IgE (Derby et al., 2005). For people who are allergic to sesame, the main symptoms include skin redness, urticaria, respiratory distress, angioedema, and gastrointestinal discomfort. In severe cases, the allergy can be life threatening (Li et al., 2017; Sarinho et al., 2017). According to epidemiological investigations, most patients with sesame allergy are infants and young children. According to the Bock survey, 21% of allergic patients are under 1 year old, 44% are between 1 and 1.5 years old, 23% are between 1.5 and 2 years old, and up to 50% are between 2 and 3 years old (Bock, 1987). In a survey of the prevalence of sesame allergy in several countries, it was found that 0.1–0.8% of children were allergic to sesame. The prevalence was 0.1% in the United States and Canada, 0.2% in Israel, and 0.8% in Australia (Dalal et al., 2002; Fox et al., 2013; Osborne et al., 2011; Soller et al., 2015; Sicherer et al., 2010). In Israel, sesame is the third most common food allergen after eggs and milk (Muraro et al., 2014). There are also reports of sesame allergy in many countries in Europe (e.g., Italy, Britain, Sweden, Switzerland) and Asia (Japan) (Asero et al., 2014; Della-Torre et al., 2013; Segal et al., 2016; Teodorowicz et al., 2016).

The main allergen proteins in sesame seeds, as confirmed by the International Union of Immunological Societies (IUIS), include 2S albumin (Ses i 1 and Ses i 2), 7S globulin (Ses i 3), oleosin (Ses i 4 and Ses i 5), and 11S globulin (Ses i 6 and Ses i 7) (Beyer et al., 2007; Beyer et al., 2002; Elide et al., 2001; Leduc et al., 2006; Wolff et al., 2003). 2S albumin accounts for approximately 25% of total sesame allergens and is rich in arginine, glutamine, asparagine, and cysteine residues (Mills et al., 2004; Wolff et al., 2004; Rajendran et al., 1988; Tai et al., 1999). Ses i 3 is the main allergen protein in sesame 7S globulin and has a molecular mass of 45 kDa (Orruno et al., 2011) Ses i 4 and Ses i 5 with a MW of 15–17 kDa are composed of 166 and 153 amino acids, respectively (Leduc et al., 2010). Ses i 6 (MW: 53 kDa) and Ses i

7 (MW: 56.6 kDa) consist of 459 and 497 amino acids, respectively. For sesame allergens Ses i 1 to Ses i 7, it is known that their sensitization reactions are mainly determined by their epitopes (<http://www.allergen.org/index.php>).

At present, the most commonly used detection methods for sesame allergens include nucleic acid detection, mass spectrometry (MS), enzyme-linked immunoassay (ELISA), and real-time fluorescent polymerase chain reaction (PCR) (Anfossi et al., 2018; Gerda et al., 2010; Lubber et al., 2014; Ma et al., 2019; Röder et al., 2013). Although these methods provide good detection limits and accuracy, their disadvantages include high detection costs, long detection times, and the requirement for expensive equipment and trained professionals.

In previous studies, gold nanoparticles have been used as nanocatalysts for signal amplification, achieving enhanced visualization and sensitivity (Bai et al., 2010; Qin et al., 2015; Zhan et al., 2010). Wang et al. found that sodium thiosulfate could effectively prevent the amplification reaction of gold nanoparticles, and developed a control immunogold amplification method to detect aflatoxin B1 (AFB1) (Wang et al. 2015). However, the actual samples tested were only for corn and not highly processed foods. Moreover, the effects of food matrix and food acidity in highly processed food on the seed growth liquid and the aggregation of gold nanoparticles were not studied. The detection time of the sensor, which was also not optimized, was 50 min, and may have resulted in low detection efficiency.

Therefore, to adopt the advantages from the signal amplification of gold nanoparticles and the efficiency of magnetic separation beads, in this experiment, the seed growth solution used cetyltrimethylammonium bromide (CTAB) as a surfactant and ascorbic acid (AA) as a reducing agent, to control the growth of AuNPs in solution. For the first time, we investigated the effect of ionic strength and pH on the agglomeration of gold nanoparticles. This was also successfully applied to the detection of four types of highly processed foods.

2. Materials And Methods

2.1 Materials and instruments

Carboxyl-functionalized magnetic polystyrene microspheres (MPMs) were purchased from Beisile Co., Ltd. (Tianjin, China). N-hydroxysuccinimide (NHS) and ethyl-3-[3-(dimethylamino)propyl] carbodiimide (EDC) were purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). The BCA protein quantification kit was obtained from Solarbio Science and Technology Co., Ltd. (Beijing, China). Chloroauric acid was purchased from Sigma-Aldrich Co., Ltd. (Shanghai, China). White sesame seeds were purchased from Tianjin, China. Sesame allergen monoclonal antibody was prepared in our laboratory. CTAB, AA, Na₂S₂O₃, NaCl, KCl, CaCl₂, and MgSO₄ were purchased from Sinopharm Group Co., Ltd. (Tianjin, China). The commercial sesame allergen ELISA kit was purchased from Preibang Co., Ltd. (Shandong, China). β-Lactoglobulin was purchased from Jiangtian Chemical Co., Ltd. (Tianjin, China). Peanut protein, walnut

protein, casein, and lupin protein were prepared in our laboratory. Bread, biscuits, almond beverage, and energy bars were purchased from a supermarket from Tianjin, China.

A UV spectrophotometer and microplate reader were used to detect the absorbance of gold nanoparticles. The TEM images of prepared AuNPs were obtained by a transmission electron microscope (TEM, FEI Tecnai F20 SuperTwinFEG, USA, <https://www.fei.com/>).

2.2 Preparation of signal probes and capture probes

The signal probes were synthesized based on a previously reported method (Sheng et al., 2011). First, 10 μL of $0.2 \text{ mol L}^{-1} \text{ K}_2\text{CO}_3$ was added to the prepared colloidal gold solution (1 mL) to adjust the pH. Then, 15 μL of anti-sesame antibody (4 mg mL^{-1}) was added into the solution and the mixture was kept at 4°C for 1 h. To prevent non-specific binding to the AuNPs, 20 μL of 20% BSA in the PBS solution and 10 μL of 20% PEG-20000 in the PBS solution were added to the mixture and incubated at room temperature for a further 30 min. After a centrifugation step (15 min, 425 rcf, 4°C), the supernatant was collected and centrifuged again (30 min, 10,621 rcf, 4°C). Finally, the precipitated AuNPs-Ab complexes were resuspended in working buffer and stored at 4°C until use.

The sesame allergen protein-modified MPMs were used as capture probes (Zhang et al., 2020). After being washed twice with MEST buffer (pH 5.5), 800 μL of 5 mg L^{-1} EDC/MES buffer and 800 μL of 5 mg L^{-1} NHS/MES buffer were added into 1 mL of MPMs/PBS solution. The solution was then adjusted to 2 mL with MES buffer and mixed at 37°C for 1 h. Subsequently, the activated MPMs were added to sesame allergens and reacted at 37°C for 3 h to obtain the MPMs-SA complex. Finally, the capture probes were blocked to non-specific binding by incubation in 5% BSA PBS buffer for 20 min, washed, and resuspended in PBS before storage at 4°C .

2.3 Preparation of growth solution

The preparation method for the seed growth liquid was reported by Man (Man et al., 2019). First, 182.2 mg CTAB was dissolved in 5 mL of water, and 12.5 μL of 0.1 M chloroauric acid was added and stirred until the solution turned to an orange color. Then, 75 μL of 0.1 M AA was dissolved in the solution and mixed until the solution became colorless. The entire reaction was completed under 37°C . The optimized conditions of the growth solution were shown in Table S1 in the supporting material.

2.4 Test procedure

The colorimetric immunoassay was conducted based on the competition detection format, and its schematic diagram was shown in the Scheme. Briefly, 50 μL of sample solution and 50 μL of capture probes in PBS solution were mixed together. Subsequently, 6 μL of the signal probe solution was added to the solution and gently mixed for 15 min. During the test, sesame allergens competed with the capture probes to bind to the signal probes and form the immune complex (capture probe@signal probe). Subsequently, the immune complexes were magnetically separated and removed, and 100 μL of seed

growth solution was then added to the supernatant for 10 min. The reaction was stopped by the addition of 20 μL $\text{Na}_2\text{S}_2\text{O}_3$. The microplate reader was used for quantitative detection.

2.5 Sample preparation

In this study, bread, biscuits, almond beverage, and energy bars were purchased from local supermarkets. Homogenized bread (10 g), biscuits (10 g), energy bars (10 g), and almond beverage (10 mL) were supplemented with different amounts of sesame allergens and placed in an ice-bath for 30 min. The four samples were degreased twice with acetone in the ice bath, and then each sample was subjected to protein extraction after the addition of PBS buffer (0.01 mol L^{-1} , pH 6.8). Finally, the supernatant was obtained by centrifugation, and the sample extract was obtained by passing through a $0.22 \mu\text{m}$ filter.

3. Results And Discussion

3.1 Characterization of AuNPs-Ab

AuNPs-Ab was prepared as the signal probe for the assay. The AuNPs were approximately 21 nm in size and uniform (Quesada-González et al., 2015; Wang et al., 2008) (Fig. 1A and Table S2). The AuNPs and AuNPs-Ab were characterized by the ultraviolet spectra. The maximum absorption wavelength of AuNPs and AuNPs-Ab was 520 nm and 524 nm, respectively (Fig. 1B). As the AuNPs bound to the antibodies, the particle size increased, which led to a redshift in the absorption peak, indicating that the AuNPs were successfully modified by the C2 antibody.

3.2 Mechanism of seed growth

In the presence of CTAB, Au^{3+} formed a stable $\text{CTA}^+[\text{AuBr}_4]^-$ complex with CTAB, shown by its orange color (step 1 in Fig. 2A and Fig. 2B). After the addition of AA, the $\text{CTA}^+[\text{AuBr}_4]^-$ complex was quickly reduced to $\text{CTA}^+[\text{AuBr}_2]^-$, and the solution turned colorless (step 2 in Fig. 2A and Fig. 2B). As shown in Fig. 2C, HAuCl_4 had no absorption between 300 nm and 600 nm. In the absence of CTAB, Au^{3+} ions could be directly reduced to Au^0 by AA, and an absorption peak appeared at 553 nm, indicating the formation of Au^0 . In the presence of CTAB, a significant absorption peak appeared at 394 nm, and the shoulder peak was located near to 462 nm. This may have been due to the ligand exchange between HAuCl_4 and CTAB. Finally, the AuNPs-Ab conjugate acted as a self-catalyst by accepting electrons and transferring them to Au^+ (step 3 in Fig. 2A). Au^+ ions were reduced to Au^0 , which was deposited on the surface of AuNPs-Ab, resulting in an increase in the size of the AuNPs. Finally, the addition of $\text{Na}_2\text{S}_2\text{O}_3$ stopped the growth of AuNPs by forming a stable complex of Au^+ and $\text{Na}_2\text{S}_2\text{O}_3$.

3.3 Optimization of the CTAB concentration in seed growth solution

CTAB can stabilize reduced Au^+ and prevent the aggregation of amplified AuNPs. Therefore, the concentration of CTAB is critical for assay optimization. As shown in Fig. 2D, when the CTAB

concentration was 12.5 mM, the absorption peak was significantly red-shifted by 16 nm. The absorption spectrum of amplified AuNPs-Ab (25 mM–75 mM) also had a slight red shift (2–3 nm). This might be because when the concentration of CTAB was low, the higher surface energy caused the aggregation of AuNPs-Ab. At a CTAB concentration of 100 mM, the UV absorption value of the amplified AuNPs-Ab was the highest and the solution was red (Figure S1A), indicating that no aggregation of AuNPs-Ab occurred. When the CTAB concentration increased from 125 mM to 225 mM, the UV absorbance of AuNPs-Ab decreased and the absorbance intensity consequently decreased. Therefore, 100 mM CTAB was chosen for further experiments.

3.4 Optimization of the HAuCl_4 concentration in seed growth solution

The effect of HAuCl_4 concentration on the amplification of AuNPs-Ab was studied, and the results were shown Fig. 2E. As the concentration of HAuCl_4 increased from 0.025 mM to 0.45 mM, the UV absorbance of AuNPs-Ab increased rapidly. When the HAuCl_4 concentration reached 0.25 mM, the UV absorbance of AuNPs-Ab was around 1.0, which could be used in further experiments. However, although the absorbance increased from 0.25 mM to 0.45 mM, a slight redshift occurred. This may have been due to the large size of the amplified AuNPs-Ab when the HAuCl_4 concentration was high (the OD value was 1.2–3.1), and the high absorbance ($\text{OD} > 1.2$) resulted in inaccurate experimental results. The color changes in the experiment with different concentrations of HAuCl_4 are shown in Figure S1B. The addition of 5 μL of HAuCl_4 (0.25 mM) in the solution resulted in a burgundy appearance. The addition of 6–9 μL of HAuCl_4 (0.3–0.45 mM) was dark red, and the AuNPs may be slightly coagulated, which will affect the experimental results. Therefore, 0.25 mM HAuCl_4 was used for further experiments.

3.5 Optimization of the AA concentration in seed growth solution

During the AuNP amplification, $\text{CTA}^+[\text{AuBr}_4]^-$ was reduced to $\text{CTA}^+[\text{AuBr}_2]^-$ with AA, and $\text{CTA}^+[\text{AuBr}_2]^-$ was further reduced to Au^0 in the presence of AuNPs-Ab. As shown in Fig. 2F and Figure S1C, as the concentration of AA increased from 1.0 mM to 1.5 mM, the UV absorbance increased rapidly. However, the absorption spectrum in the range of 1.6–2.6 mM had a slight redshift and the absorbance was unchanged. The color change in the experimental results with different concentrations of AA is shown in Figure S1C; 1.5 mM AA was selected for further study.

3.6 Optimization of incubation time of seed growth solution in AuNPs-Ab amplification

The incubation time for the seed growth solution to amplify AuNPs-Ab was optimized (Fig. 2G). As the reaction time increased from 0 min to 10 min, the UV absorbance of AuNPs-Ab gradually increased, and the maximum UV absorbance displayed a gradual redshift. The reason may be that the increase in the AuNPs size caused a change in the refractive index and aggregation. When the reaction time was

increased from 11 min to 13 min, the absorbance of the amplified AuNPs-Ab did not change, so the optimal reaction time was set as 10 min. The absorbance of AuNPs-Ab remained unchanged after the addition of 20 μL of 10 mM $\text{Na}_2\text{S}_2\text{O}_3$.

3.7 Stability of seed growth solution

To eliminate the effect of different batches of seed growth liquid on gold nanoparticles, we evaluated the stable growth of gold nanoparticles by seed growth solutions ($n = 6$). The results showed that the signal amplified by AuNPs-Ab was stable each time (Fig. 2H). For the six batches, the absorbance of AuNPs-Ab with and without seed growth solution was constant.

3.8 Optimization of the amount of sesame modified on MPMs

Different amounts of sesame allergens were added to 100 μL of activated MPMs in PBS solution and incubated for 3 h, and the resulting solution was separated magnetically. The concentration of sesame allergens in the supernatant was measured using a BCA kit, as calculated in Figure S2. The amount of conjugated coated sesame allergens (mc) was calculated from the difference between the total amount of coating sesame allergens (mt) and the amount of sesame allergens in the supernatant. The formula for calculating the conjugation rate was as follows: conjugation rate (%) = $(\text{mc}/\text{mt}) \times 100\%$. The relationship between the amount of added sesame allergens to the amount conjugated and the ratio of sesame allergens on MPMs are shown in Fig. 3A. When the amount of sesame allergens was 20 μg , the conjugation amount was saturated and the conjugation ratio was 95%. When the modified amount of sesame allergens was more than 20 μg , the conjugation ratio started to decrease. In Fig. 3B, MPMs with 10, 15, 18, and 20 μg sesame allergens were utilized to optimize the assay sensitivity. The results showed that the assay of 20 μg sesame allergen-modified MPMs had a high sensitivity. The LODs of the assay for 10, 15, 18, and 20 μg of sesame allergen-modified MPMs were $95.68 \mu\text{g L}^{-1}$, $96.79 \mu\text{g L}^{-1}$, $46.23 \mu\text{g L}^{-1}$, and $45.59 \mu\text{g L}^{-1}$, respectively. Therefore, 20 μg of sesame allergens modified on MPMs with the lowest LOD was selected for further experiments.

3.9 Optimization of the amount of AuNPs-Ab for the assay

The amount of signal probes (AuNPs-Ab) was optimized. First, 50 μL of $400 \mu\text{g L}^{-1}$ sesame sample solution and 50 μL of capture probes in PBS solution were mixed together. AuNPs-Ab solutions ranging from 1 μL to 10 μL were then added into the mixture. After magnetic separation, the supernatant was added to 100 μL of seed growth solution. Finally, the reaction was terminated with $\text{Na}_2\text{S}_2\text{O}_3$ and the UV absorption spectrum was measured. As shown in Fig. 3C, the absorbance values increased as the volume of AuNPs-Ab solution increased (from 1–9 μL) and became saturated with 10 μL of AuNPs-Ab solution. Subsequently, the standard curves of the assay with 5, 6, 8, and 10 μL of AuNPs-Ab solution were used for the optimization of the assay sensitivity. In Fig. 3D, the standard curves show that the LODs with 6, 8, and 10 μL of AuNPs-Ab were 45.52, 45.63, and $45.92 \mu\text{g L}^{-1}$, respectively. The LOD with 5 μL of AuNPs-Ab was $95.73 \mu\text{g L}^{-1}$, which was much higher than the others. However, the linear range of the standard

curve with 10 μL of AuNPs-Ab solution was 50–400 $\mu\text{g L}^{-1}$, which was narrower than that with 6 μL of AuNPs-Ab (50–800 $\mu\text{g L}^{-1}$). Therefore, 6 μL of AuNPs-Ab was used for the subsequent experiments.

3.10 Optimization of ionic strength for the assay

A solution of AuNPs is a relatively stable, uniform, and single-dispersed suspension liquid. Electrolytes can disrupt the stable state of the colloids, causing the colloidal gold particles to coagulate (Holgate, 1983). To eliminate the influence of ionic salts in food (such as Na^+ , Cl^- , K^+ , Ca^{2+} , Mg^{2+} , SO_4^{2-}) to the assay, different concentrations of NaCl, KCl, CaCl_2 , and MgSO_4 (0 M, 0.01 M, 0.1 M, and 1 M) were selected for the ionic strength optimization. The effect of ionic strength on the experiment is shown for sesame sample concentrations of 100 $\mu\text{g L}^{-1}$ (Fig. 4A), 300 $\mu\text{g L}^{-1}$ (Fig. 4B), and 400 $\mu\text{g L}^{-1}$ (Fig. 4C). The results showed that the assay absorbance with 0.01, 0.1M NaCl, KCl, and CaCl_2 were consistent with the results of the control group (no ionic salts added). However, 1M NaCl, KCl, and CaCl_2 had a greater impact on the assay sensitivity, causing a significant reduction. This might be because the high ionic strength destroyed the surface tension of proteins and the stability of the hydrogen and disulfide bonds. For MgSO_4 , both low ionic strength (0.01 M) and high ionic strength (1 M) affected the sensitivity of the assay. In general, the molar concentration of ions is much higher than that usually found in food and beverages. In most cases, the performance of the analysis method for these foods would not be affected by the presence of these common ions.

3.11 Optimization of pH for the assay

When the pH is equal to or slightly greater than the isoelectric point of a protein, the protein is neutral. The electrostatic interaction between the protein molecules and AuNPs was very small, whereas the surface tension of the protein molecules in the water was very large. Therefore, they could easily adsorb on the surface of the AuNPs and form a protein layer to prevent aggregation (Karyakin et al., 2000; Smita et al., 2010; Tassel et al., 2006; Wang et al., 2014). The pH of the sample solution may affect the sensitivity and the selectivity of the assay. Therefore, sesame allergens sample solution (100, 300, and 400 $\mu\text{g L}^{-1}$) with different pH were considered for assay optimization. As shown in Fig. 4D, the final sample solutions with pH values of 3.5, 5.5, and 7.4 inhibited the absorbance of the assay. The sample solutions with pH 8.5 and 9.5 caused nanoparticle aggregation and reduced the assay sensitivity. Therefore, the optimal pH of the sample solutions was 3.5–7.4.

3.12 Optimization of incubation time from the assay

Signal and capture probes were prepared under optimized conditions, and the incubation time for the experiment was optimized. First, 50 μL of sesame allergen solution (200, 400, and 800 $\mu\text{g L}^{-1}$) and 50 μL of capture probe were mixed together in PBS solution. Then, 6 μL of the signaling probe was added and mixed gently for different times (5, 10, 15, and 20 min). As shown in Fig. 4E, the absorbance value reached highest value (0.17, 0.23, 0.31) for 15 min with different concentrations of sesame allergens (200, 400, and 800 $\mu\text{g L}^{-1}$). Therefore, 15 min was selected as the incubation time for the assay.

3.13 Sensitivity of the assay

Under the optimized conditions above, different concentrations of sesame allergen solutions (1600, 1500, 800, 400, 300, 200, 100, 50, 25, 20, and 12.5 $\mu\text{g L}^{-1}$) were added to the assay for measurement. After the reaction, the sesame capture probe@signal probe immune complex was magnetically separated. The supernatant was added to 100 μL of seed growth solution, and $\text{Na}_2\text{S}_2\text{O}_3$ was added to stop the reaction after 10 min. As shown in Fig. 5A and 5B, the linear range of the assay for sesame allergen detection was 50–800 $\mu\text{g L}^{-1}$, and the LOD was 45.53 $\mu\text{g L}^{-1}$. Currently, the reported detection methods for sesame allergens include TaqMan qPCR (Brzezinski, 2007), indirect competitive ELISA (Husain et al., 2010), double-antibody sandwich ELISA (Gerda et al., 2010), liquid chromatography coupled with mass spectrometry (LC–MS) (Ma et al., 2019), and liquid chromatography–tandem mass spectrometry (LC–MS/MS) (Gallien et al., 2013). Compared with these methods, our method had higher sensitivity and a shorter detection time (25 min) (Table 1).

Table 1
Comparison between this method and other reported methods for detection of sesame allergens

Analytical method	Label or probe	LOD (PBS)	LOD (samples)	Detection time (min)	References
TaqMan qPCR	Dual fluorescent labeled DNA	5 pg DNA	50 mg kg^{-1} (biscuit)	120	(Brzezinski, 2007)
Indirect competitive ELISA	HRP-goat anti-mouse antibody	5000 $\mu\text{g kg}^{-1}$	5 mg kg^{-1} (bread) 30 mg kg^{-1} (biscuit)	150	(Husain et al., 2010)
Double antibody sandwich ELISA	HRP-goat anti-mouse antibody	500 $\mu\text{g kg}^{-1}$	0.5 mg kg^{-1} (bread)	150	(Gerda et al., 2010)
LC-MS	–	10000 $\mu\text{g kg}^{-1}$	10 mg kg^{-1} (biscuit, bread)		(Ma et al., 2019)
LC-MS/MS	–	0.4 $\mu\text{mol/L}$	0.02 mmol L^{-1} (hotpot dipping sauce, spicy chili sauce, cookie, cake, candy)		(Gallien et al., 2013)
Magnetic separation colorimetric immunoassay	AuNPs-Ab MPMs-SP	45.529 $\mu\text{g L}^{-1}$	0.4 mg kg^{-1} (biscuit and bread), 0.3 mg kg^{-1} (almond beverage), 0.6 mg kg^{-1} (energy bar)	25	This work
– means no description					

3.14 Specificity of the assay

Several common allergen proteins were selected (walnut protein, β -lactoglobulin, peanut protein, lupin protein, and casein) to evaluate the assay specificity. The assay was used to analyze $800 \mu\text{g L}^{-1}$ of sesame allergens and 4 mg L^{-1} of other selected allergen proteins. The results showed that, with the exception of walnuts and peanuts, there was almost no cross-reaction with the other three allergens (Fig. 6A). The reason might be that the main allergens in walnuts and peanuts contain 2S albumin and 7S globulin. The sesame allergen protein extracted in this experiment contains 2S albumin Ses i 1, among which Ses i 1 and walnut 2S albumin Jug r 1 share 38.56% homology (Wolff et al., 2004). Peanuts also contain 2S albumin and 7S globulin. The phylogenetic tree in Fig. 6B supported this result. In addition to the low cross-reactivity to walnut and peanut allergen proteins, this method had good specificity for the other three proteins (β -lactoglobulin, lupin protein, and casein).

3.15 Matrix effects

The sesame allergen protein was extracted from different samples (bread, biscuits, almond beverage, and energy bars) with PBS to study the matrix effect. As shown in Fig. 7A–D, the standard curves of the assay in 8-fold diluted biscuits and bread sample solutions overlapped with the standard curve in PBS. This indicated that there was no matrix effect. To eliminate the food matrix effect, the energy bar sample solution was diluted 12 times and the almond beverage sample solution was diluted six times.

3.16 Spike and recovery study

To evaluate the reliability and practicability of the assay, we used a commercial ELISA kit and the developed assay to detect sesame allergens in four real food samples (bread, biscuits, almond beverage, and energy bars). Sesame allergens were added to food samples that did not contain sesame as an ingredient to make final concentrations of sesame allergens of 50, 150, 300, 600, and $800 \mu\text{g L}^{-1}$ or $\mu\text{g kg}^{-1}$. At the same time, these samples were tested using the commercial ELISA kit to verify the accuracy of the method. As shown in Table 2, the test results of this method were the same as those of the ELISA. The recovery rates were between 82.50% and 116.67%. The LODs of the bread and biscuit samples were all 0.4 mg kg^{-1} . The LODs of the almond beverage and energy bar samples were 0.3 and 0.6 mg kg^{-1} , respectively. These results showed that the method was accurate and reliable, and can be presented as an alternative tool for the analysis of sesame in baked goods and plant-based beverages.

Table 2

Spike and recovery results of magnetic separation colorimetric immunoassay method and ELISA kit

Sample	Spike level ($\mu\text{g kg}^{-1}$ or $\mu\text{g L}^{-1}$)	Colorimetric immunoassay		ELISA kit	
		Mean $\pm$ SD ^a (mg kg^{-1} or mg L^{-1} , n = 3)	Recovery (%)	Mean $\pm$ SD (mg/kg or mg L^{-1} , n = 3)	Recovery(%)
Bread	0.0	NDb	—	ND	—
	0.4	0.45 ± 0.007	112.50	0.45 ± 0.026	112.50
	1.2	0.99 ± 0.003	82.50	1.36 ± 0.091	113.33
	2.4	2.61 ± 0.008	108.75	2.76 ± 0.015	115.00
	4.8	5.18 ± 0.100	107.92	5.61 ± 0.144	116.87
	6.4	6.05 ± 0.08	94.53	6.69 ± 0.020	104.53
Biscuits	0.0	ND	—	ND	—
	0.4	0.44 ± 0.003	110.11	0.43 ± 0.030	107.50
	1.2	1.22 ± 0.010	101.67	1.40 ± 0.042	116.67
	2.4	2.25 ± 0.039	93.75	2.30 ± 0.059	95.83
	4.8	4.16 ± 0.004	86.67	5.04 ± 0.006	105.00
	6.4	7.20 ± 0.020	112.50	7.11 ± 0.002	110.09
Almond beverage	0.0	ND	—	ND	—
	0.3	0.35 ± 0.003	116.67	0.34 ± 0.022	113.33
	0.9	0.88 ± 0.012	97.78	0.83 ± 0.008	92.22
	1.8	1.80 ± 0.015	100.00	1.70 ± 0.004	94.44
	3.6	3.37 ± 0.015	93.61	3.54 ± 0.006	98.33
	4.8	5.30 ± 0.016	110.42	5.27 ± 0.013	109.79
Energy bar	0.0	ND	—	ND	—
	0.6	0.66 ± 0.007	110.00	0.71 ± 0.005	118.33
	1.8	1.58 ± 0.002	87.77	1.68 ± 0.007	93.33
	3.6	4.02 ± 0.014	111.67	3.93 ± 0.009	109.17
	7.2	6.70 ± 0.012	93.05	7.87 ± 0.021	109.30
^a SD, standard deviation.					
^b ND, not detected.					

9.6	10.88 ± 0.014	113.33	10.07 ± 0.014	104.90
^a SD, standard deviation.				
^b ND, not detected.				

4. Conclusion

We had developed a magnetic separation colorimetric immunoassay. Sesame Ab was modified to AuNPs as signal probes (AuNPs-Ab). The seed growth liquid for AuNPs-Ab was prepared and optimized to improve the assay sensitivity. Under optimal conditions, the linear range of the proposed immunoassay was 50–800 µg L⁻¹, and the LOD was 45.529 µg L⁻¹. The total detection time of this assay was 25 min. The assay had good selectivity to sesame allergens with no-cross reactivity to β-lactoglobulin, lupin protein and casein. Applying this strategy to the sesame sample matrix (bread, biscuits, almond beverage and energy bars), the recovery rate was 82.50-116.67%. In general, this sensor could highly efficiently extract analytes from the sample and shorten the detection time.

Declarations

Funding

The authors gratefully acknowledge the financial support from Tianjin Food Quality and Health Key Experiment Open Project from Tianjin University of Science and Technology (TJS202104).

Data Availability

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

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.

ORCID iD authorship contribution statement

Xiaopan Linghu: Conceptualisation, Data curation, Formal analysis, Investigation, Methodology, Writing-original draft, Writing –review & original draft; **Wentao Liu:** Investigation, Methodology, Writing-original draft, Writing –review & original draft; **Shasha Wang:** Investigation, Methodology, Writing –review & original draft; **Rui Wang:** Data curation, Investigation, Methodology, review & original draft; **Yang Lu:** Conceptualisation, Supervision, Data curation, Investigation, Methodology, Project administration, Software, Writing –review & original draft

Acknowledgments

We are very much thankful for the support and guidance of Dr. Xiao Yunjie from Tianjin University.

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Schemes

Scheme 1 is available in the Supplementary Files section

Figures

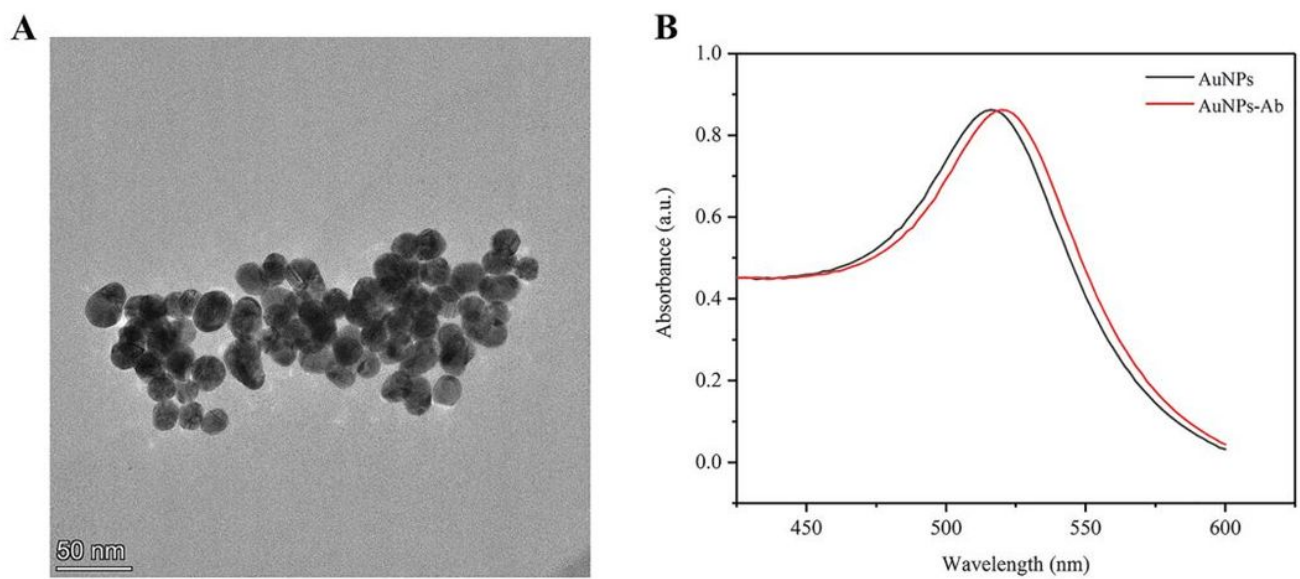


Figure 1

Characterization of AuNPs and AuNPs-Ab. TEM of AuNPs (A) and absorbance spectrum of AuNPs-Ab (B).

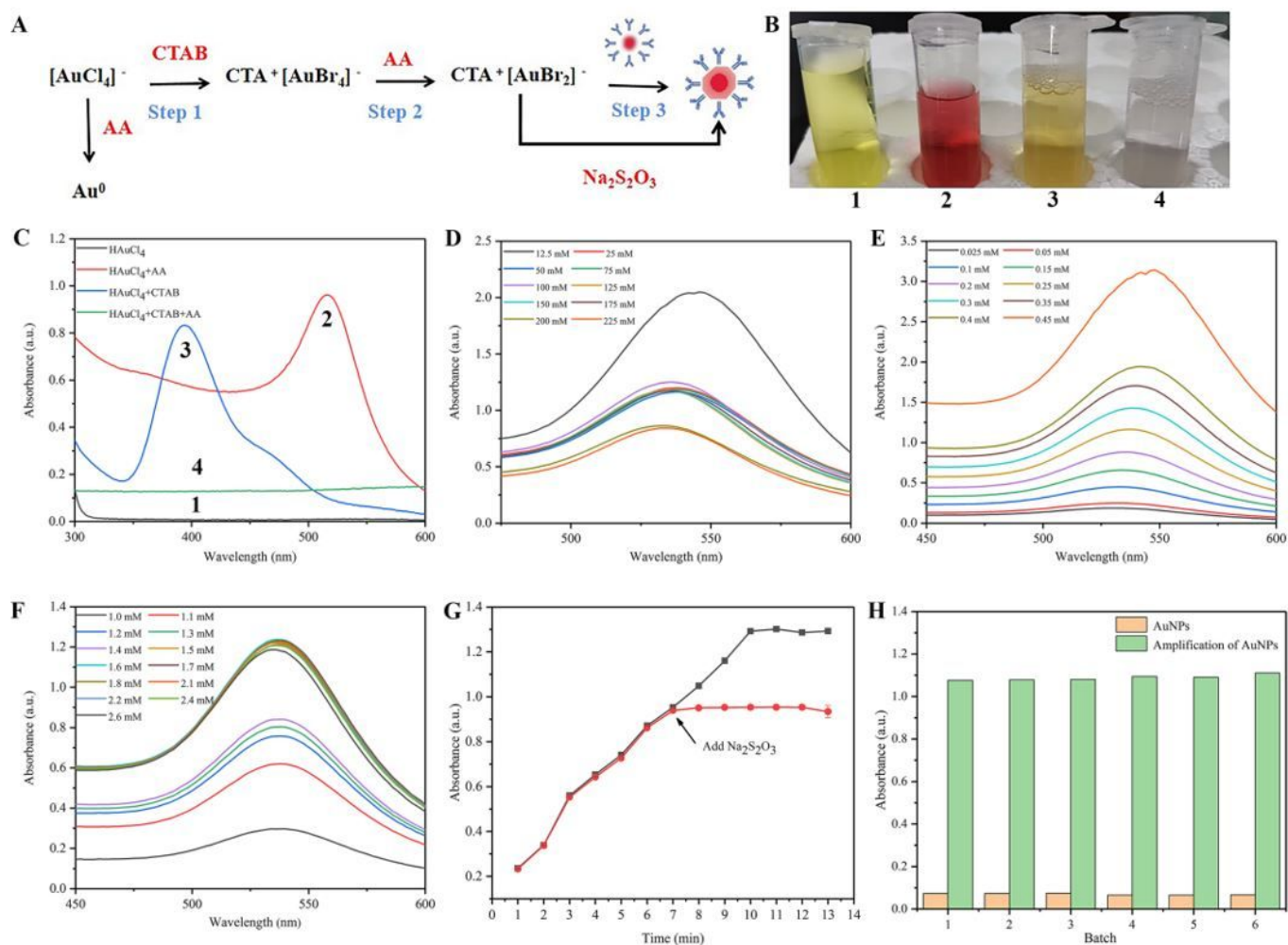


Figure 2

Optimization of working parameters of seed growth liquid. Schematic diagram of seed growth solution (A); Color diagram of HAuCl_4 , HAuCl_4+AA , $\text{HAuCl}_4+\text{CTAB}$, $\text{HAuCl}_4+\text{CTAB}+\text{AA}$ (B); UV absorption spectrum of CHAuCl_4 , HAuCl_4+AA , $\text{HAuCl}_4+\text{CTAB}$, $\text{HAuCl}_4+\text{CTAB}+\text{AA}$ (C); The absorbance values of seed growth solution with different concentrations of CTAB (D); The absorbance values of seed growth solution with different concentrations of HAuCl_4 (E); The absorbance values of seed growth solution with different concentrations of AA (F); The absorbance values of AuNPs and seed growth solution over 13 min (with $\text{Na}_2\text{S}_2\text{O}_3$ (red dot) and without $\text{Na}_2\text{S}_2\text{O}_3$ (black dot)) (G); The absorbance value of six batches of seed growth solution (H).

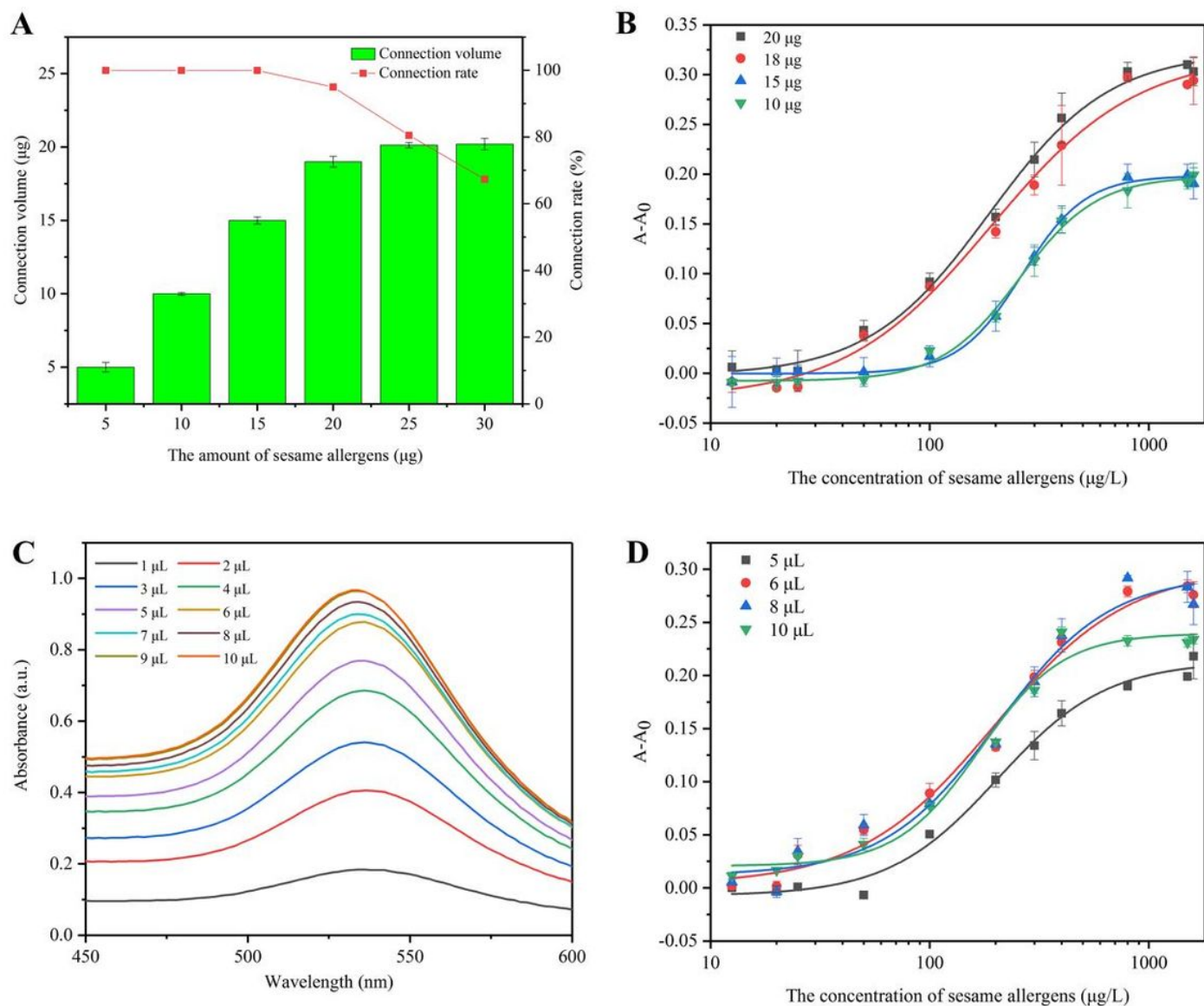


Figure 3

Optimization of the amount of capture probes (MPMs-SA) and signal probes (AuNPs-Ab). The connection volume and connection rate of the MPMs-SA complex with different amounts of additional sesame allergens (A); The standard curve of the assay with different amounts of MPMs-SA (10, 15, 18, and 20 μg) (B); The absorbance value of the assay with different volumes of AuNPs-Ab (C); The standard curve of the assay under different AuNPs-Ab addition (5, 6, 8, and 10 μL) (D).

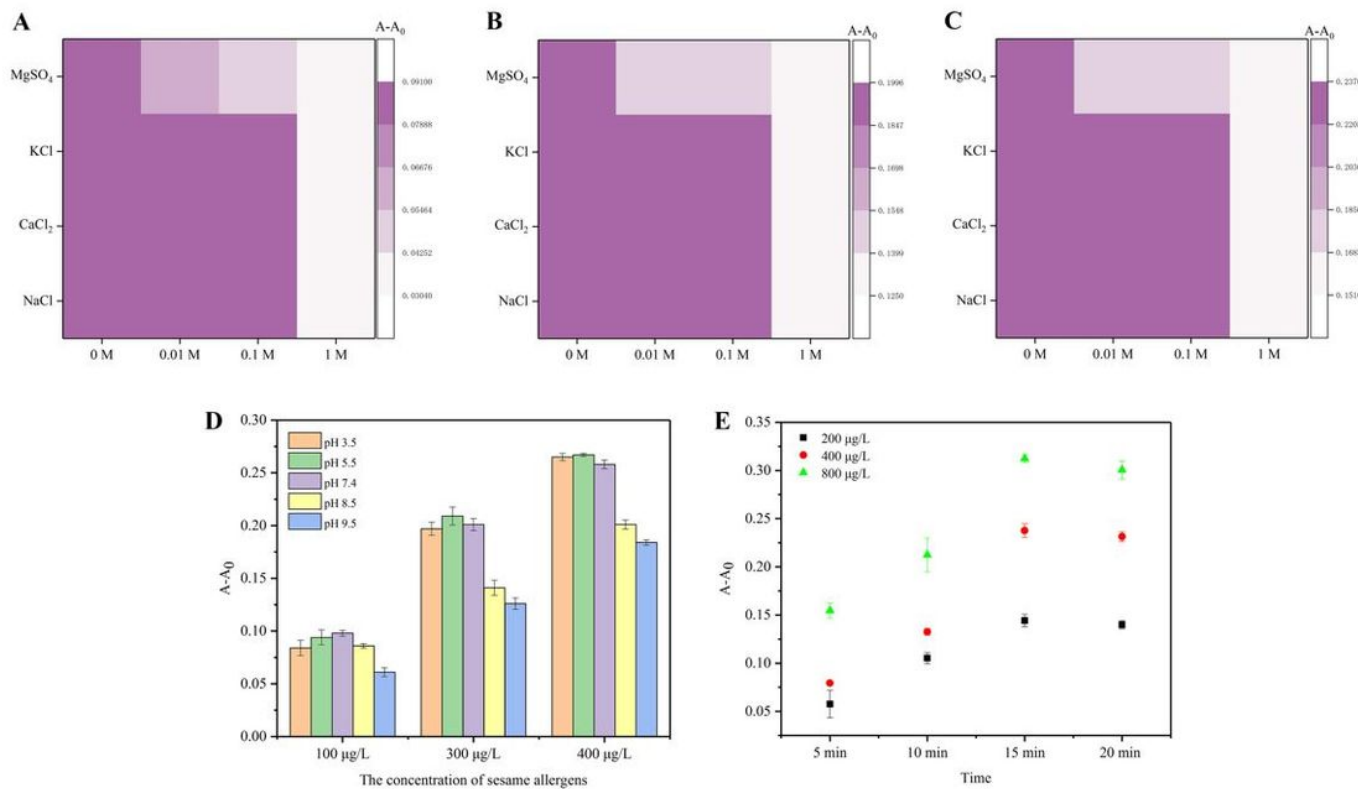


Figure 4

Optimization of working parameters. The absorbance value ($A-A_0$) of the assay under different concentrations of ions (the concentrations of NaCl, KCl, CaCl_2 , and MgSO_4 were 0, 0.01, 0.1, and 1 M, respectively, and sesame allergen concentration was 100 $\mu\text{g L}^{-1}$) (A); The absorbance value ($A-A_0$) of the assay under different concentrations of ions and 300 $\mu\text{g L}^{-1}$ sesame allergen (B); The absorbance value ($A-A_0$) of the assay with different ion concentrations (the sesame allergen concentration was 400 $\mu\text{g L}^{-1}$) (C). The absorbance value ($A-A_0$) of the assay with different concentrations of sesame allergens (100, 300, and 400 $\mu\text{g L}^{-1}$) and at various pH values (3.5, 5.5, 7.4, 8.5, and 9.5) (D). The absorbance value ($A-A_0$) of the assay with different concentrations of sesame allergen (200, 400, 800 $\mu\text{g L}^{-1}$) under various incubation times (5, 10, 15, and 30) (E).

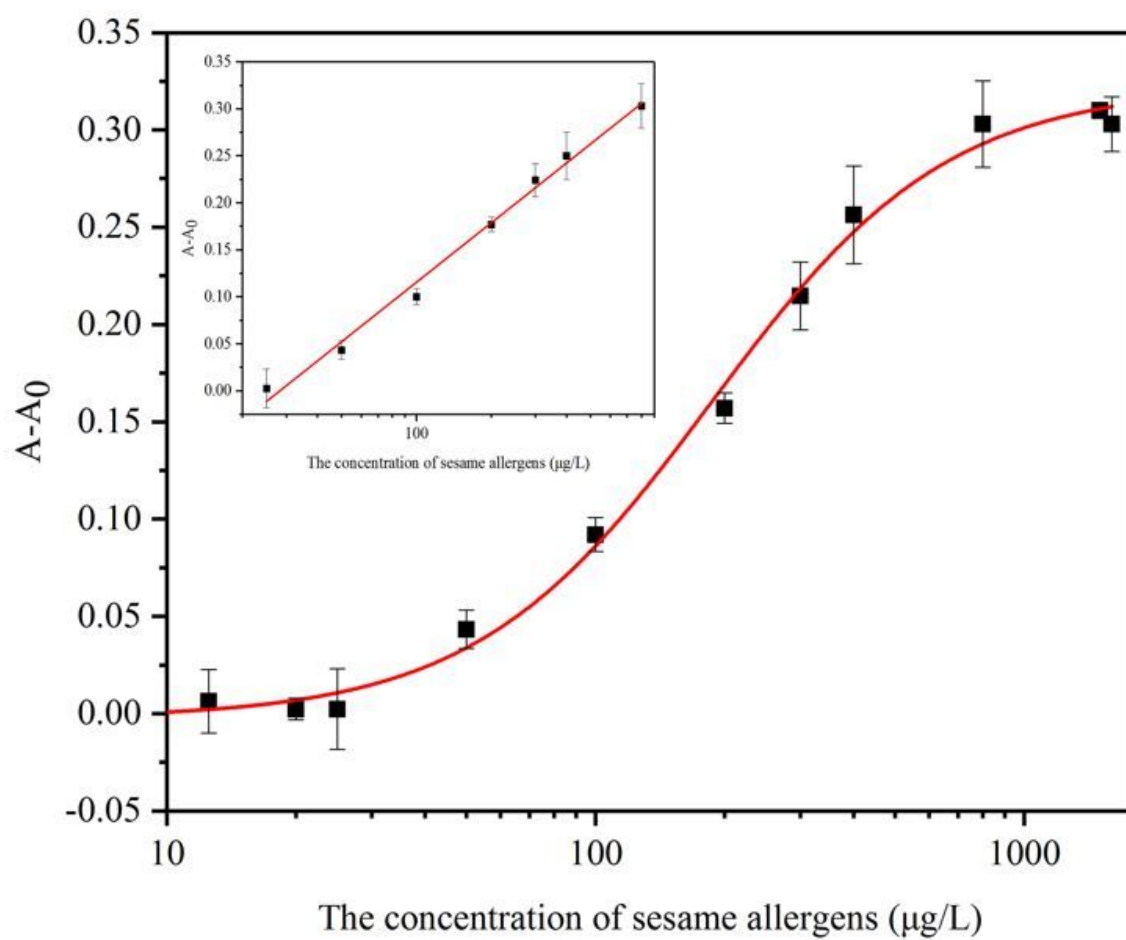


Figure 5

The standard curve of the immunocolorimetric assay based on amplified gold nanoparticles and magnetic separation beads. Sesame allergen concentrations were 1600, 1500, 800, 400, 300, 200, 100, 50, 25, 20, and $12.5 \mu\text{g L}^{-1}$.

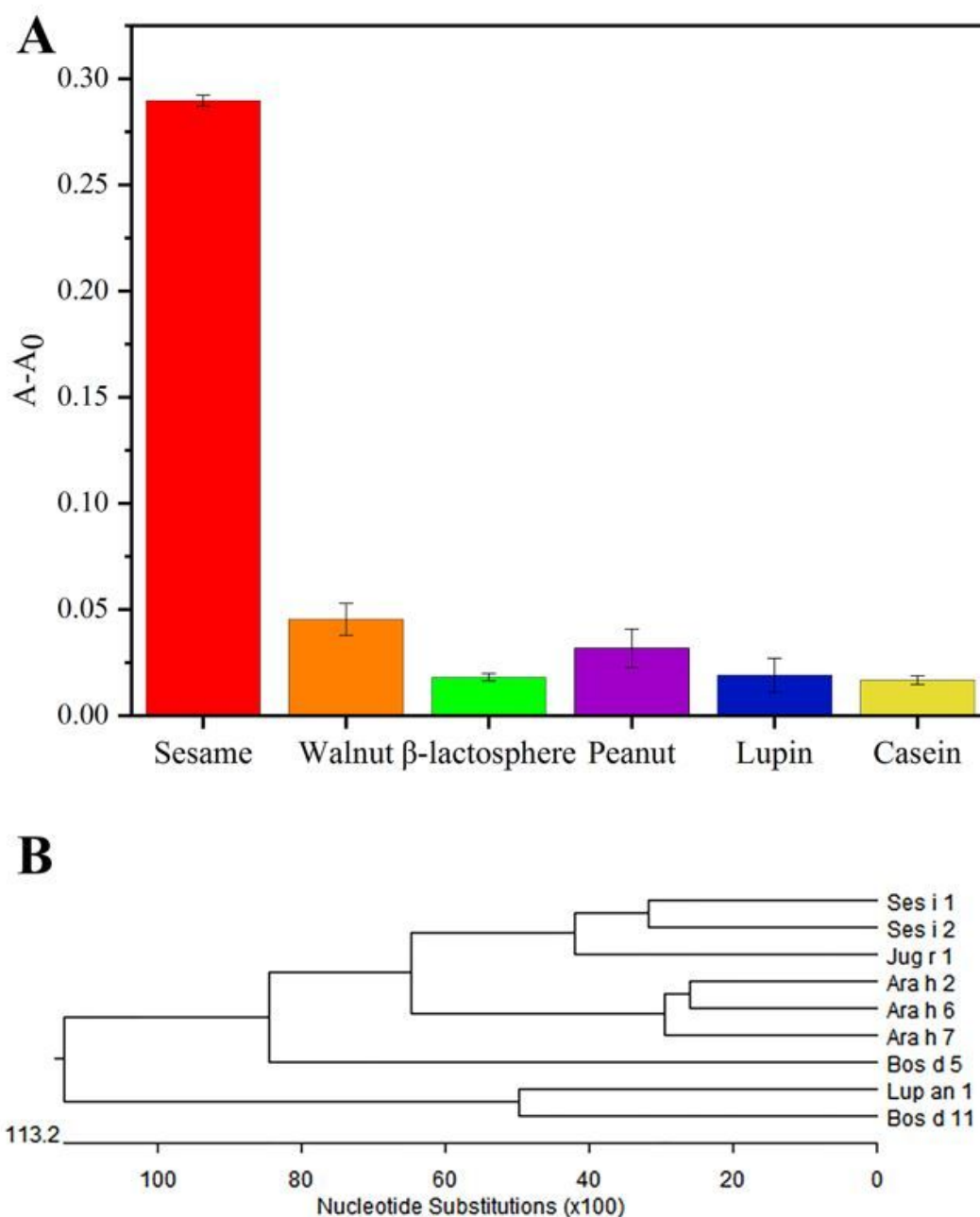


Figure 6

The specific of immunocolorimetric assay based on amplified gold nanoparticles and magnetic-separation beads. The absorbance value ($A-A_0$) of the assay for the detection of different allergens (the sesame allergen concentration was $800 \mu\text{g L}^{-1}$; the concentration of walnut, β -lactoglobulin, peanut, lupin, and casein allergens was 4 mg mL^{-1}) (A). The DNASTAR phylogenetic tree of the different allergens: Ses i 1, Ses i 2, Jug r 1, Ara h 2, Ara h 6, Ara h 7, Bos d 5, Bos d 11, and Lup an 1 (B).

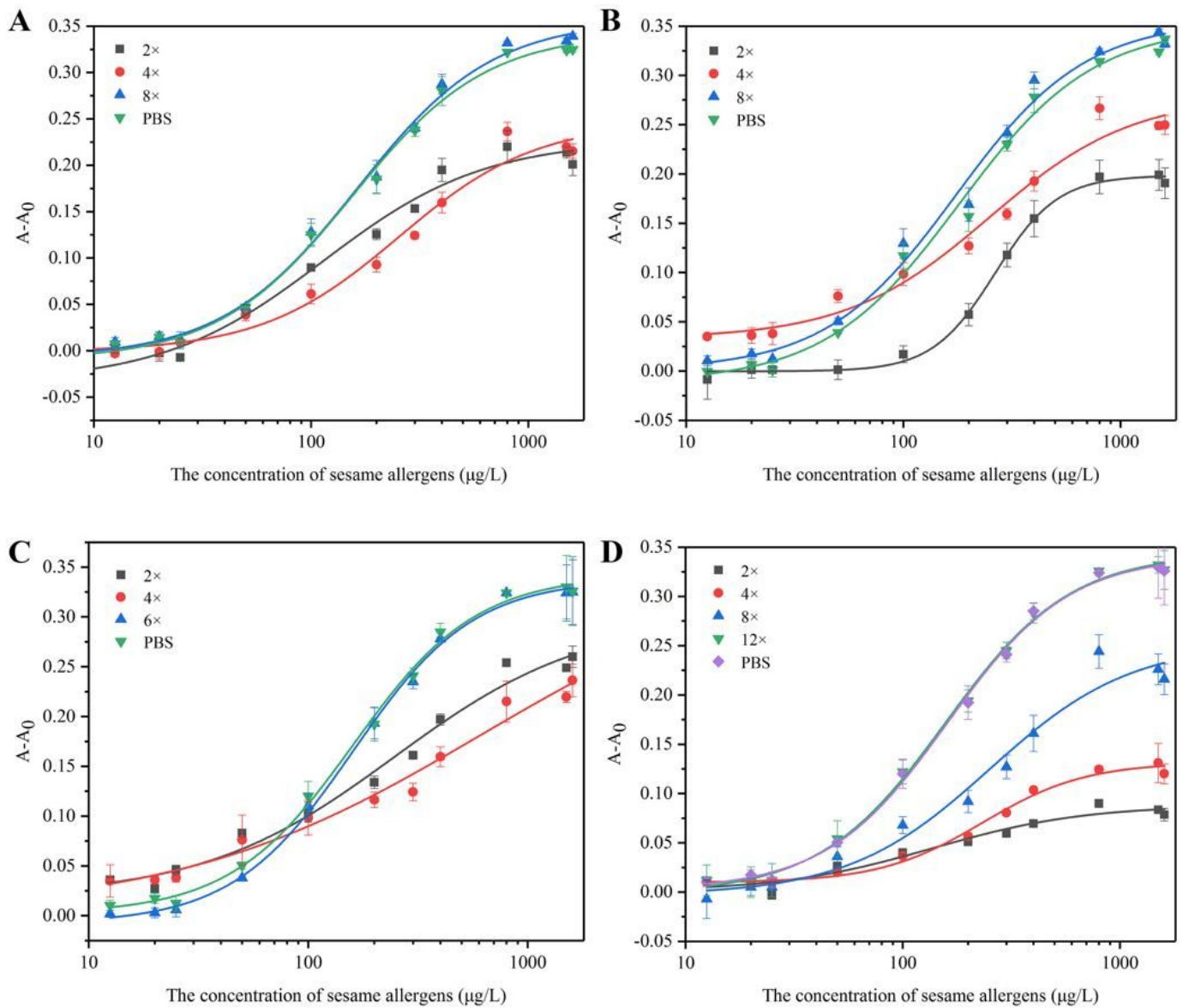


Figure 7

Matrix effect. The standard curves of the assay in 2-, 4-, and 8-fold diluted bread sample solutions and PBS (A). The standard curves of the assay in 2-, 4-, and 8-fold diluted biscuit sample solutions and PBS (B). The standard curves of the assay in 2-, 4-, and 6-fold diluted almond beverage sample solutions and PBS (C). The standard curves of the assay in 2-, 4-, 8-, and 12-fold diluted energy bar sample solutions and PBS (D).

Supplementary Files

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