

# Preparation of pH-sensitive polymer by thermal initiation polymerization and its application in fluorescence immunoassay

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## Abstract

A fluorescence immunoassay for human IgG (Ag) was developed using a pH-sensitive polymer prepared by thermal initiation or redox initiation polymerization as a carrier. In the competitive immunoassay, appropriate quantity of Ag was immobilized on the polymer and the standard Ag (or sample) solution, and a constant amount of fluorescein isothiocyanate labeled goat anti-human IgG antibody (Ab-FITC) was added. Immobilized Ag and the standard (or sample) Ag competed for binding to the Ab-FITC in 37 °C in homogeneous format. After changing the pH to separate the polymer-immune complex precipitate, it was re-dissolved and determined by fluorescence method. The results showed that the immobilization efficiency, immunological reaction activities of immobilized Ag and phase transition pH range were improved as Ag was immobilized by thermal initiation instead of redox initiation polymerization. Under optimum conditions, the calibration graphs for the Ag in both methods, thermal initiation and redox initiation, were linear over the concentration range of 0.0–1000 ng mL<sup>-1</sup>, with detection limits 8 (thermal initiation) and 12 ng mL<sup>-1</sup> (redox initiation), respectively. Moreover, some pH-sensitive polymer prepared only in organic solvent or under high temperature could also be used as an immunoreaction carrier by thermal initiation polymerization. Thermal initiation polymerization was a better immobilization mode.

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**Keywords:** pH-sensitive phase separating polymer; Fluorescence immunoassay; Human IgG

## 1. Introduction

In immunoassay, antigen or antibody (analytes) can be precisely measured using either heterogeneous or homogeneous assays [1–2]. Generally, Homogeneous immunoassays have the advantages of being rapid, easy to perform, and readily amenable to automation. However, the drawbacks of these methods, such as poor sensitivity and being prone to interference, have limited their application. On the other hand, heterogeneous procedures are more sensitive than homogeneous assays, less prone to interference. But in heterogeneous assays, the affinity constant of the antibody and antigen is

reduced and the equilibrium of the immune reaction may require a longer time because of steric hindrance.

The problems mentioned above for both homogeneous and heterogeneous immunoassays have been solved by using certain water-soluble polymers to carry out a fast homogeneous immune reaction and a simple heterogeneous separation process. The polymers precipitate out of water when the temperature is raised above the lower critical solution temperature (LCST) [3–5]. Among the temperature-sensitive polymers, poly-*N*-isopropylacrylamide (PNIP) is known to have a LCST of about 31 °C throughout a wide concentration range. The special thermo-sensitive characteristic of PNIP was successfully exploited in immunoassays by Hoffman et al decades ago [6]. Then PNIP has been widely utilized as the separation carrier in immunoassays [7–10]. However, such

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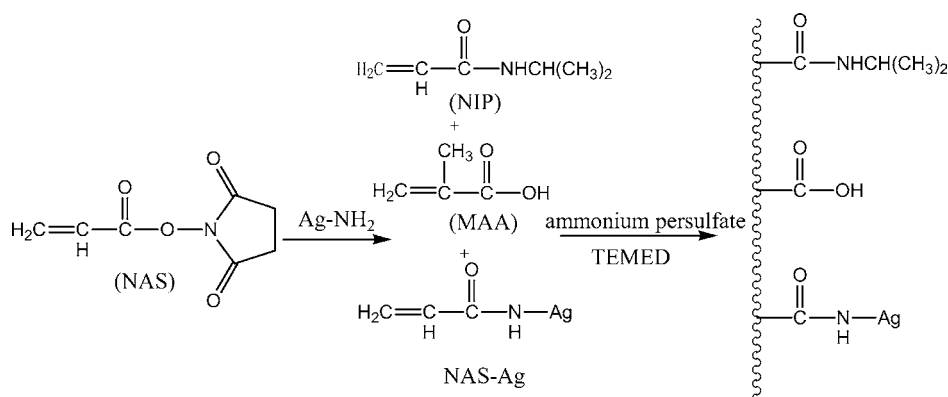


Fig. 1. Immobilization of Ag to the polymer P(NIP-MAA) by redox initiation polymerization.

assays can only be conducted under the LCST of the polymers, and therefore, this would inevitably affect the immune reaction efficiency.

pH-sensitive polymers are another kind of environmentally sensitive polymers, which contain ionizable or protonable groups and can lead to pH-dependent phase transition [11–14]. By using the polymers as a separating carrier for the reactants in immunoassay, the immunoreaction will be carried out at a physiological environment temperature of organism and the rate of immunoreaction will be faster. However, the polymers formerly developed are not fit for using as the carrier in immunoassays due to their pH values for precipitation being in low pH range [15], in which the damage to antigen–antibody immune complex would unavoidably be caused in the course of separation. We have ever prepared a pH-sensitive polymer containing *N*-isopropylacrylamide (NIP) and methacrylic acid (MAA) with phase transition pH ( $\text{pH}_{\text{tr}}$ ) of 5.6 and used it as a novel separation carrier for the reactants in immunoassay for  $\alpha$ -fetoprotein [16]. The method offers several advantages over the thermal phase-separating immunoassay. The immune process is conducted at 37 °C; thus shorter incubation time is needed. This method can be applied to the processes in which temperature change is not allowed or hardly conducted. Furthermore, this polymer can also be used as a pH-switching control of selective removal

and delivery in human bodies since the pH-dependent phase transition is conducted at 37 °C.

At present, redox initiation polymerization reaction has mainly been utilized for the immobilization of antigen or antibody to temperature- or pH-sensitive polymers in immunoassay [8,17,18]. So was our work above-mentioned. In this case, amido of antigen or antibody firstly coupled with active acrylate [e.g. *N*-hydroxysuccinimidyl acrylate (NAS)] and then was immobilized to polymer at definite temperature by using ammonium persulfate (APS) as the initiator and *N,N,N',N'*-tetramethyl-ethylene-diamine (TEMED) as the accelerator (Fig. 1). Owing to polymerization after coupling reaction, the free radicals might damage antibody or antigen and polymer chains would unavoidably wrap around the surface of antibody or antigen, which would surely lead to the decrease of immunological activities of antibody or antigen. Moreover, immobilization efficiency was not high [19] and residual initiator affected immune reaction. In the study of immobilized enzyme, thermal initiation polymerization was often used [20,21] and different immobilization mode significantly affected the bioactivity of enzyme [22]. So we intend to resolve the problem mentioned above by changing the immobilization mode.

In this work, a pH-sensitive polymer containing NIP, MAA and NAS with  $\text{pH}_{\text{tr}}$  of 5.6 was prepared by thermal initia-

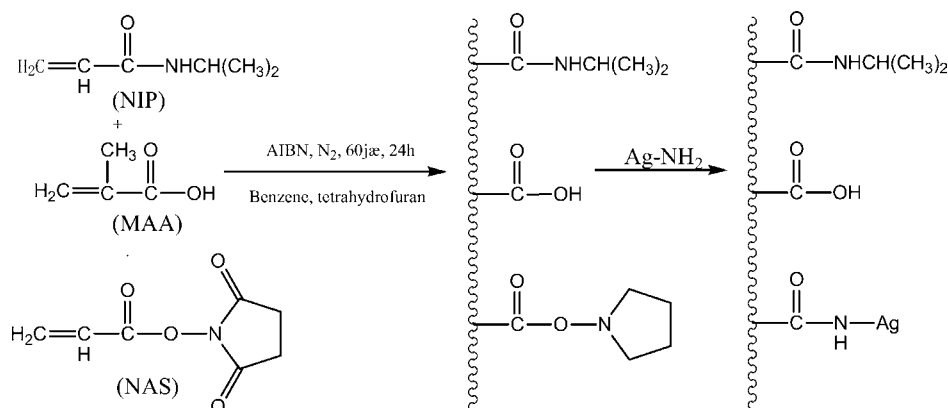


Fig. 2. Immobilization of Ag to the polymer P(NIP-MAA) by thermal initiation polymerization.

tion polymerization and used as a separating carrier for the reactants in immunoassay for the first time. The whole reaction was carried out at 60 °C with 2,2'-Azobisisobutyronitrile (AIBN) as a thermal initiator. NAS has highly reactive ester groups reacting with amino groups in human IgG (Ag). Then this polymer was conjugated to Ag by NAS (Fig. 2). And the polymer was precipitated below  $pH_{tr}$  at 37 °C and re-dissolved when the solution pH was above  $pH_{tr}$ . Thus, with the use of P(NIP-MAA) as a carrier, the immunoassay for Ag could be carried out by control of the pH. In a competitive fluorescence immunoassay, the standard (or sample) Ag and Ag immobilized to P(NIP-MAA) first reacted with the fluorescein isothiocyanate labeled antibody (Ab-FITC), then the pH of solution was adjusted below the polymer's  $pH_{tr}$  to precipitate the polymer-immune complex, and the polymer-immune complex precipitate was separated and re-dissolved by the adjustment of pH, finally the Ab-FITC in the immune complex was quantified by fluorescence measurement. At the same time, the two immobilization modes were compared. The results showed that thermal initiation polymerization was a better immobilization mode.

## 2. Experimental

### 2.1. Reagents

Human IgG (Ag), goat anti-human IgG antibody (Ab), and bovine serum albumin (BSA) were purchased from Beijing Zhongshan Biotechnology Co., Ltd. *N*-isopropylacrylamide (NIP) and *N,N,N',N'*-tetramethylethylenediamine (TEMED) were purchased from TCI-EP, Tokyo Kasei and used without further purification. Methacrylic acid (MAA), ammonium persulphate (APS) and fluorescein isothiocyanate (FITC) were obtained from Shanghai Chemical Reagent Co. MAA was separated from the reaction inhibitor, *p*-methoxyphenol, by distillation under vacuum. *N*-hydroxysuccinimidyl acrylate (NAS), 2,2'-azobisisobutyronitrile (AIBN) and *p*-hydroxyphenylacetic acid (*p*-HPA) were obtained from Sigma Co., St. Louis, MO. and used without further purification. FITC-labeled anti-human IgG antibody (Ab-FITC), FITC-labeled human IgG (Ag-FITC) and horseradish peroxidase-labeled human IgG (Ag-HRP) were synthesized in our laboratory. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>, 30%) was obtained from Shanghai Taopu Chemical Factory, and the stock solution (H<sub>2</sub>O<sub>2</sub>, 3%) was standardized by titration with a standard solution of KMnO<sub>4</sub>. Phosphate-buffered saline (PBS) solution (0.01 mol L<sup>-1</sup>, pH 7.0) was prepared. All the reagents were of analytical grade. The distilled, de-ionized water was used throughout.

### 2.2. Apparatus

A Hitachi 650-10S spectrofluorimeter (Tokyo, Japan) equipped with a plotter unit and a 1-cm quartz cell was used for recording fluorescence spectra and making fluorescence

measurements. The absorption spectrum was obtained on a Beckman DU-7400 UV/VIS diode array spectrophotometer (Fullerton, CA, USA). A TGL-16G supercentrifuge (Shanghai, China), and a SHZ-88 water-bath vibrator (Jiangsu, China) were used.

### 2.3. Procedures

#### 2.3.1. Preparation of the pH-sensitive polymer P(NIP-MAA) and Immobilization of Ag by thermal initiation polymerization

The polymer was prepared by dissolving 18 mg NAS, 552 mg NIP and 11 mg MAA in 30 mL of 50:50 (v/v) of tetrahydrofuran and benzene mixture. After removing oxygen by nitrogen purging, 5 mg of AIBN was added as an initiator to start polymerization under nitrogen. The polymerization process was continued for 24 h at 60 °C. Petroleum ether was added at the end of reaction to precipitate the polymer. After removal of the supernatant, the polymer was dissolved in dichloromethane, and then the polymer was re-precipitated by pouring petroleum ether. This procedure was repeated three times. The product was collected by filtration, dried in vacuum, and stored in a desiccator. The P(NIP-MAA) was named A.

A 100 mg sample of polymer was dissolved in 3 mL Na<sub>2</sub>CO<sub>3</sub>-NaHCO<sub>3</sub> buffer (0.01 mol L<sup>-1</sup>, pH 9.2) and added to 1 mL carbonate buffer containing 1 mg Ag. The solution was mixed by stirring for 16 h. The pH of the solution was maintained at 9.0–9.5 by the addition of 0.2 mol L<sup>-1</sup> Na<sub>2</sub>CO<sub>3</sub> during the coupling reaction. Then the P(NIP-MAA)-Ag conjugate was precipitated by addition of a constant amount of 0.1 mol L<sup>-1</sup> HCl and separated by centrifugation at 37 °C for 5 min (4000 rpm). After the supernatant was removed, the precipitate was dissolved in PBS (0.01 mol L<sup>-1</sup>, pH 7.0). 200 µL of 0.2 mol L<sup>-1</sup> HAC-NaAc buffer (pH 5.0) was added into the PBS solution. The precipitate was produced again. The above-mentioned procedure was repeated three times. Finally, the conjugate was dissolved in 5 mL of PBS (pH 7.0) and stored at 4 °C. The conjugate A-Ag was gotten.

#### 2.3.2. Preparation of the pH-sensitive polymer P(NIP-MAA) and Immobilization of Ag by redox initiation polymerization

pH-sensitive polymer was prepared using 975 µL of 1 mol L<sup>-1</sup> NIP and 250 µL of 0.1 mol L<sup>-1</sup> MAA. In addition, 5 mg APS was used as the initiator and 10 µL TEMED as the accelerator. After polymerization for 2 h, the polymer was precipitated by adding a constant amount of 0.1 mol L<sup>-1</sup> HCl and separated from unreacted monomers by centrifugation at 37 °C for 5 min (4000 rpm). The P(NIP-MAA) was called B.

Redox polymerization was also used to immobilize Ag. A 1.8 mL of PBS (0.01 mol L<sup>-1</sup>, pH 7.0) containing 1 mg of Ag and 0.2 mL of 0.1% (m/v) NAS was mixed and vibrated in a 37 °C water bath for 60 min in order to form the monomer conjugate, then the mixture was dia-

lyzed three times against PBS at 4 °C. The solution was diluted to 4 mL with PBS, and then 975  $\mu\text{L}$  of 1 mol L<sup>-1</sup> NIP, 250  $\mu\text{L}$  of 0.1 mol L<sup>-1</sup> MAA, 5 mg APS and 10  $\mu\text{L}$  TEMED were added and the mixture was allowed to stand for 2 h to carry out the copolymerization reaction. The P(NIP-MAA)-Ag conjugate was precipitated by adding 200  $\mu\text{L}$  of 0.2 mol L<sup>-1</sup> HAc-NaAc buffer (pH 5.0) and separated by centrifugation at 37 °C for 5 min (4000 rpm). After removing the supernatant, the precipitate was dissolved in PBS (pH 7.0). The precipitation-centrifugation-dissolution procedure above-mentioned was repeated three times. Finally, the conjugate was dissolved in 5 mL of PBS (pH 7.0) and stored at 4 °C. The conjugate B-Ag was gotten.

### 2.3.3. Measurement of $\text{pH}_{\text{tr}}$ of polymer

A and B can be rapidly precipitated from the solution when the pH of the solution is lowered to below their  $\text{pH}_{\text{tr}}$ , and these inevitably result in a large decrease of the transmittance of the polymer solutions. Therefore, the change in transmittance was utilized to measure the  $\text{pH}_{\text{tr}}$ . In the measurement of  $\text{pH}_{\text{tr}}$ , transmittance of aqueous polymer solution at 450 nm was monitored against the pH of solution, at 37 °C using a Beckman DU-7400 UV/VIS diode array spectrophotometer.

### 2.3.4. Comparison of the immunological reaction activities of Ag, A-Ag and B-Ag

The immunological activities of Ag, A-Ag and B-Ag were tested with ELISA method based on competitive immunoassay format. A 100  $\mu\text{L}$  of Ab solution (1:5000 dilution of the original Ab solution (10 mg mL<sup>-1</sup>) with 0.05 mol L<sup>-1</sup> sodium carbonate buffer, pH 9.0) was transferred into the wells to coat the plate at 4 °C overnight. The plate was washed three times with Tris-HCl (pH 7.4, containing 0.05% Tween-20) and blocked with 100  $\mu\text{L}$  of 0.1 mol L<sup>-1</sup> PBS (pH 7.4) in the presence of 1% BSA at 37 °C for 1 h, then the wells were washed three times with Tris-HCl buffer. Known amounts of Ag, A-Ag and B-Ag (the amount of Ag contained in A-Ag and B-Ag conjugates is the same as that of free Ag) were added to the different wells, respectively, followed by adding 10  $\mu\text{L}$  Ag-HRP solution in each well and incubating the mixture for 2 h at 37 °C. The plate was washed three times with Tris-HCl buffer, then 100  $\mu\text{L}$  of 0.05 mol L<sup>-1</sup> NaH<sub>2</sub>PO<sub>4</sub>-NaOH buffer (pH 5.8), 40  $\mu\text{L}$  of  $9.0 \times 10^{-5}$  mol L<sup>-1</sup> H<sub>2</sub>O<sub>2</sub> and 40  $\mu\text{L}$  of 0.01 mol L<sup>-1</sup> *p*-HPA solution was added to the wells. Mixtures were allowed to stand for 15 min at room temperature followed by the addition of 20  $\mu\text{L}$  of 0.2 mol L<sup>-1</sup> NaOH. And then, the fluorescence intensities of the final solutions were measured at 410 nm with the excitation at 320 nm.

### 2.3.5. Competitive fluorescence immunoassay for Ag

Ag standards with different concentrations were prepared in 0.01 mol L<sup>-1</sup> PBS (pH 7.0, containing 1% BSA). A Ab-FITC solution (0.5 mg mL<sup>-1</sup>) was diluted by 1:5 in PBS/BSA.

To a 1.5 mL plastic centrifuge tube, the standard Ag solution (or normal human blood serum), 20  $\mu\text{L}$  A-Ag (or B-Ag) and 100  $\mu\text{L}$  BSA (5%) was added and diluted to 1.0 mL with 0.01 mol L<sup>-1</sup> PBS solution (pH 7.0). Then 30  $\mu\text{L}$  of diluted Ab-FITC was added. The mixture was mixed thoroughly by shaking and stirred by vibration for 5 min at 37 °C to carry out the homogeneous immune reaction. Immobilized Ag and standard Ag competed for binding to the Ab-FITC. And then 50  $\mu\text{L}$  of 0.2 mol L<sup>-1</sup> HAc-NaAc buffer (pH 5.0) was added to precipitate the polymer with antigen-antibody complex. The resultant precipitate was separated by centrifugation at 4000 rpm for 5 min at 37 °C and re-dissolved in 1.0 mL of 0.01 mol L<sup>-1</sup> PBS (pH 7.0), then 50  $\mu\text{L}$  of HAc-NaAc buffer was added to precipitate the polymer again. This procedure was repeated three times, and the final precipitate obtained was dissolved in 1.0 mL of PBS. The fluorescence intensities of the solutions were measured at 525 nm with the excitation at 495 nm. The scheme of immunoassay based on the pH-sensitive phase separating polymer was shown in Fig. 3.

## 3. Results and discussion

### 3.1. The effect of polymer composition on $\text{pH}_{\text{tr}}$

Polymers were formed in both methods, thermal initiation and redox initiation, with compositions ranging from 0 to 20% MAA. The  $\text{pH}_{\text{tr}}$  was determined according to the procedure described in Section 2.3.3. The polymers with 2.5% or higher contents of MAA displayed sharp phase transitions as a function of pH. Polymers with higher  $\text{pH}_{\text{tr}}$  were obtained by using the less amounts of MAA. The polymer containing 2.5% MAA had the highest  $\text{pH}_{\text{tr}}$  of 5.6, a pH that should not greatly affect the antibody-antigen binding. This polymer was therefore conjugated with Ag and used as a carrier of the reactants for immunoassay.

### 3.2. Phase transition characteristics of A and B

The phase transition behavior for A and B in aqueous solution was studied based on their percent transmittance changes at various pH (Fig. 4). It can be seen that A and B dissolved in water when pH was higher than 5.6, and percent transmittance of the solutions was very high. However, if pH was below 5.6, A and B rapidly precipitated from the solutions, resulting in a large decrease of percent transmittance of the solutions. From Fig. 4, it can be seen that the  $\text{pH}_{\text{tr}}$  of A and B is almost the same.

Error bars represent the standard deviations of 3 percent-age transmittance readings.

According to the literature [23], the number-average molecular weight of P(NIP-MAA) using AIBN as initiator was about 100,000. The molecular size of polymers was independent of the mode of initiation [24]. Hence, we

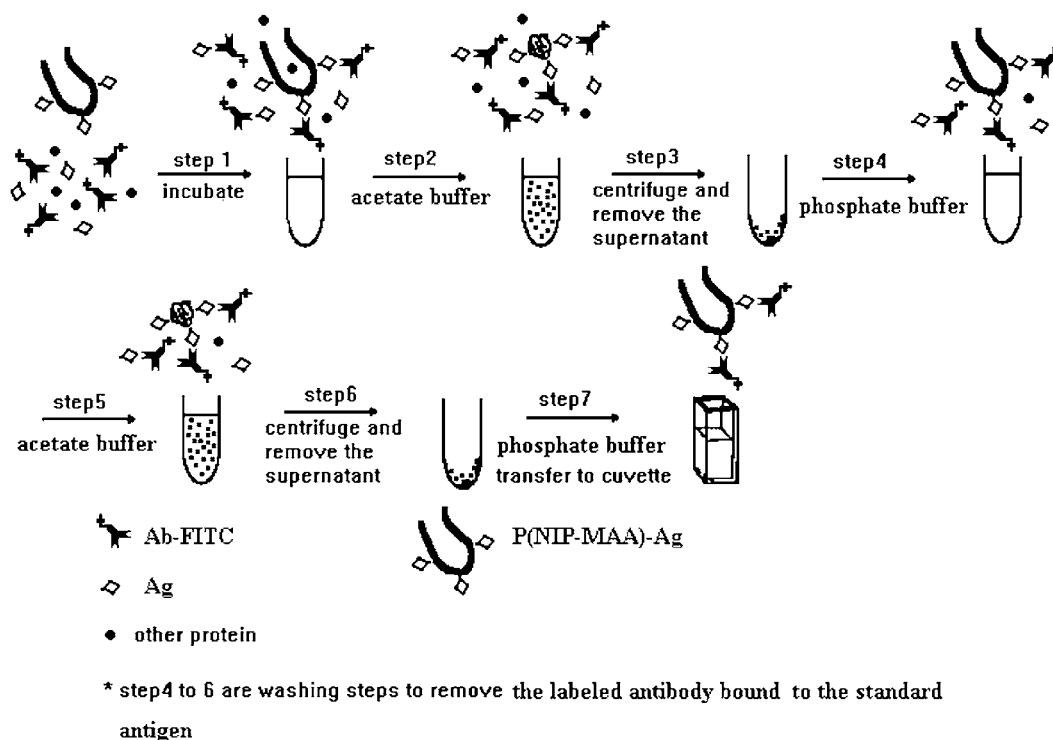


Fig. 3. Protocol for the pH-sensitive phase separating immunoassay method.

thought that there was not much difference between molecular weights of A and B, which led to almost same  $pH_{tr}$ . However, the soluble–insoluble phase transition pH range of A was narrower than that of B. It is suggested that the molecular weight distribution of the copolymer by thermal initiation polymerization be narrower because the residual product of AIBN is simpler than that of APS and TEMED. The narrower the phase transition pH range of polymer, the easier the adjustment of precipitation and dissolution is. Therefore, A is more suitable for using as a carrier in immunoassay.

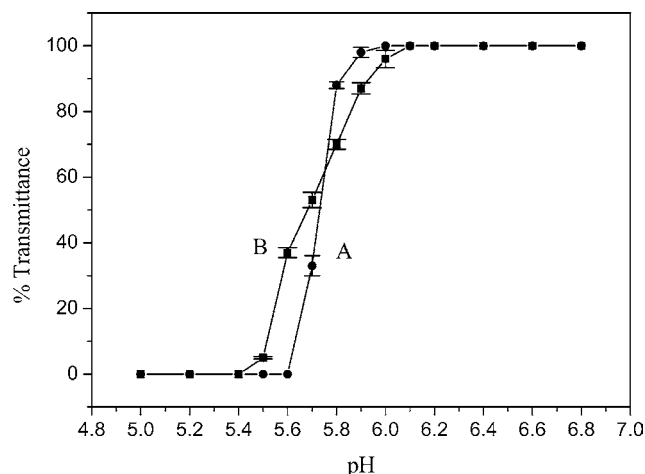


Fig. 4. Change of percent transmittance in A and B as a function of pH at 37°C.

### 3.3. Immobilization of Ag to P(NIP-MAA)

In order to determine immobilization efficiency of Ag to P(NIP-MAA), we used Ag-FITC instead of Ag. The immobilization of Ag-FITC to P(NIP-MAA) was performed according to the procedure described in Sections 2.3.1 and 2.3.2, respectively. The relative fluorescence intensity was compared with that before centrifugation. From Fig. 5, it was found that about 62% of the fluorescence was reserved in

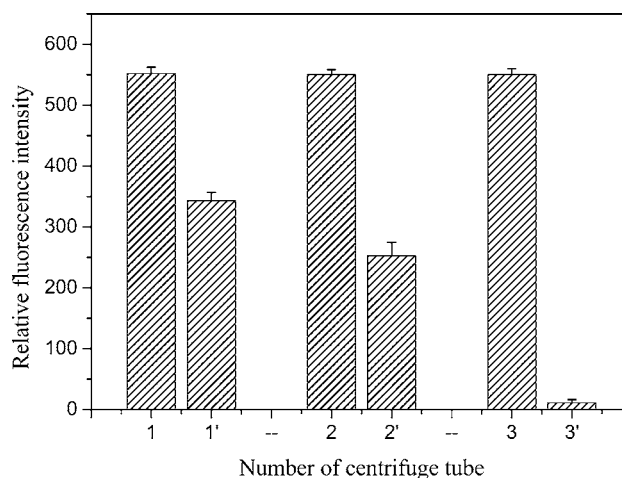


Fig. 5. The fluorescence of polymerisation of Ag-FITC with A(1,1'), Ag-FITC with B(2,2') in contrast to the mixture of free Ag-FITC and P(NIP-MAA) without NAS (3,3'). Error bars represent the standard deviations of three fluorescence readings. 1,2,3: Before centrifugation; 1',2',3': after centrifugation.



the A–Ag–FITC after centrifugation, which indicated that Ag–FITC could be successfully immobilized on A and immobilization efficiency was 62%. On the other hand, about 46% of Ag–FITC remained in the B–Ag–FITC conjugate. Whereas, only 2% of Ag–FITC remained in P(NIP-MAA) for the mixed solution of P(NIP-MAA) and Ag–FITC without NAS. These experiments demonstrated that Ag–FITC was chemically conjugated with A or B rather than absorbed. The non-specific adsorption of Ag–FITC on P(NIP-MAA) was weak. Furthermore, the immobilization efficiency in thermal initiation way was higher than that in redox initiation way.

### 3.4. Comparison of the immunological reaction activity of free Ag, A–Ag and B–Ag

According to literature [25], the immunological activities of Ag immobilized to A and B were tested with ELISA method based on competitive immunoassay format. In the immunoassay, Ab was coated on a 96-well plate (polystyrene), then constant amounts of Ag–HRP and free Ag (or A–Ag or B–Ag conjugates) were added to compete for binding to the plate-bound Ab. After the immunoreaction, the immunochemically adsorbed Ag–HRP moiety was determined by measuring the fluorescence produced from a solution containing *p*-HPA and hydrogen peroxide. For these different types of Ag mentioned above, the higher the activity of immunological reaction, the more the quantity of corresponding Ag bound to the plate-bound Ab will be, thus the less the amount of Ag–HRP bound to the plate-bound Ab will be, which leads to the less fluorescence intensity of final solution.

The results showed that A–Ag conjugate maintained 59% immunological reaction activity of free Ag, while B–Ag conjugate only maintained 44% immunological reaction activity of free Ag. The reason possibly is that thermal initiation polymerization effectively avoids polymer chain's wrapping around the surface of Ag since the coupling reaction occurs after the polymerization, which would surely maintain more immunological activities. Thus, A is more suitable for using as the carrier of Ag in pH-sensitive phase separation fluorescence immunoassay.

### 3.5. Optimization of polymerization conditions.

Although the structure of active acrylate NAS is similar to those of NIP and MAA, the pH-sensitive character will disappear if there is too much NAS. The experiment showed that the mol % of NAS in the polymer should be controlled below 3%. Polymer A (containing about 2.1% NAS) demonstrated not only sharper phase transition characteristics, but also higher immobilization efficiency. The effect of the polymerization time of A was also investigated. The results showed that polymerization was completed in 24 h. The effect of varying polymerization temperature (40–80 °C) was examined. The lower the temperature, the longer will be the polymerization time. The tempera-

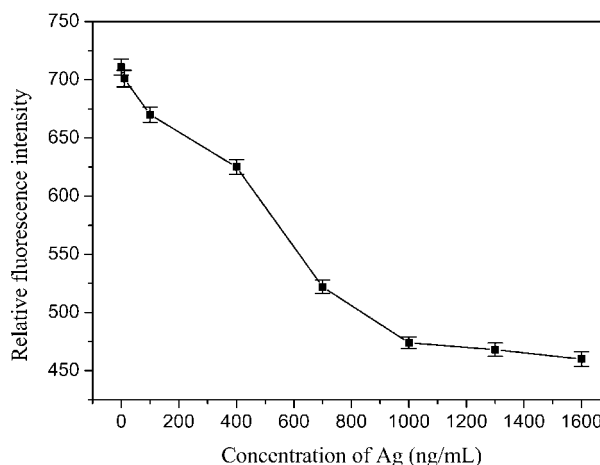


Fig. 6. The calibration curve for human IgG in A system.

ture of 60 °C was selected as the polymerization temperature. The polymer B was polymerized at room temperature for 2 h.

### 3.6. Optimization of immunoassay conditions.

In the competitive immunoassay for Ag using A or B as a carrier, the results showed that the optimal volume used for the Ab–FITC was in the range 20–40  $\mu\text{L}$ , thus, 30  $\mu\text{L}$  was recommended. The influence of the amount used for A–Ag or B–Ag conjugate was studied. Different volumes (10, 20, 30, 40  $\mu\text{L}$ ) of immobilized Ag were chosen to get the different calibration curves for the determination of Ag. The linear range was 500–2000  $\text{ng mL}^{-1}$  for 30 and 40  $\mu\text{L}$  of immobilized Ag and the fluorescence intensity hardly changed with concentrations below 500  $\text{ng mL}^{-1}$ . For 10 and 20  $\mu\text{L}$  of immobilized Ag, the linear range was 0–1000  $\text{ng mL}^{-1}$ . However, the sensitivity of the latter was better than that of the former. In the next experiments, 20  $\mu\text{L}$  of immobilized Ag was adopted.

The results showed that the immune reaction would reach equilibrium within 4 min, which is much shorter than that of solid immunoreaction [26], and immunoassays modulated by temperature-sensitive polymers [6–10].

### 3.7. Calibration graph for Ag

In A system, the calibration curve for the determination of Ag is shown in Fig. 6. It could be treated as a straight line within 1000  $\text{ng mL}^{-1}$ . The linear regression equation was  $F = 704.46 - 0.24 [\text{Ag}]$ , where  $F$  meant relative fluorescence intensity and the unit of  $[\text{Ag}]$  was in nanograms per milliliter. The detection limit ( $3\sigma/S$ ) for Ag was 8  $\text{ng mL}^{-1}$  calculated from the standard deviation of blank ( $n = 9$ ). The correlation coefficient was 0.993. In B system, the calibration curve for the determination of Ag is shown in Fig. 7. It also could be treated as a straight line within 1000  $\text{ng mL}^{-1}$ . The linear regression equation was  $F = 587.40 - 0.23 [\text{Ag}]$ . The detection

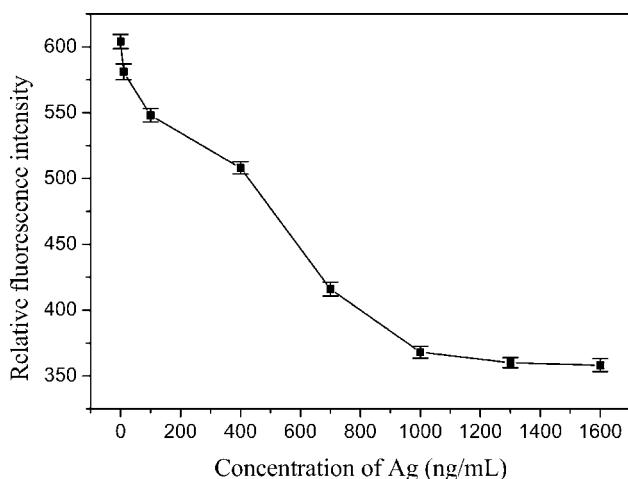


Fig. 7. The calibration curve for human IgG in B system.

Table 1  
Determination of human IgG in human sera

Sample no.	Human IgG levels <sup>a</sup> (mg mL <sup>-1</sup> )	Added (ng)	Found (ng)	Recovery (%)	R.S.D. (%)
1	13.8	200	195	97.5	4.5
2	13.6	200	210	105.0	6.1
3	12.6	200	206	103.0	6.2

<sup>a</sup> Mean of six determinations.

limit ( $3\sigma/S$ ) for Ag was  $12 \text{ ng mL}^{-1}$  ( $n = 9$ ). The correlation coefficient was 0.990.

### 3.8. Immunoassay of human IgG in human blood sera by thermal initiation polymerization

The method by thermal initiation polymerization was used for the determination of human IgG in healthy human sera. The results obtained are summarized in Table 1 and coincident with the human IgG levels in normal human sera ( $10\text{--}14 \text{ mg/mL}$ ) [27]. The relative standard deviation within a batch of determinations was below 7% ( $n = 6$ ). The possibility of using the proposed method for the analysis of samples was further confirmed by determining the recovery of known amounts of IgG added to the sample. The results in Table 1 show that the proposed method is feasible in practice.

## 4. Conclusion

Compared to redox initiation polymerization, the sensitivity of thermal initiation polymerization was higher. Immobilization efficiency was improved, and the conjugate maintained higher natural immunological reaction activity of Ag. The polymer showed a sharper transition in solution and precipitation around pH 5.6, which was more suitable

for using as the carrier in immunoassay. Furthermore, some pH-sensitive polymer prepared only in organic solvent or under high temperature could also be used as an immunoreaction carrier by thermal initiation polymerization. Thermal initiation polymerization was a better immobilization mode.

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