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Synthesis and properties of polymer latex with carboxylic acid functional groups for immunological studies

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Abstract

The poly(methyl methacrylate) (PMMA)/poly(methyl methacrylate)–poly(methyl acrylate acid) copolymer (PMMA–PMAA) composite polymer latex were synthesized by the method of soapless seeded emulsion polymerization. The morphology of the composite polymer latex was core–shell structure. The core was PMMA and shell was PMMA–PMAA copolymer. Because the PMMA–PMAA copolymer was shell, the carboxylic acid functional groups (COOH) of MAA distributed on the surface of composite polymer latex. The concentration of carboxylic acid groups distributed on the surface of composite polymer latex could be controlled by the amount of MAA. Antigens (Bovine Serum Albumin (BSA) or Anti-human IgG) were chemically bound onto the surface of PMMA/PMMA-PMAA core–shell composite latex by the method of either pre-activation or pre-adsorption to form the protein-coated latex (immunolatices). The more the carboxylic acid groups on the latex, the more the antigens were bound onto the surface of PMMA/PMMA–PMAA core–shell composite latex. The immunolatices had the higher stability than the parent composite latex due to the effect of steric hindrance of the antigens. Moreover, the sensitivity of the immunological agglutination of immunolatices was significantly influenced by the amount of covalently bound antigens and temperature.

Keywords: Core–shell latex; Immunolatices; Immunological agglutination

1. Introduction

In recent years, the antigen-coated (or antibody-coated) latex particles (immunolatices) have been applied in the field of biotechnology and medicine. The agglutination was a simple and rapid method to detect the antigens (or antibody) in biological fluids based on the specific agglutination of antigen-coated latex particles and the antibody or the specific agglutination of antibody-coated latex particles and the antigens.

The conventional method that was used to immobilize antigen (or antibody) on the surface of latex particle was physical adsorption [1–4]. However, since the physical bonding between latex particles and proteins was weak adsorption, the proteins partly dissociated from the latex particles to interfere with specific antigen–antibody reactions.

In order to enhance the adsorption between latex particles and proteins, the latex particles that had functional groups to chemically bond with proteins had been studied. Suzuki et al. [5] and Suzuta et al. [6] examined the possibility of using a methacrylic acid latex and nylon particles. But, both of the latex particles had been found unsuccessful due to particle aggregation and non-specific adsorption [7]. Besides, Rembaum et al. [8,9] had prepared similar latex particles that had hydroxyl, carboxyl or other functional groups on their surface by the method of emulsion copolymerization or Co γ radiation in the presence of a crosslinking agent. The results showed that these latex particles covalently bonded with the antibodies exhibit specific agglutination by corresponding antigens. Kondo et al. [10] pointed out that these latex particles, which covalently bonded with the antibodies had higher sensitivity of specific agglutination than the latex particles that physical adsorbed with the antibodies.

According to our previous work [11–14], the morphology of the composite polymer latex synthesized by the method of soapless seeded emulsion polymerization was core–shell structure. In order to increase the amount of functional groups on the surface of latex particles and avoided that the emulsifier polluted the latex particles. In this present article, the PMMA

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Table 1
Ingredients and conditions for the synthesis of seed latex (first stage)

Methyl methacrylate (MMA) (g)	120
Initiator ($K_2S_2O_8$) (g)	0.866
Deionized water (g)	1100
Temperature (°C)	80
Stirring rate (rpm)	300
Reaction method	Bath
Reaction time (min)	60

(core)/PMMA–PMAA (shell) core–shell composite polymer latex was synthesized by the method of soapless seeded emulsion polymerization. Either bovine serum albumin (BSA) or human immunoglobulin G (Human IgG) was covalently bound onto the surface of latex particles to form the immunolatices. The properties and the immunological agglutination of the immunolatices were investigated.

2. Experimental

2.1. Material

Methyl methacrylate (MMA) and methyl acrylic acid (MAA) were distilled under nitrogen atmosphere and reduced pressure prior to polymerization. The other materials were analytical grade and used without further purification. Distilled and deionized water was used throughout the work.

2.2. Synthesis of core–shell composite polymer latex

The PMMA/PMMA–PMAA core–shell composite polymer latex was synthesized by the method of soapless seeded emulsion polymerization.

This method was divided into two stages. The first stage was to synthesize the PMMA seed latex by the method of soapless emulsion polymerization. The ingredients and conditions of the first stage were listed in Table 1. The synthesis of seed latex was carried out at 70°C under nitrogen atmosphere. The stirring rate was controlled at 300 rpm. After reacting for 1 h, the first stage was completed, then the seed latex was quenched to room temperature. The second stage was to synthesize the core–shell composite polymer latex. In the second stage reaction, adding quantitative MAA and MMA into the seed latex emulsion, and swelling the seed latex at room temperature for 24 h, and then the reaction system was heated in a water bath. After the reaction system reached the reaction temperature, the aqueous solution of $K_2S_2O_8$ was added into the reactor to start the second stage reaction. The ingredients and conditions of the second stage reaction are shown in Table 2. The shell composition and symbols of the composite polymer latex are shown in Table 3.

Table 2
Ingredients and conditions for the synthesis of core–shell composite polymer latex (second stage)

Seed latex emulsion (g)	500
MMA monomer (g)	78.4 or 68.6
MAA monomer (g)	19.6 or 29.4
Initiator ($K_2S_2O_8$) (g)	0.251
Deionized water (g)	310
Temperature (°C)	70 or 80
Stirring rate (rpm)	300
Reaction method	Bath

Table 3
Symbols and shell composition of core–shell composite latex

Symbol	Weight percentage of MAA of shell composition (%)	Reaction temperature of second stage (°C)
CS-70-73	30	70
CS-70-82	20	70
CS-80-73	30	80
CS-80-82	20	80

In order to purify the latex emulsion, the dialysis molecularporous membrane tube was used to dialyze the latex emulsion. The latex emulsion was poured into the dialysis molecularporous membrane tube and then put in the deionized water for one week. After the process, the residual monomer and $K_2S_2O_8$ in the emulsion product could be dialyzed.

2.3. Measurement of the concentration of carboxylic groups on the composite latex

The composite latex was added into the NaOH solution, the conductivity of NaOH solution decreased abruptly due to the decrease of ionic concentration of NaOH solution upon the neutral reaction of COOH groups distributed on the composite latex's surface and NaOH. Then the conductivity of NaOH solution decreased slowly due to the neutral reaction of the COOH groups that is hidden inside the composite latex and the NaOH. The composite latex was added into the NaOH solution, a digital conductivity meter immediately measured the abrupt decrement of conductivity of NaOH solution. The amount of COOH groups distributed on the surface of composite latex was obtained from the decrement of ionic concentration of NaOH solution by using the calibration curves representing the relationships between the ionic concentration and the conductivity. On the other hand, the surface's area of the composite latex was measured by BET analysis (Micromeritics ASAP-2100). The concentration of COOH groups distributed on the surface of composite latex was obtained from the result of the amount of COOH groups divided by the surface's area of composite latex.

2.4. Preparation of immunolatices

The proteins such as antigens or antibodies were

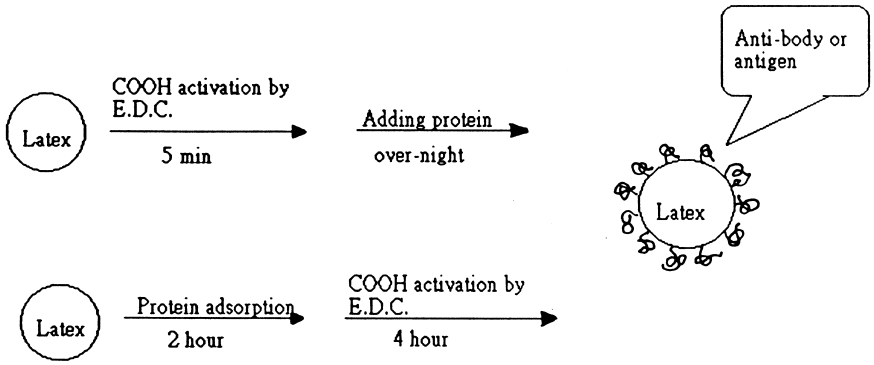

Fig. 1. The process of pre-adsorption and pre-activation.

covalently bonded onto the surface of the composite latex by the method of either pre-adsorption or pre-activation to form the immunolatices. The process of pre-adsorption and pre-activation was shown in Fig. 1.

2.4.1. The pre-activation method

A mixture of 150 mg composite polymer latex and 10 mg 1-ethyl-3-(3-dimethylamine propyl) carbodimide (EDC) was added into the phosphate buffer saline (PBS) solution at pH 5, and then the active reaction was allowed to proceed for 5–10 min. Then the antibody or antigens was added to proceed the covalently bonding reaction overnight.

2.4.2. The pre-adsorption method

A mixture of 150 mg composite latex and quantitative antibody or antigens was added into the PBS solution at pH 5 to proceed the adsorptive reaction for 2 h. Then 10 mg of coupling agent (EDC) was added into the mixture to activate the COOH groups and reacted with the antigens or antibody for 4 h.

After the reaction of either pre-activation or pre-adsorption, the mixture was then centrifuged for 30 min at 12,000 rpm and the precipitate was washed with glycine buffer (pH 11) by the method of centrifugation to separate the immunolatices from the unbound antigens. Finally, the immunolatices were re-suspended in the glycine buffer solutions (pH 7.4) and then sodium azide (NaN_3) was added to these suspensions of immunolatices and stored at 4°C. The amount of bound antigens was estimated by subtracting the amount of unbound antigens that consisted in the supernatant fluid as well as the washings from the initial amount of antigens. The amount of unbound antigens was obtained from the calibration curve representing the relationships between the amount of antigens and the absorption of UV spectrometer at a wavelength of 280 nm.

2.5. The stability of immunolatices

Based on the principle that the unstable particle was easy to coagulate, the hydrodynamic diameter of the unstable immunolatices was larger than that of stable immunolatices. The stability of the immuolatices was decided by the method that the latex particles were suspended in PBS solution and shocked by sonicator for 5 min. Later, laser light scattering was used to measure the average diameter of the latex sample. Since the hydrodynamic diameter of the particle is time-dependent, all the values of particles' hydrodynamic diameters are taken only after the values of hydrodynamic diameters are stable, and do not vary with time. The time for these values of hydrodynamic diameters of the particles to be stable was about 5 min.

2.6. Immunological agglutination of immunolatices

The immunolatices suspension of a desired amount was diluted to a final concentration of 150 μg/ml with PBS solution (ionic strength 0.02, pH 7.4) and the diluted suspensions were sonicated for 5 min. Besides, the antigen (BSA or anti-human IgG) was dissolved in PBS solution (ionic strength 0.02, pH 7.4) to form the antigen solution.

Mixing the diluted immunolatices suspension (300 μl) and the antigen solution (100 μl) to proceed the reaction of immunological agglutination for 20 min, UV spectrometer was then used to measure the absorbance of the mixture at a wavelength of 360 nm. Besides, the mixture of the suspension of parent composite latex and the antigen solution was used as control experiment. The control experiment was measured by UV spectrometer at a wavelength of 360 nm. The difference between the absorbance of immunolatices suspension and the absorbance of control experiment was due to immunological agglutination. The change in absorbance of the immunolatices suspension due to immunological agglutination was employed as a measure of the agglutination reaction.

2.7. Immunological studies

The immunolatices (bonding with anti-human IgG) was mixed with a patient's blood and a healthy human's blood at a temperature of 37°C to proceed the immunological reaction for 20–30 min. After the immunological reaction, the immunolatices that did not bond on the blood cells were separated from the blood cells by the method of washing

Table 4
Concentration of COOH groups distributed on the surface of composite latex and the MAA shell composition of composite polymer latex

Composite latex	Concentration of COOH groups distributed on the surface of composite latex (mol/m^2)	Ratio of concentration of COOH groups distributed on the surface of composite latex	Ratio of MAA shell composition
CS-70-73	3.96×10^{-7}		
CS-70-82	2.15×10^{-7}		
CS-80-73	5.02×10^{-7}		
CS-80-82	3.24×10^{-7}		
CS-70-73/CS-70-82		1.58	1.5
CS-80-73/CS-80-82		1.55	1.5

the reaction mixture with the PBS solution by centrifugation. Finally, the blood cells were fixed and dried, and then observed by scanning electron microscope.

3. Results and discussion

3.1. Concentration of COOH groups on the surface of composite polymer latex

Based on the principle that the conductivity of NaOH solution could be decreased by the neutral reaction of COOH groups and NaOH, the concentration of COOH groups distributed on the surface of composite latex was obtained as shown in Table 4. The ratio of the concentration of COOH groups distributed on the surface of CS-70-73 to that distributed on the surface of CS-70-82 was approaching the ratio of MAA weight percentage of the shell composition of CS-70-73 to that of CS-70-82, as shown in Table 4. Thus, the COOH groups distributed on the surface of composite polymer latex could be adjusted by the amount of MAA in the reaction mass. Besides, in order to understand if the temperature of reaction could influence the concentration of COOH groups distributed on the surface of composite polymer latex, the composite polymer latex was synthesized at 80°C. The result showed that the ratio of the concentration of COOH groups distributed on the surface of CS-80-73 to that distributed on the surface of CS-80-82 was also approaching the ratio of MAA weight percentage of the shell composition of CS-80-73 to that of CS-80-82, as shown in Table 4. Thus, the COOH groups distributed on the surface of composite polymer latex that was synthesized at a temperature of either 70 or 80°C could be varied by the amount of MAA in the reaction mass.

3.2. Effect of covalently binding method on the amount of antigen bound onto the surface of composite latex

Fig. 2 shows the relation between binding method and the amount of BSA bound to the composite latex. The result showed that the pre-adsorption method was better than the pre-activation method at high concentrations of added BSA. The anti-human IgG bound to the composite polymer latex had a similar result that is shown in Fig. 3. During the process of pre-activation method, the composite latex must have reacted with coupling agent (EDC) first, then

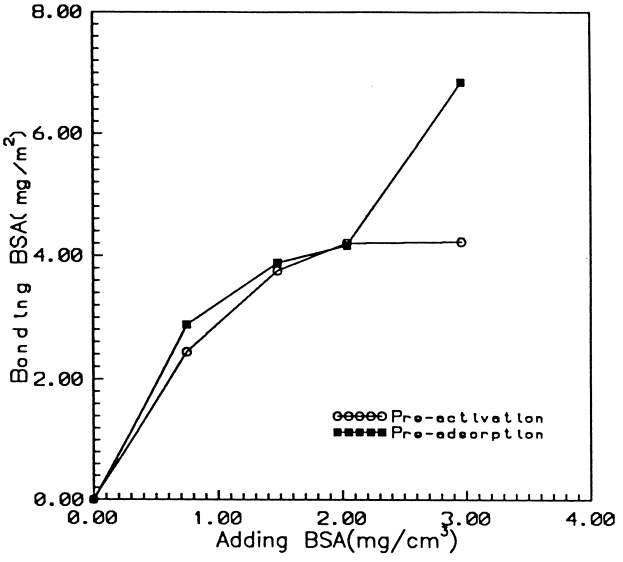

Fig. 2. Effect of covalently bonding method on the amount of antigen (BSA) bonded to the composite latex (CS-70-73).

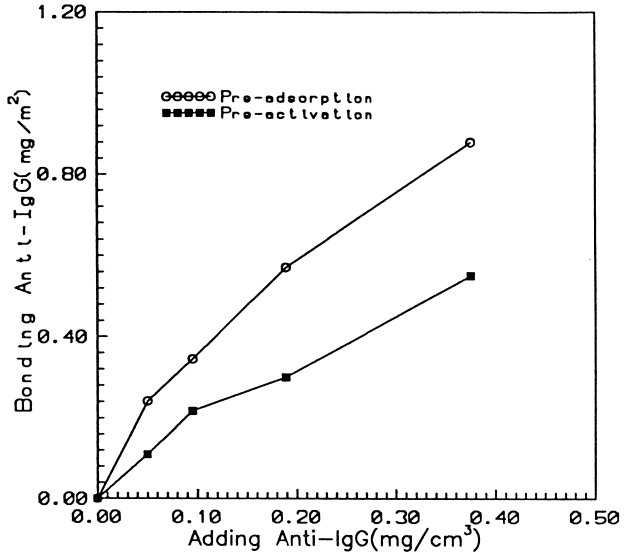

Fig. 3. Effect of covalently bonding method on the amount of antigen (anti-IgG) bonded to the composite latex (CS-70-73).

Table 5
Concentration of COOH groups distributed on the surface of composite latex and the amount of BSA bonding on the surface of composite latex

Composite latex	Concentration of COOH groups distributed on the surface of composite latex (mol/m^2)	Bonding BSA by pre-activation (mg/m^2)	Bonding BSA by pre-adsorption (mg/m^2)
CS-70-73	3.96×10^{-7}	4.47	6.98
CS-70-82	2.51×10^{-7}	2.88	4.5
CS-80-73	5.02×10^{-7}	4.73	6.8
CS-80-82	3.24×10^{-7}	2.98	4.4

reacted with antigen. After the EDC covalently bound to the composite latex, the amino groups (NH_2) of antigen were not easy to react with the COOH groups distributed on the composite latex due to the steric hindrance of EDC [15]. Besides, the molecular weight of antigen was large (the molecular weights of BSA and anti-human IgG are 70,000 and 150,000, respectively), the steric hindrance occurred between antigen and antigen too. However, during the process of the pre-adsorption method, the composite polymer latex was allowed to mix with antigen first, and then to react with EDC. In this way, although the steric hindrance might occur not only between EDC and EDC but also between antigen and EDC, the NH_2 groups of antigen comes closer to the COOH groups of the composite polymer latex due to the process of pre-adsorption. After the COOH groups were activated by EDC, the NH_2 groups easily reacted with COOH groups, so that the antigen made it easy to bind to the composite polymer latex by the method of pre-adsorption than that by the method of pre-activation.

3.3. Effect of the concentration of carboxylic acid groups on the amount of covalently bound antigens

Table 5 shows the relationships of the amount of antigen, which covalently bound on the composite polymer latex, and the concentration of COOH groups distributed on the surface of composite latex. The result appeared that to increase the amount of COOH groups distributed on the surface of composite latex would lead to an increase in the amount of antigen that covalently bound on the composite latex. Thus, the amount of COOH groups that located on the surface of the composite latex could be controlled by the amount of MAA at the shell of the composite latex. Therefore, by increasing the amount of MAA shell composition of the composite latex, the amount of antigens covalently bound on the composite latex would also increase.

Besides, the ratio of the amount of antigens bound on the composite latex of CS-70-73 to the amount of antigens bound on the composite latex of CS-70-82 was approaching the weight ratio of MAA shell composition of the composite latex, as shown in Table 6. Thus, the amount of antigens bound on the polymer latex were controllable by adjusting the MAA shell composition of the composite latex.

3.4. Effect of the covalently bonding antigen on the stability of the composite latex

The unstable polymer latex easily undergoes flocculation, and so the hydrodynamic diameter of the unstable polymer latex was larger than that of the stable polymer latex, due to the effect of flocculation with time. The hydrodynamic diameter of the immunolatices was shown in Table 7. The results appear that the hydrodynamic diameter of the immunolatices was smaller than that of the parent particle. That is to say the immunolatices are more stable than the parent particle. The stability of the particle with polymer chains on the surface is dependent on the amount of the polymer chains [16]. If the particle carries a less amount of polymer chains on the surface, the polymer chains may generate the bridging flocculation effect, which decreases the stability of the particles. But if there is a large amount of polymer chains distributed on the particle surface, the effect of bridging flocculation becomes insignificant, yielding significant effect of steric stabilization to increase the stability of the particles [16]. Thus, the stability of the immunolatices is larger than that of the parent particles due to the effect of steric stabilization, which generates from the larger amount of antigens that is distributed on the surface of immunolatices. Hence, the hydrodynamic diameter of the immunolatices bonded with antigens was smaller than the parent composite latex.

Table 6
Ratio of the amount of MAA shell composition and the ratio of the BSA bonded to the composite latex

Latex	Ratio of MAA shell composition	Ratio of bonding BSA by pre-activation	Ratio of bonding BSA by pre-adsorption
CS-70-73/CS-70-82	1.5	1.55	1.55
CS-80-73/CS-80-82	1.5	1.58	1.54

Table 7
Hydrodynamic diameter of immunolatices measured by laser light scattering

Latex	Hydrodynamic diameter (nm)
Parent composite latex	1024
Latex-BSA	955
Latex-anti-IgG	946

3.5. Effect of immobilized amount of antigens on immunological agglutination of immunolatices

Fig. 4 shows the effect of immobilized amount of BSA on the absorbance changes of immunolatices measured by UV spectrometer at a wavelength of 360 nm. This absorbance change of immunolatices was the indication of immunological agglutination. The results showed that the rates of the immunological agglutination increased with increasing anti-BSA concentration. Besides, the agglutination rates of the antigen-immobilized immunolatices increased with increasing amount of immobilized antigens.

3.6. Effect of temperature on immunological agglutination of immunolatices

The effect of temperature on immunological agglutination of immunolatices was analyzed by UV spectrometer at a wavelength of 360 nm. Fig. 5 shows the changes in absorbance of the mixture of anti-BSA and immunolatices bound with BSA at different temperatures. The result showed that the immunological agglutination was strongly affected by temperature. The immunological agglutination was more sensitive at a temperature of 37°C than at 25°C. Moreover, the effect of temperature on the stability of immunolatices

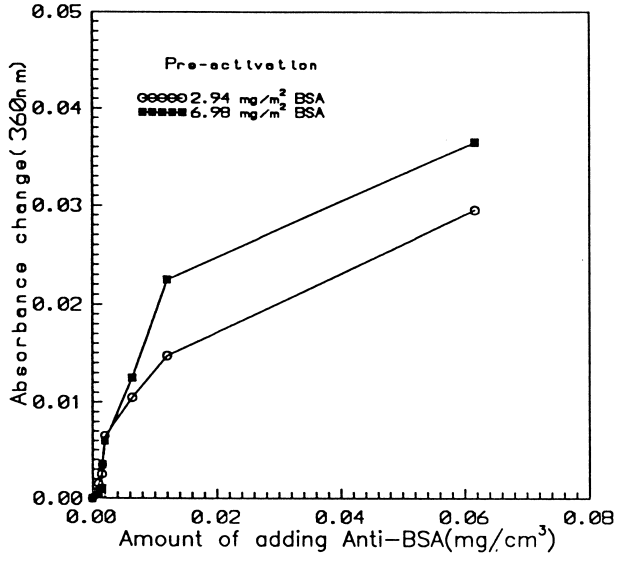

Fig. 4. Effect of the bonding amount of BSA (antigen) on the absorbance change of immunolatices (latex-BSA) suspensions at pH 7.4, ionic strength 0.02 and temperature 25°C.

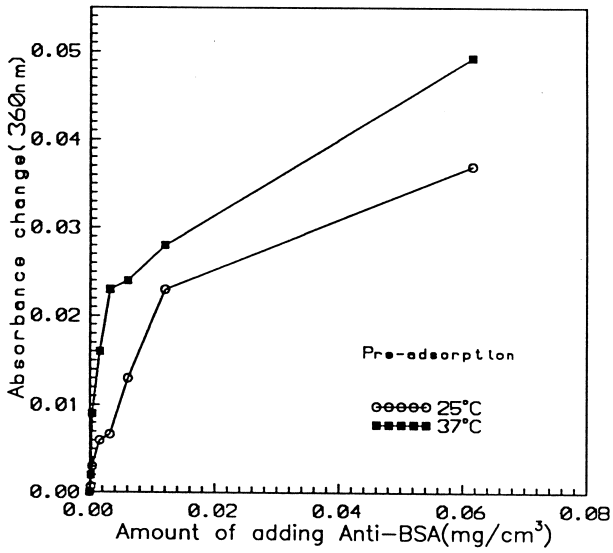

Fig. 5. Effect of temperature on the absorbance change of immunolatices (latex-BSA) suspensions at pH 7.4, ionic strength 0.02.

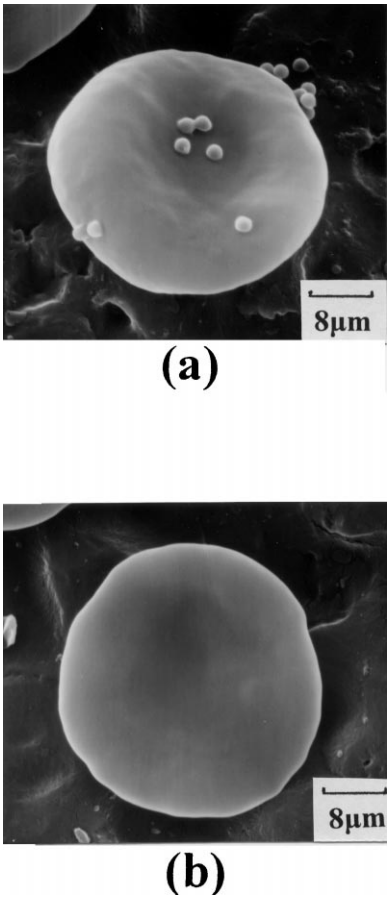

Fig. 6. The SEM photographs of: (a) patient's red blood cells and (b) healthy human's red blood cells to react with the immunolatices, which immobilized anti-human IgG.

was insignificant. Thus, the effect of temperature on the agglutination of immunolatices was not due to the decrement of stability of immunolatices. The agglutination of immunolatices generated from the reaction of BSA bind on to the surfaces of immunolatices and anti-BSA [8–10]. The reaction is more sensitive at higher temperatures, and thus the immunological agglutination was more sensitive at a temperature of 37°C than at 25°C.

3.7. Immunological study

The immunolatices which immobilized with anti-human IgG reacted with a patient's red blood and a healthy human's red blood, respectively. The result showed that there were some immunolatices adsorbed on the surface of patient's red blood cells, as shown in Fig. 6(a), but the immunolatices could not adsorb on the surface of healthy human's red blood, as shown in Fig. 6(b). This was due to the presence of human IgG distributed on the surface of patient's red blood cells, but not on the surface of healthy human's red blood cells. When the human IgG reacted with the anti-human IgG that bound on the surface of immunolatices, the immunolatices could bind with the patient's red blood cells. On the contrary, there was no human IgG distributed on the surface of healthy human's red blood cells, and so the immunolatices was not able to bind on to the surface of healthy human's red blood cells.

4. Conclusion

It was concluded that the PMMA/PMMA–PMAA core–shell composite polymer latex could react with proteins such as antigens and antibodies by the method of pre-adsorption or pre-activation through carboxylic acid functional groups that distributed on the surface of latex particles to form the immunolatices. The amount of antigens or antibodies that bound on the surface of latex particles by the method of pre-adsorption was larger than that by the method of pre-activation. Adjusting the MAA shell composition of the composite latex could control the amount of proteins that was bound to the composite latex. The stability of the immunolatices that bound with proteins was better than that of the parent composite polymer latex that did not bind with proteins. Besides, the immunological agglutination rate of the immunolatices increased by increasing the amount of immobilized antigens. The sensitivity of immunological agglutination was strongly affected by temperature. The immunological reaction was sensitive at a temperature of 37°C. Immunolatices derived from the PMMA/PMMA–PMAA core–shell composite polymer latex may therefore be used to work in immunological studies and diagnostic tests.

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