Development of Immunolatex Particle for Detection of Malaria Antigen

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Abstract:
In order to enhance the sensitivity of malaria detection based on the latex agglutination test (LAT), immunolatex particles containing magnetic nanoparticles (MNP)\textsuperscript{s} embedded in poly(styrene/divinylbenzene/acrylic acid) (P(St/DVB/AA)) matrix or magnetic polymeric nanoparticles (MPNPs) were synthesized \textit{via} the miniemulsion polymerization, and then surface functionalized by malaria antibody. After FTIR, TGA and TEM analysis of MNPs, MNPs modified by oleic acid and MPNPs stabilized by PAA, the spherical monodispersed P(St/DVB/AA) MPNPs (170 nm) with 25\% magnetic content were used as seeds for preparing non-spherical (ns) MPNPs having oval/dumbbell shape. With the multi-light scattering, the ns-MPNPs having high opacity will facilitate the visibility of immunolatex particles in the LAT when binding with malaria antigen. The magnetic and optical properties of the prepared ns-MPNPs will be further improved for increasing the sensitivity and specificity of the detection.

1. Introduction
Embedding magnetic nanoparticles (MNP)\textsuperscript{s} having superparamagnetic behavior into a functionalized polymer latex particle producing magnetic polymeric nanoparticles (MPNPs) can greatly enhance the effectiveness of latex agglutination test (LAT). As a simple and low cost technique, the LAT has been developed for detection of \textit{Plasmodium (P.) falciparum}, causing acute malaria and death, in remote area. By using spherical polystyrene copolymerized with acrylic acid (P(St/AA)) particles, synthesized by the soap-free emulsion polymerization and functionalized with malaria antibody (Ab)\textsuperscript{1}, the LAT was detected \textit{via} naked eyes within a few minutes after mixing with plasma of patients. Although the high sensitivity (90\%) was obtained, the selectivity between \textit{P. falciparum} and \textit{P. vivax} infections was low. To improve the specificity\textsuperscript{2}, a colored monomer, i.e., 2,3,6,7-tetra (2,2’-bithiophene)-1,4,5,8-naphthalenetetra carboxylic-N,N’-dit(2-methylallyl)-bisimide (ALN8T) was copolymerized with P(St/AA). After immobilizing Ab on the PS/AA-ALN8T particles by using affinity binding, the excellent sensitivity (100\%) for \textit{P. falciparum} detection was noticed with the highest specificity of 23.3\%. Specificity and detection limit can be significantly improved using a combination of MNP\textsuperscript{s} and polymerase chain reaction (PCR)-enzyme-linked gene assay (MELGA). The detection limit for \textit{P. falciparum} gametocyte is in the femtogram (10^{-15}) level.\textsuperscript{3} Therefore, MNP\textsuperscript{s} embedded in P(St/divinyl benzene (DVB)/AA) matrix producing MPNPs having high magnetization (17-27 emu/g) were further synthesized.\textsuperscript{4} The particles did not inhibit PCR and provided high specificity and sensitivity of malaria gametocyte detection comparable to conventional PCR.\textsuperscript{7} In this work, spherical P(St/DVB/AA) MPNPs with high magnetic content were prepared \textit{via} miniemulsion polymerization. The MPNPs obtained were used as seeds for preparing non-spherical (ns) MPNPs having oval/dumbbell shape and doublet
immunolatex particles formed under applying magnetic field. It has been hypothesized that ns morphology with high scattered light may increase the sensitivity of antigen detection via naked eyes. After Ab binding, the immunolatex particles will be developed for simply use in remote area.

2. Materials and Methods
2.1 Materials
Ammonium hydroxide solution (NH₄OH) 25%, iron (II) chloride tetrahydrate (FeCl₂·4H₂O), iron (III) chloride hexahydrate (FeCl₃·6H₂O), oleic acid (OA), styrene (St), sodium dodecyl sulfate (SDS), divinyl benzene (DVB), acrylic acid (AA), hexadecane (HD), potassium persulfate (KPS), polyvinyl alcohol (PVA), methanol (MeOH), azobisisobutyronitrile (AIBN) and deionized (DI) water were applied.

2.2 Synthesis of MPNPs
MNPs were synthesized by mixing FeCl₂·4H₂O (1.76 g) and FeCl₃·6H₂O (3.52 g) in DI water (80 mL) under N₂ as previously reported. Subsequently, NH₄OH was added into the mixture and the black suspension obtained was continuously stirred for 30 min before centrifugation at 5000 rpm for 15 min. After adding OA (10% v/v) and St (15 mL) while stirring for 10 min, the OA-MNPs-St was centrifuged at 5000 rpm before further use. Spherical MPNPs were synthesized by mixing OA-MNPs-St (7 g) with St (1.5 g), AA (3% wt of St), HD (0.3 g), DVB (1.0 g) and aqueous solution of SDS (0.044 g). After stirring for 1 h, the mixture was ultrasonicated at 60% amplitude in an ice bath. The polymerization was started at 72°C with addition of KPS solution and the reaction was continued for 22 h.

2.3 Synthesis of ns-MPNPs
An emulsion of St (4 mL)/AIBN (0.02 g) in 1% w/v PVA aqueous solution (16 mL) was added into spherical crosslinked MPNPs (1 g in 5 mL PVA solution) and stirred for 20 h. After polymerization at 70°C for 8 h, the suspension was centrifuged at 9000 rpm for 30 min and washed thrice with DI water.

2.4 Characterizations
The particles were characterized by using dynamic light scattering (DLS) (Malvern Instruments, 3000HS), Fourier Transform Infrared Spectroscopy (FTIR) (Perkin Elmer, Spectrum GX), thermogravimetric analysis (TGA) (Simultaneous DSC-TGA TA instruments, SDT 2960). Their morphologies were observed under Transmission Electron Microscope (TEM).

3. Results & Discussion
3.1 Morphologies of particles
TEM images of MNPs, OA-MNPs and MPNPs are presented in Fig. 1 a), b), c), respectively.

Figure 1 TEM images of a) MNPs, b) OA-MNPs and c) P(St/DVB/AA) MPNPs

In Fig.1a), the shape of MNPs is irregular. The particle size of ca.10 nm indicates the superparamagnetic behavior. TEM image in Fig.1b) shows the bigger size of OA-MNPs compared to MNPs. The MNPs (dark area) were surrounded by OA (light area) acting as stabilizer. TEM micrograph in Fig.1c) reveals that OA-MNPs were encapsulated into P(St/DVB/AA) particles whose average hydrodynamic size was 169.2 ± 29.5 nm.
Their zeta potential of -38.4 mV indicates good stability of MPNPs. Particle sediments due to the large particle size were observed. Although the saturated magnetization (Ms) of MPNPs was low (4 emu/g), their response time under an applied magnet was within 5 min.

Next, the spherical P(St/DVB/AA) MPNPs were used as seeds to prepare ns particles. Fig.2 shows dumbbell-shaped MPNPs having particle size of 308.9 ± 14.2 nm (length) and 202.8 ± 99.3 nm (width). New lobe of PS (non-magnetite) was separated from seed P(St/DVB/AA) MPNP due to elastic stress building of excess St and temperature as driving force. However, the absolute zeta potential of < 30 mV indicates low particle stability because of charge neutralization between AA and St and the existence of polymeric stabilizer, PVA, on the particle surface.

![Figure 2 TEM image of ns-MPNPs](image)

3.2 Analysis of particles
FTIR spectrum a) in Fig.3 confirms the existence of Fe-O at 625 and 449 cm⁻¹ while C-H bond of OA appears at 2848 and 2912 cm⁻¹ in spectrum b). The peak of O-H stretching at 2912 cm⁻¹ supports carboxyl group of AA and that of C-H stretching at 3023 and 3056 cm⁻¹ in spectra c)-d) confirm the presence of benzene ring.

![Figure 3 FTIR spectra of a) MNPs, b) OA-MNPs, c) MPNPs and d) ns-MPNPs](image)

TGA thermogram a) in Fig.4 exhibits 15% weight loss of MNPs. The curve b) of OA-MNPs shows that OA losses ca.13 % at 100 - 400°C and 15% at 400 - 500°C, i.e., the magnetic content remains 72%. After encapsulating MNPs in P(St/DVB/AA), the weight percentage declines to 25% as indicated in curve c). In curve d), the remained weight of magnetic in ns-MPNPs was reduced to 6.5% because of the presence of PS lobe which is non-magnetic material.

![Figure 4 TGA thermograms of a) MNPs, b) OA-MNPs, c) MPNPs and d) ns-MPNPs](image)

4. Conclusions
Spherical MPNPs (ca. 170 nm) and ns-MPNPs (dumbbell shape with 308 nm in
length and 202 nm in width) were prepared via miniemulsion and seed emulsion polymerization, respectively. The chemical composition of particles was confirmed by FTIR spectra. The magnetic content determined from TGA analysis was 25% for spherical MPNPs whereas the ns-MPNPs contained only 6.5%. Both magnetic property and stability of ns-MPNPs will be improved before using as immunolatex particles for malaria detection.

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References