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Effect of physiochemical property of Fe_3O_4 particle on magnetic lateral flow immunochromatographic assay

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ABSTRACT

In this study, Fe_3O_4 nanoparticles were prepared as bio-probes in lateral flow immunochromatographic immunoassay (LFIA) system for rapid and quantitative detection of *Vibrio parahaemolyticus* (VP). The relationship was discussed between the assay performance and material characteristics of the probe, including particle size, size distribution and magnetic property. Comparison data with two commercial magnetic particles showed that the bio-probe based on homemade Fe_3O_4 particle produced the highest magnetic resonance, smaller size and good colloidal stability, a key to obtain good detection performance. Better quantitative signals were achieved by the Fe_3O_4 particles with small particle size and spherical geometry, that yielded a detection limit of 1×10^5 CFU/mL VP. Whereas a larger diameter greater than 1.0 μm and a broad size distribution obviously impede the fluid dynamic behaviors during the chromatography on the test strip surface, causing a false-positive result. Our study shows that physiochemical property of Fe_3O_4 particle is key control element for developing high performance LFIA. Magnetic bio-probe in LFIA shows great promise in highly sensitive, rapid qualitative and quantitative on-site test of hazardous substances.

1. Introduction

Vibrio parahaemolyticus is a Gram-negative bacterium. This bacterium is a human pathogen that occurs naturally in the marine environments and frequently isolated from a variety of seafoods including codfish, sardine, mackerel, flounder, clam, octopus, shrimp, crab, lobster, crawfish, scallop and oyster. Consumption of raw or undercooked seafood, particularly shellfish, contaminated with *V. parahaemolyticus* may lead to development of acute gastroenteritis diagnosed with diarrhea, headache, vomiting, nausea, abdominal cramps, and low fever. The bacterium is recognized as the leading cause of human gastroenteritis associated with seafood consumption in the United States and an important seafood-borne pathogen throughout the world [1]. It is generally accepted that molecular biology detection is the gold standard for the detection of *V. parahaemolyticus*, such as PCR, Real-Time PCR assay, and so on. These techniques require specialized skills and equipment. They are unable to meet the needs of early and rapid diagnosis of *V. parahaemolyticus* for their complicated operation procedures [2].

The lateral flow immunochromatographic assay (LFIA), also known as the lateral flow tests, is a solid-phase immunoassay combining the principles of thin layer chromatography and immune recognition reaction. It is an extended application of enzyme-linked immunosorbent assay (ELISA). In comparison with the conventional molecular biology detection method, LFIA is a simple, fast-response, and inexpensive technique useful for medical diagnosis [3,4], home testing [5], point of care testing [6], and detection of various environmental and agricultural contaminations [7–10]. LFIA is suitable for individual samples and on-site detection of antibodies or antigens [11–13].

The tracers used in LFIA include colloidal gold nanoparticles, phosphorescent particles, and fluorescent microspheres [14–16]. The LFIA strip has drawn increasing attention for its sensitivity and specificity. Currently, colloidal gold nanoparticles [17] and

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fluorescent microspheres [18] have been used for detection of food-borne pathogenic bacteria. Recently, magnetic nanoparticle as a signal indicator has been reported to exhibit high sensitivity in clinical diagnosis when compared to the fluorescent labels [19–24]. In the magnetic lateral flow immunochromatographic assay, the physiochemical properties of the magnetic nanoparticles are essential for detection efficiency. In this study, a unique lateral flow immunochromatographic assay was developed using the Fe_3O_4@silica composites that were especially designed and architectured for detection of *V. parahaemolyticus*. By comparing with some of the commercial magnetic materials, the homemade Fe_3O_4@silica composites exhibit high magnetization, stable surface carboxyl groups, and good colloidal stability which are ideal properties for developing high sensitivity LFIA. The study was focused on the effect of particle size, size distribution, surface bio-modification, magnetization, and colloidal stability on the detection performance such as sensitivity and accuracy.

2. Experimental

2.1. Reagents and materials

1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxy-sulfosuccinimide sodium salt (sulfo-NHS) were purchased from Medpep Co., Ltd. (Shanghai, China). Anti-*V. parahaemolyticus* polyclonal antiserum and *V. parahaemolyticus* (33874) were prepared in our laboratory. Goat anti-rabbit IgG was purchased from Luoyang Biotechnology Co., Ltd. (Henan, China). The nitrocellulose membrane (Sartorius CN 140), glass fiber, and absorbent paper were purchased from Jiening Biotech Co., Ltd. (Shanghai, China). 2-[N-Morpholino] ethane sulfonic acid (MES), Bovine serum albumin (BSA), polyoxyethylene sorbitan monolaurate (Tween-20), and sodium tetraborateand boric acid were purchased from Sangon Biotech Shanghai, Co., Ltd. (Shanghai, China). Bangs Fe_3O_4 nanoparticle was purchased from Bangs Laboratories, Inc. (IN, USA) Ademtech Fe_3O_4 nanoparticles and Magnetic Assay Reader (MAR) were purchased from MagnaBioScience, LLC (CA, USA).

2.2. Preparation of magnetic composite particles

Magnetic particles were prepared via a solvothermal reaction [25]. 0.12 M of $FeCl_3{\cdot}6H_2O$, 0.07 M of sodium citrate and 1.0 M of urea were dissolved in 30 mL of ethylene glycol under vigorous magnetic stirring. The obtained homogeneous yellow solution was transferred to the Teflon-lined stainless-steel autoclave and heated at 200 °C for nearly 8 h. After that, the autoclave was cooled to room temperature. The products were washed with ethanol and DI water each for three times and redispersed into DI water for use. These magnetite particles were coated with silica generated from the hydrolysis and condensation of tetraethyl orthosilicate (TEOS) [25]. Subsequently, the magnetic silica particles were grafted with aminopropyltri(ethoxysilane) (APS), which provided chelate sites (amino groups) on the surface of magnetic silica particles. Carboxyl groups were immobilized on the particles by chelation for the final complete structure that is ready for conjugation with antibody and lateral flow immunochromatographic assay.

2.3. Preparation of magnetic composite particles–antibody conjugates

The conjugates of Fe_3O_4 nanoparticles and anti-*V. parahaemolyticus* polyclonal antibody were prepared by EDC and NHS method as described earlier [26]. Typically, 5 μL EDC (0.5 g/mL) and 10 μL sulfo-NHS (0.25 g/mL) were mixed with 1 mg of Fe_3O_4 nanoparticles in 500 μL of 50 mM borate buffer (BS), pH 9.0, containing 0.05% (w/v) Tween-20 (BST). After reaction for 30 min, the particles were washed with BST buffer for three times. 100 μg antiserum against *V. parahaemolyticus* was added and reacted for 3 h at room temperature under gentle mixing. Following washing with BST for three times, the resultant mixture was blocked by 500 μL 1% BSA in PBS for 30 min. Finally, the obtained antibody-modified Fe_3O_4 particles were dispersed in the BST solution containing 1% NaN_3, 0.1% BSA and stored at 4 °C till using. The conjugation reactions between different commercial Fe_3O_4 particles and antibodies were carried out in a same way as previously introduced. In the following, probe 1-PA refers to the homemade Fe_3O_4 antibody–particle conjugate. Probe 2-PA refers to Ademtech antibody–particle conjugate. Probe 3-PA refers to Bangs antibody–particle conjugate.

2.4. Characterization

Dynamic light scattering was carried out at 298.0 K with a Zetasizer Nano-ZS (Malvern, UK) equipped with a solid-state He Ne laser ($\lambda = 633$ nm) for monitoring the hydrodynamic size of the magnetic nanoparticles and antibody–particle conjugates. Transmission electron microscope (TEM) images were obtained from using a JEM-1230 transmission electron microscope at an accelerating voltage of 120 kV. The magnetic properties were characterized at room temperature by a Vibrating Sample Magnetometer (VSM 7407, Lake Shore, USA). The surface zeta potentials of the particles were measured using a DLS Particle Size analyzer (Zetasizer Nano-ZS, Malvern, UK).

2.5. Preparation of the LFIA strip

The LFIA strip consists of five components including a sample pad for applying sample solution, a conjugate pad for loading the particle-labeled antibody, a 25 mm nitrocellulose (NC) membrane acting as the chromatography matrix, an absorbent pad serving as the liquid sink, and a backing card for supporting all the components. At 5 mm from the absorbent pad, a band of goat antirabbit IgG (2 mg/mL) was drawn on the NC membrane as a control line (C-line), while a band of polyclonal antibody against *V. parahaemolyticus* (2 mg/mL) was drawn as a test line (T-line) by a spraying machine. After drying for 2 h at 37 °C, the well-assembled card was covered with a transparent film and cut to 5 mm width test strips for detection (Fig. 1.).

2.6. Magnetic LFIA for Vibrio parahaemolyticus

Serial dilutions of *V. parahaemolyticus* with concentrations of 0, 1×10^5, 1×10^6, 2.5×10^6, 5×10^6 CFU/mL in borate buffer were prepared and used in the following detection experiments. 3% sucrose (0.35 g/mL), 1.7% BSA (0.2 g/mL), 0.4% Tween-20, and 6 μL antibody–particle conjugate (2 mg/mL) were mixed in the *V. parahaemolyticus* dilutions and applied on the conjugate pad. After chromatography for 10-15 min at room temperature, the qualitative results were observed by naked eyes and magnetic signals at T- and C-lines were measured by a MAR system.

2.7. Detection of Vibrio parahaemolyticus in raw seafoods

Fresh seafood samples (shrimp, clam, razor clam) purchased from a local seafood market (Shanghai, China) were firstly boiled for 30 min to inactivate the native bacteria. A 10 g portion of cooked samples was aseptically weighed into a sterile stomacher bag, and 90 mL sterile BS buffer was added to obtain a 10-fold dilution. The suspension was homogenized for 2 min with a Stomacher 400 (Bag-Mixer400, Interscience, France). Next, the suspensions were

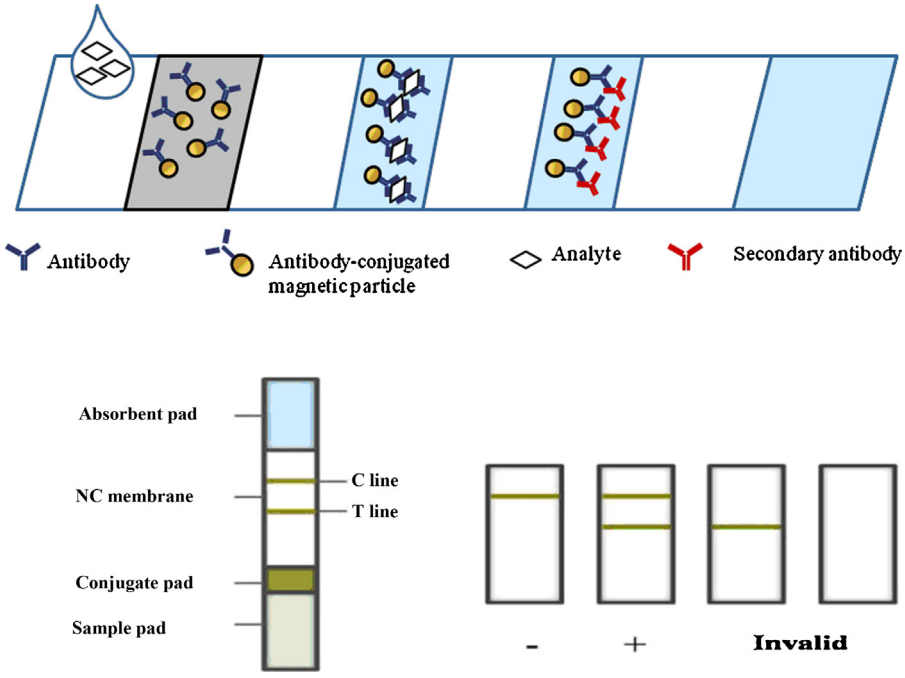

Fig. 1. Schematic illustration of magnetic immunochromatographic strip for detection. LFIA principle based on Fe_3O_4 particles (upper panel), and the structure of a LFIA test strip with the detecting results indicated (lower panel).

centrifuged at 400 rpm for 10 min, the obtained supernatants were inoculated with *V. parahaemolyticus* and diluted with BS to various bacterial concentrations as detection samples for LFIA. Correspondingly, the supernatants without bacteria inoculation were used as negative samples for LFIA. On the other hand, the aliquots of each sample was diluted by TCBS agar and incubated for 24 h at 37 °C. The precise number of CFU in each dilution sample was obtained by a standard plate counting methods using TCBS agar plates.

3. Results and discussion

3.1. Magnetic LFIA of Vibrio parahaemolyticus with three different Fe_3O_4 particles

3.1.1. Detection principle

In this study, the *V. parahaemolyticus* detection was performed by using a sandwich model test strip. As shown in Fig. 1, when an aqueous solution of target analyte is applied onto the sample pad, the antibody–particle conjugates are rehydrated. Consequently, it is released into the migrating liquid that is to migrate across both the T-line and C-line due to a capillary force. As a result of antigen-antibody interaction, the analyte, namely *V. parahaemolyticus* (VP) in solution is captured by the specific antibody pre-immobilized on the T-line, and the immunomagnetic particles are captured by the secondary antibody on the C-line. The color intensity of the T-line is an intuitive result of the target analyte, which is proportional to the amount of the captured magnetic particles. In the same fashion, the color of the C-line indicates the effective release of the antibody–particle conjugates from the conjugate pad, which is a validity mark of the test results.

Typically, a negative sample shows C-line only. Both testing lines (C- and T-lines) must be valid for a positive sample. The strip is considered invalid when only the T-line is observed or both C- and T-lines are invisible. The quantitative detection is achieved by the magnetic signal ratio of the positive to negative sample (S/N ratio) on the T-line, where S and N each presents the magnetic signal of the positive and negative sample. $S/N \geqq 2.1$ and $S/N < 2.1$ respectively gives the positive and negative testing results.

3.1.2. LFIA based on three different magnetic probes for Vibrio parahaemolyticus

In this study, three different Fe_3O_4 particles were used, including the one developed in our laboratory and two commercial particles. For the LFIA detection of VP, a series of VP dilutions of 0, 1×10^5, 1×10^6, 2.5×10^6, 5×10^6 CFU/mL in 50 mM borate buffer were prepared and used in LFIA. The quantitative magnetic signals (MSs) on both T- and C-lines are averaged for six parallel runs measured by MAR. For probe 1-PA and 2-PA, the MS of T-line is decreased with decreasing VP concentration under 2.5×10^6 CFU/mL. For probe 3-PA, the MS of T-line is much lower than the other two probes above 1×10^6 CFU/mL VP. However, a high MS is observed from the negative sample, indicating a false-positive reaction (Table 1). Also, weak MSs are observed on C-line for VP concentrations of 1×10^6, 2.5×10^6, and 5×10^6 CFU/mL, showing invalid detection of these VP samples. It was observed that probe 3-PA was more frequently blocked on the sample pad when the VP concentration is above 1×10^6 CFU/mL. The blockage was generally found to be improved at lower VP concentrations. No blocking occurred for probes 1-PA and 2-PA. Thus, serious block was considered to be the main cause of invalid test.

For the S/N ratios of the MSs on T-line from test strips based on 3 probes, as shown in Table 1, probe 3-PA is not suitable for LFIA in the VP test due to its strong false positive signal. For the same VP concentration above 1×10^6 CFU/mL, the MS from probe 1-PA is much stronger than that from probe 2-PA. The S/N ratio is very close at 1×10^5 CFU/mL VP. However, a faint test line was observed by naked eye for 1-PA, whereas no test line is observed for 2-PA. In addition, 1-PA shows much higher S/N ratio and MSs at high VP concentrations. Thus, relative to the weak MS of 2-PA, 1-PA is highly preferred for LFIA of VP.

The specificity of the immunoassay is closely related to the antigen-antibody reaction. However, non-specific adsorption of

Table 1
Comparison of the magnetic signals from lateral flow test strips with different Fe_3O_4 antibody–particle probes.

Probe 1-PA	T	CV%	C	CV%	S/N[a]
–[b]	45.0 ± 1.7	3.8%	5510.1 ± 12.1	0.2%	/
1×10^5 CFU/mL	308.4 ± 4.8	1.6%	5491.0 ± 24.0	0.4%	6.9
1×10^6 CFU/mL	5501.7 ± 94.8	1.7%	5920.9 ± 62.0	1.0%	122.3
2.5×10^6 CFU/mL	5613.9 ± 45.5	0.8%	1245.7 ± 44.5	3.6%	124.8
5×10^6 CFU/mL	4776.7 ± 40.1	0.8%	1134.2 ± 28.0	2.5%	106.1
Probe 2-PA	**T**	**CV%**	**C**	**CV%**	**S/N**
–	9.4 ± 0.5	5.3%	333.1 ± 2.6	0.8%	/
1×10^5 CFU/mL	58.3 ± 1.2	2.1%	479.7 ± 28.3	5.9%	6.2
1×10^6 CFU/mL	153 ± 1.9	1.2%	756.9 ± 17.5	2.3%	16.3
2.5×10^6 CFU/mL	325.1 ± 8.8	2.7%	815.3 ± 15.7	1.9%	34.6
5×10^6 CFU/mL	365.3 ± 2.1	0.6%	887 ± 16.1	1.8%	38.9
Probe 3-PA	**T**	**CV%**	**C**	**CV%**	**S/N**
–	132.8 ± 0.6	0.5%	292.9 ± 4.3	1.5%	/
1×10^5 CFU/mL	261.2 ± 6.1	2.3%	255.7 ± 6.7	2.6%	Is[c]
1×10^6 CFU/mL	402.7 ± 6.0	1.5%	56.7 ± 0.2	0.4%	Is
2.5×10^6 CFU/mL	307.8 ± 3.3	1.1%	19.3 ± 0.6	3.1%	Is
5×10^6 CFU/mL	360.2 ± 9.1	2.5%	18.1 ± 0.7	3.9%	Is

[a] S value presents the magnetic signals of T-line from the positive samples, N value presents the magnetic signals of T-line from the negative samples.
[b] "–" means negative sample.
[c] Is: insignificance.

the nanoparticle often takes place in an indiscriminate fashion before binding reaction on the membrane of the test strip. In turn, this behavior will ultimately influence the detection specificity. Table 2 shows the quantitative results from the lateral flow assay of three different Fe_3O_4 particles. The high MS from sample 3 on T-line characterizes it as a high likelihood of false positive diagnosis. The MSs of samples 1 and 2 are comparable on T-line, although the MS of sample 2 is higher on C-line. This difference implies insignificant effects of these two samples on detection specificity.

3.2. Comparison of physiochemical properties of three different Fe_3O_4 particles

From the above assay data, one can see that sample 1 (homemade Fe_3O_4 particles) exhibits most effective LFIA detection of VP, attributable to the unique physiochemical properties of the particles and probes. The physiochemical properties include the particle size, size distribution, surface potential, structure, and magnetization of the particles. In order to investigate the effects of the size and size distribution before and after antibody coating on VP detection, DLS and TEM experiments were carried out for the magnetic particles (samples 1, 2 and 3) and the antibody-labeled probes (1-PA, 2-PA, and 3-PA).

As shown in Fig. 2, samples 1 and 2 showed the similar diameters of 140 and 200 nm respectively, and comparable narrow size distribution. Whereas sample 3 has a large size more than 1.0 μm, and a broad size distribution. According to the TEM images of the magnetic particles (Fig. 3), samples 1 and 2 both exhibit the monodispersed particle sizes with spherical shape. Sample 3 is, however, aggregated with irregular geometries. The characteristic irregularities and large clusters of sample 3 would impede the fluid dynamic behaviors during the transport process on the strip surface. Consequently, the slow drag exerted on probe 3-PA, which may come from the interactions between its antibodies and the

Table 2
Comparison of non-specific magnetic signals from test strips with different Fe_3O_4 particle samples.

Fe_3O_4 particle[a]	T	CV%	C	CV%
Sample 1	60.6 ± 2.2	3.6%	36 ± 3.5	9.7%
Sample 2	50.1 ± 0.1	0.2%	103.3 ± 7.3	7.1%
Sample 3	502.8 ± 6.5	1.3%	36.5 ± 0.9	2.5%

[a] Sample 1 presents the homemade Fe_3O_4 particle; sample 2 presents the Ademtech Fe_3O_4 particle; sample 3 presents the Bangs Fe_3O_4 particle.

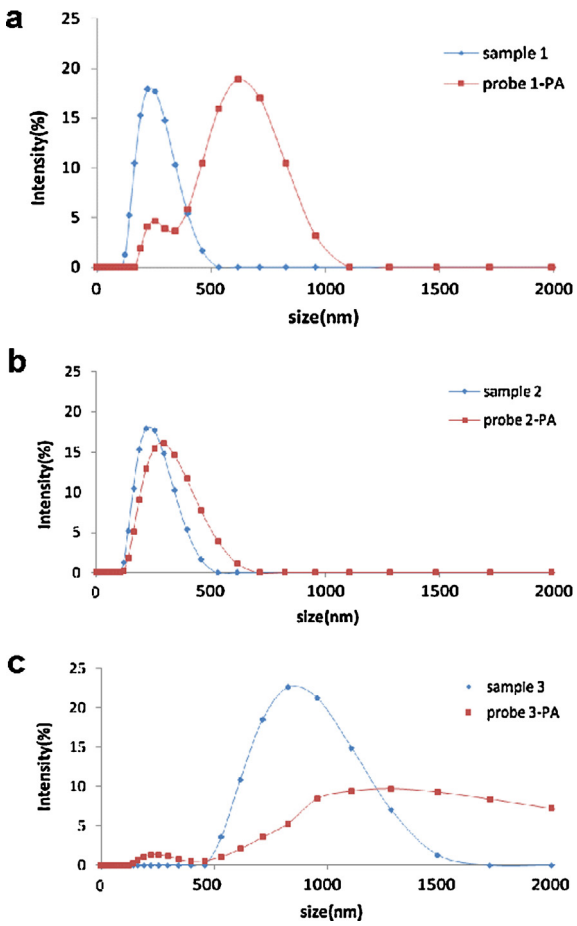

Fig. 2. Hydrodynamic size distribution profiles of different particle samples recorded before (denoted as samples 1, 2, 3) and after conjugation with antibody (denoted as probes 1-PA, 2-PA, and 3-PA, respectively).

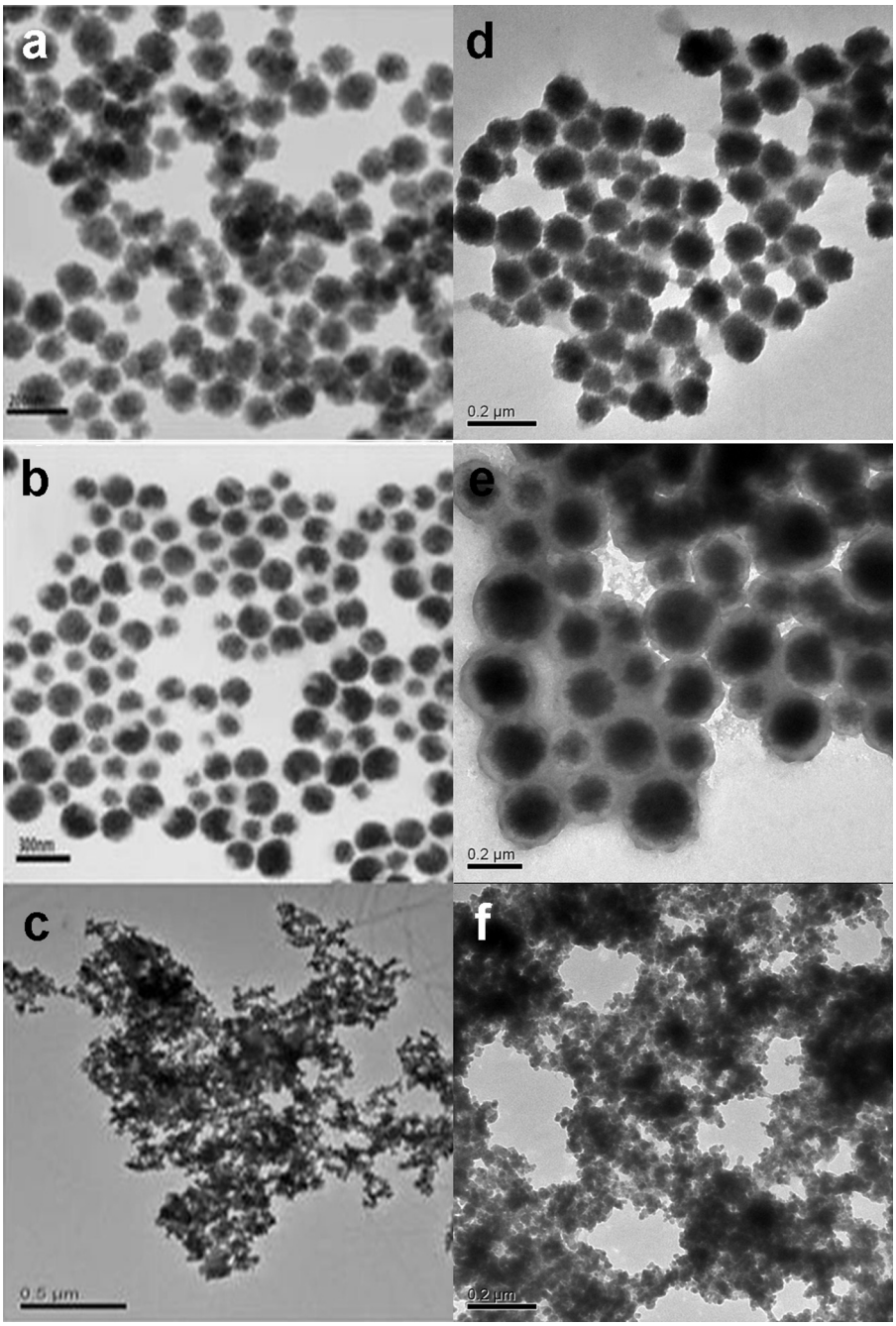

Fig. 3. TEM images of the magnetic particles (a, b, c) and the antibody–particles conjugates (d, e, f). (a) sample 1; (b) sample 2; (c) sample 3; (d) probe 1-PA; (e) probe 2-PA, and (f) probe 3-PA. The scale bars correspond to 200 nm (a, d, e, f), 300 nm (b) and 500 nm (c), respectively.

NC membrane of the strip, tends to immobilize the probes on the T- and C-lines, making a false positive diagnosis.

After coating with the antibody on the surface of the magnetic particles, the hydrodynamic size of probes 1-PA and 3-PA are much larger than samples 1 and 3. The main hydrodynamic peaks of sample 2 and probe 2-PA showed little shift, however, a size increasing is still obvious. The size shift phenomenon of particles before and after antibody coating showed an effective antibody immobilization on the particle surface. As can be seen in TEM data, average diameter of samples 1 and 2 particles is 140 and 200 nm, respectively. After antibody conjugation, the probes 1-PA and 2-PA exhibit less aggregation, however, the 3-PA shows obvious aggregation. Consequently, both the TEM and DLS data displayed that samples 1 and 2 have small size and good colloidal stability. We also examined the surface potential of three magnetic particles and the corresponding particles–antibody conjugations by measurement with DLS instrument (Zetasizer Nano-ZS, Malvern, UK). In Table 3, the surface potential values of all samples are shown. It is found that three magnetic particles all have negative surface charges and are kept negative surface charges after antibody conjugation. For sample 2, there is only small change of Zeta potential due to antibody conjugation, and over −20 mV surface potential can contribute to good colloidal stability. For sample 1 and 3, obvious decreasing of the absolute surface potential values after antibody conjugation is consistent with the increase of average size.

When these 3 probes were applied into LFIA for VP, it was found that test strip based on probe 1-PA gives the highest test signal on T-line (Table 1). Also, a high S/N ratio (6.9) at VP of 1×10^5 CFU/mL is

Table 3
Zeta potential results for the Fe_3O_4 particles (samples 1–3) and the Fe_3O_4 particles–antibody conjugations (probes 1–3 PA).

	Sample 1	Probe 1-PA	Sample 2	Probe 2-PA	Sample 3	Probe 3-PA
Zeta potential (mV)	−23.1	−19.2	−24.5	−25.3	−31.7	−14.9

observed, implying that a detection limit under 1×10^5 CFU/mL VP may be achieved. In order to investigate the application feasibility of the developed magnetic LFIA, raw seafood samples contaminated with VP were further tested. The results were compared with culture isolation method. Table 4 shows the log (CFU/mL) generated from culture isolation, and the magnetic signals obtained by LFIAs based on three different probes. For probes 1-PA and 2-PA, the MSs of T-line were gradually decreased with the decreasing of VP concentration. However, the magnetic gradient difference of probe 3-PA does not change significantly with the various VP concentrations. In addition, false positive reaction is observed for the negative samples of probe 3-PA, and the S/N ratio is much lower than those from other two probes. The results indicated that probe 3-PA is not suitable for application.

As shown in Table 4, it is clear that the MSs at T lines and the S/N values from probe 2-PA at each concentration of different samples are much lower than those of 1-PA. Because the S/N ratio above 2.1 means a positive result, thus, the sensitivity of test strip from 1-PA is highest, capable to reach less than 5×10^5 CFU/mL. However, one thing should be noted that no blocking and rapid flowing is observed at each sample when the test strips from 2-PA were used for detection. Whereas faint false positive reaction and weak blocking from probe 1-PA is observed for the calm negative sample. This phenomenon is consistent with the different dispersibility of the two probes described previously. In addition, we found that food matrix might also contribute to the false positive result because of different sample viscosity, which may influence the flowing time onto the surface of test strip. As we known, longer flowing time gives stronger MS. Besides of this, the magnetic content of probes also greatly influence the detection performance. The magnetization of sample 1 is found to be higher than other 2 particles (Fig. 4). As magnetic moment (or magnetization) is proportional to the volume fraction of the magnetite, that is a key to obtaining strong detection signal [19]. Therefore, Fe_3O_4 particle and probe 1-PA developed in this study has shown satisfying qualities for high sensitivity and low background LFIA in terms of materials characteristics and magnetic properties.

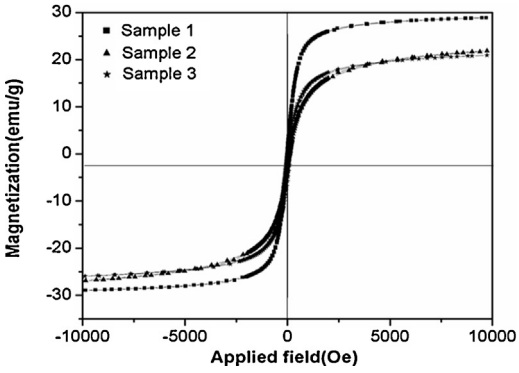

Fig. 4. Magnetization curves of the three different Fe_3O_4 particles.

Table 4
Quantitative detection of *V. Parahaemolyticus* in seafoods and comparison of results obtained by conventional culture isolation method.

Sample	Culture isolation method (CFU mL^{-1})	Magnetic signals of lateral flow immunoassay (LFIA)			
			Probe 1-PA	Probe 2-PA	Probe 3-PA
Shrimp	0 (negative)	T line	50.1 ± 0.67	21.6 ± 0.67	154.2 ± 1.51
	2.5×10^6	T line	3288.7 ± 40.07	366.67 ± 2.6	360.3 ± 3.47
		C line	694.9 ± 31.49	594.8 ± 25.3	48.5 ± 1.31
		S/N	65.64	16.98	2.34
	1.25×10^6	T line	1911.7 ± 24.87	273.97 ± 13.0	341 ± 3.27
		C line	2491.8 ± 86.96	569.67 ± 10.36	68.9 ± 2.42
		S/N	38.16	12.68	2.21
	5×10^5	T line	1049.2 ± 29.16	163.9 ± 6.56	315.2 ± 8.44
		C line	2888.9 ± 13.82	445.4 ± 8.58	113.5 ± 6.62
		S/N	20.94	7.59	2.04
Clam	0 (negative)	T line	178.2 ± 2.49	54.3 ± 1.87	332 ± 3.13
	2.5×10^6	T line	2270.8 ± 7.62	194.6 ± 1.56	484.4 ± 14.93
		C line	1522.8 ± 32.67	583.1 ± 50.42	130.5 ± 8.78
		S/N	12.74	3.58	1.46
	1.25×10^6	T line	1153.7 ± 6.49	147.1 ± 1.16	668.9 ± 17.44
		C line	2377.3 ± 49	495.5 ± 12.09	340.3 ± 9.38
		S/N	6.47	2.71	2.01
	5×10^5	T line	519.0 ± 13.38	87.1 ± 1.02	581.4 ± 16.78
		C line	2173.3 ± 79.84	347.7 ± 13.53	685.3 ± 27.76
		S/N	2.91	1.60	1.75
Razor clam	0 (negative)	T line	45.7 ± 2.67	26.3 ± 1.42	273.0 ± 1.22
	2.5×10^6	T line	2302.7 ± 51.16	191.8 ± 3.36	385.2 ± 3.62
		C line	1344.7 ± 54.87	639.7 ± 64.71	140.9 ± 2.11
		S/N	50.39	7.29	1.41
	1.25×10^6	T line	1271.0 ± 63.22	106.3 ± 2.29	467.4 ± 8.78
		C line	1543.1 ± 29.84	462.2 ± 13.69	370.2 ± 4.02
		S/N	27.81	4.04	1.71
	5×10^5	T line	776.7 ± 22.64	64.4 ± 2.64	350.3 ± 13.53
		C line	1601.6 ± 26.98	337.5 ± 16.89	416.3 ± 16.78
		S/N	17.00	2.45	1.28

4. Conclusions

For LFIA detection of *V. parahaemolyticus*, a magnetic LFIA model has been developed using different Fe_3O_4 particles. Physiochemical properties of all three Fe_3O_4 particles are characterized with surface conjugated antibody. When they were applied to LFIA, it has been found that the detection performance is highly dependent on the materials characteristics such as size, size distribution and magnetic property. The probe 1-PA based on homemade Fe_3O_4 particle exhibits a good size distribution and the strongest magnetic signal for its large volume fraction of magnetite. Accordingly, it shows a good sensitivity and colloidal stability. It is also found that the probe 3-PA flowing on the membrane of the test strip can be significantly hindered by low monodispersity of particles. Therefore, particle dispersion is an important material factor that must be optimized to obtain the best LFIA performance. Additionally, magnetite content is a key factor for high sensitivity LFIA. The relationship between the physiochemical properties of the employed probes and LFIA performance is established in terms of particle size, size distribution, magnetite content and test signal. The experimental results in this study have shown a promising magnetic particle-based LFIA for rapid and sensitive detection of hazardous substances.

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