Nanoparticles: synthesis and applications in life science and environmental technology*

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Abstract
This work focuses on the synthesis, functionalization, and application of gold and silver nanoparticles, magnetic nanoparticles Fe3O4, combination of 4-ATP-coated silver nanoparticles and Fe3O4 nanoparticles. The synthesis methods such as chemical reduction, seeding, coprecipitation, and inverse microemulsion will be outlined. Silica- and amino-coated nanoparticles are suitable for several applications in biomedicine and the environment. The applications of the prepared nanoparticles for early detection of breast cancer cells, basal cell carcinoma, antibacterial test, arsenic removal from water, Herpes DNA separation, CD4+ cell separation and isolation of DNA of Hepatitis virus type B (HBV) and Epstein–Barr virus (EBV) are discussed. Finally, some promising perspectives will be pointed out.

Keywords: gold nanoparticles, silver nanoparticles, magnetic nanoparticles, functionalization
Mathematics Subject Classification: 4.02

1. Introduction

Nanoparticles are of great interest because of their technological and fundamental scientific importance. These materials often exhibit fascinating properties which cannot be achieved by their bulk counterparts. Their applications, or potential applications, are in many fields [1–5 and references therein]. Nanoparticles have advantages in application in life science and the environment. Their particle size is comparable with the dimension of small molecules (about 1–10 nm) or of viruses (about 10–100 nm). This allows nanoparticles to attach to biological entities without changing their functions. Large surface area of nanoparticles permits strong bonds with surfactant molecules. In the environment, the small size of nanoparticles, together with their large surface area can lead to very sensitive detection of a specific contaminant from the presence of which pollution often arises. Nanoparticles can also be engineered to actively interact with a pollutant and treat them.

In this work we focus on the synthesis, functionalization, and application of gold and silver nanoparticles, magnetic nanoparticles Fe3O4, combination of 4-aminothiophenol (4-ATP)-coated silver nanoparticles and Fe3O4 nanoparticles.

2. Experimental

2.1. Synthesis of nanoparticles

2.1.1. Gold nanoparticles. Gold nanoparticles with a size of about 40 nm have been synthesized by a chemical reduction method using sodium borohydride (NBH4) [6]. HAuCl4 and NBH4 are stirred in water with appropriate time and the ratio of gold to sodium borohydride. Gold nanoparticles with sizes ranging from 2 to 5 nm were also prepared by seeding method using surfactant of cetyltrimethylammonium bromide (CTAB) [7].
2.1.2. **Silver nanoparticles.** Silver nanoparticles have been prepared by a modified sonoelectrodeposition method [8]. The modification is that a silver plate was used as the cathode instead of silver salts to avoid unexpected ions. This method allows producing Ag nanoparticles (AgNP) with the size of 4–30 nm dispersed in a non-toxic solution.

2.1.3. **Magnetic nanoparticles.** Magnetic Fe$_3$O$_4$ nanoparticles with size 10–15 nm were synthesized by using coprecipitation from iron (III) chloride and iron (II) chloride solutions with the assistance of aqueous ammonia solution, as described in [9, 10]. Coprecipitation is a facile and convenient way to synthesize magnetite nanoparticles from aqueous Fe$^{2+}$/Fe$^{3+}$ salt solutions.

2.1.4. **Combination of 4-ATP-coated silver nanoparticles and Fe$_3$O$_4$ nanoparticles.** Silver nanocolloids were synthesized by wet chemical reduction method using NaBH$_4$ with the presence of surface activator polyvinylpyrrolidone (PVP), then was coated by 4-ATP to form Ag-4ATP nanoparticles. These nanoparticles were combined with the above-mentioned Fe$_3$O$_4$ nanoparticles to form multifunctional nanoparticles by inverse microemulsion method [11]. The inverse microemulsion was created by mixing hydrophobic phase of toluene and hydrophilic phase that was made from the mixture of Ag-4ATP solution after 4 months storage and Fe$_3$O$_4$ solution right after synthesis. Under sonic bath, different mass rates of Ag-4ATP/Fe$_3$O$_4$ were moderated for surface groups. The head group functionality -NH$_2$ is for triethoxysilane (APTS). APTS is a bifunctional molecule, allowing producing Ag nanoparticles (AgNP) with the size of 4–30 nm dispersed in a non-toxic solution.

2.2. **Functionalization/coating of nanoparticles**

Nanoparticles need to be functionalized in order to conjugate with biological entities such as DNA, antibodies and enzymes. The most widely used functional groups are amino, biotin, streptavidin, carboxyl and thiol groups [12].

2.2.1. **Functionalization of gold nanoparticles.** For application to detect breast cancer cells, gold nanoparticles synthesized by a chemical reduction were functionalized with 4-aminothiophenyl (4-ATP). For basal cell carcinoma detection, different amounts of 4-ATP solutions were added to gold nanoparticles coated by CTAB. CTAB on the surface of gold nanoparticles was replaced by 4-ATP to form gold nanoparticles functionalized with 4-ATP (Au-4ATP).

2.2.2. **Functionalization of magnetic nanoparticles.** Fe$_3$O$_4$ nanoparticles were functionalized using 3-aminopropyl triethoxysilane (APTS). APTS is a bifunctional molecule, an anchor group by which the molecule can attach to free -OH surface groups. The head group functionality -NH$_2$ is for conjugating with biological objects. The amino-NP is ready to conjugate with the DNA of the Herpes virus and with the antiCD4 antibody.

2.2.3. **Silica coating of magnetic nanoparticles.** Maintaining the stability of magnetic nanoparticles for a long time without agglomeration or precipitation is an important issue (see, for instance, [4]). The protection of magnetic nanoparticles against oxidation by oxygen, or erosion by acid or base, is necessary. One of the ways to protect magnetic nanoparticles is coating them with silica. A silica shell not only protects the magnetic cores, but can also prevent the direct contact of the magnetic core with additional agents linked to the silica surface that can cause unwanted interactions. The coating thickness can be controlled by varying the concentration of ammonium and the ratio of TEOS to H$_2$O. The surfaces of silica-coated magnetic nanoparticles are hydrophilic, and are ready modified with other functional groups [13]. We have prepared Fe$_3$O$_4$/SiO$_2$ nanoparticles by coating magnetic nanoparticles with silica using TEOS [10]. Silica layer has a thickness of about 2–5 nm.

3. **Applications**

3.1. **Application of gold nanoparticles for detecting breast cancer cells**

Gold nanoparticles are potential candidates for cell imaging and cell-target drug delivery [14–18], cancer diagnostics and therapeutic applications [19–21]. Nowadays, a number of biomarkers which are expressed at a high level on the surface of breast cancer have been reported, for example human epidermal growth factor receptor (HER) belonging to a member of the epidermal growth factor (EGF) family of tyrosine kinase receptors. These include HER1, HER2, HER3, and HER4. While HER1, HER3, and HER4 are overexpressed in various types of cancer cells, such as head, neck, brain, stomach, breast, colon, gast, prostate, and so on, HER2 is a biomarker which is more specific for breast and ovarian [22, 23]. HER2 is super-expressed with several hundred folds higher in cancer cells of 20–30% breast cancer patients than in normal cells. Therefore, HER2 is an interesting target for therapy of breast cancer. Anti-HER2 with generic name trastuzumab or trade name herceptin is a humanized monoclonal antibody (mAb), which has been approved by the FDA since 1998 for treatment of metastatic breast cancer [19, 20, 24]. In this study we conjugated the gold nanoparticles with anti-HER2 antibody (trastuzumab) through either non-covalent or covalent linkages. The trastuzumab-conjugated gold nanoparticles were then used to specifically label breast cancer cells, KPL4 line, for imaging of the cells.

As seen from figure 1, in the case of the gold nanoparticles without conjugation with trastuzumab, the gold nanoparticles could not find the cancer cells and nothing was observed in the dark-field microscopy image (A2). When the gold nanoparticles were directly conjugated with trastuzumab, the gold nanoparticles concentrated on the cancer cells and...
these cancer cells were clearly observed in the dark-field microscopy image (A4) by means of the scattering light of the gold nanoparticles. When the amino-gold nanoparticles (amino-GNP) were covalently conjugated with trastuzumab through 1-ethyl-3-(3-dimethylaminopropyl) ethylcarbodiimide (EDC) connection, the gold nanoparticles concentrated on the cancer cells as well, but these cancer cells were observed with slightly lower intensity in the dark-field microscopy image (A6) in comparison with those in the image A4. However, the gold nanoparticles directly conjugated with trastuzumab were able to be stored in a freezer for only about two weeks before they lost their activity; while the gold nanoparticles covalently conjugated with trastuzumab were stable for storage for about two months.

3.2. Basal cell carcinoma fingerprinted detection

Recently, the surface enhanced Raman scattering (SERS) has attracted much interest in the field of bio-labeling due to the significant enhance of the labeling signals of molecular vibrations on the surface of metallic nanoparticles. In this experiment, we investigated SERS signal of 4-ATP that linked to surface of gold nanoparticles while being conjugated with the skin carcinomas cell antibody BerEP4. The Au-antibody solutions were dropped on the surface of the tissue and the SERS signals were collected and analyzed [7]. Figure 2 shows the fingerprinted landscape of SERS signals of Au-antibody on a basal cell carcinoma (BCC) tissue. Figures 2(A) and (B) show the colored and micro spectroscopy image of the tissue, where the cancer cell area may be the dark colored regions, for example, region A1, A2, B1 and B2. Figure 2(C) shows the result of SERS signal analyzed using principle component analysis [7]. Figure 2(D) shows the result of the SERS signal analyzed using only the intensity of SERS peaks at 1075 cm\(^{-1}\). The antigen–antibody coupling oriented the Au-antibody colloids close to the BCC surface. The carcinomas sections should be considered as a dock where distributed high concentration of Au-antibody particles, then the SERS peak intensity at 1075 cm\(^{-1}\) will higher in these areas. Figure 2(D) shows the results of using the peak...
height at 1075 cm$^{-1}$ to mapping the Au-antibody appearing areas in 40 $\times$ 40 $\mu$m$^2$ region. In comparison, using the principle component analysis method, where the SERS signals were compared with each other, then the difference of the SERS spectra from the average spectrum is mapped in figure 2(C). In figure 2(C), the yellow to the red colored areas such as C1 and C2 areas can be considered as cancer regions. However, the area D1 in figure 2(D) does not show the high intensity of the peak at 1075 cm$^{-1}$ while the others such as D2 area indicate very high intensity of the peak at 1075 cm$^{-1}$. From all the figures, only A2, B2, C2 and D2 regions can be surely considered as the cancer areas, while A1, B1, C1 and D1 may be assigned as the position of a skin hole where the cell concentration is higher than in other parts. By principle component analysis, only those regions were highlighted which differ from other regions and the non-carcinomas can also be observed. However, in some special regions, one can make a mistake during the diagnosis. In addition, according to the collecting time of each spectrum being nearly 5 s, the whole SERS map collecting time should be longer than 2 h. In order to shorten the collecting time, if the collected band is only limited by a narrow band around the 1075 cm$^{-1}$ peak, the collecting time of each spectrum may decrease to 0.1–0.2 s. Then, the fingerprinted image using peak height at 1075 cm$^{-1}$ can be observed in around 5 min, hence, this can be the solution for quick diagnostics during an operation.

3.3. Antibacterial test using silver nanoparticles

The quantitatively antibacterial study of AgNP in Luria–Bertani (LB) broth is shown in figure 3, which presents the dynamics of *Escherichia coli* (*E. coli*) growth in only LB broth (negative control), LB broth supplemented with 120 $\mu$l trisodium citrate (TSC) solution (TSC control) and LB broth supplemented with AgNP (AgNP antibacterial tests). The amount of AgNP was adjusted to have the concentration from 2 to 200 $\mu$g ml$^{-1}$. Vertical axis represents optical density at

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**Figure 2.** Fingerprinted landscape of SERS signals of Au-antibody on BCC tissue. (A) Gram staining picture of a BCC tissue where A1 and A2 are the suspected area; (B) microscope picture of BCC tissue. Areas B1 and B2 are the same position on the tissue with A1 and A2, respectively; (C) principle component analyzed SERS signal landscape. Areas C1 and C2 are the same position on the tissue with A1 and A2, respectively; (D) the landscape of intensity of SERS peaks at 1075 cm$^{-1}$. Areas D1 and D2 are the same position on the tissue with A1 and A2, respectively. In figure D, the difference of D1 and D2 show that only red colored D2 (and the similar color area) is the infected area and D1 is not.
close to the MIC, normally ranging from 1 to 16 μg ml⁻¹, of antibiotics used for the treatment of *E. coli* [28].

### 3.4. Magnetic nanoparticles

#### 3.4.1. Arsenic removal from water

The arsenic adsorption abilities of the magnetite, Fe₁₋ₓCoₓFe₂O₄ (Co-ferrites) and Fe₁₋ₓNiₓO₂Fe₂O₃ (Ni-ferrites) were studied with different conditions of stirring time, concentration of nanoparticles and pH [29]. Table 1 shows the stirring time dependence of arsenic removal for 1 g l⁻¹ of Co-ferrites at neutral pH. The starting concentration of 0.1 mg l⁻¹ was reduced about 10 times down to the maximum permissible concentration (MPC) of 0.01 mg l⁻¹ after a stirring time of few minutes (the standard deviation is about 10%). The removal process did not seem to depend significantly on the concentration in the sample. The probe DNA sequence of the Herpes virus was HSV-1 of 5' -AT CAC CGA CCC GGA GAG GGA C-3'. We also studied the effects of the weight of the nanoparticles on the removal process. The stirring time was fixed to 3 min and the weight of samples was changed from 0.25 g l⁻¹ to 1.5 g l⁻¹ in steps of 0.25 g l⁻¹. The results showed that, after 3 min, the optimal weight to reduce arsenic concentration down to a value lower than the MPC was 0.25 g l⁻¹ for magnetite and 0.5 g l⁻¹ for Co- and Ni-ferrites.

The arsenic adsorption was reported to be independent of pH in the range of 4 to 10. However, at high pH, the adsorption was reduced significantly. Arsenic was desorbed from the adsorbent at alkaline pH [30]. Our reported results were conducted at a pH of 7. After arsenic adsorption, the nanoparticles were stirred under a pH of 13 to study the desorption process. Nanoparticles were collected by using a magnet and the arsenic concentration in the solution was determined by using atomic absorption spectroscopy. The results showed that 90% of the arsenic ions were desorbed from nanoparticles. The nanoparticles after desorption did not show any difference in arsenic re-adsorption ability. The adsorption–desorption process was repeated four times, which proved that the nanoparticles could be reused for arsenic removal.

#### 3.4.2. Herpes DNA separation

Herpes simplex virus causes extremely painful infections in humans [31]. The determination of the presence of this virus is important. An electrochemical sensor is a simple and fast way to recognize the presence of the DNA of the virus. However, electrochemical sensors have a limit of sensitivity, so they cannot measure concentrations lower than a few tens of nM 1⁻¹ [32]. Therefore, a DNA separation before the measurement by using the electrochemical sensor needs to be carried out to increase the concentration of the DNA. To do that, we used a DNA sequence, which is representative of the Herpes virus, as a probe to hybridize with the target DNA in the sample. The probe DNA sequence of the Herpes virus was HSV-1 of 5' -AT CAC CGA CCC GGA GAG GGA C-3' (Invitrogen). The phosphate group in the 5' of the probe DNA

595 nm (1 optical density at 595 nm, OD₅₉₅, equals the concentration of 1.7 x 10⁹ cells ml⁻¹). The initial number of *E. coli* inoculated into 2 ml LB medium of the tested tube was 1.7 x 10⁹ cells ml⁻¹. For the negative control and the TSC control, *E. coli* bacteria grew normally. The concentration of *E. coli* after 30 h in the TSC control (OD₅₉₅ = 2.5) is higher than that in the negative control (OD₅₉₅ = 1.5) which suggests that TSC was not toxic to *E. coli* and may be even enabled for the bacterial growth. The situation is different with the presence of AgNP because of the well-known antibacterial property of this metal [25]. When AgNP concentration was 2 μg ml⁻¹, the result was similar to the result of the negative control because the low value of AgNP could not inhibit bacteria growth. With higher AgNP concentration, the inhibitory effect occurred within 8 h even at low AgNP concentration of 4 μg ml⁻¹. This value is about twofold lower than the threshold concentration of 8 μg ml⁻¹ reported for Ag-loaded activated carbon in another research [26] and slightly higher than a value of 2–3 μg ml⁻¹ reported for the complicated Tolllens process [27]. The minimal inhibitory concentration (MIC) is defined as the lowest concentration of a drug that will inhibit the visible growth of *E. coli* after a period of time long enough for the growth of single colony to a turbid bacteria culture observable to the naked eye. Commonly it is overnight incubation. For longer incubation time, i.e., 24 and 30 h, *E. coli* grew in the broth tubes with AgNP concentration < 12 μg ml⁻¹ and inhibited in the broth tubes with AgNP concentration > 16 μg ml⁻¹. Therefore, the MIC of AgNP against the growth of *E. coli* is 16 μg ml⁻¹ which is

| Time (min) | x = 0.05 | x = 0.1 | x = 0.2 | x = 0.5 |
|-----------|----------|----------|---------|---------|
| 1         | 10       | 11       | 6       | 6.5     |
| 3         | 6        | 5        | 8.5     | 7       |
| 7         | 10       | 9        | 4.2     | 7.8     |
| 15        | 9        | 12       | 5       | 6.9     |
| 30        | 12       | 4.5      | 5       | 11.2    |
| 60        | 4.5      | 5        | 8       | 9.8     |

**Table 1.** Arsenic concentration (μg l⁻¹) remained in water after removal by 1 g l⁻¹ of the Co-ferrites as a function of the stirring time.
sequence needs to be activated in order to conjugate with the amino group of the amino-NP surface. The probe DNA after being activated with EDC and 1-methylimidazole (MIA) was mixed with the amino-NP to have nanoparticles with the probe DNA on the surface. The DNA-NP was heated in deionized water at 37 °C for 18 h. The products of this process were nanoparticles with the probe DNA sequence on the surface (DNA-NP).

The DNA separation was conducted as follows: 1 ml of the solution containing 2 wt.% of DNA-NP was mixed with 2–20 ml of a solution with 0.1 nM \(1^{-1}\) of the Herpes DNA. The hybridization of the probe DNA and the target DNA occurred at 37 °C for 1 h; then, by using magnetic decantation, the nanoparticles with hybridized DNA were collected and redispersed in 0.1 ml of water. The dehybridization of the nanoparticles with the probe and target DNA occurred at 98 °C. Removing the DNA-NP from the solution by using magnetic decantation, we obtained a solution with a high concentration of the DNA of the Herpes virus. When all the target DNA was separated, the concentration of the DNA had increased from 20–200 times [29].

Figure 4 presents the dependence of the output signal on the initial volume before and after magnetic separation.

![Figure 4](image.png)

### 3.4.3. CD4+ cell separation

The prepared nanoparticles have been used for CD4+ cell separation [29]. The amino-nanoparticles were coupled with the antiCD4 monoclonal antibody (antiCD4, invitrogen). In some samples, an amount of fluorescent isothiocyanate (FITC) labeled antiCD4 monoclonal antibody (FITC-antiCD4 or antiCD4*, excitation/ emission: 480 nm/520 nm; Exiobio) was additionally added with various amounts of non-labeled antiCD4 for interaction with the amino-NP via carbodiimide. Two types of nanoparticles were suspended in phosphate saline buffer (PBS) containing 0.1% bovine serum albumin (BSA). One type was coated with non-label antiCD4 (antiCD4-NP) and the other was coated with a mixture of non-labeled and FITC-labeled antiCD4 (antiCD4*-NP).

Several 200 \(\mu\)l tubes of blood were gently centrifuged to remove the serum and to obtain the blood cells. After that, each tube was incubated with either 0.2 mg of antiCD4-NP or 0.2 mg of antiCD4*-NP. 1.3 ml of hypotonic buffer (5 mM Tris pH 7.0, 10% glycerol) was added to burst the red blood cells to form ghost cells. The antiCD4-NP or antiCD4*-NP-coated cells were then magnetically separated from the ghost cells.

In a parallel experiment, for direct labeling of the CD4+ T cells by the FITC-antiCD4 monoclonal antibody, 20 \(\mu\)l of FITC-antiCD4 monoclonal antibody (Exiobio) was also used to directly label the CD4+ T cells. In this experiment, the CD4+ T cells were collected, together with other cells in blood, by centrifugation. Finally, the collected cells were resuspended in 50 \(\mu\)l of storage buffer (PBS containing 10% glycerol) to be observed under a Carl Zeiss Axio plan microscope.

The FITC-antiCD4 monoclonal antibody emits green light (520 nm) when being excited by blue light (480 nm). Figure 5 presents a visualization of individual CD4+ T cells under white light and under blue light excitation, after being labeled with the FITC-labeled antiCD4 monoclonal antibody. We could observe many cells, including red blood cells and white blood cells, under white light illumination (figure 5(A)), but under the blue light excitation, only two of the white blood cells emitted green fluorescent signals (figure 5(B)) in an area of about 10^4 \(\mu\)m^2, indicating that they are the CD4+ T cells. The white cells that did not emit fluorescent signals were not CD4+ T cells, but were other types of white cells. The average relative intensity of the FITC labeled CD4+ T was estimated to be 137 000 ± 45 000 arbitrary unit (mean ± standard deviation). Based on the average counted number of CD4+ T cells on 10^4 \(\mu\)m^2 vision areas, we estimated the relative number of CD4+ T cells in 1 \(\mu\)l of two blood samples from healthy people to be about 670 and 810 cells \(\mu\)l^-1, respectively. As the normal count in a healthy, HIV-negative
adult can vary but is usually between 600 and 1200 CD4+ T cells \( \mu l^{-1} \), the measured numbers of the CD4+ T cells in our experiment were acceptable as they fell in the standard range. Nevertheless, we suspected that elimination of the background in fluorescent detection might have caused the fairly low numbers of the CD4+ T cells in the two blood samples.

We attempted to develop an alternative method to primarily separate the CD4+ T cells by using an external magnetic force before counting the cell number by using a fluorescence microscope. For that purpose, it was essential to prepare magnetic nanoparticles that had stable and specific links between the monoclonal antibody and the particular receptor CD4 on the membranes of the CD4+ T cells. Therefore, the nanoparticles were functionalized with free amino group (amino-NP) for covalent linking with the carboxyl group of the antiCD4 monoclonal antibody to obtain antiCD4 antibody modified nanoparticles (antiCD4-NP). The antiCD4-NPs were used as a material to conjugate with CD4+ T cells for the magnetic separation. In fact, we tried with various amounts of antiCD4 from 1–100 \( \mu g \) and found that 20 \( \mu g \) was enough for conjugating with 0.4 mg of nanoparticles. The magnetically sorted cells were observed under a conventional microscope, as shown in figures 5(C) and (D). Here, the FITC-labeled antiCD4 plays as a signal for detection of CD4+ T cells under a fluorescence microscope. All of the cells bound with a single layer of antiCD4*-NP emitted high average fluorescent intensities of 356 000 ± 64 000 (arbitrary unit), which were about 2.6 times higher than that observed when using FITC-antiCD4 directly, as shown in figure 5(B). We did not observe white cells without fluorescent signals due to the magnetic separation. Our data confirmed that a combination of magnetic separation and the detection of the fluorescent signal improved the signals compared to that of direct labeling of CD4+ T cells by FITC-antiCD4 monoclonal antibody. Counting the exact number of antiCD4*-NP coated cells still had some challenges: (a) number of nanoparticles attached to the cells, contribution to the background which largely interferes with the signals of antiCD4*-NP bound cells; (b) nonuniform distribution of the cells in the vision area; (c) a certain percentage of antiCD4-NPs bound cells (about 20%) was not attracted by the magnetic field as we could observe the fluorescene emitting cells in the supernatant after separation.

**Figure 5.** Visualization of the blood cells under white-light (A, C) and under blue light excitation (480 nm) (B, D), after being coupled with the antiCD4 antibody and antiCD4-NP*s and being separated by using a magnet.
3.4.4. Detection of pathogenic viruses

3.4.4.1. Purification of DNA of Hepatitis virus type B (HBV) using silica-coated magnetic nanoparticles and optimized buffers. Before testing the DNA purification procedure with real serum samples, we measured the efficiency of DNA recovery of the Fe3O4/SiO2 nanoparticles and the optimized buffers using standard pure pGEM-HBV plasmid at tenfold diluted concentrations ranging from 4 × 10^9 copies ml^-1 to 4 × 10^2 copies ml^-1. The enriched DNA solutions were used as templates for amplification of 434 bp fragment of S gene specific for HBV [10]. The results indicate that Fe3O4/SiO2 nanoparticles and the optimized buffer could successfully enrich DNA from solution and that the purified DNA was qualified for further PCR-based detection of HBV at a sensitivity of 4 × 10^2 copies ml^-1.

We then used Fe3O4/SiO2 nanoparticles and the buffers to isolate DNA of HBV in six real serum samples (one negative, figure 6, lane 5 and five positives, figure 6, lanes 1–4, 6). As a result, we could observe faint specific bands of 434 bp for HBV in samples in lanes 1 and 3, and very bright bands of 434 bp for HBV in samples in lanes 2, 4, and 6. Meanwhile, no band was observed in the sample in lane 5. The data indicates that six real serum samples had different concentration of virus copies, of which the sample in lane 6 had the highest virus load. Our data were in good agreement with those confirmed by the hospital where the samples were collected. In parallel, we performed similar experiments with these six serum samples using the commercialized silica-coated magnetic microparticles Dynabeads® myone™ silane (short name: Dynabeads, Life Technologies). As shown in figure 6, clear bands of 434 bp for HBV were observed in the samples in lanes 2’, 4’, and 6’. However, intensities of those bands were weaker compared to those in the same samples in lanes 2, 4, and 6 obtained in the case of Fe3O4/SiO2 nanoparticles. We could not observe the specific PCR-amplified bands in the samples in lanes 1’ and 3’, possibly due to the low levels of purified template DNA obtained when using Dynabeads. We conclude then that Fe3O4/SiO2 nanoparticles may be more efficient than Dynabeads in DNA isolation of HBV from serum.

3.4.4.2. Purification of DNA of Epstein–Barr viruses (EBV) using silica-coated magnetic nanoparticles and optimized buffers. Fe3O4/SiO2 nanoparticles and the buffers were then used to isolate DNA of EBV in real serum samples, in comparison to Dynabeads [10]. Among 10 suspected EBV infected serum samples, we could detect clearly 250 bp-specific bands for EBV in samples 7 and 10 using both Fe3O4/SiO2 nanoparticles and Dynabeads. However, the brighter signals were observed when using Fe3O4/SiO2 nanoparticles (not shown here), indicating that the DNA isolation efficiency of EBV by Fe3O4/SiO2 nanoparticles was higher than that using Dynabeads. The result in table 2 indicates that higher concentrations of EBV (copies/ml) in both samples were measured with using Fe3O4/SiO2 nanoparticles to purify DNA compared to those with using Dynabeads. The increase in DNA isolation efficiency by Fe3O4/SiO2 nanoparticles is likely due to a larger total surface of silica-coated magnetic nanoparticles. During the process of DNA isolation, we have found that the time required for magnets to attract completely the Dynabeads from solution was much longer, about 2–3 min, compared to 15–20 s for Fe3O4/SiO2 nanoparticles. This phenomenon is probably also due to the fact that Fe3O4/SiO2 nanoparticles have a larger total surface area compared to that of the Dynabeads.

4. Conclusion and perspective

This work reviews numerous methods of synthesis and functionalization of gold and silver nanoparticles, magnetic nanoparticles Fe3O4, combination of 4-ATP-coated silver nanoparticles and Fe3O4 nanoparticles. Some applications of the prepared nanoparticles in life sciences and the environment are discussed.

It is expected that new fabrication approaches in an environmental friendly way will be introduced. Efforts will be made for improving nanoparticles manufacturing that requires less energy and fewer toxic materials (‘green manufacturing’) which sometimes is referred to as ‘green nanotechnology’. An example of ‘green nanotechnology’ is the development of aqueous-based microemulsion or inverse microemulsion described above. As the functionality of nanoparticles becomes more complex, the major trend in further

Table 2. Quantitation of EBV load in serum using DNA templates purified by Fe3O4/SiO2 nanoparticles and Dynabeads [10].

| Sample number | Material for DNA isolation | C_t (threshold cycle) | Virus load (copies ml^-1) |
|---------------|---------------------------|-----------------------|--------------------------|
| 7             | Fe3O4/SiO2 nanoparticles  | 36.2                  | 7.17 × 10^3             |
|               | Dynabeads                 | 40.62                 | 6.53 × 10^2             |
| 10            | Fe3O4/SiO2 nanoparticles  | 26.78                 | 1.18 × 10^6             |
|               | Dynabeads                 | 27.73                 | 7.04 × 10^5             |
development of nanoparticles is to make them multifunctional which has the potential to integrate various functionalities, and use them for manufacturing nano-devices.

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