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Isolation of DNA from *Arthrospira platensis* and whole blood using magnetic nanoparticles ($\text{Fe}_3\text{O}_4@OA$ and $\text{Fe}_3\text{O}_4@OA@SiO_2$)

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Abstract

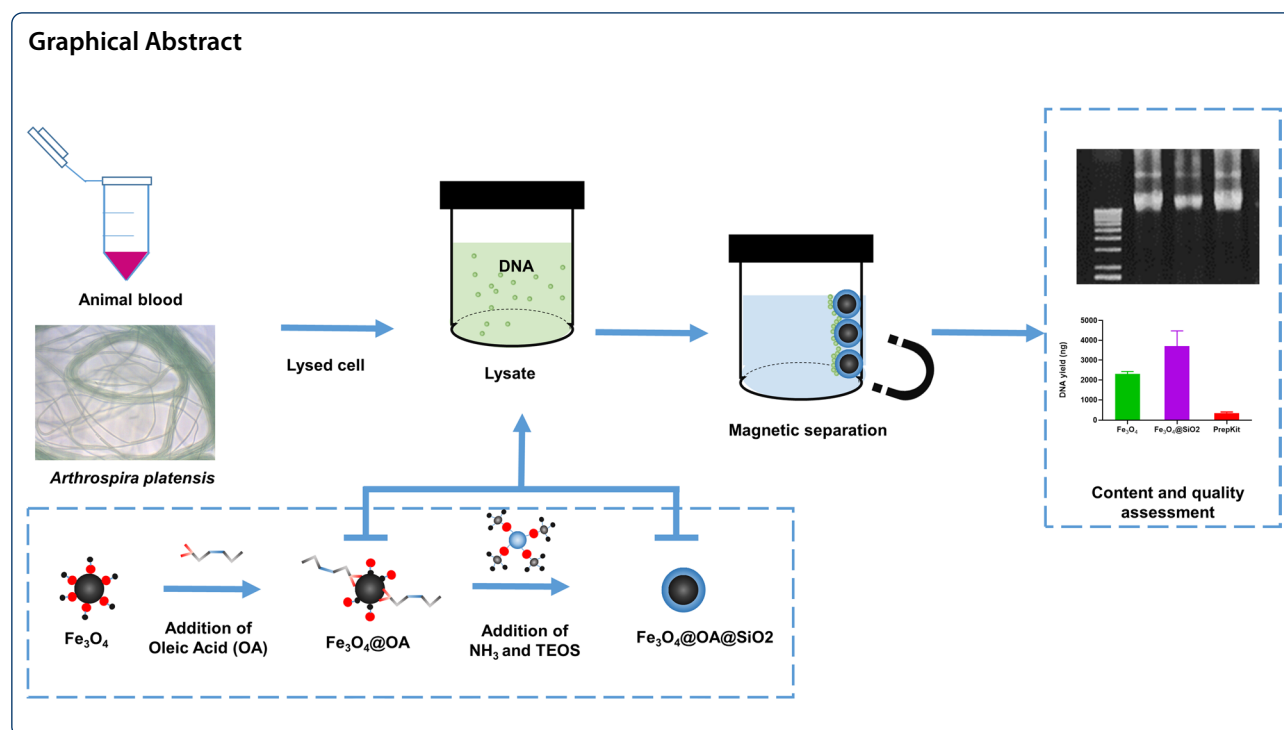
Magnetic nanoparticles (MNPs) provide a fast, cost-effective, and organic-free method for DNA isolation. In this paper, we synthesized MNP coated with oleic acid ($\text{Fe}_3\text{O}_4@OA$) and silica nanoparticles ($\text{Fe}_3\text{O}_4@OA@SiO_2$), characterized the properties of MNP using TEM, VSM, and FTIR, and investigated their efficiency in DNA isolation from cyanobacteria. The yield and quality of isolated DNA were evaluated and compared with those from animal blood and those obtained by the silica column or organic solvents. The results showed the successful preparations of $\text{Fe}_3\text{O}_4@OA$ and $\text{Fe}_3\text{O}_4@OA@SiO_2$ with superparamagnetic behaviors and a mean diameter of 7 nm and 106 nm, respectively. The FTIR spectra of $\text{Fe}_3\text{O}_4@OA$ confirmed the bonding of OA to the surface of iron oxide, while those of $\text{Fe}_3\text{O}_4@OA@SiO_2$ showed the exposed silanol groups. Although MNPs yielded a lower quantity of DNA compared with phenol/chloroform extraction, they showed the potential protection of the integrity of DNA against centrifugal and shear forces. $\text{Fe}_3\text{O}_4@OA@SiO_2$ favored more nucleic acid absorption than $\text{Fe}_3\text{O}_4@OA$, producing a 1.2 and 1.6 times greater amount of DNA from *Arthrospira platensis* and animal blood respectively. The purity of DNA isolated from *Arthrospira platensis* was also higher than that of animal blood. These findings indicate a new and simple approach for the isolation of DNA from *Arthrospira* genus.

Keywords: Magnetic nanoparticles, Oleic acid coating, Silica coating, DNA isolation, *Arthrospira platensis*, Whole blood

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Introduction

DNA isolation is a central procedure in life science and has significant effects on downstream studies such as DNA sequencing and PCR amplification (Ruggieri et al. 2016; Kovačević 2016). There are two principle methods for DNA purification: liquid- and solid-phase separation (Ruggieri et al. 2016). As for the former, organic separation using phenol and chloroform was considered the gold standard (Price et al. 2009) for its high yield and purity of isolated DNA. However, it requires cumbersome procedures which involve many steps of pipetting, centrifugation, also the use of toxic organic solvents (Zhou et al. 2013; Min et al. 2014; Corchero and Villaverde 2009).

Solid-phase separation was developed based on the reversible binding acid nucleic to silica particles (Vogelstein and Gillespie 1979), resulting in the commercial application of spin column-based extractions (Ruggieri et al. 2016). Some of the commercially available kits featuring these techniques from Zymo Research, Qiagen, Life technologies have been widely applied to purify DNA due to its high consistency, productivity, and free of toxic chemicals (Price et al. 2009; Tan and Yip 2009). Magnetic nanoparticles (MNPs) provide the alternative solid-phase purification of DNA (Hawkins et al. 1994; DeAngelis et al. 1995), which has also been well characterized in various studies (Tang et al. 2020; Horak et al. 2007). This method not only shares the similar advances of the spin column over organic separation, but also has the potential for automation, dismissing of the

cross-contamination, and exerts fewer effects on DNA structure due to free of centrifugal and shearing forces (Zhou et al. 2013; Tang et al. 2020; Berensmeier 2006).

Iron oxides smaller than 30 nm in diameter usually possess superparamagnetic properties (Xu et al. 2006), enabling them to be well dispersed in lysate solution and evenly absorb DNA in the absence of magnet field (Kovačević 2016). Naked magnetic nanoparticles (Fe_3O_4) could also directly absorb DNA from plasmids (Davies et al. 1998), mammalian cells, and tissues (Saiyed et al. 2006) without reports of being toxic like cobalt ferrite (Lee and Kim 2012) or titanium oxide (Anas et al. 2008). However, the naked MNPs are often agglomerated due to the attractive forces (Berensmeier 2006), which could reduce their biocompatibility and lead to undesirable interactions (Tang et al. 2020; Horak et al. 2007). Thus, surface modifications of MNPs are necessary to maintain stability and facilitate the adsorption of DNA (Horak et al. 2007; Li et al. 2011).

MNPs have been surface-modified by using surfactant, chitosan, silica, cellulose, or polystyrene (Kovačević 2016; Li et al. 2011). Surface coating usually decreases the magnetization of NPs, which is related to the decreased agglomeration and net moment of particles (Ghosh et al. 2011) or lattice defects (Wang et al. 2010). Among them, oleic acid (OA) and silica are two common capping materials because of their good stability, biocompatibility, and straightforward synthetic methods (Li et al. 2011; Ghosh et al. 2011; Chen et al. 2016). Oleic acid is a surfactant with a long hydrocarbon tail. The surface-modified OA

results in the reduced size, decreased agglomeration, and nonpolar solubility of MNPs (Horak et al. 2007; Ghosh et al. 2011; Vogt et al. 2010). Alternatively, silica has also been studied for DNA isolation due to its ability of fast binding and elution of nucleic acid in the presence of chaotropic salts or water, respectively (Rittich et al. 2006; Melzak et al. 1996). The chaotropic agent dehydrates the nucleic acid backbone, forming the cation bridge between nucleic acid and silica surface which can be reversed by adding water and allowing for the elution of absorbed nucleic acids (Tan and Yiap 2009; Melzak et al. 1996). Silica-coated magnetic nanoparticles have also been widely applied for the adsorption of protein and immune cells (Ta et al. 2016) or isolation of DNA from bacteria (Chen et al. 2016), carcinoma cells (Wang et al. 2017), human whole blood (Li et al. 2011), and soil samples (Sebastianelli et al. 2008). However, there is little information reporting the application of coated MNPs to isolate DNA from fungi or cyanobacteria.

Arthrospira genus, multicellular and filamentous cyanobacteria, has gained popularity in food supplements, functional food, and nutraceuticals (Belay 2013; Morin et al. 2010). *Arthrospira* genus contains a high amount of phenolic compounds and proteins but a small amount of genetic material (Ciferri 1983; Xu et al. 2016), which leads to the fluctuating efficiency in DNA isolation. Isolation methods that utilize silica membranes appear to result in an inefficient yield of DNA, while procedures that rely on mechanical beating of the cells by beads seem to exert a strong shearing effect on DNA, rendering DNA degradation (Morin et al. 2010). Other methods mainly applied organic solvents to extract and precipitate DNA. In contrast to *Arthrospira platensis*, DNA from blood samples has been widely isolated by MNPs. However, the high levels of protein in blood can inhibit nucleic acid separation (Ruggieri et al. 2016).

Although MNPs have been widely applied to purify DNA from various samples, less research is on using MNPs to isolate DNA from *Arthrospira* cells. In this study, we first applied $\text{Fe}_3\text{O}_4\text{@OA}$ and $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ to purify DNA from *Arthrospira platensis*. The quantity and purity of DNA isolated from *Arthrospira platensis* were evaluated and compared with that from animal blood and other methods including phenol/chloroform and silica-based columns.

Materials and methods

Materials

Arthrospira platensis and animal blood were provided by the Institute of Applied Technology (Thu Dau Mot University, Binh Duong, Vietnam). All analytical grade reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise mentioned.

Preparation of oleic acid-coated magnetic nanoparticles

Fe_3O_4 nanoparticles were initially synthesized through the ultrasound-assisted co-precipitation method and coated with oleic acid on the surface later (Ding et al. 2012). Briefly, ferric chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 1.03 g), ferrous chloride tetrahydrate ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, 0.01 mol), and double distilled water (ddH_2O ; 40 ml) were added into a round-bottomed flask. The mixture of iron oxide was thoroughly dissolved by ultrasonication at 50 °C for 20 min. Sodium hydroxide solution 0.8 M (40 ml) was dropped into the mixture at the rate of 1 droplet per 2 seconds. After adding NaOH, the mixture was kept sonicated for 1 h to form the black precipitate of magnetic nanoparticles (Fe_3O_4). The magnetic nanoparticles were further coated with oleic acid by the addition of 20 ml of the surfactant and continuous sonication for 1 h. After coating, the obtained $\text{Fe}_3\text{O}_4\text{@OA}$ was washed by absolute EtOH five times by centrifuging for 30 min at 10,000 rpm 4 °C (Hermle Centrifuge Z 216 MK, Germany) and dried at 50 °C for 15 h.

Preparation of silica-coated magnetic nanoparticles

Silica-coated magnetic nanoparticles were further prepared through the Stöber process (Stöber et al. 1968). $\text{Fe}_3\text{O}_4\text{@OA}$ (40 mg) was dispersed in ddH_2O (16.8 ml) at room temperature for 20 min. Subsequently, EtOH (64 ml), NH_3 (4 ml), and tetraethyl orthosilicate (TEOS; 4 ml) were added to the solution under continuous ultrasonic vibration to form silica-coated magnetic nanoparticles. The obtained $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ was rinsed by absolute EtOH five times by centrifuging for 30 min at 10,000 rpm 4 °C (Hermle Centrifuge Z 216 MK, Germany) and dried at 50 °C for 15 h.

Characterization of magnetic nanoparticles

The morphology of synthesized $\text{Fe}_3\text{O}_4\text{@OA}$ and $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ was observed using transmission electron microscopy (TEM, JEM-1400, JEOL, Japan). The size distribution of MNPs was analyzed from TEM photographs using ImageJ (1.52, National Institutes of Health, USA). The Fourier-transform infrared (FTIR) spectra were recorded on a spectrophotometer model (Tensor 27, Bruker, Bremen, Germany). Magnetic nanoparticles were measured at room temperature using vibrating sample magnetometer (VSM, EZ9 MicroSense, USA).

Preparation of crude cell lysates of *Arthrospira platensis*

Crude cell lysates of *Arthrospira platensis* were prepared by the modified procedure (Rittich et al. 2006). Briefly, *Arthrospira platensis* cells (600 μl) were lysed by lysis buffer (60 μl of sodium dodecyl sulfate (SDS; L3771, Sigma-Aldrich, USA), 15 μl of proteinase K 20 mg/ml

(AM2546, Thermo Fisher Scientific, Invitrogen TM)) at 55 °C for 4 h. After lysis, the insoluble particles were removed by centrifugation ($14,500 \times g$ for 5 min), while the soluble polysaccharides were further precipitated with 170 μ l of NaCl (5 M) and 135 μ l of 10% cetyltrimethylammonium bromide (CTAB, Sigma-Aldrich, USA) at 65 °C for 30 min. The precipitate was subsequently discarded by centrifugation ($10,000 \times g$ for 5 min) to obtain the crude cell lysate.

Preparation of crude cell lysates of animal blood

A sample of animal blood (200 μ l), 10% SDS (100 μ l), and 10 μ l of proteinase K (20 mg/ml) were sequentially added to the 1.5 ml Eppendorf. The mixture was then gently mixed and incubated at 65 °C for 30 min to lyse the blood cell.

DNA isolation on magnetic particles

The procedure for DNA isolation was based on the modified reported process (Hawkins et al. 1994). Briefly, lysate samples of *Arthrospira platensis* were mixed with 1 mg of $\text{Fe}_3\text{O}_4@\text{OA}$ or $\text{Fe}_3\text{O}_4@\text{OA}@\text{SiO}_2$ and that of animal blood was mixed with 2 mg of $\text{Fe}_3\text{O}_4@\text{OA}$ or $\text{Fe}_3\text{O}_4@\text{OA}@\text{SiO}_2$. The quantity of MNPs was optimized for each type of samples (Additional file 1: Table S1). Binding buffer (165 μ l of 1.25 M NaCl and 0.5% Tween 80 with the ratio of 9:1) was subsequently added to each sample and gently mixed. The mixture was incubated at room temperature for 5 min to form the complexes of nanoparticles and DNA. These complexes were then separated by a magnet (Promega, Madison, WI, USA), followed by the discarding of the supernatant. The immobilized DNA was subsequently rinsed with 100 μ l of 70% EtOH thrice. After thorough evaporation, the sample was well agitated in 100 μ l of TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) at 65 °C for 10 min to elute DNA. The purified DNA was collected and the nanoparticles were magnetically removed afterward.

DNA isolation on silica column

Silica column kits, AccuRive Plan/Food DNA Prekit (KT biotech, Vietnam) and AccuLite Blood DNA Prekit (KT biotech, Vietnam), were used to extract DNA from the crude cell lysates of *Arthrospira platensis* and animal blood, respectively. The procedures involved lysis steps, centrifugation, washing, and elution, which were performed according to the manufacturing instructions.

DNA isolation by organic solvents

The DNA from cell lysate was extracted through the addition of an equal volume of the solution of phenol/chloroform/isoamyl with the ratio of 25:24:1. The sample was further kept on ice for 20 min, followed by centrifugation

($10,000 \times g$ for 5 min) at 4 °C. The supernatant was then collected and transferred to a fresh Eppendorf. Absolute EtOH was further added to precipitate DNA at -20 °C for 30 min. The nucleic acid was collected by centrifugation ($10,000 \times g$ for 5 min) at 4 °C, followed by discarding the supernatant. The DNA pellet was air-dried and further dissolved in 100 μ l of TE buffer.

Characterization of isolated DNA

Isolated DNA was analyzed by gel electrophoresis. The sample (10 μ l) and Gel Red (1.6 μ l; Sigma-Aldrich, St. Louis, MO, USA) were mixed and loaded on a 1% agarose gel in TAE buffer (0.04 M tris-acetate and 0.001 M EDTA). The gel was electrophorized at 100 V for 50 min and photographed by UV light at 305 nm. In addition, the content and quality of the eluted DNA were further assessed by ultraviolet spectrophotometry (NanoDrop 2000c, Thermo Scientific, USA). The content of DNA was measured by spectrophotometric quantification at 260 nm. The overall quality was estimated by the absorbance ratio at 260/280.

Statistical analysis

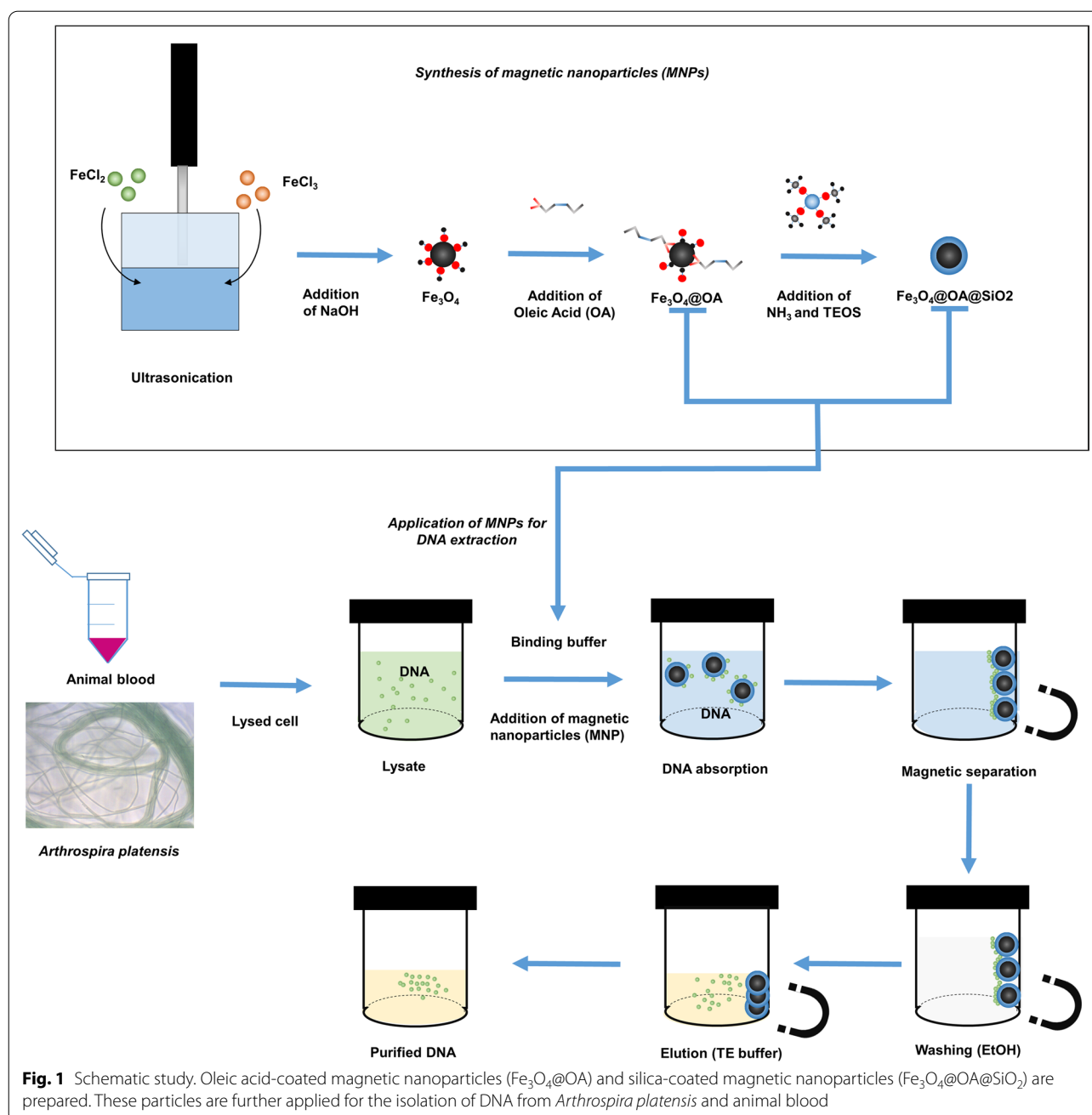
All experiments were conducted at least three independent times whose experimental values were presented in means \pm SD. The analysis was performed by GraphPad Prism (8.4.2, GraphPad Software, San 153 Diego, California, USA).

Results

In this study, we synthesized magnetic nanoparticles by co-precipitating divalent and trivalent iron ions under ultrasound vibration and controlled temperature. MNPs were further coated with oleic acid ($\text{Fe}_3\text{O}_4@\text{OA}$) and silica nanoparticles ($\text{Fe}_3\text{O}_4@\text{OA}@\text{SiO}_2$). The obtained magnetic nanoparticles with modified surfaces were evaluated for size distribution, magnetization, and surface through TEM, vibrating sample magnetometer, and FTIR. Two types of magnetic particles were further used as adsorbents for the isolation of genetic DNA from *Arthrospira platensis* and animal blood (Fig. 1). The quantity and quality of DNA extracted by $\text{Fe}_3\text{O}_4@\text{OA}$ and $\text{Fe}_3\text{O}_4@\text{OA}@\text{SiO}_2$ were determined by gel electrophoresis and ultraviolet spectroscopy and compared with that isolated by organic separation and silica column.

TEM images

The morphology of $\text{Fe}_3\text{O}_4@\text{OA}$ and $\text{Fe}_3\text{O}_4@\text{OA}@\text{SiO}_2$ was evaluated by TEM images that at least 100 MNPs were measured from each sample using ImageJ. $\text{Fe}_3\text{O}_4@\text{OA}$ displayed the nanosphere shape with a narrow distribution (Fig. 2A). The diameter of $\text{Fe}_3\text{O}_4@\text{OA}$ was



ranging from 5 to 12 nm with the average diameter of approximately 7 nm and 66.7% of the distribution in the range of 6–10 nm (Fig. 2B). TEM image of $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ displayed a core-shell structure in which the multicores of Fe_3O_4 were observed as dark dots in the center and the silica shells covered as the gray area outside (Fig. 3A). The average diameter of $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ was roughly 106 nm, which mainly distributed in the range from 85 to 115 nm (Fig. 3B).

FTIR spectra

The surfaces of $\text{Fe}_3\text{O}_4\text{@OA}$ and $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ were examined using FTIR (Fig. 4). The FTIR spectra of $\text{Fe}_3\text{O}_4\text{@OA}$ and $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ showed the peaks at 1632 cm^{-1} and 3437 cm^{-1} that corresponded to $-\text{OH}$ bonds and 575 cm^{-1} which were attributed to $\text{Fe}-\text{O}$ vibrations of Fe_3O_4 (Li et al. 2011; Ta et al. 2016). The FTIR spectra of $\text{Fe}_3\text{O}_4\text{@OA}$ displayed additional peaks at 2851 cm^{-1} and 2922 cm^{-1} which were assigned to the stretching vibrations of CH_3 and CH_2 groups in

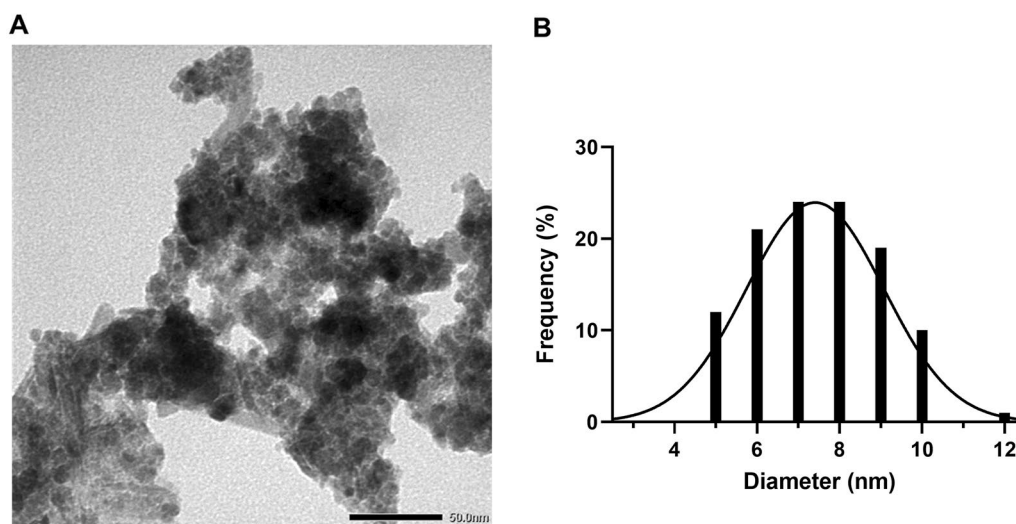


Fig. 2 Transmission electron microscopy (TEM) image and size distribution of oleic acid-coated magnetic nanoparticles ($\text{Fe}_3\text{O}_4\text{@OA}$). (A) TEM image, scale bar: 50 nm. (B) Size distribution, $n = 100$

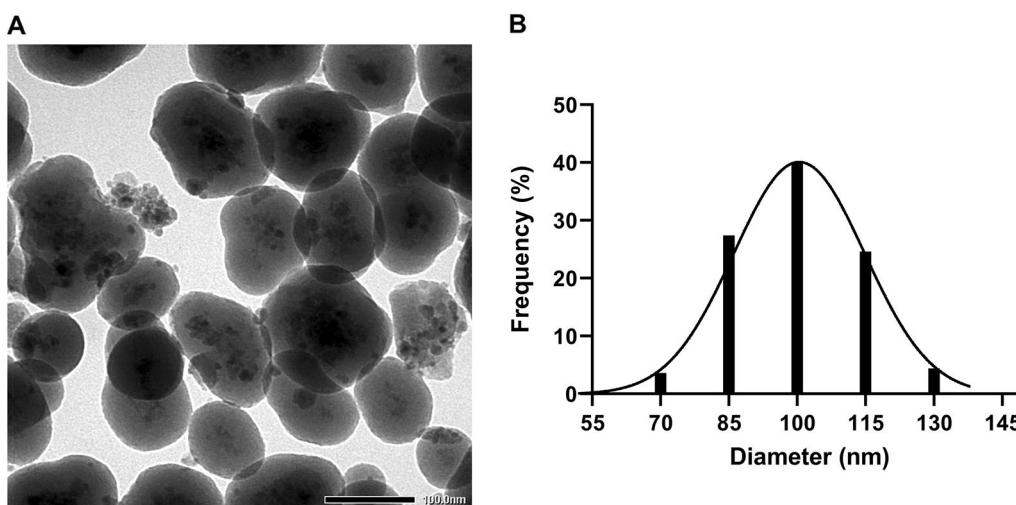


Fig. 3 Transmission electron microscopy (TEM) image and size distribution of silica-coated magnetic nanoparticles ($\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$). (A) TEM image, scale bar: 100 nm. (B) Size distribution, $n = 100$

the carbon backbone of OA (Ghosh et al. 2011; Vogt et al. 2010; Nalle et al. 2019). A new band appeared at 1464 cm^{-1} corresponding to the stretching vibration of COO^- . FTIR spectrum confirmed the capping of oleic acid onto the surface of iron oxide nanoparticles. $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ showed new peaks at 467 cm^{-1} , 802 cm^{-1} , 959 cm^{-1} , and 1099 cm^{-1} which were assigned to the asymmetric vibration of Si–O–Si bonds, the bending vibration of the Si–O–Si, the symmetric stretching of Si–OH bonds, and the symmetric stretching of the Si–O–Si, respectively (Li et al. 2011; Vogt et al. 2010; Ta et al. 2016). The FTIR spectra confirmed that silica

nanoparticles were successfully coated on the surface of Fe_3O_4 .

Magnetic properties

The magnetic properties of synthesized MNPs were measured at room temperature using VSM (Fig. 5). The magnetization curves showed the typical superparamagnetic behavior with zero coercivity and remanence on the magnetization loops. The saturation magnetization values (Ms) of $\text{Fe}_3\text{O}_4\text{@OA}$ and $\text{Fe}_3\text{O}_4\text{@OA@SiO}_2$ were approximately 80.0 emu/g and 19.5 emu/g, respectively.

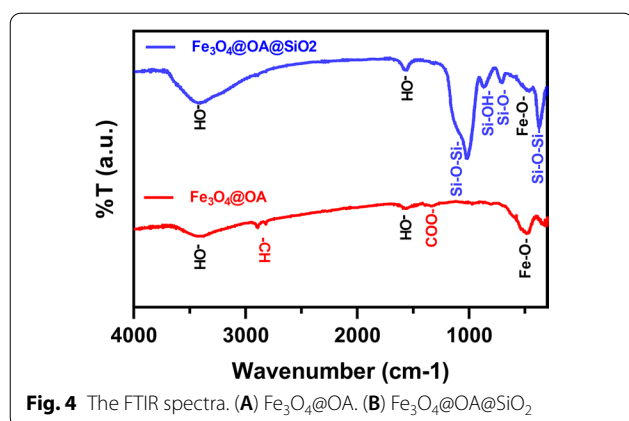


Fig. 4 The FTIR spectra. (A) $\text{Fe}_3\text{O}_4@OA$. (B) $\text{Fe}_3\text{O}_4@OA@SiO_2$

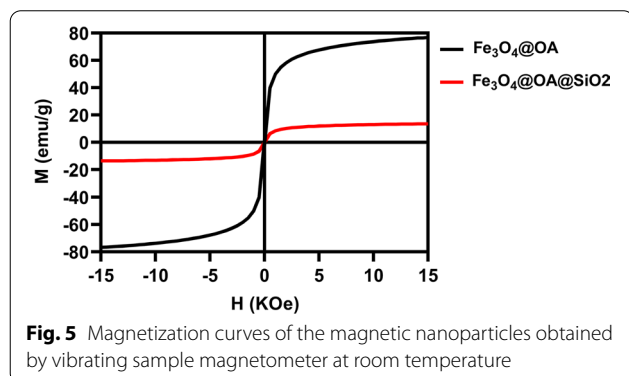


Fig. 5 Magnetization curves of the magnetic nanoparticles obtained by vibrating sample magnetometer at room temperature

The decrease in the M_s value of $\text{Fe}_3\text{O}_4@OA@SiO_2$ was attributed to the coating of silica on the magnetic properties of the NPs.

Extraction of DNA from *Arthrosira platensis*

Molecular weight and the degradation of isolated DNA were evaluated through gel electrophoresis. The gel displayed a large size of isolated DNA which was roughly 15–20 kb (Fig. 5A). The DNA isolated by phenol/chloroform, $\text{Fe}_3\text{O}_4@OA$, and $\text{Fe}_3\text{O}_4@OA@SiO_2$ exhibited sharp patterns, while that by the commercial silica column showed smeared fragments (Fig. 5A). The yields of DNA isolated were further determined using ultraviolet spectrophotometry (Table 1). Phenol extraction yielded the highest DNA content (approximately 20.5 μg), which

was consistent with the brightest electrophoresis patterns. The average yields of DNA isolated by $\text{Fe}_3\text{O}_4@OA$, $\text{Fe}_3\text{O}_4@OA@SiO_2$, and silica column were 2.14, 2.61, and 2.89 μg , respectively. In addition, the absorbance ratio (OD 260/280) of DNA isolated from $\text{Fe}_3\text{O}_4@OA$ and $\text{Fe}_3\text{O}_4@OA@SiO_2$ was approximately 1.5, while that from phenol/chloroform and silica columns were 2.0 and 1.9, respectively.

Extraction of DNA from animal blood

Gel electrophoresis indicated the large molecular sizes of the extracted DNA, which were greater than 20 kb (Fig. 6A). DNA extracted by phenol/chloroform was degraded into smears and small fragments, whereas this was not observed in those extracted by $\text{Fe}_3\text{O}_4@OA$, $\text{Fe}_3\text{O}_4@OA@SiO_2$, and silica columns. The phenol/chloroform method isolated the highest content of DNA (roughly 10.4 μg), while $\text{Fe}_3\text{O}_4@OA$, $\text{Fe}_3\text{O}_4@OA@SiO_2$, and silica column yielded 2.3, 3.7, and 0.36 μg of DNA content, respectively (Table 2). The absorbance ratios of DNA isolated from $\text{Fe}_3\text{O}_4@OA$, $\text{Fe}_3\text{O}_4@OA@SiO_2$ were low, approximately 1.1 in comparison with those extracted from phenol/chloroform and silica columns were 2.1 and 1.9, respectively (Table 2).

Discussion

We successfully synthesized OA-coated and silica-coated MNPs and applied them to purify DNA from cyanobacteria and animal blood (Fig. 1). Silica was more favorable for the binding of nucleic acid than OA. That may be due to the hydrophilic property of the silica surface and the aided binding conditions which were created by adding chaotropic salts to the lysates. In addition, MNPs isolated higher purity of DNA from *Arthrosira platensis* than the blood samples which could be attributed to the different nature of the samples. MNPs resulted in the lower yield of DNA than the phenol/chloroform. However, the structure of DNA isolated by MNPs was well maintained than the solvent extraction and spin column methods.

OA-coated magnetic nanoparticles dispersed in a narrow distribution with an average diameter of 7 nm (Fig. 2). That was smaller than the previously reported size of Fe_3O_4 , roughly 10–16 nm (Unal et al. 2010; Mehta et al. 1997). The smaller size could be attributed to the coating of OA, which might contribute to stabilizing,

Table 1 Comparison of methods to separate DNA from *Arthrosira platensis*

	Method			
	Phenol/chloroform	$\text{Fe}_3\text{O}_4@OA$	$\text{Fe}_3\text{O}_4@OA@SiO_2$	Silica column
DNA yield (μg)	20.53 ± 1.6	2.14 ± 0.39	2.61 ± 0.24	2.89 ± 0.10
A260/A280	2.03 ± 0.01	1.49 ± 0.01	1.43 ± 0.01	1.9 ± 0.01

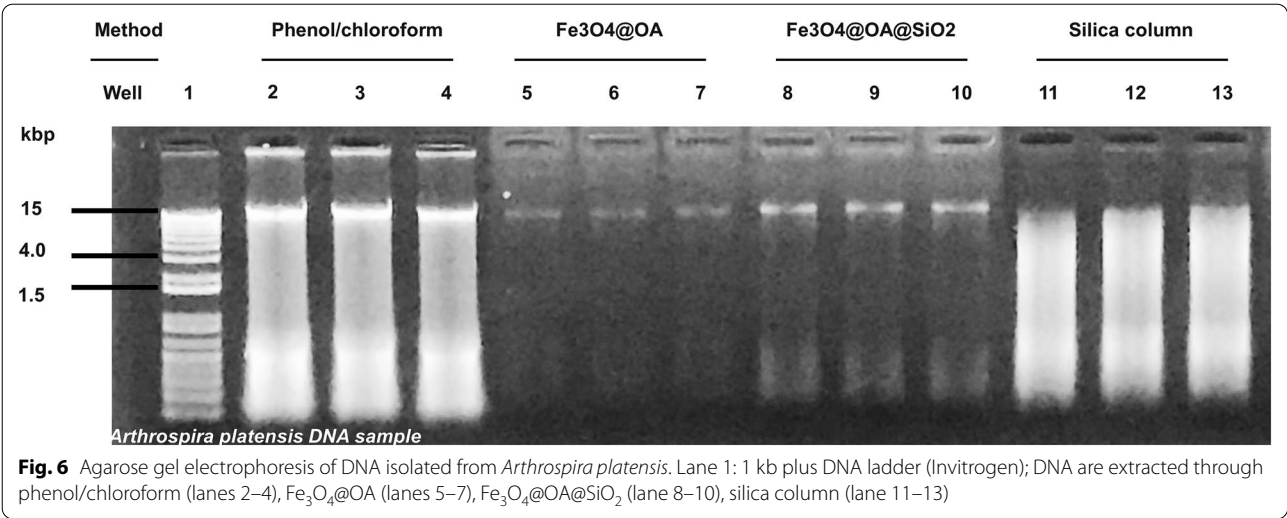
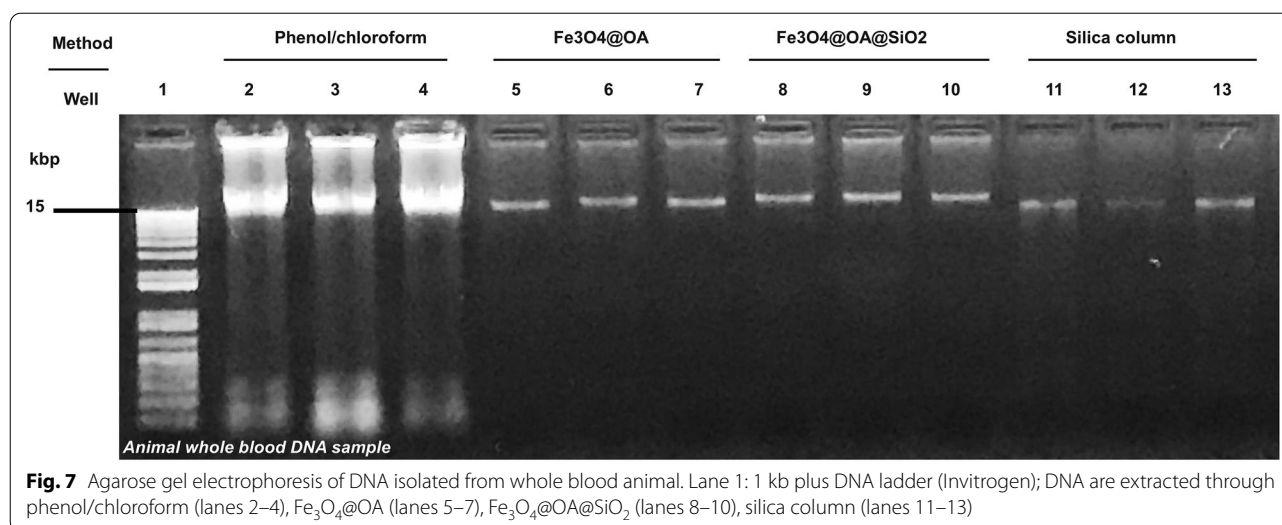


Table 2 Comparison of methods ro isolate DNA from whole blood sample

Method				
	Phenol/Chloroform	Fe ₃ O ₄ @OA	Fe ₃ O ₄ @OA@SiO ₂	Silica column
DNA yield (μg)	10.32 ± 5.80	2.3 ± 0.13	3.7 ± 0.77	0.36 ± 0.05
A260/A280	2.05 ± 0.09	1.13 ± 0.06	1.05 ± 0.01	1.94 ± 0.23

reducing the size and agglomeration of MNPs (Ghosh et al. 2011; Nalle et al. 2019; Zhang et al. 2006). The FTIR spectra showed the stretching vibrations of the CH-bond at 2851 cm⁻¹ and 2922 cm⁻¹ and the COO- bond at the new peak of 1464 cm⁻¹ (Fig. 4). These spectra indicated that OA formed carboxyl bonds with the surface of MNPs (Wang et al. 2010; Nalle et al. 2019; Zhang et al. 2006). The Diameter of MNPs increased to 106 nm after coating silica, which was mainly due to the increase in the size of the shell structure. The silica shell was amorphous with no formation of crystallographic planes (Fig. 3). Furthermore, the FTIR spectra showed the presence of silanol groups (peak at 802 cm⁻¹) on the surface of MNP (Fig. 4). The silanol group makes Fe₃O₄@OA@SiO₂ hydrophilic and facile surface modification, while the hydrophobic properties of OA could limit their bio-applications (Vogt et al. 2010). Coating silica further decreased the magnetization of Fe₃O₄@OA from 80.0 to 19.5 emu/g (Fig. 5). The previous studies showed that Ms values of surface-modified MNPs were often lower than that of naked MNPs (92 emu/g) (Li et al. 2011; Han et al. 1994). Another study also reported that the Ms value of core-shell magnetic particles was inversely corresponding to the thickness of silica shells (Vogt et al. 2010). This

could be attributed to the reduction in agglomeration of particles, leading to the faster spin relaxation, in turn, decreasing magnetization (Vogt et al. 2010). However, the value Ms of Fe₃O₄@OA@SiO₂ was considered practical for bio-application (Vogt et al. 2010; Ta et al. 2016). We further applied coated nanoparticles for the solid-phase supported isolation of DNA from *Arthrospira platensis* and animal blood. Gel electrophoresis showed a molecular weight of 15–20 kb for *Arthrospira platensis* (Fig. 6) and of greater than 20 kb for a blood sample (Fig. 7). Those were consistent with the previous report on the molecular size of DNA from *Arthrospira platensis* (Morin et al. 2010) and animal blood. Fe₃O₄@OA@SiO₂ isolated a higher amount of DNA compared with Fe₃O₄@OA, specifically 1.2-fold and 1.6-fold greater in the case of *Arthrospira platensis* and animal blood, respectively (Tables 1, 2). This might be because silica favored the binding of DNA molecules (Li et al. 2011; Chen et al. 2016; Wang et al. 2017). Previous studies showed that the fast binding of DNA to the silica surface occurred under the condensed condition caused by adding chaotropic salts. A chaotropic agent dehydrates the nucleic acid backbone, forming the cation bridge between nucleic acid and silica surface and can be reversed by adding



water and allowing for elution of nucleic acid (Tan and Yiap 2009; Melzak et al. 1996). In this study, we applied a binding buffer containing salts and surfactant (165 µl of 1.25 M NaCl and 0.5% Tween 80 with the 9: 1) to facilitate the binding of DNA. In addition, DNA could bind to silanol group on the surface of silica due to the hydrogen bonding in the presence of chaotropic salts (Kovačević 2016; Rittich et al. 2006). Otherwise, OA capping would result in oil soluble nanoparticles (Ghosh et al. 2011; Vogt et al. 2010). The hydrophobicity of the OA coating may lead to a decrease in DNA absorption to the surface of NPs, resulting in a lower DNA yield of DNA than Fe₃O₄@OA@SiO₂.

Naked MNPs and silica are widely studied for their biocompatibility (Kovačević 2016; Vogt et al. 2010). Saiyed Z et al. applied Fe₃O₄ to purify DNA from tissues and whole blood, producing 1.8–2.0 µg of DNA and 1.3 times higher than the Qiagen spin column (Saiyed et al. 2006). That quantity of DNA was similar to the yield of DNA isolated from the blood sample (2.3–3.7 µg) which was also higher than the yield of the spin column (0.36 µg) (Table 2). These results might indicate that MNPs can purify DNA from high protein samples better than the spin column, which could be due to the well disperse of MNPs in the lysate supporting the absorption of DNA in comparison to the silica membrane. Sebastianelli et al. also studied the application of silica nanomagnetite particles to purify DNA from the soil sample. The results were reported that silica-coated MNPs have approximately two times higher quantity than the phenol/chloroform method and almost four times less expensive than another extraction kit (Sebastianelli et al. 2008). Compared to our study, MNPs yielded a lower quantity than phenol/chloroform extraction (Table 1). This may

be due to the nature of *Arthrospira cells*. This genus contains approximately 4% nucleic acid per dry weight while that of *Bacillus subtilis* was approximately 20% (Ciferri 1983). Furthermore, *Arthrospira platensis* is also rich in polyphenols and polysaccharides, which could co-absorb on MNPs (Philippis and Vincenzini 1998), resulting in a low quantity of DNA isolation. However, DNA structure showed the sharp band without smear like phenol/chloroform extraction or degraded as spin column (Fig. 6), whereas previous studies also reported the degradation of DNA from *Arthrospira platensis* isolated from glass bead and silica membrane (Morin et al. 2010), suggesting that these methods induced more negative effects of shear force on DNA structure than MNPs.

Conclusion

In summary, we successfully synthesized modified surface magnetic nanoparticles through co-precipitation and solgel process under ultrasonic vibration. Both Fe₃O₄@OA and Fe₃O₄@OA@SiO₂ proved the capability in adsorption and isolation of DNA from *Arthrospira platensis* and animal blood, in which Fe₃O₄@OA@SiO₂ provided a higher binding efficiency than Fe₃O₄@OA. The extracted DNA maintained integrity due to the free shearing and centrifugal force procedure. The optimized procedures in lysed cells and DNA adsorption would be further studied for a higher quality of isolated DNA.

Abbreviations

MNPs: Magnetic nanoparticles; TEM: Transmission electron microscopy; FTIR: Fourier-transform infrared spectroscopy; OA: Oleic acid; TEOS: Tetraethyl orthosilicate; ddH₂O: Double distilled water; UV: Ultraviolet; VSM: Vibrating sample magnetometer.

Supplementary Information

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Additional file 1. Tables S1. DNA yields corresponding to the quantity of magnetic nanoparticles.

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Author contributions

L-TTN contributed to conceptualization, methodology, and project administration. NHTL and HKTT performed investigation. KDN was involved in investigation, formal analysis, writing—original draft, and writing—review and editing. All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests

The authors declare that there are no competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

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