Comparative study of three magnetic nano-particles ($FeSO_4$, $FeSO_4/SiO_2$, $FeSO_4/SiO_2/TiO_2$) in plasmid DNA extraction

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Abstract

Recent updates on Magnetic Nano-Particles (MNPs) based separation of nucleic acids have received more attention due to their easy manipulation, simplicity, ease of automation and cost-effectiveness. It has been indicated that DNA molecules absorb on solid surfaces via hydrogen-bonding, and hydrophobic and electrostatic interactions. These properties highly depend on the surface condition of the solid support. Therefore, surface modification of MNPs may enhance their functionality and specification. In the present study, we functionalized Fe_3O_4 nano-particle surface utilizing SiO_2 and TiO_2 layer as Fe_3O_4/SiO_2 and $Fe_3O_4/SiO_2/TiO_2$ and then compare their functionality in the adsorption of plasmid DNA molecules with the naked Fe_3O_4 nano-particles. The result obtained showed that the purity and amount of DNA extracted by Fe_3O_4 coated by SiO_2 or SiO_2/TiO_2 were higher than the naked Fe_3O_4 nano-particles. Furthermore, we obtained pH 8 and 1.5 M NaCl as an optimal condition for desorption of DNA from MNPs. The result further showed that, 0.2 mg nano-particle and 10 min at 55 °C are the optimal conditions for DNA desorption from nano-particles. In conclusion, we recommended $Fe_3O_4/SiO_2/TiO_2$ as a new MNP for separation of DNA molecules from biological sources.

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Introduction

Nucleic acid separation and purification, is a basic step for many molecular biology techniques [1]. The presence of large amounts of cellular or other contaminating materials such as proteins, lipids and carbohydrates in such complex mixtures, often hinders the subsequent reactions utilized in downstream applications, e.g. detection, cloning, sequencing, amplification, hybridization, cDNA synthesis, etc [2]. Hence, efficient and reliable methods for isolation and purification of nucleic acids from the complex mixtures where they are always found are important for many nucleic acids based methods [2,3].

A range of protocols are known for the extraction of nucleic acids in the fluid phase. These conventional methods involve cell lysis by a detergent or chaotropic substance followed by the removal of all contaminants such as proteins, lipids and carbohydrates and finally isolating DNA, utilizing a series of precipitation and centrifugation steps with long durations which is difficult to carry out [2,3]. Furthermore, organic solvents such as phenol, chloroform and ethanol utilized in these methods are highly toxic and therefore require a special and expensive disposal management.

Alternative methods based on molecular-exclusion, reversed-phase, hydrophobic interaction, and anion-exchange chromatography have been developed for isolation and purification of DNA [4]. These solid-phase systems are utilized in chromatographic separation columns as DNA extraction kits. Most chromatographic separation processes are limited in terms of speed and generation of waste chemicals [2,4].

The high demands for automation and high-throughput facilities have led to the development of new technologies for easier, faster and accurate DNA extraction [3]. Magnetic separation methods applying magnetic particles have been increasingly utilized for nucleic acids extraction processes. Although uncoated magnetic particles have been utilized for the isolation and purification of plasmid and genomic DNA from different biological sources, some functionalized magnetite particles with different groups provide a higher recovery of DNA compare to those using naked particles [1,4,5,6]. In addition, carboxyl [7], dimercaptosuccinic acid [8], gelatin [1], silica [5,9], amino silane [6], polyethylenimine (PEI) [4] and gold [10,11] coated magnetic particle has been utilized as an adsorbent for the isolation and purification of bacterial genomic and plasmid nucleic acids.

In recent years, super-paramagnetic particles have received much attention due to the fact that they don't interact among themselves in the absence of a magnetic field [2,4]. It indicates that the particles are only magnetic in a magnetic field and this property (super-paramagnetic particles) does not persist when the external magnetic field is removed [2]. This property also allows easy suspension of the particles and increases the efficiency of nucleic acid extraction.

Titanium dioxide has also received great attention because of its low cost, non-toxicity, and high stability in photochemistry and photobiology [12,13]. Titanium oxide and silica are both biocompatible and resistant to corrosion.

In the context of developing an eco-friendly green chemical process, we decided to look at the utilization of deposited silica and amorphous TiO_2 on magnetic core in isolation of DNA.

In the present research, the effects of surface modification of super-paramagnetic nano-particles in isolation of plasmid DNA were studied. The surface of the magnetic nano-particles (MNPs) was functionalized using a combination of silica and titania. Thereafter, the efficiency of the coated Fe_3O_4 nano-particles with the naked Fe_3O_4 nano-particles in separation of plasmid DNA from bacterial cells was compared.

Materials and Methods

Synthesis of Fe_3O_4 nano- particles

The Fe_3O_4 particles were synthesized via a modified method (Chen et al. 2005). A solution of $FeCl_2$ (5.4 g) and $FeCl_3$ (2 g) in aqueous hydrochloride acid (2 M, 25 ml) at room temperature under argon atmosphere, was stirred until the salts were completely dissolved. Aqueous ammonia (25%, 30 ml) was added slowly over 20 min to the mixture under argon atmosphere at room temperature, and then stirred for 30 min with a mechanical stirrer. The Fe_3O_4 nano-particles were separated with an external magnet and washed three times with de-ionized water and ethanol. The final product was obtained after drying under vacuum at 60 °C for 12 h.

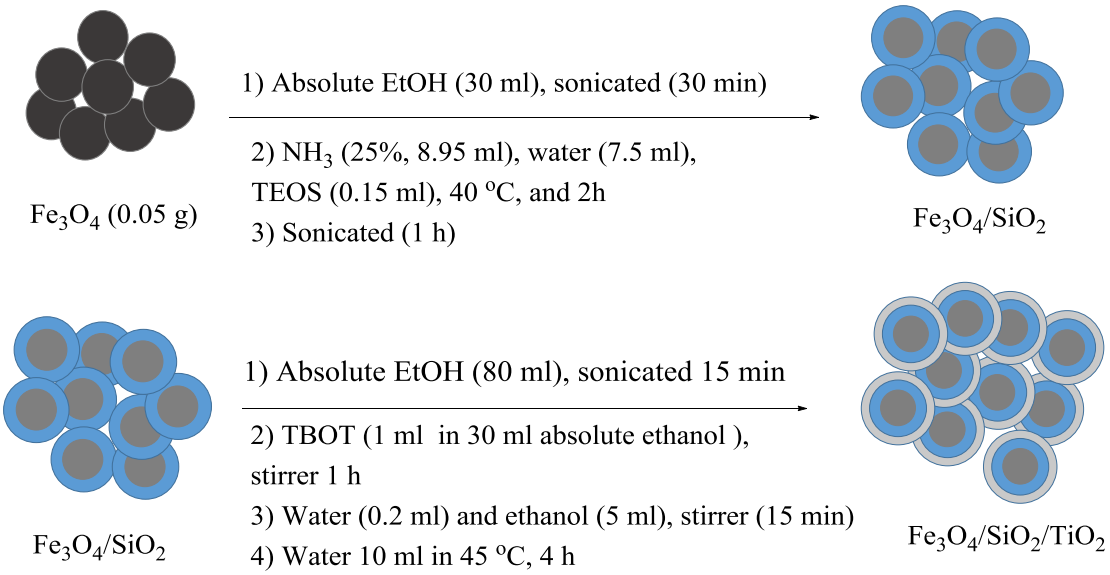
Preparation of Fe_3O_4-SiO_2

Fe_3O_4 (0.05 g) powder was dispersed in 20 ml absolute EtOH by sonication for 30 min, and thereafter ammonia (25%, 8.95 ml), water (7.5 ml), and Tetraethyl orthosilicate (TEOS) (0.15 ml) were gradually added into the generated magnetic nano-particle solution, maintained at 40 °C in a water bath, the mixture was then stirred vigorously for 2 h, followed by sonication for 1 h, to coat a thin layer of SiO_2 onto the surfaces of the MNPs. The nano-particles were rinsed with ethanol (3 ×30 ml), and then re-suspended in ethanol (30 ml). This suspension was heated under reflux at 60 °C for 12 h. The resulting Fe_3O_4/SiO_2 microspheres were isolated with the help of a magnet and washed with de-ionized water three times, then dried under vacuum at 60 °C for 12 h**.**

Preparation of $Fe_3O_4/SiO_2/TiO_2$

The nano-particles were re-suspended in absolute EtOH (80 ml), followed by sonication for 15 min. Thereafter, a solution containing 1 ml titanium butoxide (TBOT) in 30 ml absolute

ethanol was added to it. The mixture was stirred at room temperature for 1 h. Subsequently, water (0.2 ml) and ethanol (5 ml) were added into this solution, which was then subjected to vortex-mixing for 15 min. The mixture was combined with water (10 ml) and then stirred vigorously in a water bath maintained at 45 °C for 4 h, followed by sonication for 1 h. The resulting $Fe_3O_4/SiO_2/TiO_2$ microspheres were isolated with the aid of a magnet and washed with de-ionized water three times, then dried under vacuum at 60 °C for 12 h (Scheme 1).



Scheme 1: Preparation of Fe_3O_4/SiO_2 and $Fe_3O_4/SiO_2/TiO_2$

Characterization of MNPs

The surface morphology of products was analyzed utilizing a scanning electron microscope (SEM), LEO, 1430VP at 14 and 15 kV accelerating voltage and transmission electron microscopy (TEM) images were recorded on a CM-120 microscope (Philips, 120 kV), FT-IR Bruker Vector, nitrogen sorption analysis (Belsorp, BELMAX, Japan) and characterized by energy dispersive X-ray spectroscopy (EDS, JEM-2100).

Extraction and Purification of plasmid DNA using MNPs

Extraction of plasmid from bacterial cells containing pBI121 plasmid (see Fig 1, 2. In [14]) was carried out using a modified alkaline method as described by Birnboim and Doly [16]. A single colony of *E. coli* containing pBI121 was taken from LB-kanamycin plates and were grown overnight in LB broth containing 100 mg/l kanamycin. The bacterial cells were harvested from 3 ml cell culture by centrifugation at 10000 g for 5 min. The bacterial pellet was re-suspended in Solution I (50 mM Tris-HCl buffer, pH 8.0, 10 mM EDTA). The re-suspended cells were lysed by adding and gently mixing with 20 mM NaOH containing 1% (w/v) sodium dodecyl sulfate (SDS) (Solution II). Genomic DNA and other contaminants were precipitated by addition of Solution III (3 M potassium acetate, pH 5.5). Finally, the mixture was centrifuged at 10000×*g* for 10 min at 4 ºC and the supernatant was transferred to a new 1.5 ml tube.

For purification of plasmid DNA, MNPs (Fe_3O_4, Fe_3O_4/SiO_2, $Fe_3O_4/SiO_2/TiO_2$) and an equal volume of binding buffers (1.25 mM NaCl, 10% polyethylene glycol) were added to the supernatant of bacterial lysate. The suspension was gently mixed and the tube was incubated at room temperature for 5 min. The beads were separated utilizing a permanent magnetic rack with a surface magnetization of 2000G (Gilnanogene Co, www.gilnanogene.com) and the supernatant was discarded. The MNPs were washed with 70% ethanol and the pellet was dried at room temperature. The beads were re-suspended in 50 µl TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) and the plasmid DNA was eluted by incubation at 55 ºC with agitation for 10 min. Lastly, MNPs were separated using magnetic rack and the supernatant containing purified DNA was transferred to a new tube.

Effects of NaCl concentration and pH of elution buffer

Effects of different NaCl concentrations (0, 0.5, 1.0, 1.5 and 2 M) and pH (7, 8 and 9) of elution buffer were evaluated on desorption of plasmid DNA from magnetic nano-particles.

Determination of the optimal amount of MNPs

Various amounts (0.05, 0.1, 0.15 and 0.2 mg) of three MNPs were utilized for determination of the optimal amounts of nano-particle in separation of plasmid DNA.

Interaction of MNPs amounts, elution buffer volume, time and temperature

In order to optimize the different factors on desorption of DNA from MNPs, elution buffer volume (50 and 100 µl), elution time (10, 30 min and overnight) and elution temperature (room temperature (RT), 55 and 4 ºC) were evaluated in combination with each other.

Polymerase Chain Reaction (PCR)

The authenticity of extracted DNA was checked by PCR analysis for GUS gene, present in pBI121 plasmid. The PCR reaction was carried out by 20 ng plasmid DNA in 25 µl of a PCR mix containing 50 mM KCl, 10 mM Tris–HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 µM dNTPs, 50 pM of each primer pairs (F-gus: 5´-GGT GGG AAA GCG AGA- -3´, R-gus: 5´-TGG ATT CCG GCA TAG TTA AA-3´) and 1 U Taq DNA polymerase (Roche, Germany). Amplification was performed in a thermocycler (Techne, UK) under the following conditions; 94 ºC for 4 min, then 35 cycles of 94 ºC for 1 min, 60 ºC for 1 min, and 72 ºC for 1 min 30 s, followed by a final 5 min incubation at 72 ºC.

Isolation of DNA from mammalian cells

The MNPs were used for isolating genomic DNA molecules from human blood based on Saiyed et al (2008) [3]. Briefly, 30 μl of 1% (W/v) sodium dodecyl sulfate (SDS) solution was added to 30 μl of sample (whole blood). The tubes were mixed and incubated at room temperature for 2-3 min. Thereafter, 20 μl of MNP were added to cell lysate, followed by addition 80 μl binding buffer. The steps were followed as explained in the plasmid extraction method.

Sensitivity of DNA Extraction

Serial dilutions, 1 (~6 µg/µl), 1:2 (~3 µg/µl), 1:5 (~1.2 µg/µl), 1:10 (~0.6 µg/µl) and 1:20 (~0.3 µg/µl) of plasmid DNA were used for identifying the sensitivity of DNA extraction by MNPs. An equal volume of binding buffer was added to 40 µl of each diluted sample and purification steps were followed as explained earlier in the plasmid DNA extraction method by MNPs.

Assay of DNA content and purity

The concentration of DNA in extracting solution was assayed using a nano-drop spectrophotometer instrument. The ratio of absorbance at 260 and 280 nm was utilized to assess the purity of DNA. MNPs were analyzed by CHNS analysis to determine the remaining DNA on the nano-particles before and after elution step.

Furthermore, the extracted DNA and PCR products were separated by electrophoresis on a 1% agarose gel and then visualized under UV light after post staining by Gelred.

Results and Discussion

In recent years, MNPs based separation of nucleic acids has received much attention due to their easy manipulation, simplicity, ease of automation and cost-effectiveness [8]. It has been indicated that DNA molecules are absorbed on solid surfaces via hydrogen-bonding, hydrophobic and electrostatic interactions. These properties highly depend on the surface condition of the solid supports [8, 17]. So, surface modifications of MNPs may improve their functionality and specificity. In the present study, we functionalized Fe_3O_4 nano-particle surface utilizing SiO_2 and TiO_2 layers and then compared their functionality in adsorption of plasmid DNA molecule with the naked Fe_3O_4 nano-particles.

Characterization of MNPs

FT-IR spectra of Fe_3O_4/SiO_2 magnetic nano-particles showing the description peaks at 802 and 1068 for Si-O-Si group is illustrated in Fig. 1, which indicates the presence of silica phase on magnetic particles. After coating a thin TiO_2 layer onto the surfaces of the Fe_3O_4/SiO_2 nano-particles, the Ti-O-Si stretching vibration bands appears in the FTIR spectrum at 970 cm^{-1} [18].

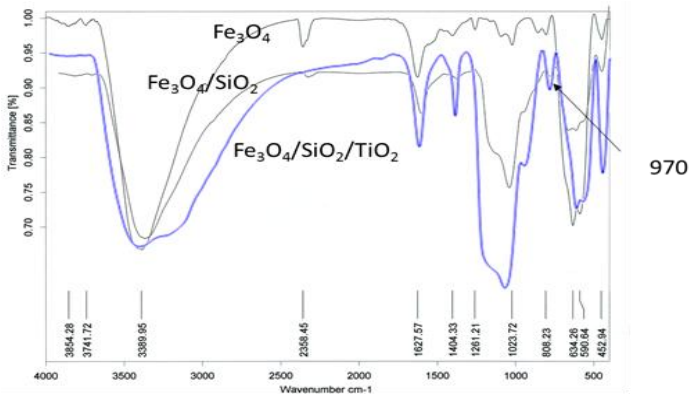


Fig. 1. FT-IR spectra for Fe_3O_4, Fe_3O_4/SiO_2 and $Fe_3O_4/SiO_2/TiO_2$

In order to further confirm of the presence of both SiO_2 and TiO_2 on the surface of the Fe_3O_4 microspheres, samples were analyzed by energy dispersive spectroscopic (EDS) (see Fig. 3 in [14]). This analysis showed the existence of Ti, Fe, and Si elements, indicating the presence of both TiO_2 and SiO_2 on the surface of the magnetic oxide microspheres.

A scanning electron microscopy (SEM) image was obtained using a JSM-6500F SEM instrument (JEOL, Japan). The SEM image in Fig. 2 displays the general spherical morphology of the Fe_3O_4/SiO_2 and $Fe_3O_4/SiO_2/TiO_2$ magnetic beads.

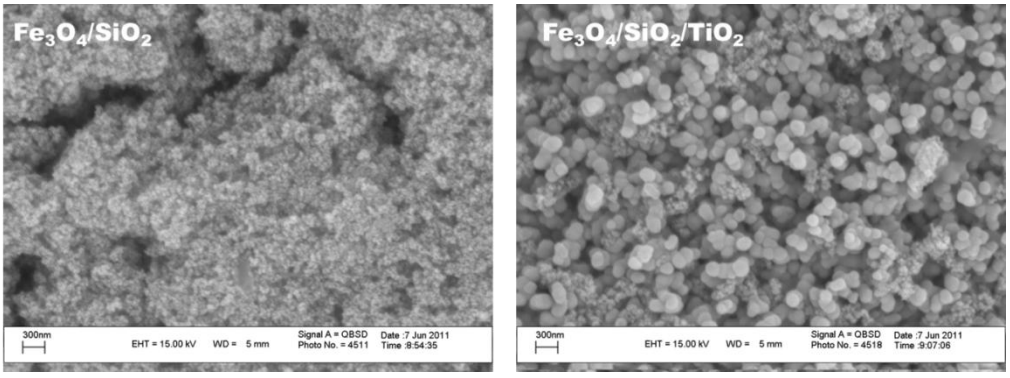


Fig. 2. SEM micrographs of Fe_3O_4/SiO_2 (A) and $Fe_3O_4/SiO_2/TiO_2$ (B)

The structure of the prepared MNPs was further verified utilizing transmission electron microscopy (TEM) images (see Fig. 4 in [14]). The particle size was estimated to be ~30 nm for Fe_3O_4/SiO_2 and ~60 nm for $Fe_3O_4/SiO_2/TiO_2$.

Moreover, N_2 adsorption-desorption at 77 K was utilized to characterize the porosity of the products (Fig. 3). It can be seen that both the Fe_3O_4/SiO_2 and Fe_3O_4 microspheres have type V isotherms (based on IUPAC classification), which suggests that they are mesoporous structures. Furthermore, $Fe_3O_4/SiO_2/TiO_2$ has type III, which is a nonporous structure. The BET surface values of the Fe_3O_4 microspheres, Fe_3O_4/SiO_2 and $Fe_3O_4/SiO_2/TiO_2$

microspheres were calculated to be 88, 22.4, and 192 m^2/g, respectively. The high specific surface area of $Fe_3O_4/SiO_2/TiO_2$ (192 m^2/g) is the result of titania particles.

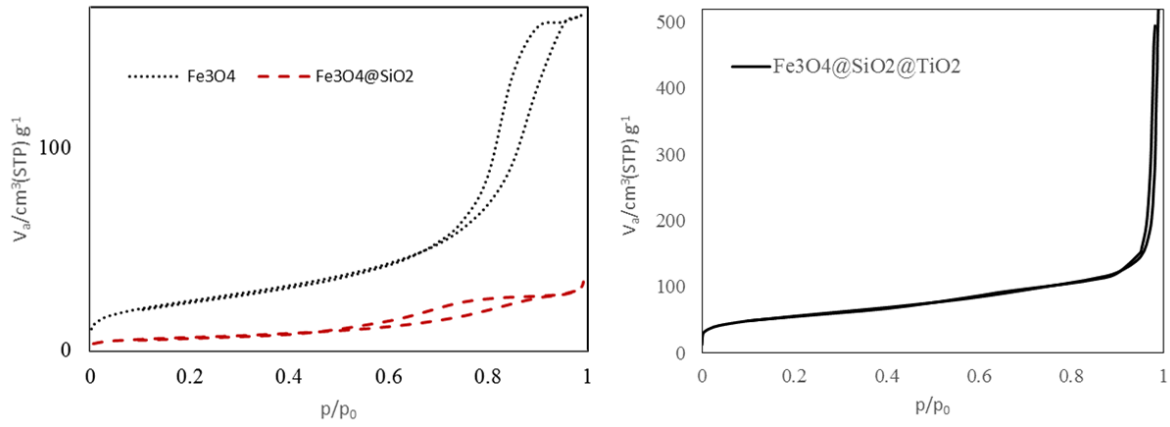


Fig. 3. N_2 adsorption-desorption isotherms of Fe_3O_4, Fe_3O_4/SiO_2 (left), and $Fe_3O_4/SiO_2/TiO_2$ (right)

DNA extraction

pBI121 plasmid is a common binary vector for plant genetic transformation. The size of the plasmid is 14758 bp. pBI121 plasmid exists as a highly supercoiled form to enable it to fit inside the cell. When the plasmid preparation is carried out; plasmid DNA can exist in three conformations, i.e. linear, open-circular and supercoiled forms. The supercoiled form of plasmid DNA sustains less friction when run in the agarose gel matrix. When a pool of plasmid sample containing supercoiled, open-circular and linear form is run on an agarose gel, supercoiled DNA will migrate faster, which is the bottom most band, next is open-circular form which is the middle band and the top most band in the gel is the linear form.

In the present study, the efficiency of DNA isolation and purification of different MNPs (Fe_3O_4, Fe_3O_4/SiO_2, $Fe_3O_4/SiO_2/TiO_2$) was compared by using them in the final steps of a routine plasmid DNA extraction (the alkaline lysis method). The results demonstrated that

Fe_3O_4/SiO_2 can separate plasmid DNA more efficiently than other nano-particles (Table 1, see Fig. 5 in [14]).

The purity and amount of DNA extracted by Fe_3O_4/SiO_2 were higher than the $Fe_3O_4/SiO_2/TiO_2$ and Fe_3O_4 (Table 1). SiO_2 layer significantly enhanced the DNA extracted by Fe_3O_4. With increase in the nano-particle size, by the addition of TiO_2 layer on Fe_3O_4/SiO_2 core, the surface-to-volume ratio decreases and therefore the resultant efficiency of DNA extraction decreased in the $Fe_3O_4/SiO_2/TiO_2$ when compared to the other ones [19].

Table 1. Physical properties of MNPs and the concentration and purity of plasmid DNA extracted by different nano-particles. BET: Brunauer-Emmett-Teller surface area.

MNPs	BET (m^2g^{-1})	Particle size (nm)	DNA (ng/µl)	A260/A280
Fe_3O_4	88	~20	981.4	2.11
Fe_3O_4/SiO_2	22.4	~30	2370.2	1.76
$Fe_3O_4/SiO_2/TiO_2$	192	~60	1137.1	1.89

Saiyed et al (2006, 2008) reported that naked Fe_3O_4 nano-particles can efficiently separate genomic DNA from mammalian cells. Surface modification of MNPs with silica has been utilized as an adsorbent for the isolation of genomic DNA from plant tissues [5]. Amino silane-coated magnetite increased the efficiency of DNA isolation than the naked particles [6]. Chiang et al (2005) utilized polyethylenimine (PEI)- modified magnetic particle for the purification of bacterial plasmid DNA. Gelatin coated magnetic particle has also been used for the purification of genomic DNA from bacterial cells [1]. This functionalized magnetic particle recovered twice as much DNA than the standard phenol-chloroform extraction method. Recently, Min et al (2014) utilized MNPs coated with dimercaptosuccinic acid (DMSA) for the isolation of DNA from human blood with a maximum yield of 86% [8]. In the present study, a new functionalized MNP, $Fe_3O_4/SiO_2/TiO_2$ was compared with SiO_2

coated and naked Fe_3O_4 nano-particles for separation of plasmid DNA. The result showed that though SiO_2/TiO_2 layer enhances naked Fe_3O_4 in plasmid DNA separation from bacterial cells, its efficiency is lower than the SiO_2 coated magnetic nano-particles. This can be attributed to the larger particle size of $Fe_3O_4/SiO_2/TiO_2$ than to Fe_3O_4/SiO_2 and to the fact that the surface-to-volume ratio reduces as particle size increases [19]. Furthermore, the purity of plasmid DNA (A260/A280) extracted by SiO_2/TiO_2 coated nano-particles was higher than the naked Fe_3O_4 particle (Table 1).

Effects of NaCl concentration and pH of elution buffer

Effects of pH and NaCl concentration of elution buffer were evaluated on desorption of plasmid DNA from magnetic nano-particles. The results showed that pH affects desorption of plasmid DNA from all three MNPs. With increase in the pH of elution buffer from 7 to 8, the amount of desorbed plasmid increased significantly (Table 2; Fig.4). On the other hand, the increased pH to 9 has no significant effect on elution of plasmid DNA from nano-particles. Nevertheless, pH 8 enhanced the purity of extracted plasmid. The effects of pH on DNA desorption may be related to the surface charge of MNPs and plasmid DNA [4]. Furthermore, the increase in desorption efficiency with the increase in pH of the elution buffer may be as a result of the increasing number of deprotonized groups and increased electrostatic repulsion [21]. The elution buffer with pH 8.0 was utilized to perform all subsequent experiments.

Table 2. The effect of pH of elution buffer on plasmid DNA desorption from nano-particles

MNPs	pH	DNA (ng/µl)	A260/A280
Fe_3O_4	7	879.6	2.1
Fe_3O_4	8	1088.2	1.91
Fe_3O_4	9	1097	2.01
Fe_3O_4/SiO_2	7	1746.6	1.65
Fe_3O_4/ SiO_2	8	2619.7	1.81
Fe_3O_4/SiO_2	9	2530.4	1.85
$Fe_3O_4/SiO_2/TiO_2$	7	1172.2	2.1
$Fe_3O_4/SiO_2/TiO_2$	8	1387.1	1.93
$Fe_3O_4/SiO_2/TiO_2$	9	1231.6	1.86

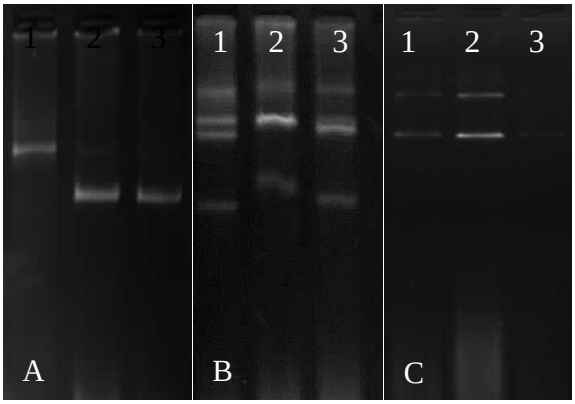


Fig. 4. The effects of pH of elution buffer on desorption of plasmid DNA from Fe_3O_4/SiO_2 (A) $Fe_3O_4/SiO_2/TiO_2$ (B), Fe_3O_4 (C). 1- pH7, 2-pH8, 3- pH9

In addition, different concentrations of NaCl (0, 0.5, 1, 1.5, 2 M) in elution buffer were utilized to determine the effect of ionic strength on desorption of plasmid DNA from MNPs. The results showed that the elevated NaCl concentrations significantly increase DNA desorption from all three nano-particles (Fig. 5, Table 3). This may be due to modulation in DNA- nano-particle interaction afforded by ionic strength and the DNA net charge [5]. Furthermore, the purity of genomic DNA indicated by *A*260/*A*280 ratio was up to about 1.8 when the final concentration of NaCl was 1.5 M (Table 3). So, 1.5 M NaCl was selected as an optimum content in elution buffer to perform all subsequent experiments.

The binding between DNA and coating layers of magnetic nano particles Fe_3O_4 is based on inter-molecular electrostatic interactions, dehydration of the layer surface and DNA and inter-molecular hydrogen bond between DNA and the contact layer [21].

So, DNA adsorption takes place mainly through negative charges of the phosphate backbone, though the bases might also have moderate contributions. Infrared spectroscopy (IR) studies indicate that the negative charge of DNA backbone is responsible for DNA adsorption on TiO_2 NPs [22, 23].

Furthermore, silica and titania are negatively charged at neutral pH [21, 24]. So, due to negatively charged DNA backbone, the electrostatic repulsion by salts has an important role to adsorb DNA to NPs [20, 24]. Zhang et al (2014) observed little adsorption of DNA to TiO_2 NPs in the absence of salt, and the rate of adsorption was significantly improved with more NaCl [25]. Subsequently, the presence of monovalent or divalent ions in the media has important effects on the DNA desorption on the silica and titania surface [254].

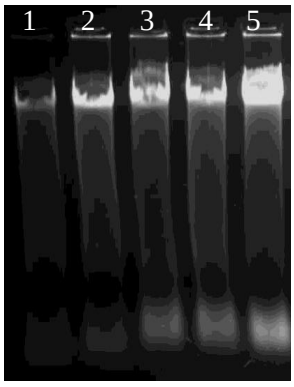


Fig. 5. Effect of NaCl concentration on elution of plasmid DNA from Fe_3O_4/SiO_2 MNPs. 1, 2, 3, 4, 5: NaCl concentration 0, 0.5, 1, 1.5, 2 M, respectively.

Tbele 3. The effect of NaCl concentration on plasmid DNA desorption from MNPs

MNPs	NaCl (M)	DNA (ng/µl)	A260/A280
Fe_3O_4	0	1076	1.67
Fe_3O_4	0.5	1081.4	1.7
Fe_3O_4	1	1097.3	1.71
Fe_3O_4	1.5	1109.7	1.85
Fe_3O_4	2	1088.7	1.97
Fe_3O_4/SiO_2	0	2226.8	2.05
Fe_3O_4/SiO_2	0.5	2740.7	1.97
Fe_3O_4/SiO_2	1	2773.1	1.96
Fe_3O_4/SiO_2	1.5	2899.7	1.84
Fe_3O_4/SiO_2	2	2564.5	1.75
$Fe_3O_4/SiO_2/TiO_2$	0	1141.3	2.09
$Fe_3O_4/SiO_2/TiO_2$	0.5	1040.4	2.1
$Fe_3O_4/SiO_2/TiO_2$	1	1190.4	1.97
$Fe_3O_4/SiO_2/TiO_2$	1.5	1415.8	1.88
$Fe_3O_4/SiO_2/TiO_2$	2	1281.6	1.95

Optimal amount of the MNPs

The effects of nano-particle amounts were studied at optimum pH (8) and NaCl concentration (1.5 M) based on the above results. The results showed that the DNA yield increases when the nano-particle content increased from 0.05 to 0.2 mg (Fig. 6, Table 4). Therefore, we recommended 0.2 mg as an optimal amount of nano-particle for efficient DNA extraction in our studies. Min et al (2014) reported that DNA isolation yields of DMSA-MNPs decreases with increasing amount of the particles after the saturation point [8]. At the higher amount of NPs, such tendency for the DNA isolation yields could be observed due to excess amounts of magnetic supports which made it difficult for the separation of DNA from the DNA-magnetic support complex [26].

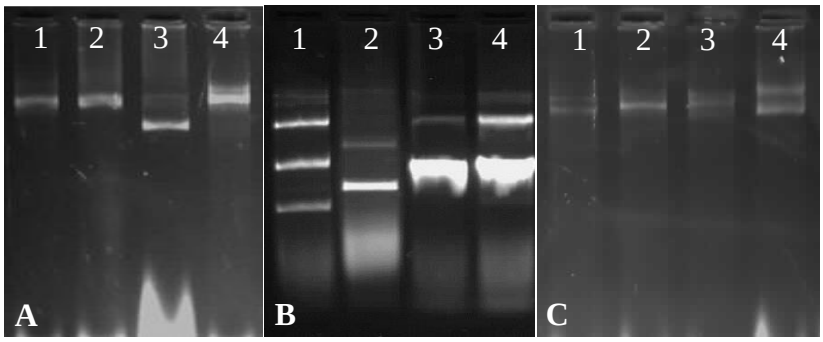


Fig. 6. The effect of the amount of MNPs on the DNA yield. A- $Fe_3O_4/SiO_2/TiO_2$, B- Fe_3O_4/SiO_2, C- Fe_3O_4. 1- 0.05 mg, 2- 0.1 mg, 3- 0.15 mg, 4- 0.2 mg.

Table 4. The effect of MNPs amount on the plasmid DNA yield

MNPs	**MNPs amount (mg)**	**DNA (ng/µl)**	**A260/A280**
Fe_3O_4	0.05	515.4	1.72
Fe_3O_4	0.1	879.4	1.87
Fe O_4	0.15	1209.4	1.91
Fe_3O_4	0.2	1296.2	2.04
Fe_3O_4/SiO_2	0.05	1457	1.82
Fe_3O_4 /SiO_2	0.1	1700.9	1.85
Fe_3O_4/SiO_2	0.15	2160.6	1.91
Fe_3O_4/SiO_2	0.2	2165	1.89
$Fe_3O_4/SiO_2/TiO_2$	0.05	674.4	1.76
$Fe_3O_4/SiO_2/TiO_2$	0.1	1141.3	1.83
$Fe_3O_4/SiO_2/TiO_2$	0.15	1579.6	1.82
$Fe_3O_4/SiO_2/TiO_2$	0.2	1608.1	1.84

Interaction of elution buffer volume, time and temperature on desorption of plasmid DNA

The combined effects of TE buffer volume (50 and 100 µl), elution time (10, 30 min and overnight) and elution temperature (RT, 55 and 4 ºC) were evaluated for optimization of the factors on desorption of DNA from MNPs.

The results showed that higher temperature of 55 ºC leads to more efficient DNA yield by elution buffer (Fig.7). Although, 30 min incubation at RT increases DNA yield, however, 10 min incubation at 55 ºC showed a higher DNA yield than the others.

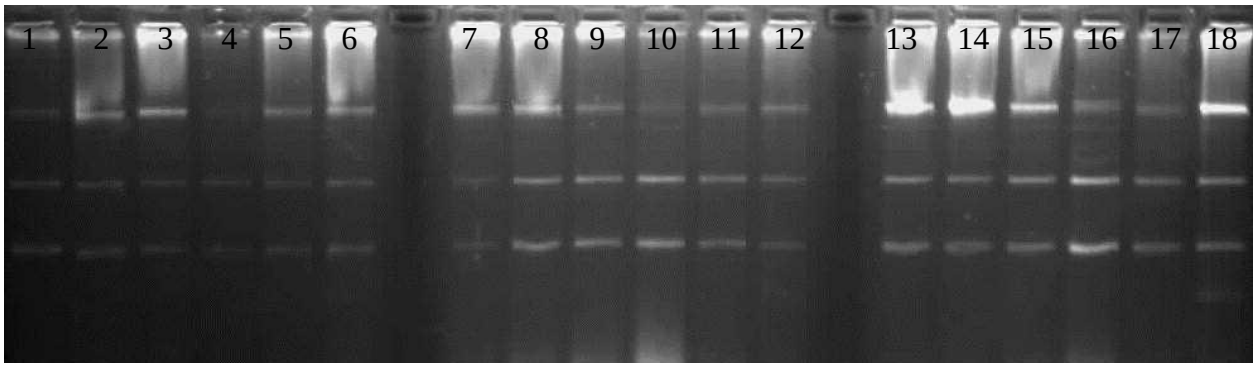


Fig. 7. The effects of elution temperature and time on desorption of DNA from MNPs.
1-7: 10 min at RT; 1, 2- $Fe_3O_4/SiO_2/TiO_2$; 3, 4- Fe_3O_4/SiO_2; 5, 6- Fe_3O_4
8-12: 30 min at RT; 7, 8- $Fe_3O_4/SiO_2/TiO_2$; 9, 10- Fe_3O_4/SiO_2; 11, 12- Fe_3O_4
13-18: 10 min at 55 ºC; 13, 14- $Fe_3O_4/SiO_2/TiO_2$; 15, 16- Fe_3O_4/SiO_2

The combined effects of elution buffer volume (50 and 100 µl), elution temperature (RT, 55 and 4 ºC) and time (10, 30 min and overnight) were studied for all three NPs. The results showed that there are no significant differences between 50 and 100 µl elution buffer in separation of DNA from nano-particles. Higher elution temperature (55 ºC) resulted in more DNA yield. Nevertheless, we observed that overnight incubation at 4 ºC can increase desorption of DNA from nano-particles (Fig. 8). Furthermore, in one experiment, the addition of methanol to elution buffer inhibited the release of DNA from nano-particles (Fig. 8, Lane 3). Smerkova et al (2013) reported the highest amount of eluted DNA from silica MNPs at 15 min at 99 ºC conditions; but at that temperature, the DNA denaturation is occurring, therefore the single stranded DNA is released to the elution solution [21].

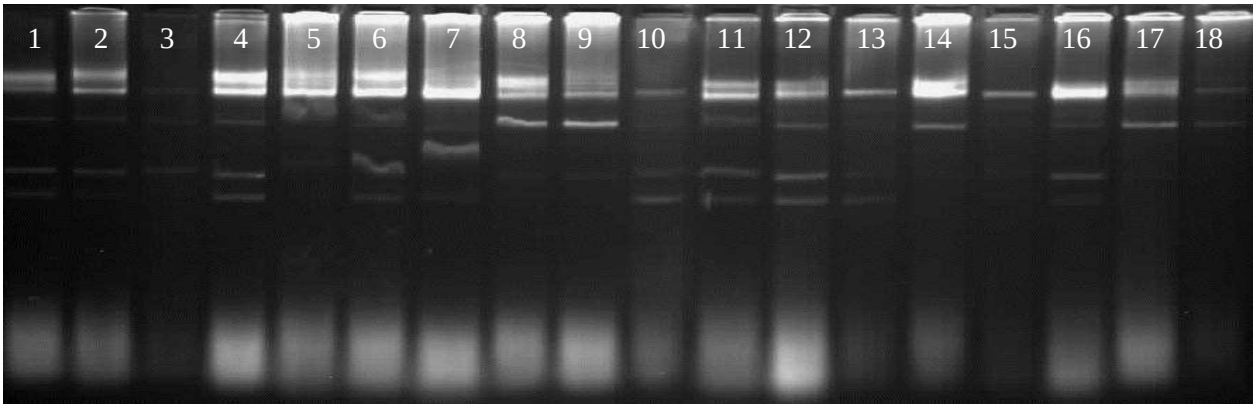


Fig. 8. Combinational effects of elution buffer volume (TE buffer), elution temperature and time on DNA desorption from $Fe_3O_4/SiO_2/TiO_2$.

1- 100 µl TE for 10 min at RT, 2- 50 µl TE for 10 min at RT, 3- 50 µl TE + 50 µl methanol for 10 min at RT, 4- 100 µl TE for 10 min at 55 ºC, 5- 50 µl TE for 10 min at 55 ºC, 6- 100 µl TE for 30 min at 55 ºC, 7- 50 µl TE for 30 min at 55 ºC, 8- 100 µl TE overnight at 4 ºC, 9- 50 µl TE overnight at 4 ºC, 10-18- nano- particles after elution steps of 1-9, respectively.

CHNS analysis

The percentages of carbon, hydrogen, nitrogen and sulfur were determined experimentally using a CHNS analysis. It shows that there are more percentages of C, H and N in all nano-particles before eluting step (Table 5). The results showed that desorption of DNA from nano-particles were not completed in the experimental conditions and a part of DNA isolated from bacteria remained on the magnetic nano-particle after eluting step (Fig. 8; Table 5). Nevertheless, the attached DNA was at minimum content at optimal condition of DNA desorption.

Table 5. CHNS data for magnetic samples. MNP: CHSN of MNP, MNP+ DNA: MNP used for DNA isolation before elution step, MNP-DNA: MNP used for DNA isolation after elution step.

Catalyst	**%N**	**%C**	**%S**	**%H**
Fe_3O_4	0.14	0.30	-	0.33
Fe_3O_4+ DNA	1.474	20.13	0.663	0.507
Fe_3O_4- DNA	0.961	5,897	0.898	1.199
Fe_3O_4-SiO_2	0.37	0.67	-	0.54
Fe_3O_4/SiO_2+DNA	1.746	16.80	0.903	1.227
Fe_3O_4/SiO_2-DNA	0.672	3.717	0.494	0.713
Fe_3O_4/SiO_2/TiO_2	0.52	0.98	-	0.98
$Fe_3O_4SiO_2$-T/iO_2+ DNA	0.883	16.75	1.28	1. 83
Fe_3O_4/SiO_2/TiO_2- DNA	0.368	2.268	0.494	0.857

PCR analysis of extracted DNA

PCR analysis revealed that all plasmid DNA extracted by nano-particles contained *gus* gene in their structures (Fig. 9). The results confirmed the authenticity of DNA extracted by nano-particles and the extracted DNA belong to pBI121 plasmid. Moreover, the purified genomic DNA was suitable for downstream applications.

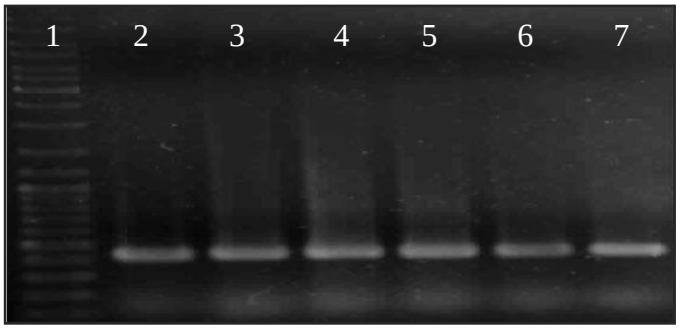


Fig. 9. PCR analysis of DNA extracted by different nanoparticles. 1- 1kb molecular weight ladder, 2, 3- plasmid DNA extracted by Fe_3O_4/SiO_2/TiO_2; 4, 5- plasmid DNA extracted by Fe_3O_4/SiO_2; 6, 7- plasmid DNA extracted by Fe_3O_4.

DNA extraction from mammalian cells

The current MNPs was tested for their potential in genomic DNA extraction from human blood cells. The yield of DNA extracted using magnetic nanoparticles was between 24.1 to

38.7 µg/µl (Fig. 10; Table 6). The yield and purity of extracted DNA were comparable with those reported by Saiyed et al [3]. The present results indicated the potential of the present MNPs for isolation of genomic DNA from mammalian cells. However, improvement of digestion solution for blood cells may increase DNA yield [27].

Table 6. Genomic DNA from fresh blood extracted using various MNPs

MNPs	DNA (ng/µl)	A260/A280
Fe_3O_4	24.1	1.65
Fe_3O_4/SiO_2	38.7	1.76
$Fe_3O_4/SiO_2/TiO_2$	31.1	1.7

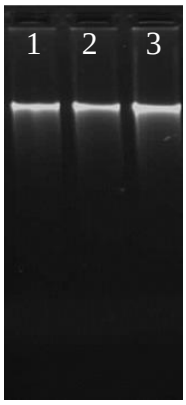


Fig. 10. Agarose gel electrophoresis of genomic DNA isolated from human blood cells using MNPs: 1- Fe3O4, 2- $Fe_3O_4/SiO_2/TiO_2$, Fe_3O_4/SiO_2

Sensitivity of DNA Extraction

Serial dilutions of plasmid DNA were used to address minimal quantity of DNA isolated by different MNPs. Fe_3O_4/SiO_2 with visible bands for 1:20 (0.2 µg/µl) plasmid DNA on agarose gel electrophoresis have the highest efficiency in recovering of DNA, followed by $Fe_3O_4/SiO_2/TiO_2$ (for 1:10 dilution) and Fe_3O_4 (for 1:5 dilution) (Fig. 11, Table 7).

Table 7. The content and purity of DNA recovered by MNPs in different dilution of plasmid DNA

Dilution	Initial DNA		MNPs					
			Fe_3O_4		$Fe_3O_4/SiO_2/TiO_2$		Fe_3O_4/SiO_2	
	DNA (ng/µl)	A260/A280	DNA (ng/µl)	A260/A280	DNA (ng/µl)	A260/A280	DNA (ng/µl)	A260/A280
1	5823	1.98	511	1.88	695	1.77	896	2.02
1:2	3137	1.84	181	2.01	412	1.9	655	2.01
1:5	1286	1.84	57	2.16	179	1.98	261	1.9
1:10	621	1.76	22	1.92	144	1.8	160	1.86
1:20	273	1.82	14	1.99	37	1.87	61	1.89

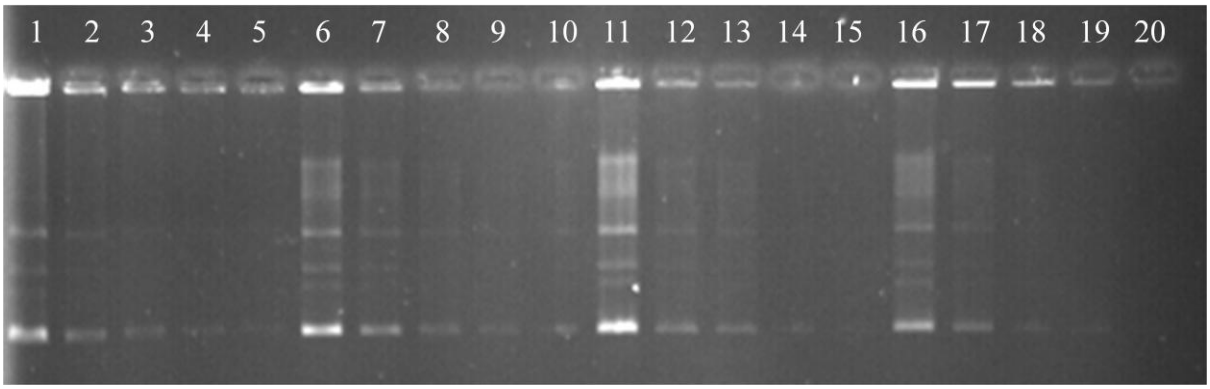


Fig. 10: DNA recovery efficiency of MNPs from different dilution of plasmid DNA. 1-5: Initial serial dilution, 6-10: plasmid DNA recovered by Fe_3O_4/SiO_2; 11-15: plasmid DNA recovered by $Fe_3O_4/SiO_2/TiO_2$; 16-20: plasmid DNA recovered by Fe_3O_4. 1, 6, 11, 16: 6 µg/µl; 2, 7, 12, 17: 3 µg/µl; 3, 8, 13, 18: 1.2 µg/µl; 4, 9, 14, 19: 0.6 µg/µl; 5, 10, 15, 20: 0.3 µg/µl DNA solution.

Conclusions

The results obtained in this study indicate that surface modification of Fe_3O_4 nano-particles using TiO_2 and SiO_2 layers can enhance their functionality and specificity in adsorption of

plasmid DNA. The result showed that ionic strength and pH of elution buffer can affect desorption of DNA from MNPs. In addition, we indicated that desorption of DNA from magnetic nano-particles is highly affected by elution time and temperature. Finally, $Fe_3O_4/SiO_2/TiO_2$ can be recommended as an alternative MNPs for separation of DNA molecules from biological sources.

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