Silica-coated natural iron ore particles for genomic DNA separation

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ABSTRACT

As an alternative way to separation of biological molecules, silica coated magnetite ore particles were prepared through two step synthetic method including ball milling of iron ore powder followed by silica coating with Stöber method. As synthesized silica coated magnetic ore particles were composed mainly of magnetite and other accompanying minerals such as hematite and Al_2O_3, ZrO_2. The particles were irregular in shape, with average size of 0.4 ± 0.3 μm as demonstrated with SEM. Field dependent magnetization showed that silica coated magnetite ore particles are soft ferromagnet with coercivity of 320Oe and remanent magnetization of 2.26 emu/g. Using these particles, genomic DNA was successfully separated from *E. coli* with sufficient yield and purity comparable to those obtained with a commercial magnetic separation kit, demonstrating their potential for bioseparation from diverse biological sources.

Keywords: Magnetic, natural ore, silica coating, genomic DNA separation

INTRODUCTION

Beyond their extensive use in traditional industries, magnetic particles have attracted a significant interest for a wide range of biological applications such as magnetic drug targeting and delivery, enzyme-based biocatalysts as well as isolation and purification of diverse biological entities [1, 2]. Most of these applications require tailor made magnetic materials, which means magnetic property, composition, morphology, functionality of the magnetic particles should be well defined according to their respective applications [3, 4]. Among the numerous explored materials, magnetic iron oxides are the primary candidate for magnetic separations due to their ease of synthesis and strong magnetic properties. Silica and polymer are extensively used for colloidal stability and binding affinity making the magnetic particles available for further conjugation with biomolecules [5, 6]. Magnetic separation of target biological entities was first reported by Robinson *et al* [7]. Since the first commercial magnetic conjugation kit of Enzacyl FEO-(M) and Magnogel in the late 1970s, magnetic separations have been taken over gradually time and labour consuming traditional methods based on several extractions with organic solvents and centrifugation steps, often results with insufficient yield and low purity [4, 8]. In this regard, a numerous studies have reported on the development of multifunctional magnetic particles for extraction and delivery of biological molecules [9]. Since the Covid-19 pandemic, particularly, it is of the utmost importance to develop a fast, reliable and cost-effective method for extracting the nucleic acid from biological materials [10]. Kang et al. extracted the nucleic acid just in 15 min from artificial SARS-CoV-2 positive throat swabs and contaminated food samples using magnetic nanoparticles in an ordinary plastic Pasteur pipette [11]. While silica coated magnetic particles are the most used platform, the quality and yield of the nucleic acid extracted from human saliva and E-coli with uncoated magnetic particles were also compatible for further downstream applications [12]. However, magnetic particles have typically been produced through bottom-up chemical synthesis using pure chemicals, which are themselves synthesized via labor- and energy-intensive processes [13, 14]. In the present work, we describe a facile method for synthesizing silica coated magnetic iron ore particles and demonstrate their performance for separating genomic DNA from *E. coli*. Unlike most of the previous studies [13, 14], natural iron ore of Bargilt-Ovoo deposit, Mongolia was used as a magnetic carrier and then coated with silica by well-known Stöber method [15] to facilitate further conjugation reaction with DNA [16]. Using these particles as solid supports, the quality and quantity of genomic DNA extracted from *E. coli* were compared with those obtained by phenol-chloroform method and a commercially available kit.

EXPERIMENTAL

Materials and methods

The iron ore sample used in this research was obtained from open-pit mining of Bargilt-Ovoo, Khentii province, Mongolia. All reagents were of analytical grade. Tetraethyl orthosilicate (TEOS) was purchased from Sigma-Aldrich. Ethanol and ammonia aqueous (25%) were purchased from Daejung Chemicals, Republic of Korea. Proteinase K and guanidinium thiocyanate were purchased from Carl Roth, Germany. Lysis buffer, wash buffer-1 and wash buffer-2 were used from Abbot mSample preparation system kit (Abbott Laboratories, USA).

Preparation of magnetic ore particles

The iron ore was grinded down to a particle size of about 50 μm and sifted through a 44 μm (pore size) sieve. Then, the particles were milled with 3 mm zirconia balls in HPM-700 planetary ball mill (Haji Engineering and Global, South Korea) for 3 hours at 500 rpm under air atmosphere. The weight ratio of the iron ore powder and the ball was 1:10. The particles were dispersed in deionized water and magnetically collected, and this process repeated for six times to remove the nonmagnetic fraction of the iron ore. The powder was dried at 74 °C in vacuum. The magnetic fraction was determined gravimetrically to be approximately 80%.

Silica coating of the magnetic ore particles

The iron ore particles were then coated with silica using Stöber method with slight modifications in basic alcohol/water mixture using ammonia as a catalyst at room temperature [15]. Briefly, 2 g iron ore particles in a solution containing ethanol (160 mL), water (40 mL) and ammonia (5 mL) were homogenized with ultrasonic bath for 10 minutes. Then, the colloidal dispersion was placed in ultrasonic bath (270 W and 40 KHz) for 2 hours after adding 1 mL of TEOS [17]. The synthesized particles were washed 3 times with deionized water and dried at 74 °C in vacuum.

Separation of genomic DNA using silica coated iron ore particles

For nucleic acid extraction, reagents, and buffers from commercially available Abbott mSample Preparation System-DNA kit (REF.06K12-24, GTIN.00849811004452, LOT.11702311) were used. The kit is based on the selective binding availability of DNA to silica coated magnetite particles in a condition with high concentration of guanidinium thiocyanate. 200 μL overnight culture of *E. coli* (BL21(DE3) strain (New England Biolabs, USA) was centrifuged, and cell pellet was suspended in 500 μL mLysis buffer

(LOT.1147076) containing proteinase K and guanidinium thiocyanate and incubated at 60 °C for 20 min. Subsequently, 50 µL of the silica coated particle suspension (50 mg/mL in water) was added to the lysate and incubated at room temperature for 5 min with shaking at 750 rpm. The particles were immobilized using external magnetic field and the supernatant was discarded and the particles were washed one time with 500 µL of wash mWash-1-DNA buffer (LOT.11296411) containing guanidinium thiocyanate and Tween 20 in TRIS buffer. Then the particles were washed two times with 500 µL of mWash-2-DNA buffer (LOT.11668321) in ethanol. The DNA was eluted with 50 µL of mElution-DNA buffer (LOT.11649721) containing nuclease free water at room temperature for 5 min. The particles were then immobilized and the DNA containing supernatant was transferred to a sterile tube. For a control, same amount of bacterial sample was subjected to genomic DNA extraction using magnetic particles (LOT.11440361) of Abbot mSample Preparation System-DNA kit by the exact same protocol that is mentioned above. For the second control, traditional phenol-chloroform method was applied for isolation of genomic DNA from same amount of bacterial sample. The phenol-chloroform method is described here [18]. The resulting DNA was visualized on 0.8% agarose gel for integrity and yield. The concentration and purity of the isolated nucleic acid were determined by measurement of absorbance at 260 nm and 280 nm using Biorad SmartSpec 3000 spectrophotometer.

Characterization

The morphology and size distribution of the particles were examined by Scanning Electron Microscopy (SEM) (Merlin, Zeiss, Germany) and Energy Dispersive X-ray spectroscopy (EDX) (X-max extreme, Oxford Instruments, UK). Polished cross-sections of the particles were prepared by argon ion milling (Cross Section Polisher, Jeol, Japan). The X-ray diffraction (XRD) pattern was obtained using Empyrean X-ray diffractometer (PANalytical, Netherlands) with Cu-Kα radiation (1.540562Å). Energy dispersive XRF was measured with PANalytical Epsilon 3XLE spectrometer. Fourier transform infrared spectra (FTIR) were measured on Bruker Alpha-T FTIR spectrophotometer (Bruker, USA) using a potassium bromide pellet. Magnetization was measured with VSM (Lake Shore Cryotronics, USA) at room temperature.

RESULTS AND DISCUSSION

Magnetic particles derived from iron ore offer a relatively unexplored route to solid carriers suitable for nucleic acid separation. Among several iron ore deposits in Mongolia, Bargilt is the finest iron ore with a content of magnetite up to 90% [19]. Starting from the iron ore particles, silica coated magnetic particles were successfully prepared through a two step

process. At first, a straightforward top-down rout was applied, involving planetary ball milling of the iron ore particles. Next, the iron ore particles coated with silica shell using a Stöber
129 method to provide silanol groups on their surface for further conjugation and avoid agglomeration between particles [4, 5]. Well dispersed magnetic iron ore particles were obtained after silica coating, providing biocompatibility and versatile surface chemistry for
132 further DNA separation, as shown schematically in Fig. 1.

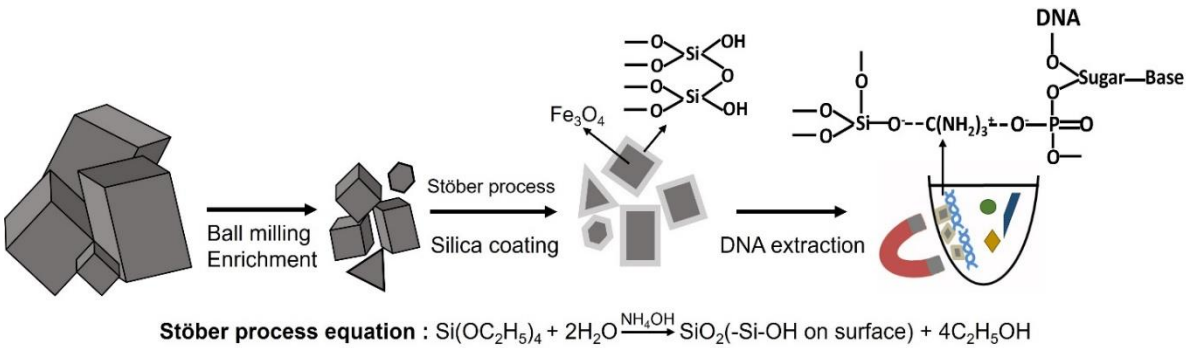
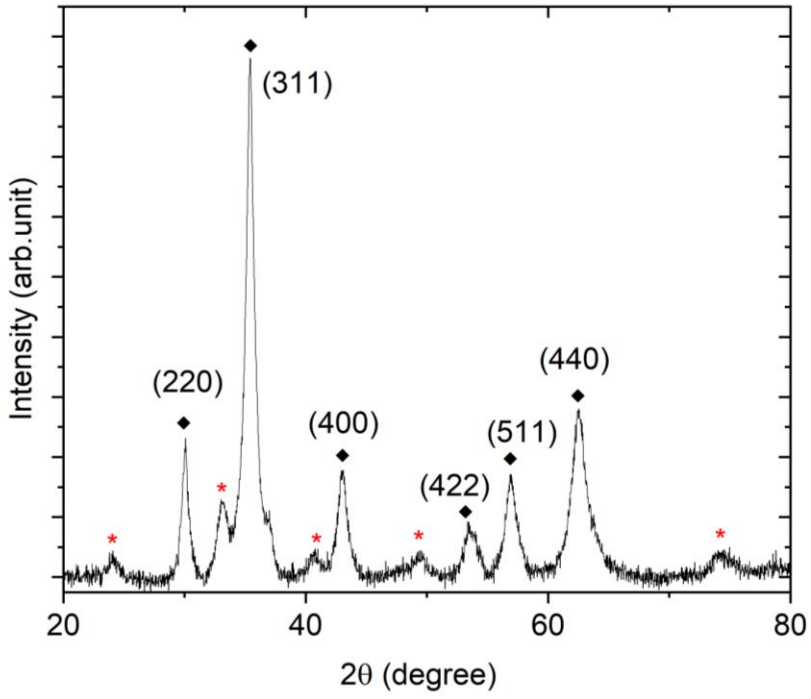


Fig. 1. Synthetic scheme for preparation silica coated iron ore particles and their application in
135 genomic DNA separation

The mineral composition of as-synthesized silica coated magnetic iron ore particles was studied with XRD. As shown in Fig. 2, the major phase was identified as magnetite by
138 indexing the characteristic peaks at 2θ values of 30.18 (220), 35.36 (311), 43.08 (400) and 56.57 (511) to the cubic spinel structure of magnetite (Fe_3O_4) (ICSD 1-089-0688).



141 Fig. 2. XRD spectrum of the silica coated magnetic ore particles: magnetite (♦) and hematite(*).

Additionally, the small peaks at 2θ values of 24.16, 33.28, 40.07 and 49.53 were assigned to hematite (α-Fe_2O_3) (ICSD 00-033-0664). Moreover, XRD pattern also highlights the broadened peaks with low background noise suggesting that presence of amorphous SiO2 and small crystallite size of the minerals. This result is consistent with XRF analysis, which shows the iron oxide dominates with 91.8%, while the minor phases are SiO_2, MgO, MnO, Al_2O_3, ZrO_2 and trace amounts of copper and zinc oxides. Since we were using natural ore, this kind of rich phase composition could be expected, but based on the above results the magnetic ore particles we used in this study are mainly considered to be magnetite.

SEM images of a polished cross section of the silica coated magnetic ore particles embedded in epoxy resin are shown in Fig. 3(a).

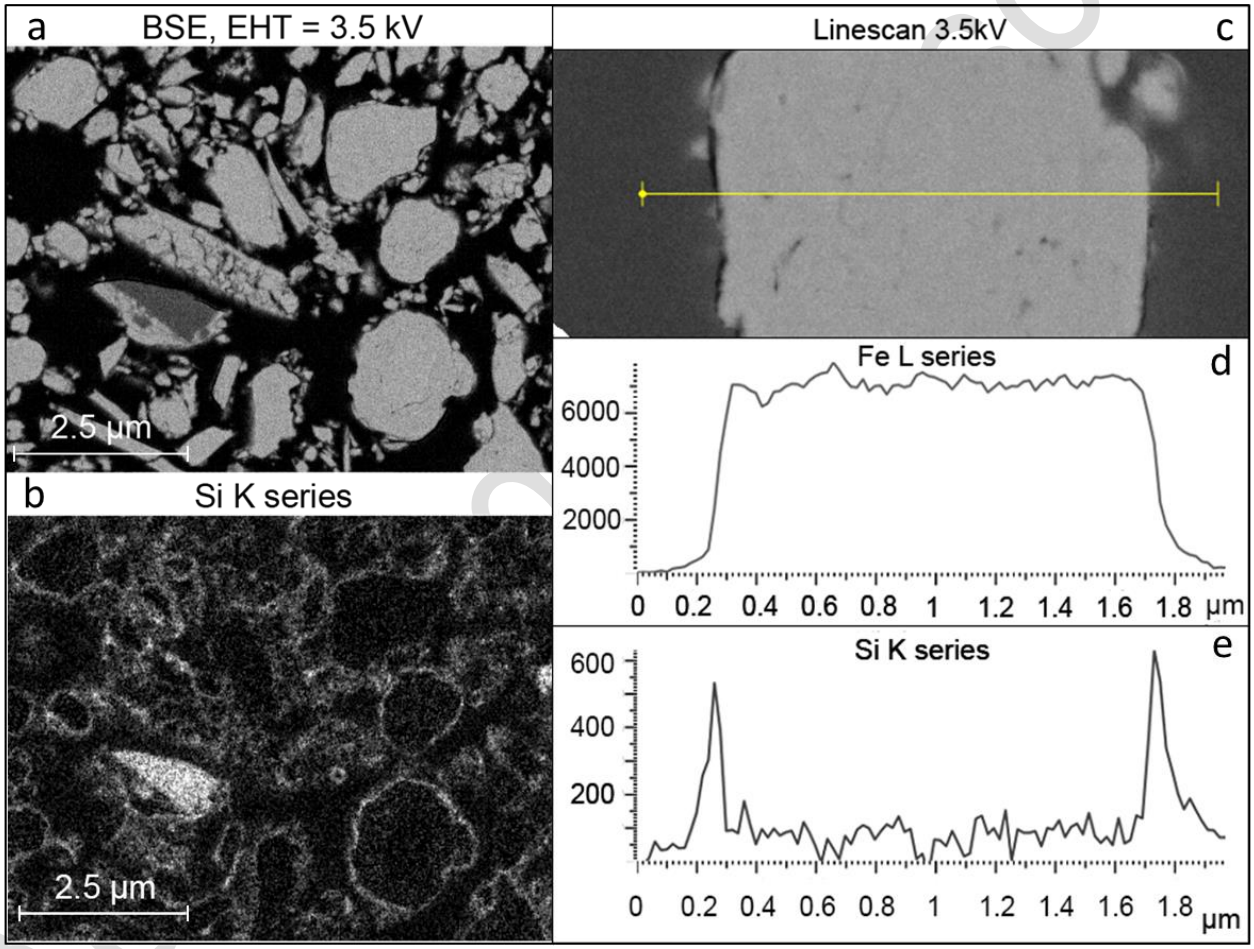


Fig. 3. (a) The cross-sectional SEM image, (b) EDX map of Si observed in the region shown in (a), (c) EDX linescan image, (d) Fe EDX signal and (e) Si EDX signal of the silica coated iron ore particles.

The thin silica layer is not visible in the SEM micrographs; however, its presence was confirmed by EDX analysis. In Fig. 3(b), an EDX elemental map of the cross section showed that all particles are covered with a thin layer of silica. The thickness of the coating cannot be accurately determined from the EDX maps due to the limited spatial resolution of this

technique. It is estimated to be in the order of some tens of nm based on EDX linescans in Fig. 3(c), (d) and (e), which show that a Si EDX signal is recorded from a region thinner than 100 nm around each particle. EDX maps of Fe and minority elements observed in the analysed region is shown in supplementary file Fig. S1.

Field dependent magnetization of silica-coated magnetic ore particles measured using VSM at room temperature is shown in Fig. 4. Magnetization curve shows typical ferrimagnetic behaviour with saturation magnetization and coercivity of 10.41 emu/g and 320 Oe, respectively. It is also seen from magnetization curve that sample has remnant magnetization of 2.26 emu/g indicating that the silica coated magnetic ore particles are soft ferrimagnetic. However, it should be noted that the magnetization of the silica coated magnetic ore particles is lower than that of magnetite particles as previously reported [9, 19]. It could be explained by the presence of non-magnetic SiO_2 on the particle surface.

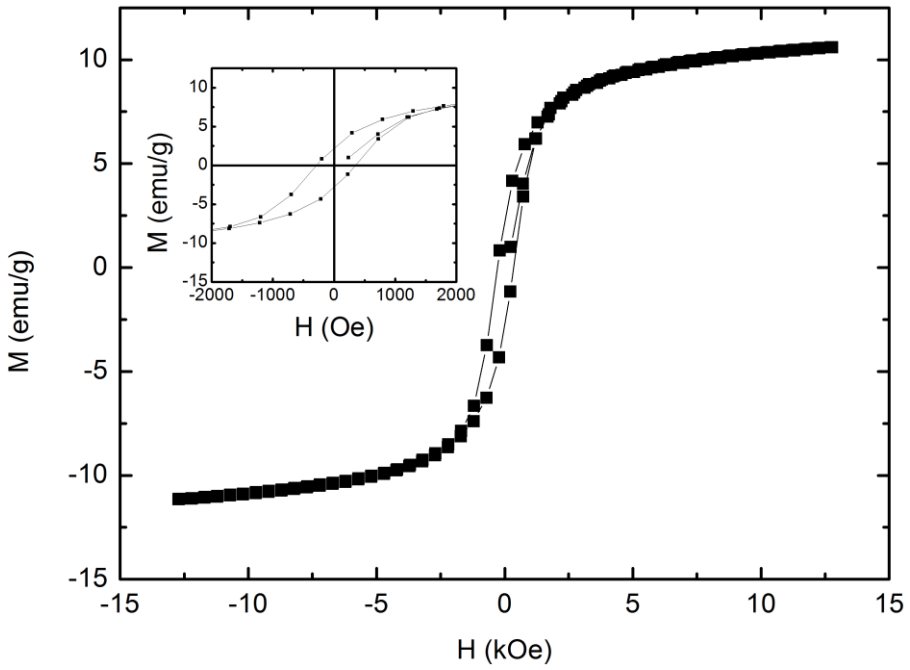


Fig. 4. Magnetization hysteresis curve of silica-coated particles from natural iron ore.

Furthermore, binding of the DNA to the silica coated magnetic nanoparticles was confirmed with FTIR measurement. As shown in Fig. 5, the silica coated magnetic particles exhibit the characteristic Si-O stretching mode at 1084 cm^{-1}, in addition to Fe-O stretching mode at around 560-580 cm^{-1} [9, 20], which can be seen in all the spectra. After conjugation with DNA, several modes corresponding to purine and pyrimidine ring vibrations, DNA sugar-phosphate vibration, asymmetric and symmetric PO2- vibration were observed at 1653 cm^{-1}, 802 cm^{-1}, 1261 cm^{-1} and 1095 cm^{-1}, respectively [21, 22], revealing that DNA was chemosorbed onto surface of the silica coated magnetite ore particles.

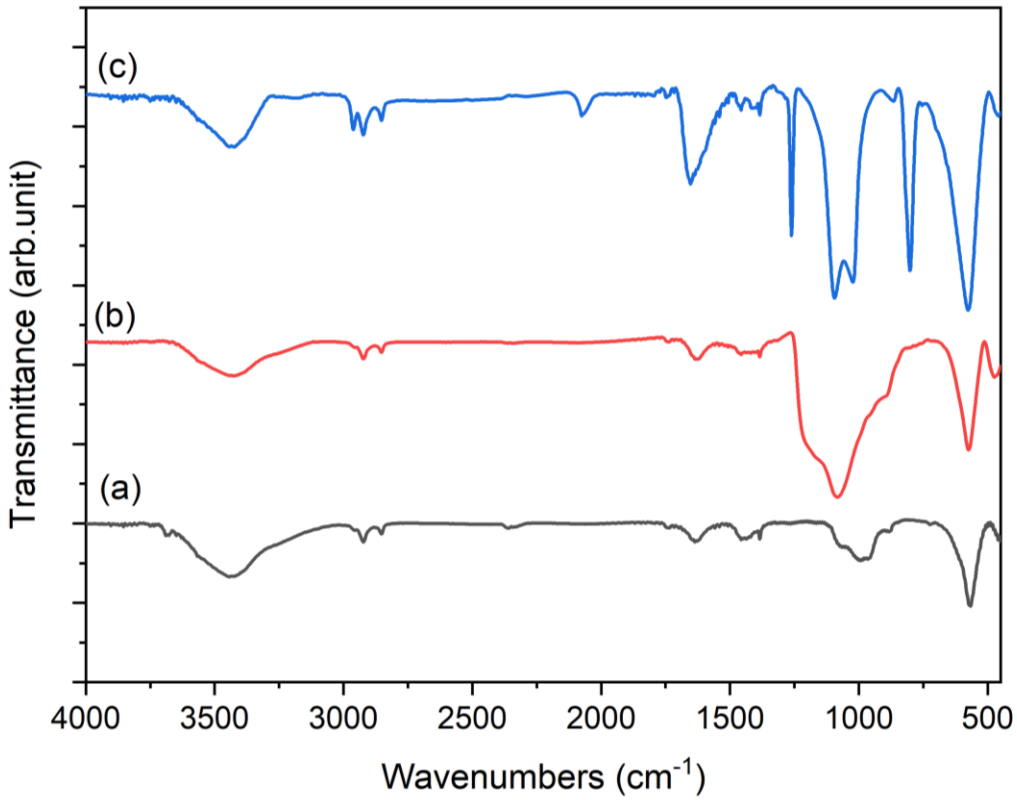


Fig. 5. FTIR spectra of iron ore particles (a), silica coated magnetic ore particles (b) and silica coated and DNA bound magnetic ore particles (c).

As quality and yield of the extracted DNA is an important prerequisite for subsequent downstream applications, we have compared the quality and quantity of genomic DNA extracted from *E. coli* by three different methods such as phenol-chloroform method, Abbot mSample Preparation System-DNA kit and the silica coated magnetite ore particles. The purity and yield of genomic DNA studied with UV-vis spectrophotometry by comparing ratio of absorbance at 260 nm and 280 nm are summarized in Table 1.

Table 1. Yield and purity of DNA isolated from *E. coli* by different methods

Magnetic particles	Concentration (ng/μL)	DNA yield (μg)	Ratio of A260/A280
Phenol chloroform method	68.5±1.35	3.42±0.67	1.76
Abbott mSample preparation system	78.47±1.07	3.92±0.53	1.87
Silica coated magnetic ore particles	88.7±0.95	4.43±0.47	1.94

The extracted nucleic acid is considered pure when the ratio of absorbance at the 260 nm and 280 nm lies between 1.8-2.0 [23]. A higher ratio of 1.94 was obtained for the silica coated magnetite particles, which was comparable to that of DNA extracted using a commercial kit. Moreover, both the yield and concentration of the DNA were significantly higher in the case of the silica coated magnetite particles compared to the phenol-chloroform

method and the commercial kit. The quality and integrity of the isolated DNA from *E. coli* was further visualized with 1% agarose gel electrophoresis.

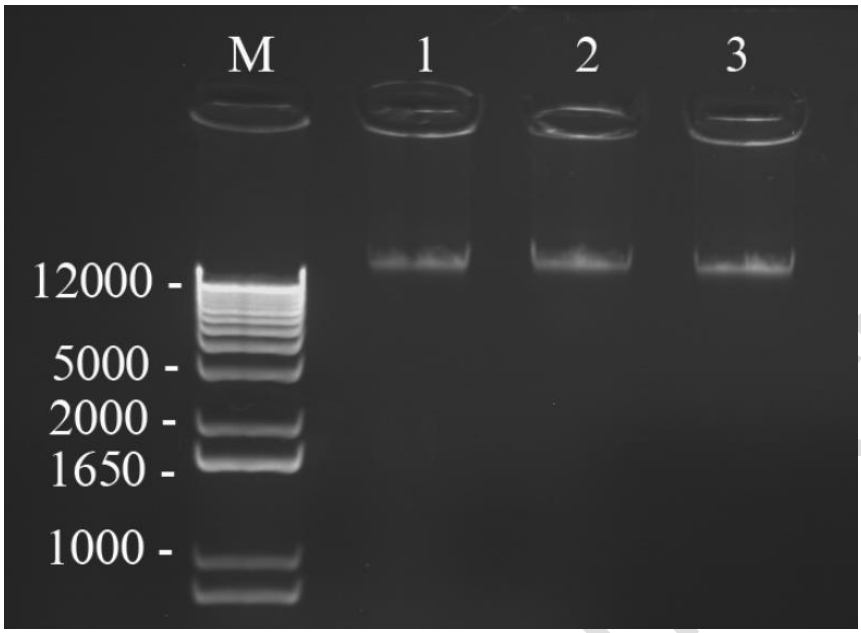


Fig. 6. Agarose gel electrophoresis of genomic DNA isolated from *E. coli* by various methods: Marker (M), phenol chloroform method (1), silica coated magnetic ore particle (2) and commercial kit (3).

As shown in Fig. 6, high molecular weight bands with no smear were observed for all the measured samples, indicating the presence of intact DNA and free from interfering compounds. To demonstrate the broad applicability of the particles, we have also extracted genomic DNA from a PCR-amplified human interferon alpha 2 gene product. Successful DNA isolation was confirmed by 1% agarose gel electrophoresis (see supplementary file Fig. S2). These findings indicate that silica coated magnetite particles derived from natural iron ore can selectively bind to DNA molecules in presence of guanidinium thiocyanate. The silica coated magnetic particles developed in this study were microsized particles with irregular shape, nevertheless, they exhibited comparable DNA extraction performance in terms of quality and purity to a well-known commercial kit and phenol chloroform method.

CONCLUSION

In conclusion, silica coated magnetic particles were synthesized through ball milling of high purity ore available in Mongolia and followed by Stöber method for silica shell. Particle size of the silica coated magnetic ore particles lies in the range of 0.4 μm and exhibit soft ferrimagnetic properties with magnetization of 10.41 emu/g and coercivity of 320 Oe. The silica coated magnetic ore particles developed here achieved genomic DNA extracts with quality and purity comparable to those obtained using a commercial kit or the phenol-

chlroform method. These findings suggest an alternative strategy towards solid magnetic support for bioseparation using inexpensive and naturally occurring, yet highly magnetic materials.

AUTHOR CONTRIBUTIONS

SO, OO and NT designed the research; SO, TT, OB, ED, SS and NT performed the experiments; SO, GC, OO and NT analyzed the data; SO and NT wrote the manuscript draft; OO author revised the manuscript. All authors approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Data Availability

The data and supportive information are available within the article

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