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Isolation of ultrapure plasmid DNA from bacterial cells with silica-magnetite nanoparticles

N. N. Volkova[a], O. N. Derjabin[a], V. V. Yanishpolskii[b] and N. O. Dudchenko[a]

[a]*Institute for Applied Problems of Physics and Biophysics, Ukrainian National Academy of Sciences, Sluzhbova 3, Kyiv 03142, Ukraine, e-mail: zoranaty@voliacable.com*

[b]*O.O. Chujko Institute of Surface Chemistry, Ukrainian National Academy of Sciences, General Naumov 17, Kyiv 03164, Ukraine*

The aim of this study was to reveal the ability of synthesized silica-modified magnetic particles to isolate DNA from the biological objects in comparison with common method used non-magnetic particles. Nanosized superparamagnetic magnetite (Fe_3O_4) particles were prepared via co-precipitation of Fe^{+2} and Fe^{+3} with NH_4OH in aqueous solution. Silica-magnetite nanocomposites were prepared via tetraethoxysilane hydrolyzation in alcohol-water-ammonia mixture. Application of these compounds for DNA isolation from different bacterial cell lysates showed significant time-savings, overall higher yields, lower RNA contamination and better PCR amplification compared to commercial available silica non-magnetic particles.

1. INTRODUCTION

Modified magnetic particles now are widely used in different biological and medical applications (enzyme and protein immobilization, cells separation and purification, MRI, targeted drug delivery, etc.) [1,2]. Due to the strong magnetic properties and low toxicity of magnetic particles, their applications in biotechnology and medicine have gained significant attention. Basically, all types of magnetic particles consist of magnetic core with inorganic or organic shell. The target molecules or cells are captured on magnetic particles coated with a target-specific surface, and separated from unbound components by the application of magnetic field. The need for quick bacterial plasmid DNA and

virus DNA/RNA preparation methods has increased the flood molecular protocols requiring highly purified genetic templates [3,4,5].

It is known that traditional methods of DNA/RNA separation are complicated, time-consuming, hazardous, labor-intensive and require the use of adsorbents, toxic substances, etc. Magnetic separation of DNA offer benefits over usual method due to rapid processing time, reduced chemical needs, the ease of separation [6]. Thus, the aim of the present study was to reveal the ability of synthesized silica-modified magnetic particles to isolate plasmid DNA from the bacterial cells in comparison with common method based on a non-magnetic sorbents.

2. EXPERIMENTAL

2.1. Materials

Ferric chloride hexahydrate, ferrous sulfate tetrahydrate, tetraethoxysilane were purchased from Sigma Chemical Co. Agarose L (low electroendosmoid) was from Amersham Biosciences (Uppsala, Sweden). Reagents for use in DNA isolation and analysis were of molecular biology grade. Ribonuclease A was obtained from "Sigma". All other chemicals and solvents used were of analytical grade. The water used throughout this work was the reagent–grade water produced by Milli-Q Ultra-Pure-Water Purification System.

2.2. Preparation of silica-magnetite nanocomposites

The magnetite particles were prepared via co-precipitation of Fe^{+2} and Fe^{+3} with NH_4OH in aqueous solution under normal conditions. Stock solutions of 1 M $FeCl_3*6H_2O$ and 2 M $FeSO_4*4H_2O$ were prepared as a source of iron by dissolving the respective chemicals in deionized water under stirring. Stock solution of 1 M NH_4OH was prepared by dilution of concentrated NH_4OH solution. The reagents solutions were mixed quickly in reaction vessel, and 50 ml of ammonium solution was added drop-by-drop to reaction mixture under slow mechanical stirring. After the reaction completing, magnetic particles were lightly dispersed using ultrasound dispergator, three times rinsed with deionized water to remove the residual surfactant and unreacted reagents.

Obtained magnetite was coated with silica via tetraethoxysilane hydrolyzation in alcohol-water-ammonia mixture. Thereto, obtained magnetic particles were dispersed in 25 ml of water using ultrasound dispergator. 100 ml of ethanol, 2 ml of concentrated NH_4OH were added to the reaction mixture at slow mechanical stirring. After that, 3 ml of tetraethoxysilane (TEOS) were added drop-by-drop to the reaction mixture. The hydrolysis of TEOS was carried out for 20 hours under normal conditions.

The resultant product was thoroughly rinsed with deionized water three times to remove the residual surfactant and unreacted reagents, and collected by magnetic separation using a permanent magnet. The silica-magnetite nanocomposite (MAGNAT) was stored in deionized water at a concentration of 10 mg/ml.

2.3. Characterization of magnetic nanoparticles

The size and morphology of magnetic nanoparticles were observed by transmission electron microscopy (TEM) using PEM-U (Sumy, Ukraine). Magnetic measurements were performed using magnetometer with Coulomb sensor (Tver University, Russia). X-ray diffraction measurements performed using diffractometer DRON-UM1 in filtered emission Co Kα with recording geometry by Bregg-Brentano.

2.4. Magnetic response characteristics

Magnetic response of synthesized magnetite nanocomposites was measured by monitoring an optical density of the magnetite adsorbent suspended in water at 600 nm. A spectrophotometer cuvette holder with attached of neodymium (S 36 grade) magnet was used.

2.5. Binding capacity of magnetite nanocomposites

Binding capacity of engineered nanoparticles was tested against Marker DNA standards with different molecular mass. Binding and recovery of Marker DNA fragments (Lambda DNA/*Hind* III with 125–23130 bp and phiX174/*Hae* III with 72–1353 bp) were titrated into 2000 mkg concentrations of nanoparticles and nonmagnetic commercial absorbent. Binding was performed in binding buffer for DNA purification, elution of adsorbed nucleic was carried out in deionized water. Eluted DNA was quantified by absorbance at 260 nm.

2.6. Purification of plasmid DNA by silica-magnetite nanocomposites

E.coli cells expressing the plasmid pGL3-Conrol-Vector were grown to log phase in culture media containing 100 mkg/ml ampicillin. Bacterial cells were harvested from 3 ml of cell culture and treated with 0.05 M Tris-HCL mkg/ml ribonuclease A. Cell lysis was performed with 0.2 M NaOH containing 1% of dodecil sulfate. Genomic DNA and other contaminants were precipitated by addition of 6M guanidine- hydrochloride, pH 5.5. After centrifugation the cell lysates were used for plasmid DNA purification with synthesized nanocomposites. Binding and elution of plasmid DNA were performed with common procedure and chemicals. The concentration of purified nucleic acids was calculated using absorbance at 260 nm.

2.7. Determination of the optimal amounts of silica-magnetite absorbent

In this set of experiments the silica-magnetite nanocomposites were added at ranges of 500–1000 mkg to bacterial lysates obtained from 3 ml of cell cultures. The yields of purified plasmid DNA were estimated by UV spectrophotometry at 260 nm.

2.8. PCR amplification

The PCR procedure had been carried out with primers targeting the insertion elements IS900 of Map. The mass of amplicons is 800 bp. The purified DNA (0 mkl) was mixed with cocktail including PCR buffer (10 mM Tris-HCL, pH 8.3) with 50 mM KCL, 1.5 mM MgCL, 5.0 pm/ml of each primers, 200 mkM ofeach dNTP, 2.5 U Tag DNA polymerase. Cycling conditions were 20 cycles of 95° C for 1 min, 60° C for 1 min, 72° C for 1 min. Molecular mass of amplified DNA fragments were detected by electrophoresis in 1% agarose with 0.5 mkg/ml ethidium bromide and 1 kb molecular mass standards (Sigma). The running buffer was TAE (49 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH 8.0). Electrophoresis was carried out at 90 V for 1 hour. Visualisation of PCR products was performed by UV illumination.

3. RESULTS AND DISCUSSION

3.1. Characterization of magnetic nanoparticles

The size and morphology of magnetic particles were characterized by TEM (Figure 1). It shows that the size of magnetic nanoparticles is about 15 nm.

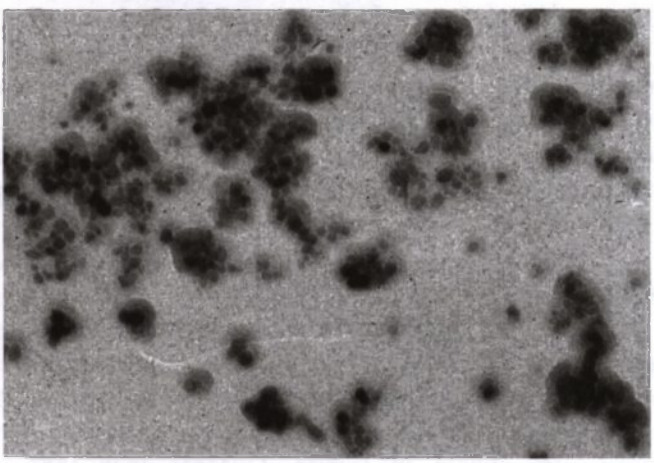


Fig. 1. Transmission electron micrographs of silica-magnetite nanocomposite (MAGNAT) *54000.

X-ray diffraction (XRD) measurements show that the magnetic core of the synthesized particles consists of magnetite (Fe_3O_4). Six characteristic peaks for Fe_3O_4 in XRD pattern (Figure 2) were observed for magnetic nanoparticles. These peaks reveal that the resultant particles were pure Fe_3O_4.

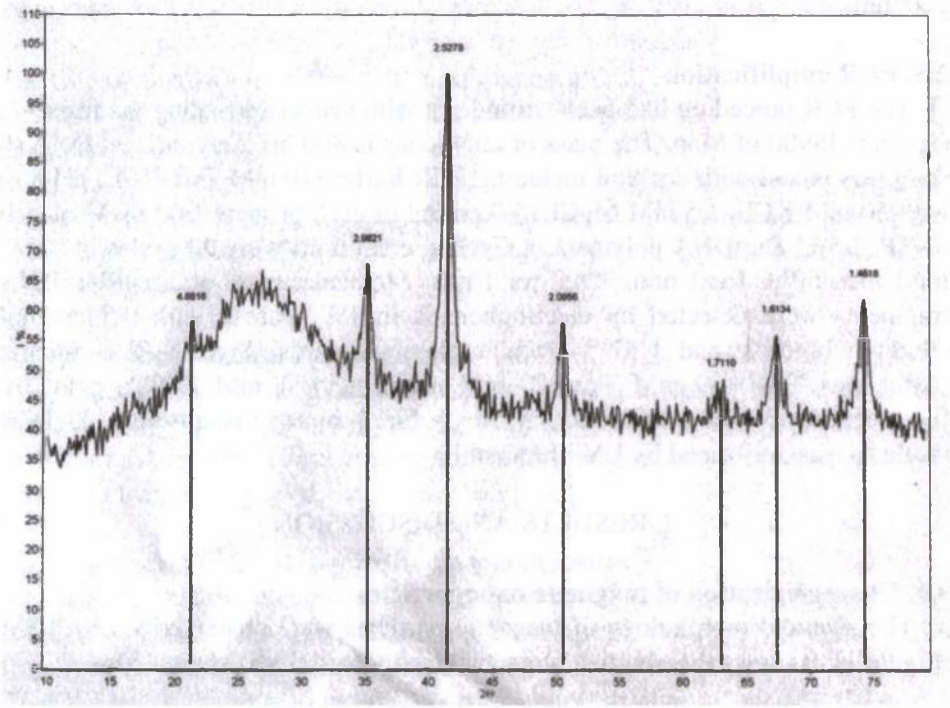


Fig. 2. Typical X-ray diffraction pattern of silica-magnetite nanocomposite (MAGNAT).

The superparamagnetic properties of the magnetic particles were verified by magnetization curve measurements. Saturation magnetization of silica-modified magnetite particles was 37 emu/g ($A \cdot m^2/kg$). This saturation magnetization of magnetic particles makes them susceptible to magnetic field and therefore makes the solid and liquid phases separate easily.

3.2. Magnetic response characteristics

Magnetic response of silica-magnetite nanoparticles has been analysed. Magnetic response was measured by placing nanoparticles in buffer solution in spectrophotometer cuvette attaching to magnet on it outside wall. Optical density of particles suspensions was measured at 600 nm over time. Concentration-dependent curves are shown in Figure 3. More than 90% of magnetic nanoparticles at concentration above 0.1 mg/ml were removed from buffer solution in less than 10 seconds after magnetic field applying. Sedimentation of

90% of nanoparticles at concentration 0.01 mg/ml were observed 25 seconds after applying of magnetic field.

The mechanism of this process could be envisaged in the following way. At the first stage of sedimentation, a few particles magnetize and self-attract to form a critical particle mass that moves toward magnet. At the required particle concentration for most molecular-biological applications, efficient removal of particles is accomplished in under 30 seconds (Promega DNA kits). In case of our nanoparticles, about 15 seconds is enough for optical clearing of solution. The standard variant of magnet was used for these investigations (Promega's MagneSil™magnetic stand which incorporate S36 grade neodymium rare earth magnet).

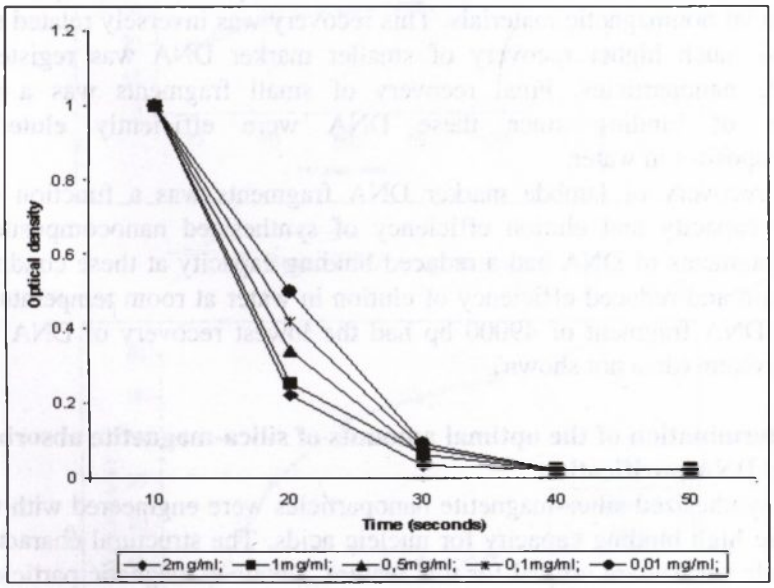


Fig. 3. Magnetic response of silica-magnetite nanoparticles.

3.3. Binding capacity of magnetite nanocomposites

Ionic strength and pH are crucial factors estimating processes binding and elution of nucleic acids by silica magnetite nanobeads. Adsorption capacity of nanoparticles could be modulated in wide ranges by the ionic strength of binding buffer system which was used for DNA purification. DNA is a polyanionic molecule due to the presence of phosphate groups and it interacts with positively charged functional groups on silica–magnetite particles' surface. In order to determine the ionic strength effect on synthesized nanocomposites, we tested several NaCL concentration ranges from 0 to 4 M in the binding buffer. It was

found that the presence of 2M NaCL and above concentrations resulted in maximal binding of plasmid DNA and marker small DNA fragments. In this study the influence of binding buffer pH on DNA adsorption by nanocomposites was also estimated. As expected, pH of binding solution had no effect on plasmid DNA adsorption by silica-magnetite nanoparticles. These results are in agreement with the data obtained by Chen-Li Chiang [6] for silica-magnetite nanoparticles with greater diameter size (about 31 nm).

Figure 4 presents the binding and recovery of marker DNA fragments with different molecular mass (small and broad DNA fragments commonly used for estimation of binding characteristics of silica adsorbents for nucleic acids).

As one could see from the illustration, nanoparticles MAGNAT resulted in the increased recovery of small DNA marker fragments in comparison with commercial nonmagnetic materials. This recovery was inversely related to DNA size and much higher recovery of smaller marker DNA was registered for magnetic nanoparticles. Final recovery of small fragments was a primary function of binding since these DNA were efficiently eluted from nanocomposites in water.

The recovery of lambda marker DNA fragments was a function of both binding capacity and elution efficiency of synthesized nanocomposites. The larger fragments of DNA had a reduced binding capacity at these conditions of adsorption and reduced efficiency of elution in water at room temperature. Full lambda DNA fragment of 49000 bp had the lowest recovery of DNA in such testing system (data not shown).

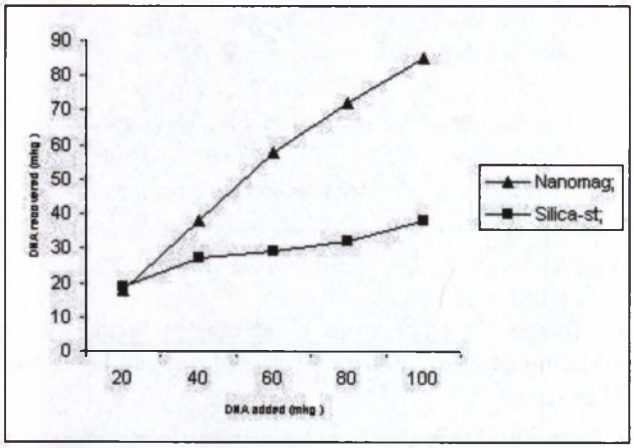
3.4. Determination of the optimal amounts of silica-magnetite absorbent for plasmid DNA purification

The synthesized silica-magnetite nanoparticles were engineered with the aim to ensure high binding capacity for nucleic acids. The structural characteristics of particle surface are critical for this feature. Obtained magnetic particles were tested for DNA isolation from *E.coli* cultures which had been transfected with some gene-engineering constructions. A set of experiments with bacterial cell lysates for measuring of adsorption capacity of synthesized nanoparticles were performed (Figure 5).

The increasing amounts of nanoadsorbent were added to bacterial cell lysates prepared from 10 ml cultures of *E.coli* containing the high copy number of pGL3-Control Vector (plasmid DNA). The traditional silica adsorbent of nucleic acids was used as a control. The result of silica magnetite nanoparticles usage was an isolation of 80 μg of plasmid DNA at 2.2 mg particles added to lysate. In contrast, 5.0 mg of commercial tradition silica adsorbent was required for isolation of equivalent amount of plasmid DNA from a 10 ml culture. The results

demonstrated that adsorbing capacity of magnetic particles was significantly higher than that of traditional non-magnetic silica-carriers.

A. Small DNA fragment size.



B. Broad DNA fragment size.

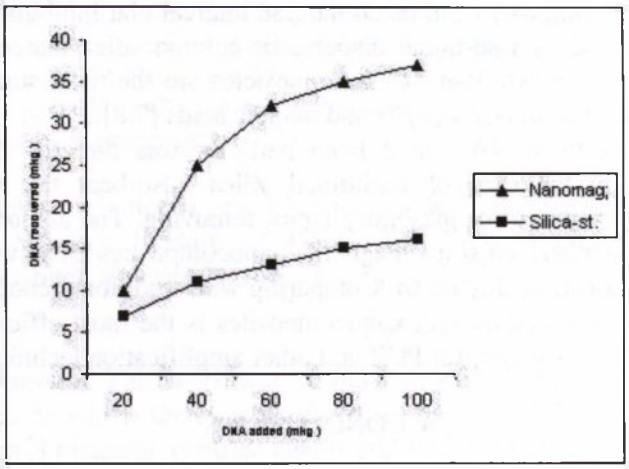


Fig. 4. Recovery of small (72–1353 bp) and broad (125–23130 bp) marker DNA fragments from magnetic nanocomposites and commercial silica adsorbent. Increasing amounts of purified marker DNA were added to a fixed concentration of magnetic nanoadsorbent and nonmagnetic microadsorbent in the binding buffer. Eluted DNA was measured by absorbance at 260 nm.

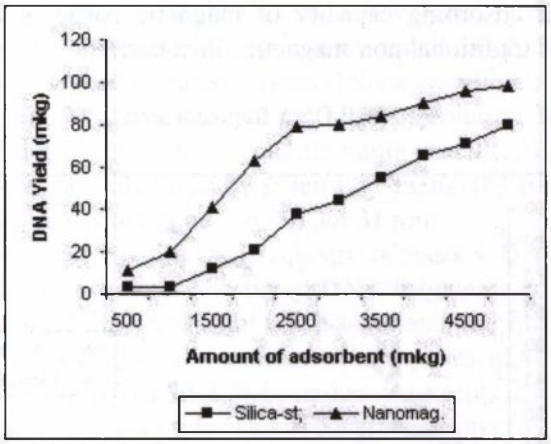


Fig. 5. Recovery of plasmid DNA from bacterial cell lysate with silica-magnetite nanoparticles and traditional silica adsorbent. Yield of plasmid DNA was determined by absorbance at 260 nm.

3.5. PCR amplification of purified DNA

PCR assays are one of the significant tests of quality estimation of purified DNA by any nucleic acid adsorbent usage. Amplification techniques are very sensitive to trace amounts of intracellular and intercellular inhibitors which often could be adsorbed by traditional disperse or column silica materials for DNA purification. It is known, that magnetic particles are the most suitable material for inhibitor-free separation of cells and nucleic acids [7,8].

In this work the DNA eluted from particles was directly used for PCR amplification. In the case of traditional silica adsorbent the solvent DNA denaturation is required for inhibitory factor removing. The amount and quality of isolated by synthesized silica-magnetite nanocomposites DNA were sufficient for DNA amplification (Figure 6). Comparing with traditional commercial silica adsorbent, the purification with nanocomposites is the most efficient and cost-effective for DNA isolation for PCR and other amplification techniques.

4. CONCLUSIONS

The results obtained in this work demonstrated the suitability of synthesized silica-magnetite nanoparticles for purification of plasmid DNA from bacterial cell lysate. The results demonstrated that both adsorption and desorption processes of plasmid DNA by nanoparticles were greatly affected by the ionic strength of binding and elution buffers. DNA binding required the presence of high salt concentration, and was recovered directly in deionized water. Using of

new nanoparticle magnetic sorbent could give the possibility of immediate downstream application native DNA without solvent precipitation. The high purity of obtained plasmid DNA purified by synthesized new silica-magnetite matrix was conformed by results of PCR amplification.

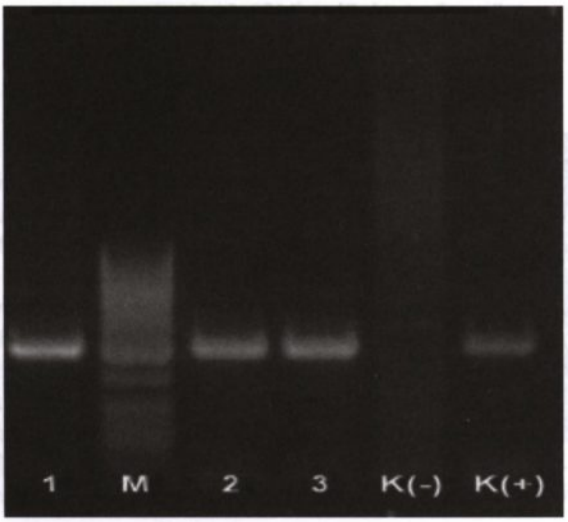


Fig. 6. Sensitivity of PCR amplification of plasmid DNA purified by silica-magnetite nanocomposites and commercial DNA isolation kit. Lines: 1 – silica-magnetite nanocomposites with 2M NaCL, pH 7.0; M – molecular markers; 2 – silica-magnetite nanocomposites with 2M NaCL, pH 8.5; 3 – commercial DNA isolation kit with alcohol precipitation; K (-) – negative control; K (+) – positive control.

So, this study presented a new nucleic acid binding matrix with optimized capasity for efficient binding of DNA. The SiO_2-encapsulated magnetite composition exibited superior performance in plasmid and small fragment DNA separation.

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CURRICULUM VITAE

Nataliia Dudchenko was born in Kiev, Ukraine in 1975. 1992–1997 – student of Kiev National University named by Taras Shevchenko, Chemical Department, Kiev, Ukraine, speciality – analytical chemistry. 1997–2000 – post-graduate course, Institute for Applied Problems in Physics and Biophysics of the Ukrainian Academy of Sciences, Department of Biophysics, Kiev, Ukraine; speciality – biophysics. 2003 – Ph.D. in biophysics from Kiev National University named by Taras Shevchenko. At present she is research associate, Institute for Applied Problems in Physics and Biophysics of the Ukrainian Academy of Sciences, Kiev, Ukraine. Research interests: magnetite nanoparticles synthesis and analysis, nonheme iron metabolism.