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FUMED SILICA-COATED MAGNETITE NANOPARTICLES FOR DNA EXTRACTION: A SAFER ALTERNATIVE TO TEOS

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DNA extraction procedures are critical in molecular biology laboratories. Magnetite nanoparticles in magnetically based DNA extraction kits often use tetraethyl orthosilicate (TEOS) as a coating agent; however, TEOS presents health risks due to its irritant properties. This study investigates the use of fumed silica as a safer alternative for coating magnetite nanoparticles. The fumed silica-coated magnetite nanoparticles (FsMNP) demonstrated ferromagnetic properties with an average size of 21.51±7.10 nm. FsMNP effectively extracted DNA from a variety of microbial strains, including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Enterobacter aerogenes*, *Shigella sonnei*, and *Staphylococcus epidermidis*. The results suggest that FsMNP can serve as a safer and more efficient material for DNA extraction. Furthermore, FsMNP-based DNA extraction may find applications in studies involving diverse biological samples, including plant, animal, and human tissues, as well as viral and environmental sources such as soil and water. This approach improves the safety and efficiency of preparing magnetic nanoparticles for DNA extraction in various biological and environmental applications.

Keywords: DNA extraction, fumed silica, magnetite, bacterial strains, nanoparticles, TEOS.

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Introduction

DNA extraction is a fundamental procedure in molecular biology laboratories, essential for genetic analysis, sequencing, and diagnostic applications. Among the available methods, magnetic nanoparticle-based kits have gained popularity due to their simplicity and convenience. These kits use magnetic fields to separate DNA from the sample matrix, typically employing silica-coated magnetite nanoparticles (Fe_3O_4) to ensure efficient DNA binding and recovery.

Tetraethyl orthosilicate (TEOS) is frequently used as a silica source [1–12]. However, TEOS poses significant health concerns due to its irritant properties, particularly affecting the respiratory system and eyes. These hazards are noted in its material safety data sheet. Fumed silica, a non-irritant silica source produced through the hydrolysis of silicon tetrachloride [13], presents a safer alternative. Its use for coating magnetite nanoparticles can reduce health risks while maintaining DNA extraction efficiency.

This study aims to synthesize and characterize fumed silica-coated magnetite nanoparticles (FsMNP) as a safer alternative for DNA extraction. The performance of FsMNPs will be evaluated through DNA extraction from various microbial species. Additionally, the compatibility of the extracted DNA with PCR will be assessed to ensure the suitability of FsMNP for downstream molecular applications.

Experimental

Materials

$FeSO_4$ and $FeCl_3$ were used as precursors and were purchased from Merck. Tetraethyl orthosilicate (TEOS), ammonia aqueous solution, NaOH, Tris, and EDTA were also obtained from Merck. Fumed silica was supplied by Wacker Chemie AG. Triton X was purchased from Vivantis, sodium dodecyl sulfate (SDS) from BASF, and polyethylene glycol 6000 (PEG 6000) from Clariant. NaCl was procured from Dominion Salt, while isopropanol and ethanol were obtained from Bratachem.

Bacterial strains

The bacterial strains used for DNA extraction were obtained from an environmental consortium, cultured overnight in the research laboratory of the Konimex Diagnostic Center, and included *Escherichia coli* ATCC 8739, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 9027, *Salmonella paratyphi* ATCC 9150, *Enterobacter aerogenes* ATCC 13048, *Shigella sonnei* ATCC 25931, and *Staphylococcus epidermidis* ATCC 12228. All bacterial cultures were purchased from Microbiologics. The medium used for bacterial growth was Tryptone Soy Broth, which was purchased from Himedia.

Magnetite synthesis and characterization

$FeSO_4$ and $FeCl_3$ were mixed in water at a molar ratio of 1:2. NaOH solution was added dropwise under stirring until the pH reached 8–10. The mixture was allowed to settle, and the supernatant was decanted. The precipitate was washed twice with water and then dried in an oven at 60–100^{0}C for two hours. The resulting magnetite was coated with fumed silica following a previously described protocol [14], in which TEOS was substituted with 20 μg per mg of magnetite. The fumed silica-coated magnetite nanoparticles (FsMNPs) were characterized using several techniques. Appearance, morphology, and size were examined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Elemental composition was analyzed by energy-dispersive X-ray spectroscopy (EDS) coupled with SEM. Crystal structure was determined using X-ray diffraction (XRD), and magnetic properties were measured by vibrating sample magnetometry (VSM).

DNA extraction evaluation

DNA extraction was performed using a general protocol in which bacterial cells were lysed with a solution containing 10 mM Tris, 1 mM EDTA, 0.6% SDS, and 0.2% Triton X. The resulting lysate was mixed with FsMNPs and 200 μL of binding buffer for approximately 3 minutes. FsMNPs and the supernatant were separated using a magnetic stand for 3 minutes, and the supernatant was discarded. The pellet was washed three times with 70% ethanol, followed by DNA elution using an elution buffer. Each DNA eluate was visualized by gel electrophoresis and amplified by PCR using the primer pair 27F and 907R.

Results and discussion

The size, shape distribution, and appearance of the resulting FsMNPs were examined using scanning electron microscopy with a JSM-IT700HR instrument (JEOL). SEM images (Fig. 1) were taken at magnifications of 1,000× (A), 3,000× (B), 10,000× (C), and 25,000× (D). The SEM visualization

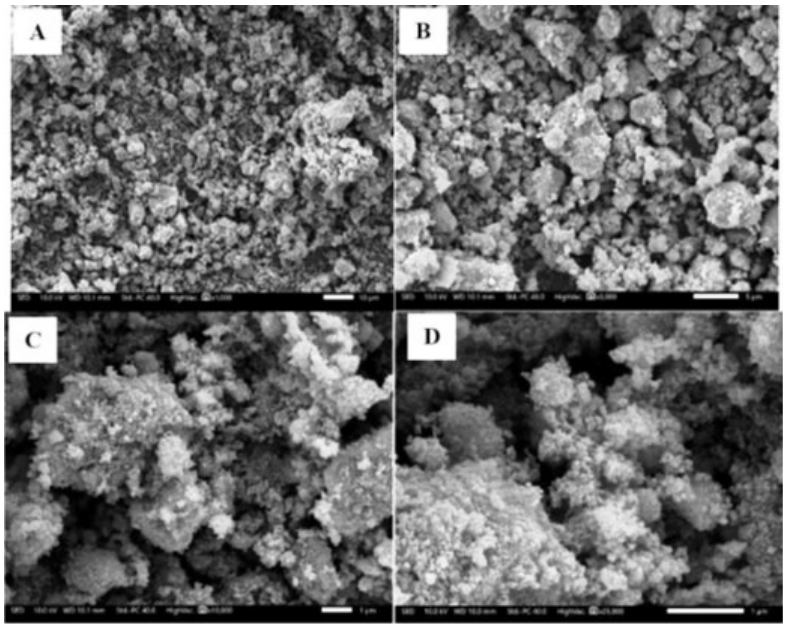


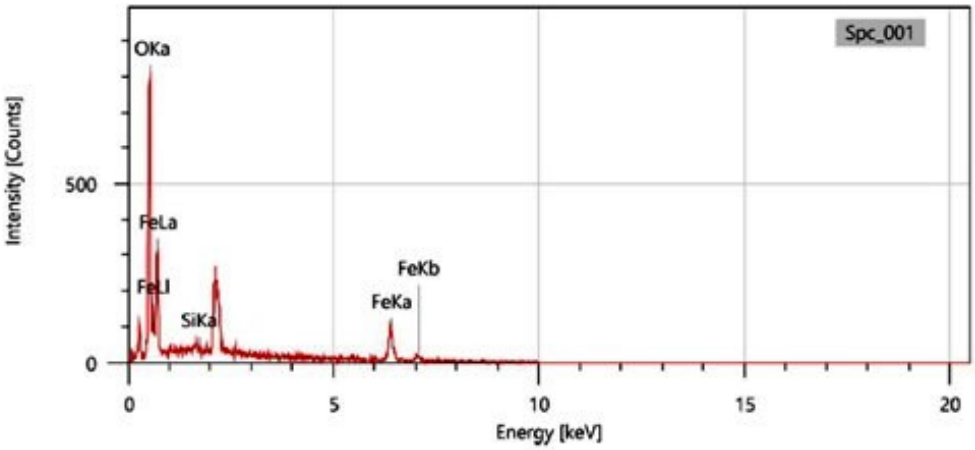
Fig. 1. SEM images of FsMNPs at different magnifications: (A) 1,000×; (B) 3,000×; (C) 10,000×; (D) 25,000×

reveals that the FsMNPs are spherical and tend to form clusters. This clustering suggests that the FsMNPs retain their magnetic properties and are capable of magnetic attraction [15].

The elemental composition of the FsMNPs was analyzed using EDS in conjunction with SEM. The detected elements were Fe (78.06±3.31 wt.%), O (21.14±0.32 wt.%), and Si (0.80±0.14 wt.%) (Fig. 2). Elemental mapping revealed the presence of silica on the particle surface, shown in purple in Fig. 3. These results confirm the successful incorporation of fumed silica into the magnetite nanoparticles.

Transmission electron microscopy observations were conducted using a JEM-1400 instrument, and the results are presented in Fig. 4. The TEM images revealed that some particles exhibited dark cores, likely corresponding to magnetite nanoparticles (MNPs), surrounded by more transparent shells, indicating the presence of a fumed silica coating. Particle size measurements were performed using ImageJ software, as shown in Fig. 4C. The average diameter of the FsMNPs was found to be 21.51±7.10 nm.

The X-ray diffraction (XRD) pattern of



Element	Line	Mass%	Atom%
O	K	21.14±0.32	48.09±0.73
Si	K	0.80±0.14	1.03±0.19
Fe	K	78.06±3.31	50.87±2.16
Total		100.00	100.00
Spc_002			Fitting ratio 0.2997

Fig. 2. EDS analysis of FsMNPs

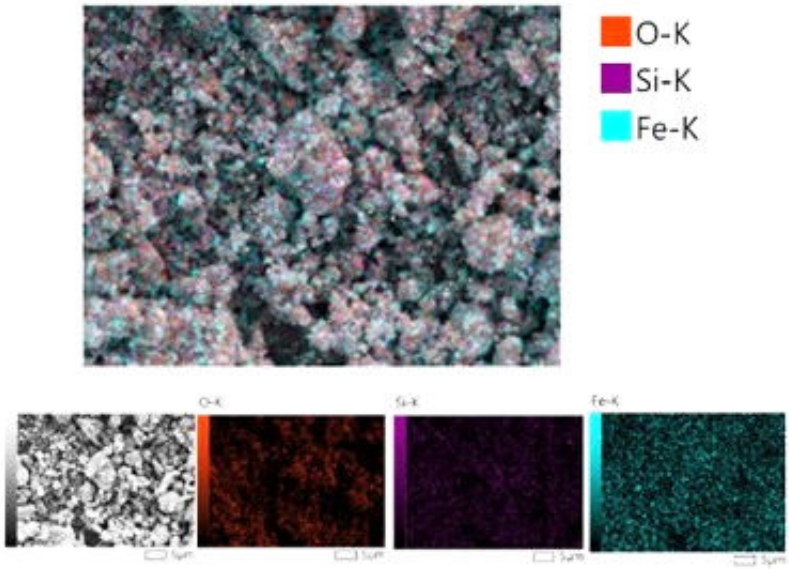


Fig. 3. EDS-mapping of FsMNPs

FsMNPs, shown in Fig. 5, was recorded using CuKα radiation (λ=1.540598 Å) on a PANalytical X'Pert 3 powder diffractometer. The XRD analysis revealed six peaks at 30.05^0, 35.51^0, 43.09^0, 53.07^0, 57.15^0, and 62.79^0, corresponding to the characteristic diffraction peaks of Fe_3O_4. The crystal planes associated with these peaks are (220), (311), (400), (422), (511), and (440), respectively. The d-values for each peak were calculated using Bragg's law. The peak at $2\theta=35.51^0$ exhibited the highest intensity and was used to calculate the crystallite size using the Scherrer equation with a shape factor K=0.94 (for spherical crystals in cubic systems), resulting in a crystallite size of 22.927 nm. These findings confirm that the MNPs were successfully coated with silica, as also observed in the TEM and EDS results.

The vibrating sample magnetometry (VSM) analysis, presented in Fig. 6, shows that the FsMNPs have a magnetic saturation value of 44.385 emu/g. The hysteresis loop is very narrow, indicating low

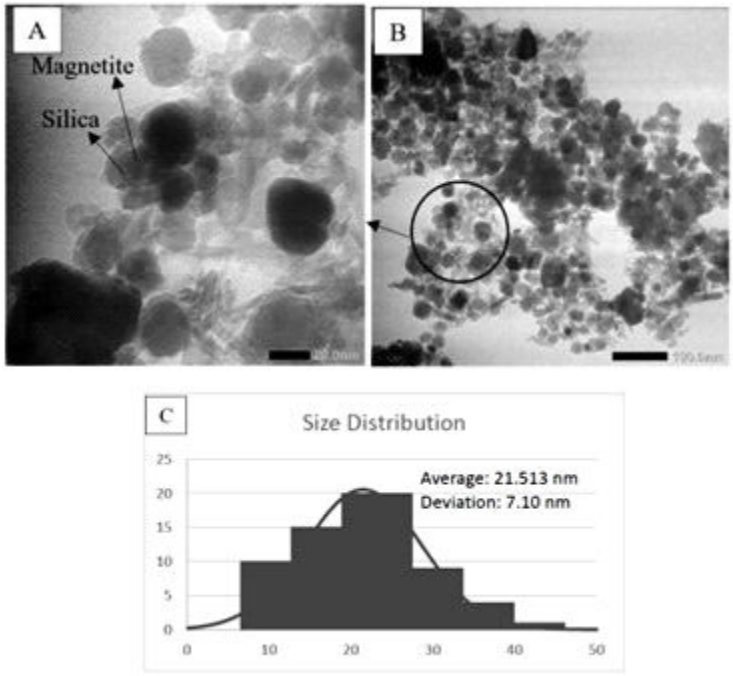


Fig. 4. TEM images of FsMNPs at magnifications of (A) 150,000× and (B) 40,000×; (C) size distribution of FsMNPs based on TEM images

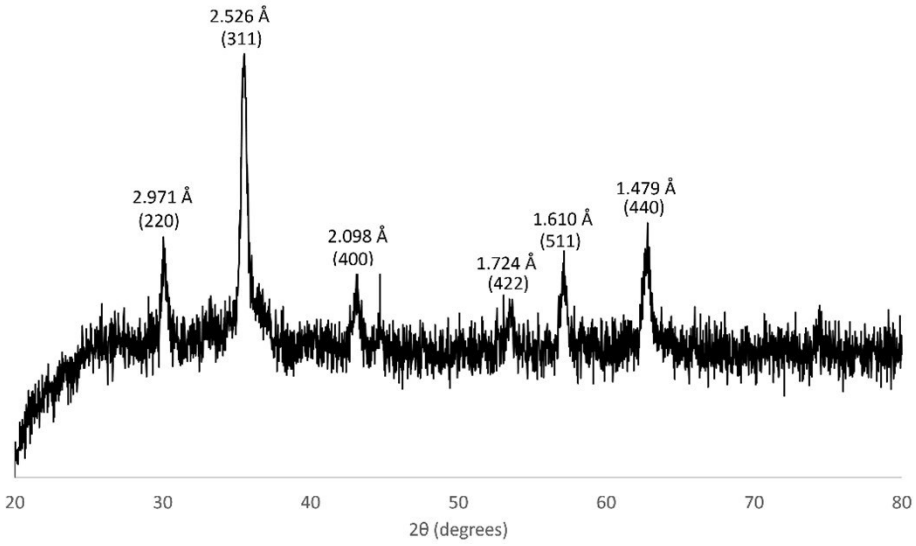


Fig. 5. XRD pattern of FsMNP

magnetic coercivity, with a value of 0.022 T, and a magnetic remanence of 11.461 emu/g. The magnetic remanence was determined from the intercept of the two points where polarity reversal occurs in the applied magnetic field. The coercivity value was calculated from the intercept between the positive and negative magnetization branches during the field reversal.

FsMNPs were used for DNA extraction from various microbial species, and the extracted DNA was visualized by agarose gel electrophoresis (0.8% w/v). The results, shown in Fig. 7, demonstrate that all lanes exhibit a single, distinct band without any smearing, indicating that the extracted DNA was intact and not fragmented.

All extracted DNA samples were successfully amplified by PCR using the parameters outlined in Table. The target amplicon of the 16S rRNA gene was approximately 1500 bp. Amplicon visualization, shown in Fig. 8, confirms that all DNA samples were successfully amplified. These results indicate that the extracted DNA was free of inhibitors that could interfere with the PCR reaction.

Conclusions

In this study, we successfully synthesized magnetic nanoparticles coated with fumed silica as a safer alternative to tetraethyl orthosilicate (TEOS) for use as a coating agent. The resulting fumed silica-coated magnetite nanoparticles (FsMNPs) had an average particle size of 21.51±7.10 nm, a crystallite size of 22.93 nm, and exhibited ferromagnetic properties. FsMNPs demonstrated effective DNA extraction performance across various bacterial species, including consortium bacteria, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Shigella sonnei*, and *Staphylococcus epidermidis*. Future research could explore the application of FsMNPs for nucleic acid extraction from additional sample types, such as viral specimens or environmental matrices like soil and water.

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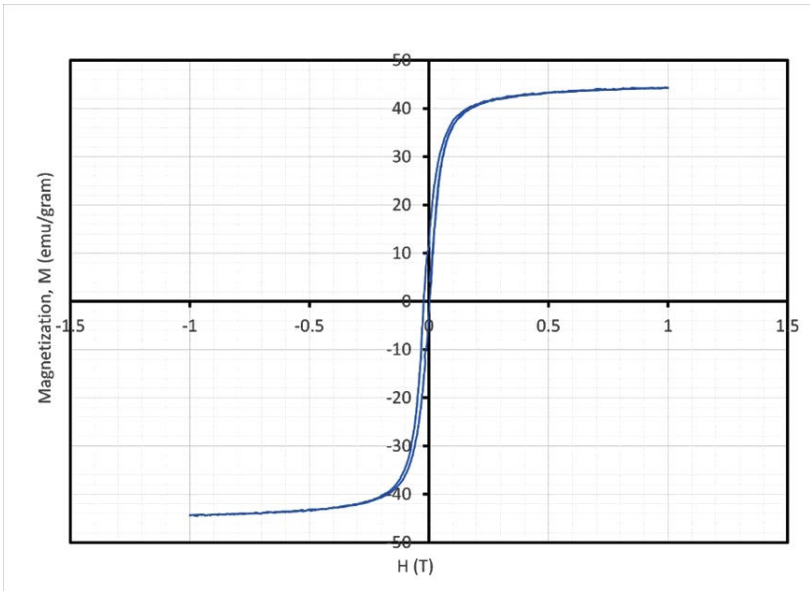

Fig. 6. VSM analysis of FsMNP

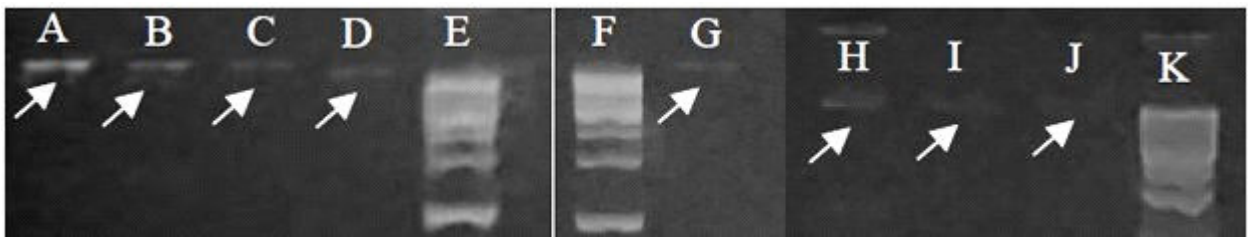

Fig. 7. Agarose gel electrophoresis of DNA extracted using FsMNPs: (A) Consortium bacteria; (B) *E. coli*; (C) *P. aeruginosa*; (D) *S. aureus*; (E) DNA ladder; (F) DNA ladder; (G) *S. paratyphi*; (H) *E. aerogenes*; (I) *S. sonnei*; (J) *S. epidermidis*; (K) DNA ladder.

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PCR parameters used in this study (pre-denaturation at 95^{0}C for 5/ min; denaturation at 95^{0}C; total of 35 cycles)

Parameter		Microbial strains				
		Consortium bacteria	*E. coli*, *E. aerogenes*, *S. sonnei, S. epidermidis*	*P. aeruginosa*	*S. aureus*	*S. paratyphi*
primer concentration (nM)		200	500	500	500	500
denaturation (s)		30	30	30	30	30
Temperature (ºC) / duration (s)	annealing	55/30	53/30	50/30	60/30	53/30
	extension	72/60	72/90	72/90	72/60	72/90
	final extension	72/300	72/300	72/300	72/300	72/300

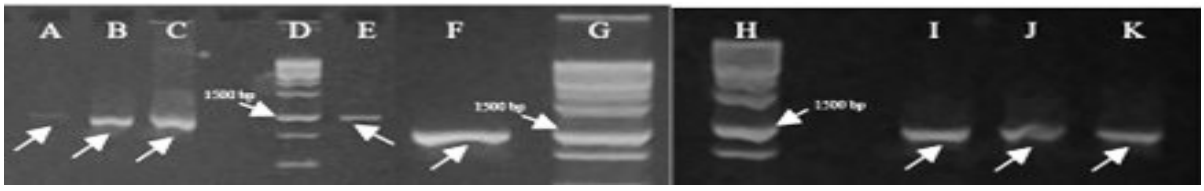

Fig. 8. Agarose gel electrophoresis of PCR products: (A) Consortium microbes; (B) *E. coli*; (C) *P. aeruginosa*; (D) DNA ladder; (E) *S. paratyphi*; (F) *S. aureus*; (G) DNA ladder; (H) DNA ladder; (I) *E. aerogenes*; (J) *S. sonnei*; (K) *S. epidermidis*

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НАНОЧАСТИНКИ МАГНЕТИТУ З ПОКРИТТЯМ ІЗ ПІРОГЕННОГО ДІОКСИДУ КРЕМНІЮ ДЛЯ ЕКСТРАКЦІЇ ДНК: БЕЗПЕЧНІША АЛЬТЕРНАТИВА TEOS

С.Е.Е. Чоа, Мудасір, Е. Сухар'яді, Б.С. Дарйоно

Процедури екстракції ДНК є критично важливими в лабораторіях молекулярної біології. Наночастинки магнетиту, що застосовуються в магнітних наборах для екстракції ДНК, часто покривають тетраетилортосилікатом (TEOS), однак ця речовина становить ризик для здоров'я через подразнювальні властивості. У цьому дослідженні розглянуто можливість використання пірогенного діоксиду кремнію як безпечнішої альтернативи для покриття наночастинок магнетиту. Одержані наночастинки магнетиту з покриттям із пірогенного діоксиду кремнію (FsMNP) виявили феромагнітні властивості із середнім розміром 21,51±7,10 нм. FsMNP ефективно екстрагували ДНК з різноманітних мікробних штамів, зокрема *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Enterobacter aerogenes*, *Shigella sonnei* та *Staphylococcus epidermidis*. Результати свідчать, що FsMNP можуть слугувати безпечнішим і ефективнішим матеріалом для екстракції ДНК. Крім того, екстракція ДНК із використанням FsMNP може знайти застосування в дослідженнях, пов'язаних із широким спектром біологічних зразків – рослинних, тваринних, людських, а також вірусних і середовищних зразків, таких як ґрунт і вода. Запропонований підхід підвищує безпеку й ефективність підготовки магнітних наночастинок для процедур екстракції ДНК у різноманітних біологічних та екологічних дослідженнях.

Ключові слова: екстракція ДНК; пірогенний діоксид кремнію; магнетит; бактеріальні штами; наночастинки; TEOS.

FUMED SILICA-COATED MAGNETITE NANOPARTICLES FOR DNA EXTRACTION: A SAFER ALTERNATIVE TO TEOS

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Keywords: DNA extraction; fumed silica; magnetite; bacterial strains; nanoparticles; TEOS.

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