

Application of magnetic nanoparticles by comparing the absorbance and stabilization of biomolecules DNA-C, L by the electrophoretic detection

Mansour Binandeh¹,
Farrokh Karimi²,
Sadegh Rostamnia¹

¹Department of Chemistry, Faculty of Science, University of Maragheh, Iran, ²Department of Biotechnology and Environmental, Faculty of Science, University of Maragheh, Iran

Address for correspondence:

Dr. Mansour Binandeh, Department of Chemistry, Faculty of Science, University of Maragheh, Iran. Tel.: +989142217299. E-mail: mansurstrong@gmail.com

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Introduction

The nanotechnology industry is one of the most important and lucrative scientific industries in the scientific community, with many projects being designed and implemented every year. One of the important branches of nanoscience is the study of the structure and function of magnetic nanoparticles. Magnetic nanoparticle structures are highly orderly and particle sized (5–100 nm), suitable for medical and pharmaceutical research, and have helped greatly to solve diseases of the body without causing any side effects. Applications of these nanoparticles include the use of magnetic imaging, measurement of red blood cells, purification of protein and DNA, and stabilization of biomolecules for targeted transfer to the target cell in the living organism to treat the damaged cell.^[1]

ABSTRACT

Objectives: The project is based on extensive studies on applied nanoparticles in biology and medicine. This study was primarily designed to investigate the role of magnetic nanoparticles by comparing the absorbance and stabilization of DNA-C, L by the electrophoretic detection.

Methods: Fe₃O₄ magnetic nanoparticles (MNPs) with core/shell structure of silica coatings were synthesized by a chemical coprecipitation method. This work is done at 15 min times with multitudes, that is, 20 numbers and nanoparticles are provided regular with good morphology which was synthesized in 20 nm in size, and its structure was analyzed by tools such as EDX analysis. Next, 20 mg of the magnetic nanoparticles were coated with silica in a heterogeneous solution at 25 µg/ml of the solution of each of the mixed DNAs (ring, linear) in separate containers. Finally, 15 minutes later, DNA was adsorbed on the surface of the nanoparticles. The amount of this adsorption was injected by spectrophotometry (UV-Vis, with 99% accuracy and optimized by the standard Tris.HCl buffer required to separate DNA from its pure solution [unabsorbed DNA]) and electrophoresis.

Results: The results showed that absorption and diffusion of DNA-C or L at the surface of nanoparticles were 95% and 85%, respectively (i.e., absorbance of DNA-C > DNA-L is) with rate of removing of on MNPs was >99%. Hence, after review, we received that a linkage of electrostatic bonding between nanoparticles and biomolecules was obtained, and the results of the EDX analysis confirmed this study.

Conclusion: In this project, nanocomposites containing magnetic nanoparticles were synthesized and their structure was identified by relevant analyzes. It was then used to stabilize the biomolecules, which yielded competitive results between the two types of DNA (linear and cyclic) at 85 and 95% adsorption, respectively.

Keywords: Electrostatic absorption, magnetic nanoparticles, spectrophotometry and electrophoresis analyses

For the latter case (i.e., the fixation of biomolecules on the nanoparticles), the surface of the nanoparticles must be suitable for this reaction. Therefore, many arrangements have been made, one of which is to modify the surface of silica-coated nanoparticles. As we know, with this coating, first, the performance of the magnetic nanoparticles surface increases, in the second, it prevents the additional oxidation of the nanoparticles in the vicinity of air oxygen.^[2] Important applications of magnetic nanoparticles include specific separation of nucleic acid,^[3] diagnosis and targeted therapy,^[4] hyperthermia,^[5] and antibacterial agents^[6,7] have been studied. One of the main techniques is that biomolecular techniques have now been established on magnetic nanoparticles. These biochemical molecules^[8,9] DNA^[10] are important for treatment and stabilization of magnetic nanoparticles. Another type of

DNA is a circular kind^[8] where nucleotide strands are twisted together to form a ring. Biomolecules^[8] (DNA) are discussed. One of the main issues is the creation of the relationship between biomolecules^[11] and the catalyst stabilized^[12,13] with magnetic nanoparticles. Usually, an electrostatic bond between magnetic nanoparticles and biomolecules, and in some cases, can also be covalent bond depending on the magnetic nanoparticle ligands.^[14] In this project, we intend to investigate the absorption and removal of two types of DNA (linear and cyclic) from silica-coated magnetic nanoparticles to an important goal being the selection of a nanocomposite designed for targeted stabilization and release of biomolecules that we can achieve *in vitro* [Figure 1].

Methods

Materials

The solvents were by completely pure. The structure of the materials was identified by EDX analysis in Tehran Laboratory. DNA marker was the standard of Chinese biotechnology. Fe^{2+} , Fe^{3+} and NaOH (German Merck), Tris/HCl solution (as buffer), argon gas, HCl, methanol, NaCl, glutaraldehyde purchased from Sino-pharm Chemical China were completely pure. The silica used to prevent the oxidation of nanoparticles was purchased purely from Sigma-Aldrich USA. DNA (as a model molecule) was prepared in double distilled water (Maragheh Laboratory, Iran).

Synthesis of silica coated with Fe_3O_4 magnetic nanoparticles

There are many methods for the synthesis of nanoparticles^[6,7,9] that we used the chemical coprecipitation method in the synthesis of MNPs. First, Fe (II) and Fe (III) were mixed with argon gas at a ratio of 1: 2 in 1 ml of deionized water at 25°C for

3 h Then, 2 ml of silica solvent was added to it, and finally, the nanocomposite was washed several times with ethanol solvent and placed in an oven at 60°C for ½ a day to dry completely.

DNA-L, C adsorption studies

Weigh 20 mg of magnetic nanoparticles and pour on 100 μl of DNA and continue the reaction at room temperature in water solvent for 15 min. The results were collected by spectrophotometric analysis.

Results

Results of DNAs loaded onto magnetic nanoparticles $\text{Fe}_3\text{O}_4/\text{SiO}_2$ by spectrophotometry

Effect of increasing the concentration of DNA studied and the results were obtained. Based on repeated, accurate, and based on the spectrophotometric analysis (its 99% accuracy and optimized by the standard Tris.HCl buffer required to separate DNA from its pure solution [unabsorbed DNA]), it can be said that increasing the concentration of DNAs gradually increases the amount of DNA-L or C in the magnetic nanoparticles, which can be said to be that all DNAs are absorbed on surface of the magnetic nanoparticles be. Hence, this is when the amount of 1 ml with a concentration of 25 $\mu\text{g}/\text{ml}$ DNA-L or C in 20 mg MNPs is dissolved in the interval of 0–30 min. Even the moment when a portion of the DNA-L or C solution was added to the container at the time of 0 min to the dissolved magnesium nanoparticles without any catalytic activity, the specimen was analyzed in a spectrophotometer and the result of 15% absorption showed. Hence, at an optimum concentration of 25 $\mu\text{g}/\text{ml}$ of DNA-L or C, the absorption rate is approximately 90% absorbed, which is close to 100% at the end. And that, the absorption results for the two types of DNAs showed that the amount of absorption in the annular type was far more than the linear one [Figure 2].

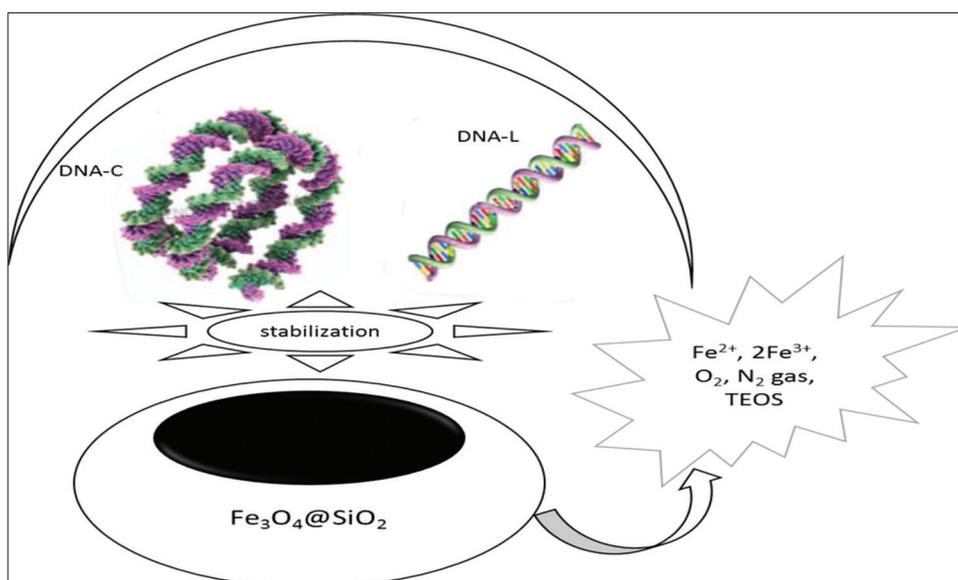


Figure 1: A representation of DNAs loaded with magnetic nanoparticles

Method of detection structure of the MNPs/silica nanoparticles

For view, structure of nanoparticles was used of EDX analysis that structure of core/shell of nanoparticles was arranged with 20–100 nm range size by %gram of anyone atomic of the MNPs nanoparticles, that is, method to detection of electrostatic bonding between MNPs with DNAs, as shown in Figure 3.

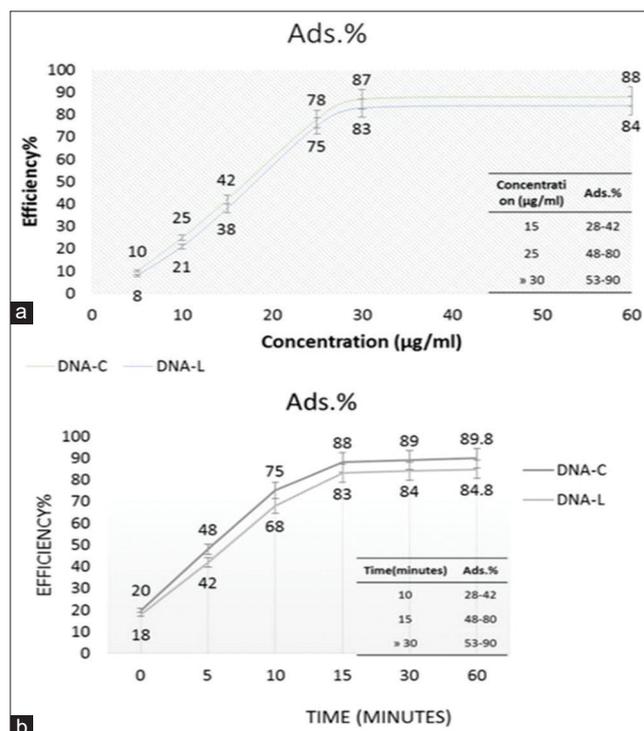


Figure 2: (a) Adsorption of DNAs (L<C); MNPs: 20.0 mg, DNA: 1.0 mL, Tris-HCl: 1.0 ml adsorption time: 15 min, (b) effect of the time for absorption DNA-L<C

Stabilization and release process of DNAs on MNPs

In this section, two patterns of different DNAs were mixed with nanoparticles. For this work first, 20 mg of nanoparticles with 100 microliter of DNAs in 1 ml distilled water were mixed. In following, in 3 times of 0, 10, and 15 min, products were gathered. Concentration of DNA in standard condition is 1 microgram per microliter that amount of 25 microgram per microliter was prepared. Then, samples were analyzed by spectrophotometry device and so results made showed that absorption of DNA C in 0–25 min time was more of DNA L, in same times. There is evidence that adsorption or release at a concentration of 25 µg/ml is absorbed or definitely released to 85% [Table 1]. The results of the discussion are that absorption 25 µg/ml maximum and above. This value of 50 µg/ml, the absorption gradient, has not been altered. Data are shown in the following Figure 2a and the measured values of the standard scale are considered. The absorption of DNA-L, C by decomposition and spectrophotometric analysis of UV–Vis was carried out. In the first analysis, the apparatus was adjusted to zero using distilled water and then calibrated with a solution containing DNAs at 280 nm. The decrease in the absorbance of the peaks (at the frequency of capture) showed that the absorbed DNAs were stable. Thus, using the obtained data, it can be concluded that the optimum concentration is 25 µg/ml, which is stabilized at the nanoparticle level in 15 min for DNA-C, L, and its release rate is higher than 90%, which is due to the corresponding buffer (Tris-HCl buffer for DNAs). As a result, its adsorption and release rates are roughly 85% higher, indicating that the purge and propagation process has been fully implemented.

Timing for absorption

The reaction was performed over an hour period and the experiment was repeated several times, and therefore, the

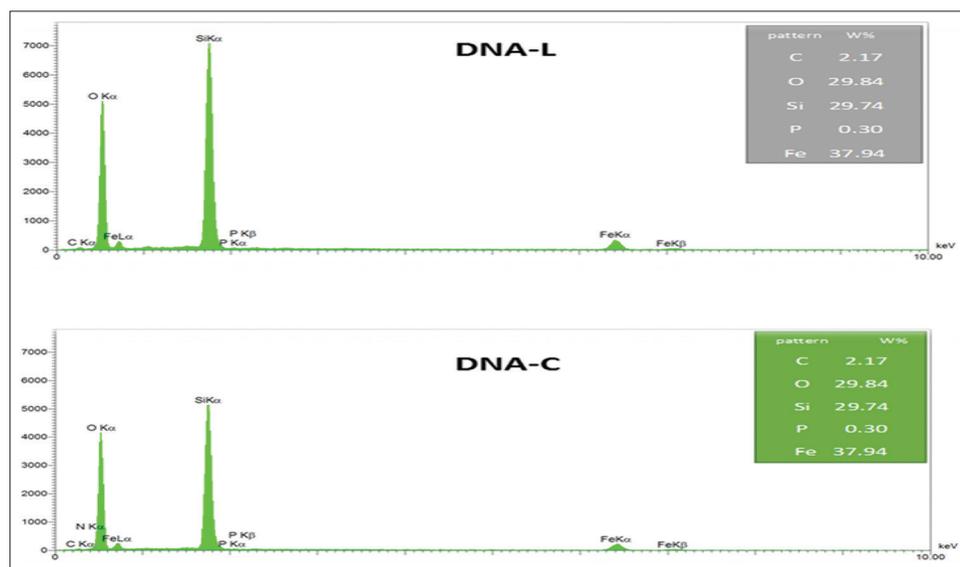


Figure 3: Results of analysis EDX showed the percentage of absorption DNA-L, C with link by magnetic nanoparticles

results showed that after 15 min, the adsorption was stabled. Adsorption was 0 min for DNA L and DNA 25 and 35, at 10 min 45 and 55, respectively, and at 15 min time was 85% and 95%, respectively. Thus, DNA C uptake was higher in 15 min than L DNA [Figure 2b].

Stability of DNA-L, C on the MNPs

The results have been summarized several times regarding the use of nanoparticles over a period of 2–120 h. The results show that nanoparticles have reduced the yield by <15%, indicating the high efficiency of nanoparticles in the stabilization of biological molecules [Figure 4a]. Thus, this stability was demonstrated in the EDX analysis,^[15] the bonding of nanoparticles with biomolecules was electrostatic bonding. The P group of DNA binds to the O factor of the -OH group, and the silica groups can provide the surface of magnetic nanoparticles for DNA adsorption.

Electrophoresis analysis

Electrophoresis analysis is one of the most important factors in determining the degree of purification and stabilization of biomolecules. In this part of the horizontal electrophoresis, we examine the degree of DNA fixation on the remaining stains on the silica gel plate. This DNA (C, L) is a model produced by a genetic researcher in the laboratory and extracted by electrophoresis with > 99.99% efficiency. First, we placed the prepared silica gel plate on a fixed table and stained each DNA (linear, circular) separately on two silica gel plates. In total, for each plate, five types of stains were performed with the first stain as ladder, the second stain as pure DNA, and the third to fifth stain, respectively, for periods of stabilization

on magnetic nanoparticles at 0–15 min. Then, we put the plates in the electrophoresis machine and gave about 4–5 h to remove the stains on the side of the plate. Finally, the results showed that, at the time of a quarter, at the ideal concentration (25 µg/ml), the fifth spot faded compared to the second spot (pure DNA) and stabilized by more than 70%. However, this rate of stabilization in the competitive reaction between the two types of DNA (linear and circular) was reported to be 85% for circular DNA and 70% for linear DNA. Evidence then shows that the rate of plasmid cyclic DNA fixation at the surface of magnetic nanoparticles is approximately 15% higher than that of linear DNA. These results can be used to repair damaged DNA (linear or circular) from living cells by targeted magnetic control, which is still in its infancy [Table 2, Figures 4b and 5].

Discussion

Our general discussion was about the efficiency of magnetic nanoparticles in the adsorption and release of DNA in an *in vitro* environment. For this purpose, the nanoparticles were first synthesized by chemical codeposition^[6,7,9] (under 20 nm) and identified by EDX analysis. Then, the powder of magnetic nanoparticles (i.e., 20 mg) is dissolved in distilled water, and at 25°C, the amount of DNA (linear and circular) is added to the nanoparticles and from zero moment (i.e., when DNA was added to the nanoparticle solution but not completely stirred) and was sampled at 5, 10, and 15 min. These samples, because the DNA was mixed inside the magnetic nanoparticles, therefore, the nanoparticles had to be separated from the solution, which was separated from the reaction solution by a magnetic field (here, the magnet) and collected for spectrophotometric detection.

Table 1: Amounts of absorption and releasing data, DNA-C>L

Pattern (µl/ml)	Time (min)	Adsorption (µg/ml) %	Releasing (µg/ml) %	Extraction of DNA absorbed from unabsorbed (50 µl (buffer (Tris.HCl)+MNPs-(DNA-L, C/wavelength (nm)
DNA-L	15	85	80	Tris-HCl/280
DNA-C	15	95	85	Tris-HCl/280

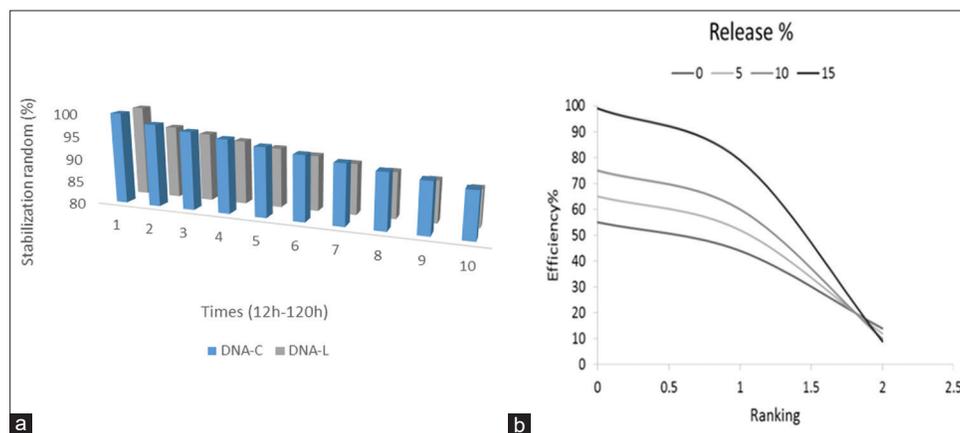
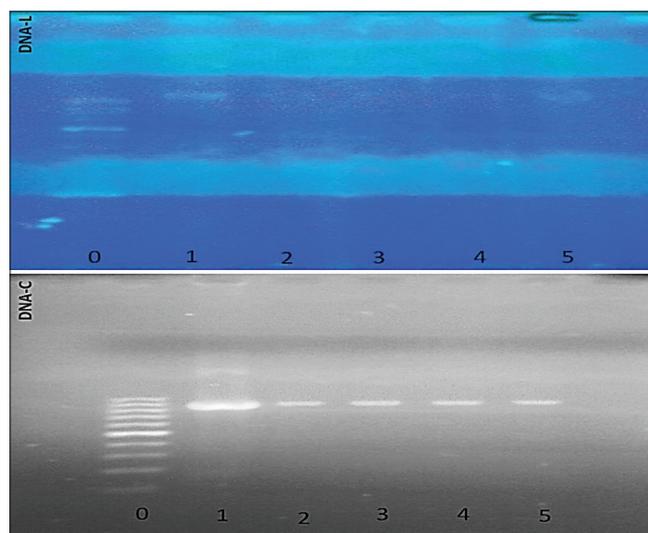


Figure 4: (a) The DNA-L<C resistance loaded MNPs overtime in 10 cycles, (b) picture of the release DNA-L<C of surface of MNPs by Tris-HCl buffer that analyzed by spectrophotometry equipment.

Table 2: Amounts of absorption and releasing data by electrophoresis and spectrophotometer analysis

Pattern 50 (μl/ml)	Ads. (μg/ml) Time: 0 min	Ads. (μg/ml) Time: 5 min	Ads. (μg/ml) Time: 10 min	Ads. (μg/ml) Time: 15 min
MNPs-DNA-C	1.210	0.62	0.29	0.147
MNPs-DNA-L	1.110	0.56	0.33	0.23
DNA-C (Ads.%)	0.496	55	75	95
DNA-L (Ads.%)	0.505	55	70	85

**Figure 5:** Electrophoresis analysis was proved of absorption the DNA-C>L on surface magnetic nanoparticles

Finally, spectrophotometric analysis showed that the amount of DNA adsorbed on the surface of nanoparticles was higher than 85% for the linear model and 95% for the ring model, and also by comparing the amount of DNA adsorbed to the pure DNA solution. It shows that more than 90% of the DNA is adsorbed on the surface of the magnetic nanoparticles and the same amount is removed from the net amount in the reaction and only less than 10% of the DNA is not adsorbed on the surface of the magnetic nanoparticles.

Comparing these results with recent research in this field shows that, in one project, DNA was placed on the surface of platinum nanoparticles and in 1 h was able to reach a standard concentration of 100 nanomoles (the lowest detection limit of about 2.6 nanomoles) and an absorption of over 65%.^[16] In another project, DNA was loaded on chitosan and injected into damaged cells (by gene therapy) with a DNA uptake rate of over 75% in 4 h.^[17] In another project, DNA was placed on the surface of gold nanoparticles, and at a temperature of 38°C for 40 min, more than 70% of the DNA was adsorbed on the surface of gold nanoparticles.^[18] Finally, in another project, the DNA was migrated to the HPMC and EC polymer and absorbed over 90% of the DNA at temperatures above 50°C for 12 h.^[19] Comparing recent projects, it can be stated that magnetic nanoparticles in terms of test conditions (molar concentration of DNA used (25 μg/μl), time (about 15 min), and temperature (room temperature) on other nanocatalysts and also other polymer substrates or even silica itself (alone)

are superior and ultimately have a high absorption of 95% for DNA (ring) and 85% for DNA (linear).

Conclusion

In this project, nanocomposites containing magnetic nanoparticles were synthesized and their structure was identified by relevant analysis. It was then used to stabilize biological molecules, which resulted in competitive results between two types of DNA (linear and circular) in 85 and 95% adsorption, respectively. Magnetic nanoparticles are used in biomedical and biochemical fields and, therefore, can be used to deliver drugs to target cells (i.e., DNA) that are infected with pathogenic viruses. The drug delivery method is simply using magnetic nanoparticles with stabilization of biomolecules (biopharmaceuticals) and then their extraction by the magnetic field outside without toxic effects. For this study, 0.002 g of MNP and 25 μg/μl of DNA (healthy) were dissolved in distilled water *in vitro* to be transferred to patient cells (by drug delivery) by magnetic field extraction for an ½ day time. Finally, MNPs are extracted by a new combination of magnets and DNA to replace damaged DNA in the human body with a healthy DNA *in vivo*, which is more than 99% efficient.

Authors' Declaration Statements

Ethics approval and consent to participate

This project was carried out without receiving any grant and was completed with the ethical approval and consent of Maragheh University.

Availability of data and material

All of data are available with corresponding author.

Competing interest

The authors have declared that no competing interest.

Funding statement

Authors did not receive any funding for this work.

Authors' Contributions

I am Mansour Binandeh (corresponding) graduated of Maragheh University, Prof. Sadegh Rostammia in Organic Chemistry, Dr.

Farrokh Karimi in biotechnology fields is a supervisor of this paper and researchers of Maragheh University.

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ORCID link of the submitting author: 0000-0003-1167-6842.

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