Silica Based Specific Adsorbents for DNA Purification

DNA Saflaştırılması İçin Silika Temelli Taşıyıcılar

Research Article

Duygu Kurt[1], Işık Perçin[2], Handan Yavuz[3], Erhan Pişkin[1], Adil Denizli[3]*

[1]Chemical Engineering Department, Hacettepe University, Beytepe, Ankara, Turkey.

[2] Biology Department, Hacettepe University, Beytepe, Ankara, Turkey.

[3]Chemistry Department, Hacettepe University, Beytepe, Ankara, Turkey.

ABSTRACT

The aim of this study is to prepare silica based affinity sorbents for the purification of plasmid DNA and genomic DNA. For this purpose, (3-aminopropyl)trimethoxysilane (APTS) is chosen for surface modification of silica particles (average size: 100-150 μm) and silica based affinity sorbent carrying NH_2 groups on its surface is obtained. APTS modified and unmodified silica particles are characterized by elemental analysis. Adsorption of genomic DNA on the APTS modified silica particles is performed in continuous and batch systems. Effect of ionic strength, pH, temperature, initial genomic DNA concentration and flow rate is investigated. In the last part of the study, pEGFP-N3 plasmid DNA is separated from *E.coli* cells by using the APTS modified silica particles and the results are compared with commercial Qiagen DNA purification kit. The results may be summarized as follows: amount of NH_2 groups on the APTS modified silica particles is in the range of 38-60 mmol/g. Maximum adsorption capacity of the APTS modified silica particles is found to be 49.3 mg/g and this value is obtained at pH 7.0. Genomic DNA adsorption capacity of the APTS modified silica particles is increased in continuous system. It is also shown that the APTS modified silica particles can be used after ten adsorption-desorption cycle without any significant decrease in adsorption capacity. The APTS modified silica particles are used for adsorption of plasmid DNA from *E.coli* cells and adsorption capacity is found to be 0.21 mg/g in continuous system.

Key words: Silica, plasmid DNA, DNA separation, pEGFP-N3

ÖZET

Bu çalışmanın amacı, plazmid DNA ve genomik DNA'nın ayrılması için silika temelli afinite sorbentlerin hazırlanmasıdır. Bu amaçla, silika partiküllerin (ortalama boyut: 100-150 μm) yüzeyinin modifikasyonu için 3-aminopropiltrimetoksisilan (APTS) seçilmiş ve yüzeyinde NH_2 grubu taşıyan silika temelli afinite sorbent elde edilmiştir. APTS ile modifiye edilmiş ve edilmemiş silika partiküller elementel analiz ile karakterize edilmiştir. APTS modifiye silika partiküller üzerine genomik DNA adsorpsiyonu sürekli ve kesikli sistemde gerçekleştirilmiştir. İyonik şiddetin, pH'nın, sıcaklığın, başlangıç genomik DNA derişiminin ve akış hızının etkisi araştırılmıştır. Çalışmanın son aşamasında APTS modifiye silika partiküller kullanılarak, pEGFP-N3 plazmid DNA *E.coli* hücrelerinden ayrılmıştır ve sonuçlar ticari Qiagen DNA saflaştırma kiti ile karşılaştırılmıştır. Sonuçlar şöyle özetlenebilir: APTS modifiye silika partiküller üzerindeki NH_2 gruplarının miktarı 38-60 mmol/g arasındadır. APTS modifiye silika partiküllerin maksimum adsorpsiyon kapasitesi 49.3 mg/g olarak bulunmuştur ve bu değer pH 7.0'de elde edilmiştir. APTS modifiye silika partiküllerin genomik DNA adsorpsiyon kapasitesi sürekli sistemde artmıştır. Adsorpsiyon kapasitesinde önemli bir düşüş olmadan APTS modifiye silika partiküllerin on adsorpsiyon-desorpsiyon döngüsünden sonra tekrar kullanılabileceği gösterilmiştir. APTS modifiye silika partiküller *E.coli* hücrelerinden plazmid DNA adsorpsiyonu için kullanılmış ve adsorpsiyon kapasitesi sürekli sistemde 0.21 mg/g olarak bulunmuştur.

Anahtar Kelimeler: Silika, plazmid DNA, DNA ayırma, pEGFP-N3

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Corresponding author: A. Denizli, Hacettepe University, Department of Chemistry, Biochemistry Division, Beytepe, 06800, Ankara, Turkey

Tel: +903122977963 Fax: +90312 299 2163 E-Mail: denizli@hacettepe.edu.tr

INTRODUCTION

In recent years, large-scale production of biomolecules such as enzymes, proteins, hormones, and nucleic acids have become important because of the developments in the field of medicine, pharmacy, biochemistry and genetic engineering [1,2]. Biomolecules produced by biotechnological techniques should be pure enough to be used in the fields related to human health. In addition to purity, purified biomolecules should be biologically active. The other challenging point is the separation of biomolecules from complex biological media. Thus, many researchers have focused on the purification of biologically important proteins. However, the mentioned points limit the methods and conditions, which are used in purification processes for biomolecules [3,4].

Nucleic acids have many important roles like storage, description and transfer of genetic information. Plasmid DNA (pDNA) is an extrachromosomal DNA and usually found in bacteria. As plasmids can replicate independently and carry specific marker genes such as antibiotic resistance, they are usually used in recombinant DNA technology [5,6]. There are many purification methods aim to purify pDNA from cell extract. However chromatographic methods have some advantages over conventional DNA purification methods. Chromatographic methods are reproducible, scalable and generally use safe chemicals. Anion exchange, hydrophobic interaction, boronate affinity and size exclusion are some of the chromatographic methods reported for the purification of pDNA [7-9].

Silica, also known as silicium dioxide, has many applications in industry. Silica gel is a porous and granular form of silica. Silica gel is used as a chromatographic material because of its hydrophilicity and high surface area [10-15]. This study presented here aims to separate pDNA based on anion exchange chromatography. Silica particles are modified with APTS and amine-rich surface is obtained for this purpose. The APTS modified silica particles are characterized by elemental analysis. In order to determine genomic DNA (gDNA) adsorption behavior of the APTS modified silica particles, effects of pH, temperature, ionic strength, initial gDNA concentration and flow rate are investigated in continuous and batch systems. The APTS modified silica particles are used to separate pEGFP-N3 pDNA from *E.coli* cell extract in the last step of this study. In order to evaluate efficiency of the APTS modified silica particles, obtained results are compared with commercial Qiagen DNA purification kit.

MATERIALS AND METHODS

DNA from herring sperm, silica (100-150 μm) and (3-aminopropyl)trimethoxysilane (APTS) is obtained from Sigma (St.Louis, U.S.A.). DNA is kept at 4°C until used. All other chemicals are analytically pure and are obtained from Merck (Darmstadt, Germany). The commercial DNA purification kit is obtained from Qiagen Inc. (Germany). Water used in the experimental procedures is processed in the high flow ROpure LP reverse osmosis unit with cellulose membrane. The water used in this study is purified by using Barnstead D 3804 NANO pure cartridge. The conductivity of pure water is 18 mS/cm.

APTS modification of silica particles

The method applied for the APTS modification of silica particles is summarized in the following: 1 g of silica (SiO_2) is stirred in 10% HNO_3 solution for 30 minutes and then the particles are filtered. Silica particles are washed with pure water until the pH of washing water is neutral. At the same time, APTS is dissolved in 10 mL solution and the silica particles are added into this solution. It is important to note that different concentrations of APTS are used (5%, 10%, 15.5%, 20%) to prepare silica particles carrying different amounts of NH_2. Then, acetic acid is added into this basic solution until the pH is 7.0. The mixture is stirred at 80°C for 4 hours. At the end of the reaction the particles are washed with pure water. Schematic representation of APTS modification of silica particles is shown in Figure 1.

Characterization of the APTS modified silica particles

The APTS modified silica particles are characterized by elemental analysis and surface area measurements. After APTS modification

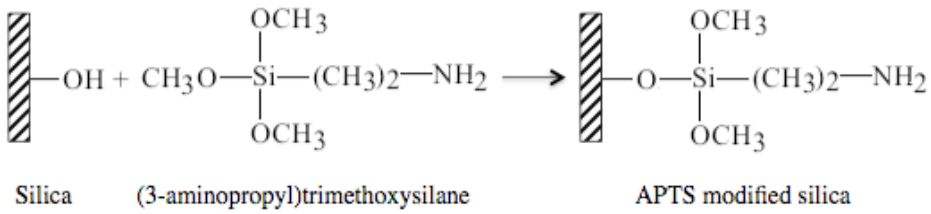

Figure 1. Schematic representation of the APTS modification of silica particles.

of silica particles, amount of NH_2 molecules on the surface of the particles are determined by elemental analysis (Leco, CHNS-932, USA) according to nitrogen stoichiometry. Percentage of carbon (C), hydrogen (H) and nitrogen (N) are measured. Brunauer, Emmett and Teller (BET) theory is used for the surface area measurements (Quantachrome NOVA 2000, USA). Liquid nitrogen is used for the gas adsorption and the APTS modified silica particles are vacuumed for 24 h before measurements.

Adsorption of gDNA from aqueous solutions

Adsorption of gDNA from aqueous solutions is performed in batch system and also in continuous system. Obtained results from these experimental systems are compared with each other. Batch system experiments are performed in 25 mL of beaker on a magnetic stirrer and the adsorption time is 20 h. Effects of pH, temperature and ionic strength on gDNA adsorption capacity of the APTS modified silica particles are investigated. Acetate (pH 4.0, 5.0 and 6.0) and phosphate buffers (pH 7.0 and 8.0) are used to adjust pH of the adsorption medium. Temperature of the adsorption medium is adjusted to 4°C, 15°C, 25°C, 37°C and change in adsorption capacity according to the temperature is recorded. In order to test effect of ionic strength on adsorption capacity, NaCl concentration of adsorption medium is changed between 0.5 and 1.5 M. In addition, effect of initial gDNA concentration on adsorption capacity is tested. Initial concentration of gDNA is changed between 0.25 and 2 mg/mL.

Continuous system experiments are achieved with a peristaltic pump. The APTS modified silica particles are filled into a Bio-Rad econo-column (5 cm long and 0.5 cm in diameter). Effect of flow rate is tested by changing it between 0.5 and 3.0 mL per minute. Concentration of initial gDNA concentration is changed between 0.25 and 2.0 mg/mL and effect of initial gDNA concentration on adsorption capacity is tested. Adsorption time is 90 min for all continuous system experiments. Desorption of gDNA from the APTS modified silica particles is achieved using Tris-EDTA (pH 8.0) buffer containing 1 M NaCl and reusability of the particles is investigated. Concentrations of all DNA samples are determined by measuring the sample absorbance at 260 nm with one unit OD_{260} corresponding to 50 µg/mL of dsDNA. DNA adsorption capacity of the silica particles is determined using Equation 1.

$$Q = (C_i - C_f)V / m \quad (1)$$

Where C_i and C_f are the initial and final DNA concentrations (mg/mL), respectively, V is the volume of the DNA solution (mL) and m is the amount of the APTS modified silica particles used (g). All the experiments are replicated at least three times.

Desorption and reusability

Desorption of gDNA from the APTS modified silica particles is performed with Tris-EDTA (ethylenediaminetetraacetic acid) solution (pH 8.0) containing 1 M NaCl. Ratio of desorption is calculated using equation 2.

$$\text{Desorption (\%)} = \frac{Desorbed\ DNA\ (\frac{mg}{ml})}{Adsorbed\ DNA\ (\frac{mg}{ml})} \times 100 \quad (2)$$

Plasmid DNA separation

In order to examine pDNA adsorption capacity of the APTS modified silica particles, pEGFP-N3 plasmid is chosen. This plasmid is responsible for encoding of green florescent protein (GFP, isolated from *Aeguorea victoria*) which is used to follow gene expression in-vitro, in-vivo and in-situ conditions. pEGFP-N3 is replicated in *E.coli* cells by using standard molecular biology techniques.

Table 1. The quantity of NH_2 of silica particles according to elemental analysis and titration results.

Ratio of APTS used in modification process (% APTS)	Amount of NH_2 (mmol/g) (Elemental analysis)	Amount of NH_2 (mmol/g) (Titration)
5.0	55.0	54.0
10.0	60.0	58.0
15.5	38.0	40.0
20.0	55.0	53.0

Alkaline lysis method is applied to extract pDNA from *E.coli* cells [16]. The buffers used in alkaline lysis method and pDNA adsorption experiments are supplied by Qiagen (plasmid buffer set). After alkaline lysis process, pDNA extract is applied to the column filled with the APTS modified silica particles.

RESULTS AND DISCUSSION

Characterization of the APTS modified silica particles

Morphology of the APTS modified silica particles are observed with optical microscope. The silica particles are irregular in shape and the particle size is around 100-150 μm. Specific surface area of the silica particles is 483 m^2/g. NH_2 quantity of the APTS modified silica particles is determined with elemental analysis and titration. The results are given in Table 1. As seen in Table 1, when 10% APTS is used in modification, maximum NH_2 loading is obtained. Maximum NH_2 loading is 60 mmol per gram of the silica particles. The particles containing 60 mmol NH_2/g are used in subsequent experiments.

Adsorption of genomic DNA from aqueous solutions

Batch system experiments

Effect of pH on gDNA adsorption capacity of the APTS modified silica particles is shown in Figure 2. Maximum adsorption is obtained at pH 7.0 and adsorption capacity is found as 49.8 mg/g. Adsorption of gDNA decreased at higher or lower pH levels. Adsorption of gDNA on unmodified silica particles is also shown in Figure 2. Non-specific gDNA adsorption is independent of medium pH and adsorption capacity is very low (0.5 mg/g) at all pH levels. This behavior indicates that the interaction involves mainly the electrostatic forces and maximum DNA adsorption capacity obtained at pH 7.0 indicates that the negatively charged gDNA molecules have the most favorable conformation to interact with positively charged APTS modified silica particles.

Figure 3 shows the effect of initial gDNA concentration on adsorption capacity. The amount of gDNA bound onto the APTS modified silica particles first increased and then reached

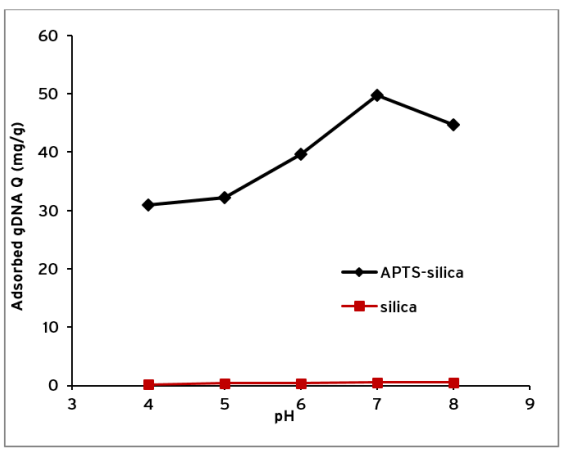

Figure 2. Effect of pH on gDNA adsorption. Concentration of gDNA: 1.0 mg/mL; adsorption time: 20 h; T: 25°C.

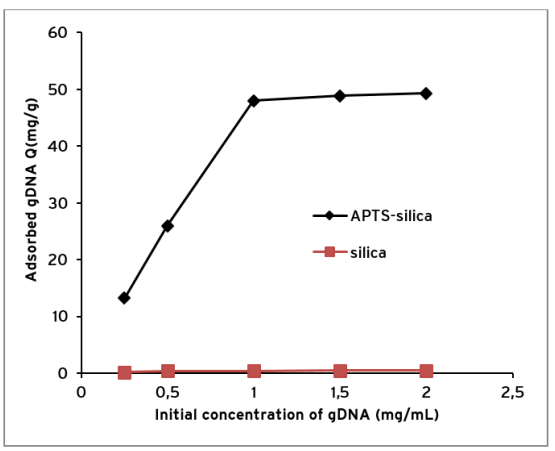

Figure 3. Effect of initial gDNA concentration on gDNA adsorption. pH 7.0 phosphate buffer; adsorption time: 20 h; T: 25°C.

a saturation value at 1 mg/mL initial gDNA concentration. The maximum adsorption capacity is 49.3 mg/g at 2.0 mg/mL of initial gDNA concentration. The adsorption isotherm has a sharp initial rise suggesting a high affinity between charged groups. When all NH_2 groups on the surface of the APTS modified silica particles are loaded with gDNA molecules the adsorption capacity reached a plateau value and remained almost the same. gDNA adsorption on unmodified silica particles is investigated, as well. As shown in Figure 3, gDNA adsorption on unmodified silica particles is very low.

Figure 4 shows the effect of ionic strength on gDNA adsorption capacity of the APTS modified silica particles. According to the results, when the concentration of NaCl is increased, gDNA adsorption capacity is decreased. The value of maximum gDNA adsorption capacity is 49.1 mg/g when concentration of NaCl is 0.0 M. However, this value decreased to 35.0 mg/g when the concentration of NaCl increased to 1.5 M. Because of the positively charged salt molecules interact with negatively charged phosphate groups of gDNA molecules, interaction between NH_2 groups of the APTS modified silica particles and phosphate groups of gDNA molecules decreases.

Effect of temperature on gDNA adsorption capacity of the APTS modified silica particles is investigated and the results are given in Figure 5. Adsorption of DNA is decreased from 61.0 mg/g to 25.8 mg/g when the temperature is increased

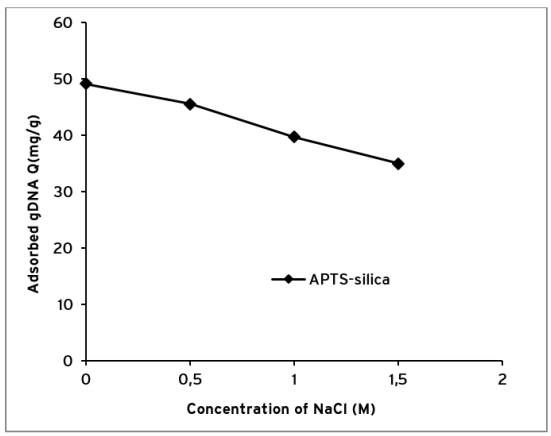

Figure 4. Effect of ionic strength on gDNA adsorption. pH 7.0 phosphate buffer; concentration of gDNA: 1.0 mg/mL; adsorption time: 20 h; T: 25°C.

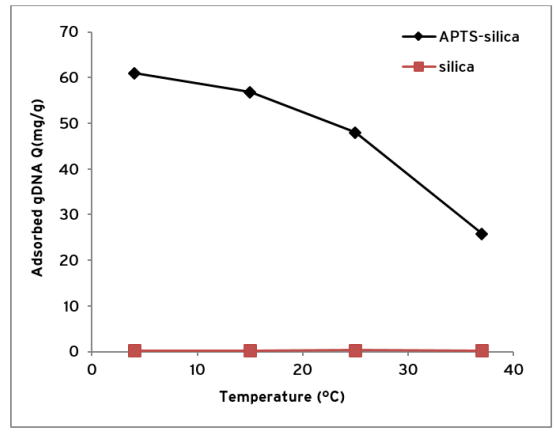

Figure 5. Effect of temperature on gDNA adsorption. pH 7.0 phosphate buffer; concentration of gDNA: 1.0 mg/mL; adsorption time: 20 h.

from 4°C to 37°C, respectively. As the adsorption of gDNA molecules onto APTS modified silica particles through electrostatic interactions is an exothermic reaction, adsorption capacity decreases at high temperatures. Nonspecific gDNA adsorption on unmodified silica particles is also given in Figure 5. Adsorption of gDNA on unmodified silica particles is very low at all temperatures.

Continuous system experiments

Effect of flow rate on gDNA adsorption in continuous system experiments is shown in Figure 6. According to the Figure 6, gDNA adsorption capacity of the APTS modified silica particles is decreased from 31.2 mg/g to 23.1 mg/g with the increase in the flow rate from 0.5 mL/min to 3 mL/min. Retention time for gDNA molecules decreases when the flow rate is high and as a result, interaction time between gDNA molecules and NH_2 molecules decreases.

Effect of initial gDNA concentration on gDNA adsorption in batch and continuous systems is given in Figure 7. When the results are compared, it is obvious that adsorption capacity of the APTS modified silica particles is higher in continuous system (32.3 mg/g) then it is in batch system (6.9 mg/g). It is easier for gDNA molecules to reach NH_2 groups on the surface of the APTS modified silica particles in continuous system. The flow makes the mass transfer easier. Adsorption capacity of unmodified silica particles is also investigated in continuous system. As it is shown in Figure 7,

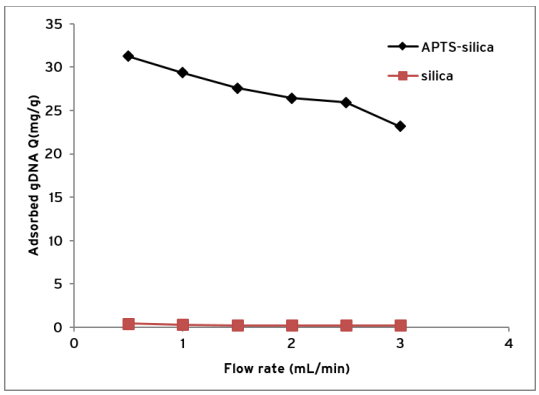

Figure 6. Effect of flow rate on gDNA adsorption. pH 7.0 phosphate buffer; concentration of gDNA: 1.0 mg/mL; adsorption time: 90 min; T: 25°C.

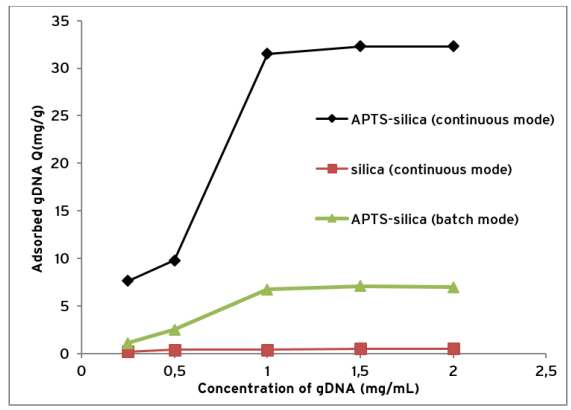

Figure 7. Comparison of batch and continuous DNA purification systems. pH 7.0 phosphate buffer; flow rate: 0.5 mL/min; adsorption time: 90 min; T: 25°C.

gDNA adsorption on unmodified silica particles is very low. Modification of the silica particles by NH_2 groups makes the adsorption process specific and increases the gDNA adsorption capacity.

Desorption and reusability

Desorption of gDNA from the APTS modified silica particles is achieved using Tris-EDTA buffer (pH 8.0) containing 1 M NaCl and 75% desorption ratio is obtained. As the interaction between NH_2 groups and gDNA molecules are electrostatic, salt containing buffers are useful as a desorption agent. Reusability of the APTS modified silica particles is also tested in continuous system and the results are given in Figure 8. The APTS modified silica particles are used 10 times for gDNA adsorption-desorption and no significant decrease is observed.

Adsorption of pDNA from *E.coli* cells

In this part of the study, the APTS modified silica particles are used to separate pDNA from *E.coli* cells. After the pDNA called pEGFP-N3 is replicated in *E.coli* cells, pEGFP-N3 pDNA is isolated from the cells by alkaline lysis. Then, pEGFP-N3 extract is given to the column filled with the APTS modified silica particles. Adsorption time is 90 min. Adsorption capacity of the APTS modified silica particles for gDNA and pDNA are compared with each other. Performance of Qiagen DNA purification kit is also tested for gDNA and pDNA and the results are presented in Table 2. When the results are evaluated, the APTS modified silica particles show a good performance close to Qiagen DNA purification kit. Although the APTS modified silica particles and Qiagen DNA purification kit adsorb gDNA, most of the gDNA is removed in the alkaline lysis step from *E.coli* cells. Therefore, adsorption of gDNA by the APTS modified silica particles and Qiagen DNA purification kit is not a big problem. Plasmid DNA adsorption capacities of the APTS modified silica particles (0.21 mg/g) and Qiagen DNA purification kit (0.35 mg/g) are very close to each other. As a result, the APTS modified silica particles are promising materials to separate pDNA from *E.coli* extract.

A comparison of the maximum adsorption capacity of the APTS modified silica particles with those of some of other affinity matrices reported in literature is given in Table 2. As seen in the table, the highest adsorption capacities

Table 2. Comparison of gDNA and pDNA adsorption capacities of the APTS modified silica particles and Qiagen DNA purification kit.

DNA source	APTS modified silica particles	Qiagen DNA purification kit
	Adsorbed pDNA (mg/g)	Adsorbed pDNA (mg/g)
pEGFP-N3 pDNA extract	0.21	0.35
	Adsorbed gDNA (mg/g)	Adsorbed gDNA (mg/g)
gDNA from herring sperm (1.0 mg/mL)	31.3	40.5

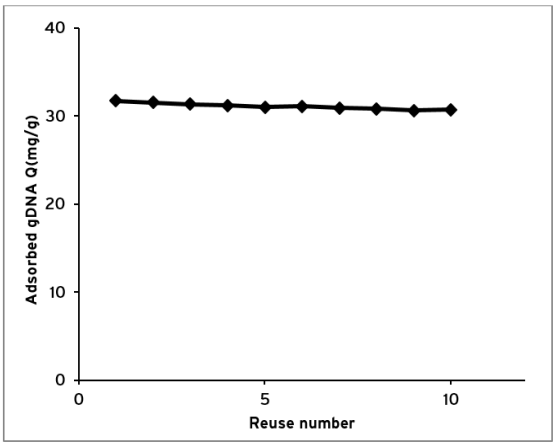

Figure 8. Reusability of the APTS modified silica particles. pH: 7.0 phosphate buffer; flow rate: 0.5 mL/min; concentration of gDNA: 1.0 mg/mL; T: 25°C.

achieved in this study for both gDNA and pDNA are quite comparable with the previous repots. Differences in adsorption amounts are results from the properties of each adsorbent in terms of bulk structure, functional groups, ligand densities, adsorbent size, porosity and surface area. These results indicate that the APTS modified silica particles have a sufficient affinity to capture gDNA and pDNA molecules.

CONCLUSION

In this paper, we described the suitability of the APTS modified silica particles for the separation of pEGFP-N3 from *E.coli* cells. In the first part of the study, gDNA adsorption capacity of the APTS modified silica particles is evaluated. In the second part, the APTS modified silica particles are used for the separation of pEGFP-N3 pDNA from *E.coli* cells. APTS modification of silica particles increased gDNA adsorption capacity. The interaction between gDNA molecules and NH_2 groups on the surface of the APTS modified silica particles are mostly electrostatic and formed the basis of the separation process. Suitability of the APTS modified silica particles for pDNA adsorption is also compared with Qiagen DNA purification kit. The pDNA adsorption capacity of APTS modified silica particles is very close to Qiagen DNA purification kit. In conclusion, the APTS modified silica particles can be used for specific separation of pDNA from *E.coli* cells.

Table 3. Comparison of gDNA and pDNA adsorption capacities of various adsorbents.

Carrier	Ligand/Interactions	Adsorption capacity	Type of DNA	Reference
Silica magnetite nanocomposite	Tetraethoxysilane	43.1 mg/g	pDNA	[10]
Magnetite-silica composite	Tetraethoxysilane	7.6 mg/g	pDNA	[17]
Magnetic hydroxyapatite nanoparticles	Hydroxyapatite	6.7 μg/mg	pDNA	[14]
Capto Adhere	Anion exchange	60.0 μg/ml	pDNA	[4]
Silanized polymeric nanoparticles	Phenylboronic acid (PBA)	672.4 mg/g	gDNA	[18]
PHEMAH cryogel	MAH	13.5 mg/g	pDNA	[19]
Magnetic PHEMAH nanoparticles	MAH	154 mg/g	pDNA	[20]
Agarose	L-histidine	0.53 mg/ml	pDNA	[21]
Poly(glycidyl methacrylate) beads	Ethylenediamine	90 μg/g	pDNA	[22]
Gold nanoparticles	Bovine serum albumin	1.8 mg/mg	gDNA	[23]
Magnetic nanoparticles	Acridine orange	161 μg/mg	gDNA	[24]
Magnetic nanoparticles	Acridine orange	119 μg/mg	pDNA	[24]
CIM monolithic column	Butyl	6.5 mg/ml	pDNA	[25]
APTS modified silica	Amino groups	32.3 mg/g	gDNA	This study
APTS modified silica	Amino groups	0.2 mg/g	pDNA	This study

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