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**ORIGINAL PAPER**

# Characteristics of pDNA-loaded chitosan/alginate-dextran sulfate nanoparticles with high transfection efficiency

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## Abstract

*In this research pDNA-loaded chitosan/alginate-dextran sulfate nanoparticles with encapsulation efficiency from 83.07 to 94.71% were prepared using modified ionotropic gelation method. Encapsulated pDNA was tightly bound to polymers and protected from hydrolysis by a BamHI nuclease. All prepared nanoparticles were non-toxic for cells of HEK298 line. Moreover, chitosan/alginate-dextran sulfate nanoparticles increased proliferation of the cells in contrast to Lipofectamin™, used as a positive control for transfection. The particles prepared were effective in transfection of pEGFP-N2 plasmid into the human cells. High homogeneity of nanoparticles, reliable protection of the encapsulated DNA, and considerably high transfection efficiency along with absence of toxicity make DNA-loaded chitosan/alginate-dextran particles a suitable gene carriers in vitro and promising candidates for design of delivery systems for gene therapy.*

**Keywords**: Biopolymeric nanoparticles, dextran sulfate, transfection efficiency, cytotoxicity, carrier

## 1. Introduction

Gene therapy is a promising approach in treatment of inherited and acquired diseases based on modifying of gene expression (NI & al [1]). Since therapeutic genes, introduced as naked DNA, have no specific targeting, show low transfection efficiency and undergo degradation by nucleases, there is a demand in development of gene carriers on the basis of non-toxic biopolymeric nanoparticles, protecting DNA and improving transfection (BOYLAND & al [2]). Chitosan is an inexpensive natural hydrophilic linear polysaccharide and the alkaline deacetylated product of chitin, which is produced from the exoskeleton of crustaceans, it is also found in fungi (MUZZARELLI & al [3]). Chitosan was shown to have advantageous biological properties, such as biodegradability, biocompatibility, antimicrobial activity, non-toxicity, good homeostatic properties and permeability enhancement (LI & al [4]; ANITHA & al [5]). Due to these advantageous properties chitosan has been used for biomedical applications (YI & al [6]), for metal adsorption, in biotechnology (BECKER & al [7]), in drug formulations (BALDRICK [8]), for waste water treatment (GERENTE & al [9]), cosmetics (HARRIS & al [10]), food science (FERNANDEZ-SAIZ & al [11]), enzyme immobilization (CHIOU & al [12]) and controlled release of drugs (BHATTARAI & al [13]). Chitosan is a cationic polymer that interacts electrostatically with phosphate backbones of DNA, which bind to primary amine groups of chitosan at acidic pH forming chitosan–DNA complexes (OPANASOPIT & al [14]). In this complex DNA is protected from degradation. It was also shown, that chitosan–DNA complex has higher efficiency for transfection in comparison to naked DNA, confirming that

cationic polymers can be used as DNA carriers for non-viral gene delivery (LUO & al [15]). Alginate is an anionic polysaccharide, which produced by marine brown algae; alginate unique specification in ionotropic gelation made possible to encapsulate proteins (vaccines, enzymes) and entire cells (MI & al [16]). Alginate is a useful biopolymer to prepare nanocapsules due to its good biocompatibility, biodegradability, non-toxicity, and mucoadhesion properties (TACHAPRUTINUN & al [17]). Alginate/chitosan polyionic complexes form due to interactions between the carboxyl groups of alginate and the amine groups of chitosan during ionic gelation. This complex protects encapsulated compounds, is biocompatible and biodegradable, and limits the release of encapsulated materials more effectively than alginate or chitosan used separately (YAN & al [18]).

Dextran sulfate is also a biocompatible and biodegradable polyanionic polymer. In drug delivery applications many drug conjugates of dextran sulfate have been reported (MITRA & al [19]). Dextran sulfate–chitosan nanoparticles are formed due to the electrostatic interaction between polymeric chitosan backbone and dextran sulfate (CHEN & al [20]). Furthermore, the combination of chitosan and dextran sulfate as formulation materials, in an optimal charge ratio, may act synergistically to incorporate, protect and release therapeutic molecules (SHARMA & al [21]). This research comprises of physicochemical characterization of DNA-loaded nanoparticles, study of DNA encapsulation efficacy, complex stability, demonstration of DNA protecting ability, cytotoxicity, and influence of prepared nanoparticles on cell viability. To our knowledge, there are no published data on DNA loaded-chitosan/alginate-dextran sulfate nanoparticles, in which transfection potential of the plasmid into a human cell line (HEK 293) was evaluated.

## 2. Materials and Methods

### 2.1. *Materials, cultures and growth conditions*

Low molecular weight chitosan ($M_w$=50 kDa, degree of deacetylation (according to analysis result DD= 94.3%)), sodium salt of dextran sulfate (molecular weight >500 kDa), chitosanase, sodium alginate of medium viscosity (3500 cps, 2% w/v aqueous solution at 25 °C), MTT and calcium chloride were purchased from Sigma-Aldrich (USA). Sodium sulfate, sodium acetate, Sodium Dodecyl Sulfate (SDS) , Tris-Cl, tryptone, yeast extract, ethanol, ammonium acetate, lysozyme, ethylene diamine tetra acetic acid (EDTA), sodium chloride, isopropanol, agarose, agar, glucose were purchased from Helicon Ltd. (Russia). Sterile syringe filter was purchased from Corning Incorporated (Germany). Acetic acid was obtained from Panreac (Spain). Lipofectamine™ 2000 Transfection Reagent was obtained from Invitrogen Inc. (Carlsbad, CA, USA), fetal bovine serum (FBS, heat inactivated), 1× trypsin-EDTA, Dulbecco's modified essential medium (DMEM), penicillin-streptomycin, were purchased from Gibco (Life technologies Inc., UK), HEK298 cells were obtained from Altogen (USA), Fastruler High Range DNA ladder, nuclease *BamH* I was obtained from Thermo Scientific (Thermo Fisher Scientific Inc., USA), 24 well cell culture plate and cell culture flask were purchased from Nunc (Nunclon$^{TM}$, Denmark). Human embryonic kidney (HEK293) cell line was obtained from Altogen (USA). HEK293 cells were cultured in fresh Complete culture medium, which comprised of DMEM, supplemented with 10%v/v FBS, 2mM L-glutamine, antibiotic solution penicillin (50U/ml) and streptomycin (50 μg/ml) at 37°C in a humidified atmosphere containing 5% $CO_2$ (Esco CelCulture $CO_2$ incubater; **Scimetrics Inc.,** Houston, USA). Cells were grown until confluent and then treated with trypsin solution (0. 25% trypsin and 0. 25% EDTA) at 37°C for 5 min. DNA of pEGFP-N2 (4700 bp) was used for preparation of all types of nanoparticles.

***2.2. Preparation of pDNA-loaded chitosan/alginate-dextran nanoparticles***

The optimum conditions for particle preparation were following: alginate/chitosan ratio of 1, $CaCl_2$/alginate ratio of 0.2% and N/P ratio of 5 at pH 5.3 (SHARMA & al [21]). Sodium alginate solution was prepared in concentration of 0.1% w/v by alginate dissolution in deionized water at room temperature for 30 min under magnetic stirring (500 rpm) and then diluted to final concentration of 0.004%w/v. Chitosan solution (0.1%w/v) was dissolved in 1% v/v of acetic acid under mechanical stirring at room temperature (~25°C) for 2h. $CaCl_2$ solution (0.1%w/v) was prepared by dissolving in deionized water. Chitosan and $CaCl_2$ were used with respect to $CaCl_2$ /alginate, alginate/chitosan and N/P ratio. Plasmid DNA was diluted in a 5 mM sodium sulfate solution and used in final concentration of 10µg/ml. Before use chitosan, alginate and calcium chloride solutions were filtered through a 0.22 µm syringe filter for removal of impurities. Biopolymeric nanoparticles were prepared using combined process of ionotropic gelation and precipitation via interactions between the carboxyl groups of alginate and the amine groups of chitosan. Briefly, 1ml of diluted $CaCl_2$ solution through insulin syringe needle at the speed of 30 mL/h was added dropwise to 3 ml of sodium alginate solution followed by adding of DNA (10 µg/ml) dropwise to alginate/$CaCl_2$ solution under magnetic stirring for 10 min. Then with respect to recommended ratio; 4ml of chitosan solution was added to pDNA-alginate/ $CaCl_2$ solution through needle as described above. Finally the solution was mixed under stirrer for 30 min to complete nanoparticles formation, and then the nanoparticles were separated from the suspension by centrifuging at 20,000 rpm for 20 min. For preparation of plasmid-loaded chitosan/alginate-dextran sulfate the dextran sulfate with a final concentration of 0.001% w/v was added to alginate solution with the same process as described above. Chitosan nanoparticles loaded with pDNA were prepared using a complex coacervation process (SAMBROOK & al [22]). pDNA was diluted in a 5 mM sodium sulfate solution. Chitosan and pDNA solutions were preheated to 55 °C separately. An equal volume of both solutions were rapidly mixed together and vortexed at maximum speed for 40 s. Finally the solution was incubated at room temperature (25 ± 1 °C) for 30 min to complete nanoparticles formation, and then the nanoparticles were separated from the suspension by centrifuging at 20,000 rpm for 20 min.

***2.3. Characterization of DNA/nanoparticles:*** *Particle size, polydispersity index, and zeta potential*

Evaluation and determination of nanoparticle size was performed using a dynamic light scattering (DLS) instrument Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK). The measurements were carried out at 25 °C using scattering angle of 173°. Each sample was measured three times using disposable sizing cuvette. The polydispersity index (PDI) was measured for each sample as a measure of particle size distribution. The zeta potential was determined Laser Doppler Velocimetry (LDV) using the Zetasizer Nano ZS with Smoluchowski as a measurement model. The measurements were performed at 25 °C in disposable folded capillary cells. Particle morphology of the selected nanoparticles was studied using scanning electron microscopy. One drop of the selected nanoparticles was placed on a thin glass and allowed to dry and then was examined with the electron microscope.

***2.4. Enzymatic treatments and Agarose gel electrophoresis***

DNA was digested using restriction endonuclease *BamH*I according to recommendation of manufacturer. To treat encapsulated DNA 5 µl of nanoparticle suspension were mixed with 2 µl of 10xbuffer and 12 µl of µQ water. Ten units of *BamH*I (1 µl) was added to previously mixed compounds. All mixtures were incubated at 37 °C for 3 h and the reaction was terminated by incubating at 65 °C for 5 min as recommended by manufacturer. DNA digestion was revealed using gel electrophoresis. To release encapsulated DNA nanoparticle

suspensions were treated with chitosanase digestion was carried out at 37 °C for 4h in sodium acetate buffer (50 mM- pH: 5.5). After all enzymatic treatments DNA release and digestion was revealed using gel electrophoresis as described above. To evaluate DNA binding capacity of chitosan, chitosan/alginate and chitosan/alginate-dextran sulfate agarose gel electrophoresis was used. For separation of DNA 1% agarose gel was prepared on Tris–borate /EDTA buffer (pH 8.0) (SAMBROOK & al [22]). The same buffer was used to perform electrophoresis. Naked pDNA and prepared nanoparticles, loaded with DNA, were mixed with a loading dye and placed into wells in the agarose gel. Electrophoresis was run at constant voltage 100 V for 40 min. The gel was stained with ethidium bromide and visualized under UV light. Electrophoresis results were documented using the gel documentation system, by Bio-Rad ChemiDoc$^{TM}$ XRS molecular imager (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

***2.5. Evaluation of encapsulation efficiency***

The encapsulation efficiency of DNA was determined by detecting both entrapped and non-entrapped DNA. The amount of plasmid DNA encapsulated in the nanoparticles was calculated by considering the difference between the total amount of added DNA and the amount of non-entrapped DNA remaining in the supernatant after the centrifugation (20000 g, 20 min) of prepared nanoparticles. For this purpose, the amount of DNA in supernatant was spectrophotometrically measured at 260 nm (MAO & al [23]).

***2.6. Evaluation of Cytotoxicity assay of nanoparticles***

Cytotoxicity of plasmid-loaded nanoparticles was evaluated by the MTT-based cytotoxicity assay in cells. MTT solution 5mg/mL was prepared in PBS and the solution was sterile-filtered through a 0.22 µm syringe filter. HEK 293 cells were seeded in 96-well plate at $5\times10^4$ cells/cm$^2$ in 100 µL of complete culture medium and incubated for 24 hours at 37°C under 5% $CO_2$ to be grow until 80% confluent. Afterward, the medium was removed and supplied with 100 µl of fresh culture medium and the cells in triplicate were exposed to the nanoparticles (with different formulation) at a dose of 5 µg pGFP-N2/ml and incubated for 48 h. Non-treated cells and cells treated with naked plasmid DNA and Lipofectamine$^{TM}$ were used as controls and incubated for the same duration of time. After 48 hours the medium was replaced with 80 µL of fresh complete DMEM and 10 µl of prepared MTT solution (5mg/ml) to reach final volumes of 100 µl and final concentration of MTT in culture medium 0.5 mg/ml was added to empty wells were filled with 100 µl of PBS and plate was covered with aluminum foil and incubated at 37˚C for 4 hours until purple precipitate becomes visible. Subsequently the medium was removed, and the formazan precipitates formed in living cells were dissolved in 100 µl DMSO per well and incubated for 15 min at 37°C. Relative viability (%) was calculated based on reading absorbance at 555 nm and also 700 nm as a reference in disposable cuvettes using a micro-plate reader TECAN, infinite® M200-PRO (Switzerland). Viability of control cells (cells incubated with DMEM alone) was arbitrarily defined as 100%. The relative cell viability was calculated as $[Abs]_{sample}/[Abs]_{control} \times 100$. Data are presented as mean±SD ($n = 3$).

***2.7. In-vitro transfection study***

Human embryonic kidney (HEK293) cell line was used for transient transfection experiments. For transfection studies of the particles, HEK293 cells were seeded into 24-well plates at a density of $5\times10^4$ cells/well and grown for 24 hours in 500µl of fresh Culture medium DMEM supplemented with 10%v/v FBS, 2mM L-glutamine, antibiotic solution penicillin (50U/ml) and streptomycin (50 µg/ml) at 37°C in a humidified atmosphere containing 5% $CO_2$. Transfection was carried out when cells reached 80% confluent. Immediately before transfection, cells were rinsed twice with PBS and the medium was replaced with 200 µl of fresh Culture medium DMEM. After dilution of particles with serum-

free medium, certain amount of plasmid DNA-loaded nanoparticles (with different formulation) having an amount of DNA equivalent to 1 µg per well were added to each cell to final volume 500 µl and incubated for 6 h at 37°C in a humidified atmosphere containing 5% CO2 and then the medium was increased to 1 ml with complete culture medium DMEM and incubated for another 48 h in humidified atmosphere (5% $CO_2$, 95% air, 37°C). Non-treated cells and cells incubated with naked plasmid DNA were used as negative controls. For positive control DNA mixed with Lipofectamine was added to cells and incubated under the same conditions. Lipofectamine/pDNA complexes were prepared according to manufacturer's protocol: pDNA and Lipofectamine were diluted in Serum-free medium separately and mixed together with a 1:1 ratio and then Lipofectamine/pDNA complexes were added to the cells and incubated in 500µl of fresh serum-free medium and after 6 h the medium was replaced with complete medium and incubated for another 48 h as described above. After transfection, the cells were washed with phosphate buffer solution. Expression of GFP was first visualized by fluorescent microscopy (Fluorescence Microscope, Axio Observer S 100 Zeiss, Germany) and then the cells were trypsinized with trypsin solution (100µl/well) and incubated at 37°C for 5 min. Afterward the medium culture was added to each well (200µl/well) and the cells were collected by centrifugation at 1400 rpm, 4 min (Hettich, Germany). Collected cells were resuspended in PBS and the percentage of the transfection efficiency was determined by flow cytometer (Guava flow cytometry easyCyte, Millipore, USA)

## 3. Results and discussion

### 3.1. *Particle size, polydispersity index, and zeta potential*

One of the important parameters that could affect the transfectivity of particles is their size. According to the Table 1 the average hydrodynamic diameters for these three different particles varied from 147.3 nm to 196.3 nm. As it can be seen in Table 1, the size of DNA-chitosan/alginate nanoparticles was smaller than those of chitosan-DNA nanoparticles. Alginate can be cross-linked with the plasmid DNA using polyvalent cations such as $Ca^{2+}$ which results in forming more compact nanoparticles in comparison to particles comprising solely of chitosan (GUO & al [24]).

**Table 1.** Particle size, size distribution, polydispersity, zeta potential and encapsulation efficiency of the prepared pDNA-loaded nanoparticles.

| Sample name | Average polydispersity (PDI) | Average particle size (nm) ± STD | Average zeta potential (mV) ± STD | Average Encapsulation efficiency (%) ± STD | Size distribution of particle by Intensity (nm) ± STD (100% of NPs) | Size distribution of particle by volume (nm) ± STD (100% of NPs) | Size distribution of particle by number (nm) ± STD (100% of NPs) |
|---|---|---|---|---|---|---|---|
| *Chitosan* | 0.135±0.001 | 182.7±9.48 | 36.1±1.82 | 83.07±2.34 | 203.4±6.102 | 222±6.66 | 127±3.81 |
| *Chitosan/alginate* | 0.161±0.005 | 147.3±6.22 | 28.9±1.24 | 91.33±1.63 | 187.1±14.96 | 196±15.68 | 108±8.64 |
| *Chitosan/alginate -dextran* | 0.146±0.002 | 196.3±11.29 | 26.8±1.04 | 94.71±2.03 | 224.9±13.23 | 234.1±15.11 | 154.7±8.60 |

Although in presence of dextran sulfate the mean particle size was increased as reported in our previous study (KHORRAM & al [25]). Particle size distribution profile of pDNA loaded chitosan/alginate-dextran nanoparticles is shown in Figure 1. Size distribution of particles was reported as polydispersity index (PDI). PDI is a measure of homogeneity in dispersed systems and ranges from 0 to 1, where homogeneous dispersion has PDI value close to zero, while PDI values greater than 0.3 suggest high heterogeneity (ZHANG & al [26]). As it is shown in Table 1

PDI values are lower than 0.2, indicating low heterogeneity of the prepared nanoparticles. Zeta potential is one of the important particle characteristics: greater zeta potential values result in stabilization of suspensions due to the electrostatic repulsion of particles with the same electric charges preventing aggregation (DIEZ & al [27]). The positive charge of particles can facilitate their adherence to negatively charged cellular membranes and therefore this may increase intracellular uptake. As it can be seen in Table 1 zeta potential of pDNA-loaded nanoparticles was positive and varied from 26.8 to 36.1 mV (measured at pH 5.3). These results may indicate that there was not an excess amount of pDNA in the mixture and also show that the binding of pDNA to polymers, was complete. Furthermore, formation of particles with residual amino groups and free amines at the surfaces would be responsible for the positive zeta potential. Zeta potential distribution of chitosan/alginate-dextran nanoparticles is shown in Figure 2.

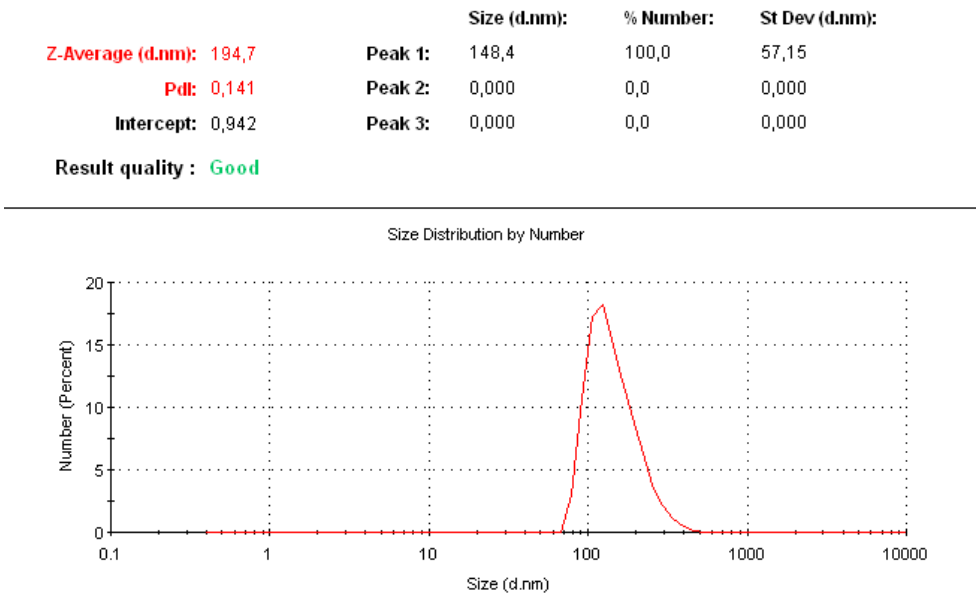


**Figure 1.** Particle size distribution of pDNA-loaded chitosan/alginate-dextran sulfate nanoparticles

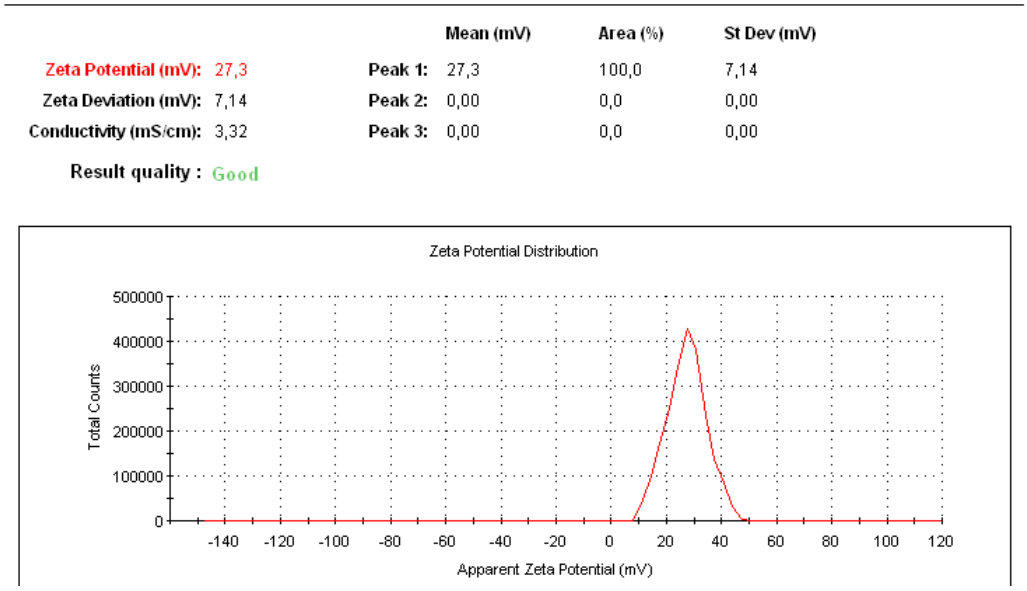


**Figure 2.** Zeta potential distribution of pDNA-loaded chitosan/alginate-dextran sulfate nanoparticles.

### *3.2. Study of encapsulation efficiency (EE)*

According to the Table 1 the EE of particles was high and varied from 83.07 to 94.71%. As shown in the Table 1 in the presence of dextran sulfate, encapsulation efficiencies substantially increased. Enhancement of EE in the presence of dextran sulfate can be a result of stronger matrix reinforcement in these nanoparticles in comparison to chitosan or chitosan/alginate nanoparticles.

### *3.3. Study of Morphology using Scanning Electron Microscopy (SEM)*

According to the SEM studies, the morphology of the pDNA-loaded nanoparticles seemed to be spherical. In contrast to data obtained using DLS aggregated nanoparticles were also observed. These discrepancies can have following explanation: in DLS analysis, the particle size was measured in aqueous suspensions, where chitosan has a tendency to swell moreover DNA requires water to form a natural helical conformation (DICKERSON & al [28]). For the SEM studies, the samples were dried, resulting in particles size reduction. This may explain the differences between the SEM and DLS results (AKTAS & al [29]). Particle morphology of the chitosan/alginate-dextran sulfate nanoparticles can be seen in Figure 3.

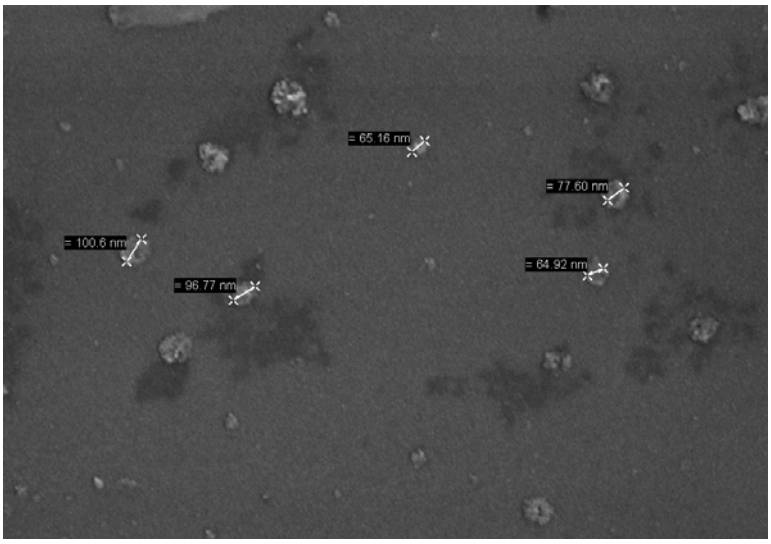


**Figure 3.** SEM of chitosan/alginate-dextran nanoparticles

### *3.4. DNA binding*

The ability of the particles to entrap pDNA and capacity of the polymers and pDNA to form nanoparticles together was studied using the agarose gel electrophoresis. The results are shown in Figure 4 and the naked plasmid DNA is seen in lane 2, while pDNA loaded-chitosan/alginate and chitosan/alginate-dextran nanoparticles were loaded in lanes 3 and 4, respectively. Figure 4 demonstrates that, pDNA loaded into the nanoparticles remains in the gel loading wells within nanoparticles, which are unable to migrate in agarose gel due to their size. This suggests strong interaction of chitosan and pDNA. Absence of the free plasmid confirms complete coupling of pDNA and polymers. These results are in agreement with the measured EE that can be seen in Table 1. According to Figure 4, digest with restriction endonuclease *Bam*HI of chitosan/alginate-dextran particles (lane 5) did not influence pDNA loaded particles. Moreover the particles digested with chitosanase after *Bam*HI treatment (lane 6) didn't show any changes in form and size of plasmid and identical to naked pDNA run in lane 2. This suggests, that plasmid DNA packed into particles is protected from *Bam*HI digestion (lane 5) and can be released being intact from particles.

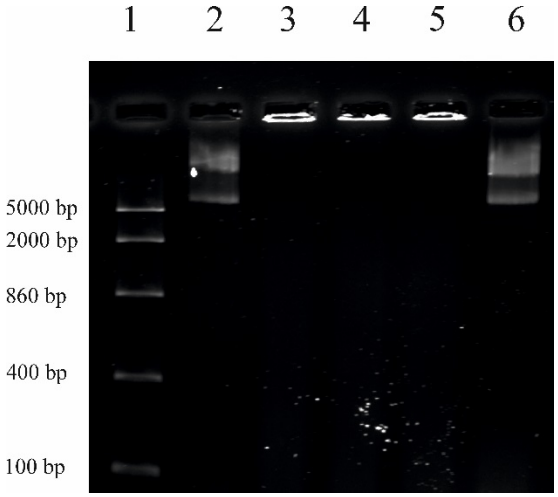


**Figure 4.** Electrophoretic mobility analysis of the free and the prepared plasmid DNA (pEGFP-N2) nanoparticles: lane 1: FastRuler Middle Range DNA Ladder (Thermo Scientific, Vilnius,Lithuania) bands size 5000 bp, 2000 bp, 860 bp, 400 bp, 100 bp; lane 2: naked plasmid DNA (pEGFP-N2); lane3: chitosan/alginate nanoparticles; lane 4: chitosan/alginate-dextran nanoparticles , lane 5: chitosan/alginate-dextran digested with BamH I (nanoparticles was incubated with BamH I at 37ºC for 1h), lane 6: chitosan nanoparticles digested with chitosanase after BamH I digestion (nanoparticles was incubated with BamH I at 37ºC for 1 h. BamH I is inactivated by incubation at 65ºC for 20 min therefore using thermal inactivation; the reaction was stopped by incubation the samples at 65ºC for 20 min. Then nanoparticles were subjected to chitosanase for 4 h).

### *3.5. Cytotoxicity assay of nanoparticles*

Cell toxicity of nanoparticles was investigated by MTT-based cytotoxicity assay in HEK293 cells. Naked pDNA and Lipofectamine/DNA complex were used as control. As shown in Figure 5, all prepared nanoparticles showed no cell toxicity in cells even at concentrations of five times more than those used for transfection studies. Moreover, results show that chitosan/alginate-dextran sulfate nanoparticles had stimulated cell proliferation in comparison to other prepared nanoparticles. In contrast, average cell viability of Lipofectamine/pDNA complex was about 60% of control. These results suggest that all nanoparticles prepared in this study, especially chitosan/alginate-dextran sulfate are non-toxic and biocompatible. This characteristic makes the nanoparticles promising candidates for developing gene transfer vectors.

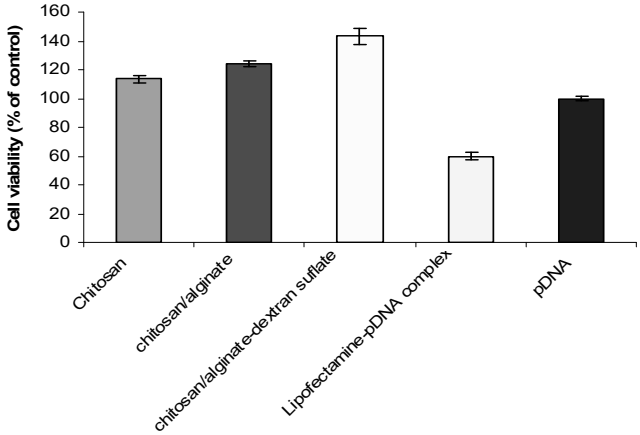


**Figure 5.** Mean value viability (± STD; n=3) of HEK293 cells after transfection with pDNA, Lipofectamine/pDNA complex, chitosan, chitosan/alginate and chitosan/alginate-dextran sulfate.

### *3.6. In-vitro transfection study*

To determine the efficiency of chitosan, chitosan/alginate and chitosan/alginate-dextran sulfate nanoparticles in transferring pDNA into cells, the pDNA-loaded nanoparticles were incubated with the HEK 293 cells for 48h as described previously. Transfected cells were analyzed under the fluorescence microscope. Transfection efficacy of the nanoparticles was compared to one of Lipofectamine which was used as positive control. Flowcytometric analysis of the transfected cells indicated 55.39%, 38.49%, 35.04% and 16.67% GFP expression for Lipofectamine, chitosan/alginate-dextran nanoparticles, chitosan/alginate and chitosan nanoparticles, respectively. Figure 6 shows the result of GFP expression in the HEK 293 cells. The transfection rate of chitosan/alginate nanoparticles was greater than one of nanoparticles prepared from chitosan solely. This may be due to alginate properties which can increase endosomal release as a proton sponge via alginate degradation. In addition, chitosan demonstrates limited ability to release DNA (GEORGE & al [30]). Elevated transfection efficiency of chitosan complexes with dextran sulfate maybe due to more intensive swelling (SONAWANE & al [31]) of the chitosan/alginate-dextran composite as compared with chitosan/alginate particles (KHORRAM & al [25]) that leads to more plasmid release into the cells. In addition alginate and dextran sulfate may reduce the electrostatic interactions between chitosan and DNA which also can increase release of plasmid. However results show that transfection efficiency of nanoparticles was less than of Lipofectamine-plasmid complexes.

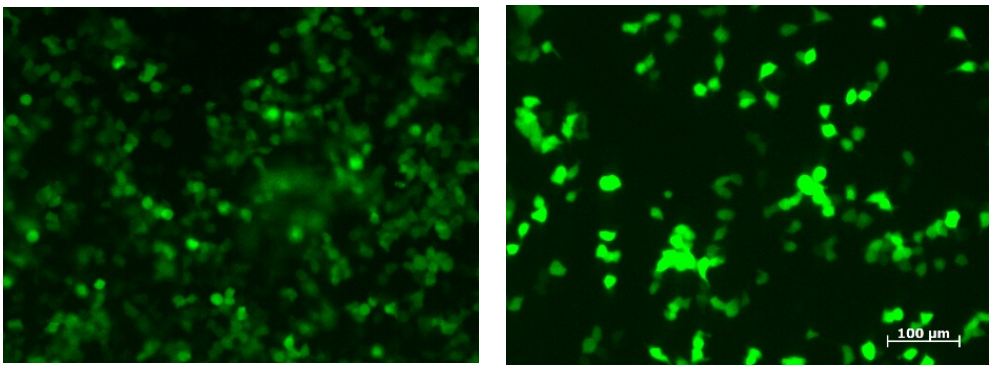


Figure 6. GFP expression in the HEK 298 cells for (left): Lipofectamine and (right): chitosan/alginate-dextran sulfate nanoparticles after 48 h

## 4. Conclusion

In the present study we designed nanoparticles using chitosan, alginate-and dextran for pDNA- encapsulation using modified method of ionotropic gelation. Nanoparticles prepared protected encapsulated plasmid DNA from degradation by endonuclease and were efficient for transfection of plasmid DNA into cells of human lines HEK293. Chitosan/alginate-dextran nanoparticles showed significantly higher transfection efficiency and more effectively stimulated cell proliferation in comparison with other prepared nanoparticles. Due to reliable protection of DNA, high transfection activity and stimulation of cell proliferation these particles can be used for plasmid delivery to the cells *in vitro*, and might be suitable gene carriers in vivo.

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