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Silanized polymeric nanoparticles for DNA isolation

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ABSTRACT

The aim of this study is to prepare silanized polymeric nanoparticles for DNA isolation. Polymeric p(HEMA)-IMEO-PBA nanoparticles around 85.7 nm diameter, was obtained by surfactant free emulsion polymerization for DNA isolation. Synthesized nanoparticles for characterization studies were realized scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), and Zeta-size. Surface area, average particle size and size distribution were also performed. The surface area of synthesized silanized polymeric nanoparticles was 2460 m^2/g. Synthesized polymeric nanoparticles were silanized with 3-(2-imidazoline-1-yl)propyl (triethoxysilane) (IMEO). After that, phenylboronic acid (PBA) which is DNA specific ligand were covalently binded to silanized polymeric nanoparticles. The amount of DNA adsorbed onto the p(HEMA)-IMEO-PBA nanoparticles first increased and then reached a saturation value at around 14.0 mg/mL of DNA concentration. The maximum adsorption was 672.41 mg/g silanized polymeric nanoparticles in the optimum adsorption medium. The maximum DNA adsorption was achieved at 4 °C. The overall recovery of DNA was calculated as 95%. In repetitive adsorption–desorption circles, it is observed not being important decrease in DNA adsorption capacities. The results were shown that silanized polymeric nanoparticles can be a good alternative for DNA isolation.

1. Introduction

Isolation of biological macromolecules has important applications in gene therapy, DNA-based sensors, and high-quality DNA purification by chromatographic techniques [1–8]. The affinity chromatography applications have been usually achieved based on complex formation between boronic acid group and cis-diol groups of biological agents [9]. Isolation and purification of a large number of biomolecules containing 1,2- or 1,3-diol groups have been accomplished using boronate affinity chromatography [10–17]. Specific interaction between boronic acid groups of the matrix and cis-diols of the biomolecule to yield an anionic boronate complex [18].

The use of deoxyribonucleic acid (DNA) for genetic analyses in population based studies is of increasing interest in the etiology of cardiovascular disease, cancer, and other diseases. Advancement in molecular biology techniques offers opportunities to study the interaction of exposure, phenotype, and genotype in epidemiological studies. The need for standardized methods of DNA isolation and storage has recently been emphasized [19–21]. Highly sensitive and selective DNA isolation and detection has attracted extensive attention for its importance in clinical diagnostics, treatment, and various genome projects [22]. To date, thousands of different diseases are known to be caused by genetic lesions such as single-point mutations in the human genome [23]. DNA is a large and highly structured biomacromolecule consisting of nucleotides as the fundamental units [24]. Its backbone is composed alternatively of a deoxyribose and a phosphate group [24].

This work reports on the isolation of DNA by using silanized polymeric nanoparticles. PBA was covalently bonded on silanized polymeric nanoparticles in optimum bonding conditions. DNA adsorption conditions onto silanized polymeric nanoparticles were optimized to improve DNA adsorption efficiencies. Various desorption buffer were applied for efficiency desorption from PBA bonded p(HEMA) nanopolymer. Efficiency desorption buffer was used desorption cycle five times with same polymeric nanoparticles.

2. Material and method

2.1. Materials

Hydroxyethyl methacrylate (HEMA), ethylene glycol dimethacrylate (EGDMA), potassium persulphate, and phenylboronic acid (PBA) were purchased from Sigma Chem. Co., St. Louis, USA. Poly(vinyl alcohol) (molecular weight: 100,000, 98% hydrolyzed) was purchased from Aldrich (Munich, Germany). DNA marker (50 bp, 1.000 μg/ml) was purchased from BioLabs (England). Deoxyribonucleic acid (from herring sperm) was purchased from Sigma Chem. Co., St. Louis, USA. All other chemicals were the guaranteed or the highest purity commercially available and were used without further purification.

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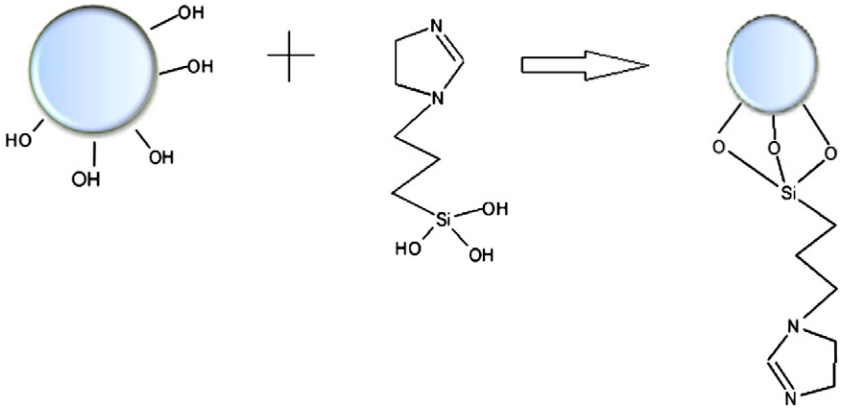

Fig. 1. Schematic presentation of reaction between p(HEMA) nanopolymer and 3-(2- imidazoline-1-yl)propyl (triethoxysilane) (IMEO).

2.2. Preparation of p(HEMA) polymeric nanoparticles

Surfactant free emulsion polymerization was carried out according to the literature procedure [25]. The stabilizer, poly(vinyl alcohol) (0.5 g), was dissolved in 100 mL deionized water for the preparation of the continuous phase. Then, the monomer mixture 0.6 mL/0.3 mL (HEMA/EGDMA) was added to the dispersion which was mixing in an ultrasonic bath for about half an hour. Potassium persulphate (KPS, initiator) concentration in monomer phase was 20 mg/mL. Prior to polymerization, initiator was added to the solution and nitrogen gas blown through the medium for about 1–2 min to remove dissolved oxygen. Polymerization was carried out in a constant temperature-shaking bath at 70 °C, under nitrogen atmosphere for 7 h. After the polymerization, the nanospheres were cleaned by washing with ethanol and distile water several times to remove the unreacted monomers.

2.3. Silanization of p(HEMA) polymeric nanoparticles

Silane is a coupling agent, and its bifunctional molecule bonds to both the exposed composite filler particles and the bonding resin [26]. The silane compounds readily react with the surface hydroxyl groups of the different supports [27]. The p(HEMA) nanopolymer activated by 3-(2- imidazoline-1-yl)propyl (triethoxysilane) (IMEO) with minor modifications. Synthesized p(HEMA) polymeric nanoparticles were mixed with IMEO (mol ratio 2:1) at 25 °C for about one day. At the end of this period, stirring was stopped. The resulting modified p(HEMA)-IMEO nanopolymer were washed with distile water [28]. As seen the Fig. 1 that was schematic presentation of reaction between p(HEMA) nanopolymer and 3-(2- imidazoline-1-yl)propyl (triethoxysilane) (IMEO).

2.4. PBA binding studies on silanized polymeric nanoparticles

As mentioned before, isolation and purification of a large number of biomolecules containing 1,2- or 1,3-diol groups have been accomplished using boronate affinity chromatography. Specific interaction between boronic acid groups of the matrix and cis-diols of the biomolecule to yield an anionic boronate complex. After the silanization of polymeric nanoparticles, PBA binding condition were studied in different conditions. The parameters (pH, temperature, initial PBA concentration) for covalently PBA binding on the silanized polymeric nanoparticles were studied. To determine the effect of pH on the adsorption, pH of the solution was changed between 5.0 and 10.0 and to determine the effect of temperature on adsoption, temperature of the solution was changed between 4 °C and 55 °C. Different initial PBA concentation was changed between 0.6 and 8 mg/mL. The following equation was used.

$$Q = \frac{(Ci - Cf) \times V}{m \times 10^{-3}} \quad (1)$$

Q, was the amount of covalently bonded PBA on the surface of silanized polymeric nanoparticles (mg/g); C_o and C were the initial and after concentration of PBA, respectively (mg/mL); V was the total volume (mL); m, was the amount of silanized polymeric nanoparticles (g).

2.5. Characterization of silanized polymeric nanoparticles

The surface morphology of silanized polymeric nanoparticles were scanned by using scanning electron microscope (SEM JEOL model JSM 5600).

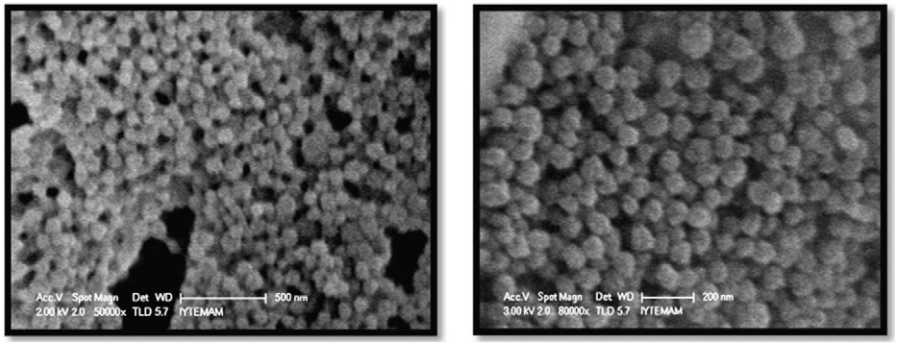

Fig. 2. SEM photograph of p(HEMA) nanopolymer.