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The generation of desired functional groups on poly(4-vinyl pyridine) particles by post-modification technique for antimicrobial and environmental applications

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ABSTRACT

Poly(4-vinyl pyridine) (p(4-VP)) particles were synthesized by a simple micro-emulsion polymerization technique using sodium dodecyl sulfate (SDS) as surfactant. The prepared p(4-VP) particles were then treated various modifying agents with different functional groups. The modifying agents used in the modification of p(4-VP) particles are N-alkyl quaternizing agents such as 2-bromo ethanol (—OH), 4-bromo butyronitrile (—CN), and 2-bromoethylamine hydrobromide ($—NH_2$). The functional groups on the modified p(4-VP) particles were confirmed by FT-IR spectrometry and zeta potential measurements. The size of p(4-VP) and modified p(4-VP) particles is between 300 and 700 nm, and the zeta potentials of modified p(4-VP) particles were varied between 2 and 45 mV. Moreover, a second post-modification was carried out on 4-bromo butyronitrile modified p(4-VP) particles by amidoximation. The modified p(4-VP) particles were also tested for their antimicrobial effects against various bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli*. It was found that p(4-VP) do not posses antimicrobial properties, whereas the modified forms especially $p(4\text{-VP})^+$ and $p(4\text{-VP})^+–NH_3^+$ showed highly bactericidal characteristics. Due to the positive charge by means of new functional groups generated on p(4-VP)-based particles by modification, the absorption of oppositely charged reagents such as fluorescein sodium salt (FSS) was increased drastically. For example, the absorption capacity of unmodified p(4-VP) was increased to 93.3, 93.5, and 93.6 form 37.6 mg for $p(4\text{-VP})^+$, $p(4\text{-VP})^+–NH_2$, $p(4\text{-VP})^+–NH_3^+$, respectively. Moreover, upon modification, except Cu(II), Co(II) and Ni(II) absorption capacities were increased from about 15.9, and 22.1 mg to 21.1 and 39,1 mg per gram particles.

1. Introduction

Hydrogels of different sizes with tunable properties, hydrophilic/hydrophobic balance, and morphology are known to be smart materials; that is, unique materials for numerous applications in biomedical fields such as drug delivery, catalysis, and the development of antimicrobial materials and advanced materials design [1–5].

Infectious diseases that arise from pathogenic microorganisms are a major problem in many biomedical fields such as healthcare products, hygienic applications, food packaging and storage, and so on. To solve the contamination problem, new classes of polymers that can show antimicrobial activity with various other functionalities are of great significance [6]. In recent years, researchers have focused on new polymeric materials with antimicrobial and/or bactericidal effect [7–10]. The accepted mechanism is via electrostatic interaction between the negatively charged bacterial cell walls and the positively charged antimicrobial polymers. Therefore, p(4-VP) and polystyrene (PS) have been used as cationic polymers that contain heterocyclic or aromatic structures in most studies. Block and random copolymers, especially, containing quaternized p(4-VP) also exhibited good antibacterial activity [11].

P(4-VP) is a neutral polymer and has very interesting properties coming from the vinyl and benzene rings with a nitrogen atom. The amphoteric properties of the pyridine rings together with the further modifiability of the nitrogen atoms by various chemistries on repeating units of p(4-VP)-based materials are crucial for advanced materials design [12–15]. To obtained particles of the same with the different functional groups may not be possible due to nonexistent the same monomers with different functional groups. Monomers such as 4-VP with the ability of chemically modifiability offer great advantages due to the nitrogen atoms on the pyridine ring for versatile applications. For example, much research has shown the antimicrobial effects of quaternized p(4-VP) by modifying with different agents such as different alkyl chain length reagents [16,17] or end groups such as $—NH_2$, and OH [18,19]. Mostly, the modification carried out with Micro-and nanomaterials is noteworthy in

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the design of multipurpose novel materials such as active agent releasing vehicles [20], targeting carriers [21], antimicrobial materials [22], detoxifying agents for environmental applications [23], and as template and reactor for the synthesis of metal nanoparticles and applications in catalysis [24,25].

In this investigation, p(4-VP) particles were synthesized in nanodimensions and modified with various functional groups containing reagents such as —OH, —NH_2 and —CN p(4-VP). The particles were characterized using FT-IR for structural confirmation, dynamic light scattering (DLS) for size determination, and zeta potential for the determination of surface charge. Most importantly, we also report the second modification of modified p(4-VP) for the first time where the p(4-VP)$^+$–CN particles were exposed to an amidoximation reaction to create amid-p(4-VP)$^+$–CN. Additionally, p(4-VP)-based particles were tested for their antimicrobial properties against three different bacteria; *Staphylococcus aureus* ATCC25323, *Bacillus subtilis* ATCC6633, and *Escherichia coli* ATCC8739. It was also shown that the modified p(4-VP) particles can be used in the removal of some dyes such as FSS.

2. Materials and methods

2.1. Materials

4-Vinyl pyridine (4-VP, 95% Acros) as monomer, ethylene glycol dimethacrylate (EGDMA, 97% Fluka) as cross-linker, ammonium persulfate (APS, 99% Aldrich) as initiator, sodium dodecyl sulfate (SDS, 95% Acros) as surfactant, 4-bromo butyronitrile (CN, 97% Aldrich), 2-bromo ethanol (OH, 95% Aldrich), 2-bromoethylamine hydrobromide (NH_2, %99 Aldrich) as modification agents, and hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$, 98% Merck) as amidoximation agent, and sodium hydroxide (NaOH, 97% Merck), and hydrochloric acid (HCl, 36.5% Sigma–Aldrich) were used as received. Fluorescein sodium salt (FSS, Sigma–Aldrich) and cobalt(II) chloride hexahydrate ($CoCl_2{\cdot}6H_2O$, 98% Aldrich), nickel(II) nitrate hexahydrate ($NiNO_3{\cdot}6H_2O$, 98.5% Aldrich), and copper(II) chloride ($CuCl_2$, 99% Acros) as metal ion sources were used in absorption studies. Dialysis tubing of cellulose membrane (Sigma–Aldrich) with average flat width 33 mm was employed to remove the surfactant form synthesized particles. Nutrient agar (agar, Merck) and nutrient broth (NB, Merck) were used for antimicrobial experiments. DI water with 18.2 MΩ cm (Millipore Direct-Q3 UV), acetone, ethanol, and methanol with highest purity available were used for solution preparation and washing of particles throughout the experiments.

2.2. Synthesis of cross-linked p(4-VP) particles

P(4-VP) particles were synthesized in accordance with procedures reported previously with some modification [26]. In short, cross-linked p(4-VP) particles were prepared using an oil-in-water micro-emulsion system employing SDS as surfactant. Into 0.1 M 150 mL SDS solution in DI water were added 26.8 mmol of 4-VP and EGDMA (10 mol% relative to the 4-VP). This mixture was vortexed until a clear solution was obtained. Then, the mixture was exposed to nitrogen gas bubbles to remove dissolved oxygen and placed in a temperature-controlled oil bath at 75 °C under 800 rpm stirring rate. The polymerization was initiated by adding 5 mL of APS aqueous (1 mol% relative to the monomer) initiator solution and allowing the reaction to proceed for 6 h. The synthesized p(4-VP) particles were purified using dialysis tubing cellulose membrane at room temperature for 3 days against distilled water. Then, the cross-linked p(4-VP) particles were collected, dried using a Freeze Dryer, and were stored for characterization and the modification experiments.

2.3. Modification of cross-linked p(4-VP) particles

Modification of p(4-VP) particles was carried out according to previous methods with various changes [27–29]. Briefly, for each modification, 0.3 g synthesized p(4-VP) particles were used, and excess amounts of modification agent (at least 1.5-fold based on repeating units of p(4-VP)) were added in a medium of 15 mL methanol in a vial. Then, the mixture was vortexed until a dispersed solution was obtained. The mixture was placed in a temperature-controlled oil bath at 50 °C under constant stirring (600 rpm) for 24 h. Upon reaching the end of this reaction time, the mixture was purified again using dialysis membrane at room temperature until all impurities were removed against distilled water, dried in a Freeze Dryer, and were stored for characterization, antimicrobial tests, and absorption studies.

2.4. Quaternization reactions of the particles

The quaternization reactions were carried out according to methods reported in the literature with some changes [30]. A certain amount of p(4-VP) particles and the modified particles e.g., p(4-VP)$^+$–NH_2 particles which were synthesized in Section 2.3, were placed in 50 mL 0.1 M HCl solution under constant mixing at 600 rpm for 12 h. The quaternized particles were also purified using dialysis membrane at room temperature until all impurities were removed against distilled water and until dialysis medium pH 7 was reached. Then, the quaternized particles were collected, dried using a Freeze Dryer, and were stored for characterization, antimicrobial tests, and absorption studies.

2.5. The second post-modification of p(4-VP)-based particles: amidoximation of nitrile group on p(4-VP)$^+$–CN

The amidoximation reaction was performed on the nitrile groups of p(4-VP)–AN using a method reported earlier with some changes [31,32]. For the amidoximation reaction, 100 mg p(4-VP)$^+$–CN, prepared as mentioned in Section 2.3, was reacted with 1.44 mmol of hydroxylamine ($NH_2OH{\cdot}HCl$ neutralized with NaOH) in 10 mL distilled water at 80 °C for 12 h. After the amidoximation reaction was completed, the amidoximated particles were taken from the reaction vial, washed with distilled water, and centrifuged at 10,000 rpm. Then, the particles were collected, dried using a Freeze Dryer, and were stored for characterization. The following particle notation will be used throughout this paper:p(4-VP)$^+$ = p(4-VP)–HCl, p(4-VP)$^+$–NH_2 = p(4-VP)–ethylamine, p(4-VP)$^+$–NH_3^+ = p(4-VP)–ethylamine–HCl, p(4-VP)$^+$–OH = p(4-VP)–ethanol, p(4-VP)$^+$–CN = p(4-VP)–butyronitrile, and amid-p(4-VP)$^+$–CN = amid-p(4-VP)–butyronitrile.

2.6. Bacterial culture: antimicrobial behaviors of p(4-VP)-based particles

The antimicrobial properties of p(4-VP)-based particles were determined according to the procedure reported earlier [1,2,33]. MIC (Minimum Inhibitory Concentration) was evaluated for *S. aureus*, *B. subtilis*, and *E. coli*. MIC tests were studied for 100, 200, 500, 1000, 5000, 10,000, and 20,000 μg/mL particles, using bacterial suspension in nutrient broth. Particles (~1–200 mg) were added to sterile 10 mL glass tubes, and the appropriate volume of a solution containing 5×10^9 CFU/mL of each bacterial suspension in N broth was added. For negative control, only inoculated broth-containing tubes were used. After 18–24 h, at 35 °C in an oven, tubes were vortexed and 1/1000 and 1/1,000,000 diluted samples prepared. From these, 10 μL samples were taken and plated on 1% agar and incubated for 18–24 h at 35 °C. Colonies were counted, and MIC values of the polymeric particles were determined.