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Chitosan and functionalized acrylic nanoparticles as the precursor of new generation of bio-based antibacterial films

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ABSTRACT

This study represents a new method for preparation of acrylic/chitosan films with antibacterial activity and non-toxic properties through an environmental friendly process containing a water-base acrylic resin and chitosan as an abundant natural polymer. Functional and positively charged acrylic particles based on butyl acrylate (BA)–methyl methacrylate (MMA)–glycidyl methacrylate (GMA) terpolymer were prepared with layered structure via semi-continuous emulsion polymerization. FTIR spectroscopy confirmed the presence of epoxy functional groups and size distribution of particles were evaluated by DLS and SEM as well. Films were prepared through mixing of chitosan solution and the prepared latex for the first time. SEM and EDX analyses revealed that chitosan has been distributed through the polymeric matrix uniformly. TGA data showed that introducing chitosan increases the maximum degradation temperature. It was found that the obtained films including positively charged chitosan reveal enhanced antibacterial activity against *Staphylococcus areus* and *Escherichia coli*. Also cytotoxicity analysis shows reasonable non-toxic behavior of the obtained composite films.

1. Introduction

In recent years, water-based coatings such as emulsion polymers are preferred to solvent-based ones due to the environmental issues [1,2]. Latex particles are extensively used in pharmaceuticals, optical devices and electronics as well as cosmetics, coatings and catalysts [3,4]. These applications depend on their morphology, size and well-defined surfaces, beside their unique physical and chemical properties. Emulsion, miniemulsion and suspension polymerization are the best methods for obtaining such particles. Some of the latex particles are produced by step-wise emulsion polymerization with various monomers, in a way that the secondary feed is polymerized in the presence of initially formed seed particles. The performance of so-called multilayer particles strongly depends on their morphology and should be controlled carefully while using them with desired properties [5–7].

Polyacrylic latexes are widely exploited according to their transparency, excellent film forming and resistance to oxidation and oils [8]. Among them, acrylic copolymers containing functional groups such as epoxy are mostly used [9–12]. Deposition of epoxy groups on the surface of latex particles would increase curing rate due to the ease and availability of these functional groups [13].

Anionic surfactants or the mixture of anionic and nonionic surfactants have been thoroughly studied in emulsion polymerization systems [14]. On the other hand, positively charged latex particles are being used in protein separation, paper industries [15,16] and polystyrene-carbon nanofibers [17]. However, there are few reports in using cationic surfactants in polymerization of styrene [18], methyl methacrylate [19] or butyl acrylate [20] monomers. There is an increasing interest toward cationic latexes since 1990 and dodecyltrimethylammonium bromide [21] and cetyltrimethylammonium bromide (CTAB) [22] are the most usual surfactants for this reason. A detailed study on emulsion copolymerization of styrene and butyl acrylate in the presence of CTAB as surfactant has been performed and the effect of anionic, nonionic and cationic initiators with different amounts of surfactants has been investigated [14].

Chitosan is obtained from chitin and is the second abundant natural polymer after cellulose. This is environmentally friend, non-toxic and non-allergic to human body [23–25]. As a cationic polymer, it shows excellent antimicrobial, antibacterial and antifungal properties via ionic interactions with the cell wall and has bactericidal effects as well [26–28].

Polyacrylates have reasonable mechanical properties and besides, chitosan is able to react with the synthetic polymers to improve their final properties [29]. In recent years, successful researches have been carried out on grafting of glycidyl methacrylate (GMA) onto chitosan for using in natural-synthesized hydrogels [30]. GMA contains two active sites i.e. epoxy and vinyl groups with susceptibility to get involve into the chemical reactions [30,31]. On the other hand, chitosan contains multi hydroxyl moieties like pentaerythritol and cyclodextrine for contributing in functionalization and increase in residual amount of the ash after burning or thermal decomposition [32,33].

Antibacterial coatings can prevent microorganisms to grow on the substrate and might be introduced as a new generation of materials

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for clinical and medical devices [34]. The combination of acrylic copolymers with improved properties (e.g. film formability, weather resistance and appropriate mechanical properties) together with chitosan; with antibacterial properties and inability of film formation; would be a novel approach to introduce a new class of antimicrobial coatings. The main issue is to provide the conditions for their efficient mixing.

To the best of our knowledge, the main issue in preparation of chitosan- based films is its inability to film formation and this has been a serious concern and limitation for its application. Also there is no report on the synthesis of epoxy-functionalized latex particles with cationic surfactants. According to the advantages of accompanying chemically-modified acrylic polymers with chitosan, this work would open up a gateway toward preparing multifunctional coatings without any phase separation as a disadvantage in these systems. Here, such layered epoxy/acrylic particles with positive surface charge are prepared and the corresponding reaction with chitosan is reported for the first time. After characterization, the antibacterial properties and toxicity of the prepared films are studied comprehensively. The results illustrate that mixing of polyacrylic particles with chitosan is a facile and efficient method for obtaining the desired antibacterial coatings with desired properties.

2. Experimental

2.1. Materials

Butyl acrylate (BA, Merck), methyl methacrylate (MMA, Merck), and glycidyl methacrylate (GMA, Sigma-Aldrich) as monomers were used as received. 4,4′-Azobis (4-cyanovaleric acid) (ACVA, Sigma-Aldrich) as the initiator and cetyltrimethylammonium bromide (CTAB, Merck) as surfactant and ethanol (Merck) were used as received. Chitosan (molecular weight of 100–300 kDa, Acros Organics) was exploited as the natural polymer. Deionized (DI) water was used in all recipes. Phosphate-buffer saline (PBS) was prepared by dissolving NaCl (5.85 g), KH_2PO_4 (0.6 g) and Na_2HPO_4 (6.4 g) (all from Merck) in distilled water and the volume reached to 1 L. The pH was then adjusted to 7.4 by HCl or NaOH solutions (0.2 M). Local pathogenic Gram-positive *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*) bacteria were received from Pasteur Institute of Iran for antibacterial tests. Mouse L929 fibroblast cells were supplied from Pasteur Institute of Iran to study antibacterial properties of the obtained films.

2.2. Emulsion polymerization

Semi- continuous emulsion polymerization technique was used for preparation of the latexes. Polymerization was performed in a 250 mL reactor equipped with a condenser and mechanically stirring at 300 rpm under nitrogen atmosphere. First, ACVA (0.08 g), CTAB (0.2 g) and 74 g DI water were added to the reactor at 70 °C. After 10 min, BA (3.42 g) and MMA (4.58 g) were added in 90 min. At the end of addition of the seed monomers, BA (1.66 g) and GMA (0.5 g) were added within 40 min as the outer layer monomers. Then the polymerization continued for 45 min for completion and reaching to high conversion. Final conversion of 98% was obtained for the latex with 10% solid content and coagulation amount was negligible in this polymerization process.

2.3. Samples preparation (AC-series)

Three typical films with different compositions of the cationic acrylic latex and chitosan were prepared (Table 1). Chitosan was added to 10 mL of DI water containing concentrated HCl (pH 4), followed by stirring at room temperature for 12 h until the complete dissolution of chitosan. This solution was mixed with the above prepared cationic latex for 15 min. Ultimately, films were obtained by casting the above acrylic–chitosan mixture at room temperature until complete drying.

Table 1
Composition of the prepared acrylic–chitosan films.

Sample	Chitosan (wt%)	Acrylic copolymer (wt%)[a]
AC-0	0	100
AC-10	10	90
AC-40	40	60

[a] Acrylic copolymer weight percent was considered based on the solid content of the latex.

2.4. Equipments

Monomer conversion was determined gravimetrically in all experiments. The sample preparation and analyses are described below. For extracting chitosan component from the films, the solid sample was immersed into to the acidic solution (HCl solution, pH 4) for about 2 h at room temperature. Then the sample was filtered off, washed with plenty of water and dried at room temperature for further analyses.

2.4.1. FTIR analysis

Fourier transform infrared (FTIR) spectra of the samples in KBr pellets were recorded on FTIR Bruker-IFS 48 spectrophotometer (Germany).

2.4.2. Thermal gravimetric analysis

Thermal gravimetric analysis (TGA) was recorded on a STA-PL instrument (England) at a heating rate of 20 °C/min under a flow of nitrogen atmosphere.

2.4.3. DLS, EDX and SEM analyses

Size distribution and zeta potential of the latex particles were determined by dynamic light scattering (DLS) on a ZEN 3600 particle size analyzer (Malvern Instrument, Canada) at 25 °C. Morphology of the samples was characterized by scanning electron microscopy (SEM, Vega II model, TESCAN Instrument, Czech Republic). For SEM sample preparation, the latex was diluted in distillated water and a drop was placed on a sample holder, dried at room temperature and then sputter-coated with gold for SEM analysis. For SEM sample preparation of the cross-section of the fractured surface, films were immersed in liquid nitrogen and broken in pieces, placed on a sample holder and then sputter-coated with gold. The distribution of nitrogen atoms in the hybrid system was obtained by the energy dispersive X-ray (EDX) analysis (INCA Model, Oxford Instron, England).

2.4.4. Antibacterial activity and cytotoxicity measurement

Antibacterial activity of the films was determined using local pathogenic *S. aureus* and *E. coli* bacteria, using agar diffusion methods. In agar diffusion test, films were exposed to *E. coli* and *S. aureus* bacteria in the solid media (nutrient agar) to observe the zone of inhibition around the films. Films were placed on an LB agar plate (Himedia) which was previously seeded with 1.09104 CFU of *E. coli* and *S. aureus* for 5–10 min. After incubation at 37 °C for 24 h, inhibition zone for bacterial growth was determined visually.

Cytotoxicity of the prepared films was studied by microscopic investigation of fibroblast cells morphology after direct contact with the samples. Samples were sterilized by immersing them in ethanol for 6 h. In this analysis, mouse L929 fibroblast cells were pre-cultured in 24-well plates (1×104 cells/well) using RPMI-1640 growth medium supplemented with 10% fetal bovine serum (FBS) at 37 °C and atmosphere of 5% CO_2 for 24 h. The cells were then exposed to the sterilized samples (5×5 mm), which were placed in the center of each well and incubated at 37 °C for another 24 h. A well containing cells and growth medium with no sample was set up as the negative control sample. The cell growth characteristics and morphological changes as a cytotoxicity sign were recorded using a TMS inverted optical microscope equipped