The synthesis and characterization of p(nBA-DEAEMA) polymer

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Abstract. In this paper, the polymer of p (nBA-DEAEMA) was polymerized and characterized. The structure of copolymer was characterized by FT-IR and ¹HNMR, and the analysis of particle size, appearance and rheology was determined too. The results showed that, the p (nBA-DEAEMA) was synthesised successful, and it could become a surface modification for the inner face of artificial trachea, after further optimization.

1. Introduction

Trachea stenosis that is caused by the tracheal diseases and wounds is a common clinical disease with high mortality rates, and for these diseases, the unique curative treatment at present is the reconstruction of tracheal defect after extensive resection [1-2]. However, there are so many complications such as the airway restenosis and serious infection in the reconstructed trachea, and this is because the inner surface of the artificial trachea is not covered by cilium integrally so the wastes could not be cleared with cilium but store in the airway for a long time to become the hotbeds for bacteria to cause the serious infection, at the same time, the smooth inside wall leads to the running of the neighbouring tissue inwards to produce the proliferation with granulation tissue even restenosis [3]. That’s the reason for the failure of reconstruction. In view of this situation, a new idea is provided to solve this problem based on the development of biomedical engineering and polymer science, which is coated a layer of polymer brush with cilia-like film cover on the inner surface of the reconstructed trachea to simulate the airway cilia layer. Combining the preparation and molding technology of polymer materials with the artificial cilia research, the clinical application of artificial trachea would have a new prospect. At present, the research of artificial cilia has shown a great charm with benefitting from its effectiveness to fluid treatment, but they still cannot meet the mechanical and biocompatible requirements as the cilia-like material. So, in addition to the function of removing the airway waste, we need to have a good biocompatibility and the ability to restrain the tissue from the surrounding tissue to the inner wall of the artificial trachea. Through the synthesis and study of the biological compatibility and mechanical properties of artificial cilia, to solve the problem of waste removal of artificial trachea, has very important theoretical significance and clinical application value.

The application of acrylate and its derivatives in the field of biomedical material had a long history, and they owned so many good properties as water resistance, strong adhesion, flexibility and elasticity [4]. As a fine material for surface modification, polymer brushes, not only in improving the biocompatibility [5] of the matrix but also in obtainment of functional material [6-9], had intensive studies yet.
In this paper, we used two acrylate monomers to synthesize a polymer and combined the molding process of Velcro to help achieving a topology of cilia-like surface. n-butyl acrylate (nBA) and 2-(diethylamino) ethyl methacrylate (DEAEMA) monomers were selected to copolymerize for preparing the polymer brush and molding a cilia-like film to lead the cilia-like behavior. Some properties of copolymer and cilia-like film were characterized, and then its function to clear away the airway wastes was explored with a preliminary research. The results showed a potential of this material to be a surface modification for the inner face of the artificial trachea.

2. Materials and methods

2.1. Chemicals

DEAEMA, nBA, cetyltrimethylammonium chloride solution (CTAC, 25 wt.% in H2O), tetrahydrofuran (THF), acetone and 0.1N volumetric standard solutions of sodium hydroxide (NaOH) and hydrochloride acid (HCl) were purchased from Sigma-Aldrich Chemical Co. 2,2’-Azobis (2-Methylpropionitrile) (AIBN, >98%), potassium bromide (KBr, spectral purity, >99%), and deuterium dimethyl sulfoxide (DMSO-d6, spectral purity, >99%) was purchased from Adamas Reagent Co. Ltd.

2.2. Synthesis of copolymer

p(nBA-DEAEMA) copolymer was synthesized using the semi continuous emulsion polymerization process that was adapted for a small-scale polymerization. At room temperature, 25mL of double dionized (DDI) water, 0.62g of CTAC, 5.6g of monomers and 0.1g of oil-soluble initiator AIBN were prepared to form pre-emulsion at 300 rpm. Then, the other reaction flask was immersed in a water bath preheated to 72 ºC and purged continuously with N2 gas, which was first charged with 27mL of DDI water. At this point, the pre-emulsion was fed at 110μL/min into the vessel over a period of 3h. After completion of pre-emulsion feeding, the reaction was continued for additional 3h. The resulting colloidal dispersion was frozen drying after cooling to ambient temperature to obtain the copolymer. p(DEAEMA) and p(nBA) homopolymers were prepared using the same method.

2.3. Characterization of copolymer

The Molecular weight was determined using GPC (PL-120) equipped with a 1515 HPLC pump and a 214 nm model refractive index detector. Each sample was precipitated in THF and eluted through a HT2 and HT6E column. Elution time was referenced against polystyrene standard, and the molecular weight and polydispersity of the copolymer were 1803289 Daltons and 1.809559. Generally speaking, higher the molecular weight of polymer was, better the mechanical and thermal properties were but weaker the process ability was. So, high molecular weight and wide polydispersity had been obtained at the same time to ensure a smooth molding and a suitable mechanical performance for the cilia-like film in this part. FT-IR spectra were collected using Lambda 900 UV/NIR/MIR spectrometer (Perkin Elmer, USA) and Proton NMR (1H NMR) spectra were acquired using the Nuclear Magnetic Resonance Spectrometer (500MHz, BRUKER OPTICS, Swiss). For FT-IR, a certain amount of freeze-drying copolymer colloids into acetone until dissolved completely, and drop a little sample solution onto the KBr blank flake with pipette, then test sample until the acetone evaporated completely. As anticipated, characteristic broadening and a shift of the carbonyl ester band at 1732 cm⁻¹ with the band due to aminomethyl groups at 1568 cm⁻¹ confirm copolymerization. For 1H NMR measurement, 2.78% (w/v) of p(nBA/DEAEMA) copolymer was dissolved in DMSO-d6, and typical resonances at 0.85 (−CH3−CH2) with shoulder peaks and 3.9-4.1 ppm (−O−CH2) arise from the copolymerization of nBA units, whereas the peaks at 1.2 (−C−CH3), 3.0(−CH2−N), 1.0(−N− (CH3)−CH3)), and 3.9-4.1 ppm (−O−CH2) are due to DEAEMA units. Particle size analysis was performed using an analyzer (zetasisernano ZS90, Malvern, UK). Auto calibration against standard buffer solutions was completed before the titration. Standard HCl and NaOH were utilized to adjust pH values of copolymer dispersions. We got the results that the particle size increased from 44nm to 53nm as the pH values increased from 2 to 3 firstly and then decreased to 39nm as the pH values increased to 12 at.
25°C. This behavior was attributed to the protonation of the tertiary amine functional groups that caused the colloidal particles to swell as the copolymer chains repel each other due to electrostatic interactions. Full protonation and swelling were achieved under acidic conditions, which was responsible for significantly larger particle sizes detected at pH value of 3. Also, morphology analysis showed the consistent size as the particle size analysis that was 40-50nm. Most studies believed that the particle size of colloids and size distribution of emulsion played a main role in the viscosity of high-solid content emulsion. The particles obtained in our paper were nanoparticles, and not only could the micro size enable to get more dense film to improve its mechanical properties, but also could get enlarger surface area of the film to make contact with the mucus fully, so more effective mechanical response behavior was produced and there was little influence on the properties of material due to the pH value. The resulting copolymer colloids were dissolved in glycerin to be prepared some equidifferent emulsions with 10-80mg/mL, and then tested under 25°C and 37°C. The rheological behavior was measured by rheometer (Gemini HRnano, Marvin, UK).

3. Results and Discussion

Figure 1 illustrated the FT-IR spectra and of p(nBA) and p(DEAEMA) homopolymers and p(nBA/DEAEMA) copolymer, respectively. As seen in trace A, it showed the FT-IR spectrum of p(nBA/DEAEMA) copolymer. As anticipated, selected bands in the 2850-3000 cm⁻¹ and 1350-1500 cm⁻¹ regions due to C-H stretching and CH₂/CH₃ deformation modes overlap with the p(nBA) and p(DEAEMA) spectra. Characteristic broadening and a shift of the carbonyl ester band at 1732 cm⁻¹ with the band due to aminoethyl groups at 1568 cm⁻¹ confirm copolymerization.

Figure 1. FT-IR spectrums of homopolymers and copolymer.

Further evidence of copolymerization was illustrated in the 1H NMR spectrum of p(nBA/DEAEMA) recorded at 25°C in DMSO. As shown in figure 2, typical resonances at 0.85 (-CH₂-CH₃) with shoulder peaks and 3.9-4.1 ppm (-O-CH₂) arise from the copolymerization of nBA units, whereas the peaks at 1.2 (-C-CH₃), 3.0(-CH₂-N), 1.0(-N-(CH₂CH₃)), and 3.9-4.1 ppm (-O-CH₂) are due to DEAEMA units. The resonance at 2.5 ppm was due to DMSO solvent and 3.4 ppm was due to the water in DMSO. These spectroscopic data demonstrate that nBA and DEAEMA monomers were copolymerized for the feed ratio of 1:1 during the copolymerization.
The particle size of this colloidal suspension was analyzed as a function of pH 2-12 at the 25°C temperature. Figure 3 illustrated the size distribution by volume of p(nBA/DEAEMA) copolymer colloidal dispersions at pH 7 and its pH responses. As seen, the particle size increased from 44nm to 53nm as the pH values increased from 2 to 3 firstly and then decreased from 53nm to 39nm as the pH values increased from 3 to 12 at 25°C. This behaviour was attributes to the protonation of the tertiary amine functional groups that caused the p(nBA/DEAEMA) colloidal particles to swell as the copolymer chains repel each other due to electrostatic interactions. Full protonation and swelling were achieved under acidic conditions, which was responsible for significantly larger particle sizes detected at pH value of 3.

The follows showed the rheological property of p(nBA/DEAEMA) colloidal particles in an solution state.

Generally speaking, the relationship should to be a linear one between the viscosity of polymer solution and its concentration, but in this article, as figure 4 shown, it’s a non-linear relationship. Whether 25°C or 37°C, there was a deflexed phenomenon within the concentration range of 60-80mg/ml. While the difference was that, when the concentration of polymer solution reached 70mg/ml, the viscosity decreased more at 25°C comparing with 37°C.
Figure 4. The change of the viscosity of polymer glycerin solution along with its concentration

Figure 5 illustrated the modulus changes of p(nBA/DEAEMA) glycerin solution with different concentration under 25°C and 37°C, respectively. Overall, the elastic modulus of polymer solution both 25°C and 37°C had no obvious transformation and maintained at the same level and its complex modulus had the same changed trends with its viscous modulus, but its viscous modulus (complex modulus) reduced by 55% approximately as a whole at 37°C, compared with 25°C. We were pleasantly surprised to see that different variation of viscous modulus was taken place at different temperature. At 25°C, the viscous modulus of polymer glycerin solution showed decreases first and then increase, and reached a minimum at the concentration of 70mg/ml. In contrast, at 37°C, its viscous modulus showed increase first and then decrease, and reached a maximum at the concentration of 40mg/ml.

Figure 5. The modulus changes of polymer p(nBA/DEAEMA) glycerin solution with different concentration under 25°C (left) and 37°C (right).
Figure 6. The flow curve (fixed-frequency: 1Hz) of polymer p(nBA/DEAEMA) glycerin solution at the concentration of 40mg/ml (up) and 70mg/ml (down).

We can see from the figure 6 that, the viscosity of polymer glycerin solution decreased along with the increase of the shear rate whether different temperature or different concentration, and it’s a pseudo plastic fluid. However, there was a rapid decrease in the range of 0-20 $s^{-1}$ but a slow reduction to steady gradually in the range of 20-100$s^{-1}$ at the concentration of 40mg/ml under 25$^\circ$C, and the situation under 37$^\circ$C was a continuous decline in the whole range of the experimental shear rate at the same concentration. In the same experiment, it showed a reverse trend at the concentration of 70mg/ml, that was, the sustaining downward of viscosity appeared at 25$^\circ$C while it stabilized from 20s$^{-1}$ at 37$^\circ$C.

4. Conclusion
We have prepared a cilia-like film with the two monomers to mimic the natural cilium successfully. The cilia-like layer could exist stably on the matrix and keep certain rigidity away from falling under the action of shear force, and it could produce some effective mechanical behaviors to propel the wastes that attached on the airway mucus. This research could provide a new idea to solve the problem that the inner surface of the artificial trachea was not covered by airway cilium integrally and it has a very important value of theory and clinical application.

Funding
This work was supported by the National Natural Science Foundation of China (31400805) and Fundamental Research Funds for the Central Universities (No.106112017CDJQJ238815).
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