

Fluorinate polyacrylic acid and its use as a potential adjuvant field

Lu Bai ¹, Chengyu Li ², Mengmeng Zhou ¹, Xiaoqi Chen ¹, Haijun Zhou ¹, Fen Zhang ¹, Xin Li ¹, Yantao Li ^{1, *}

¹ Institute of Energy Resources, Hebei Academy of Sciences, Shijiazhuang, Hebei Province 050081, PR China.

² Hebei University of Science and Technology, Shijiazhuang, Hebei Province 050000, China.

Abstract. This study describes a polymer potentially used as a vaccine adjuvant. We first synthesized a novel polyacrylic acid modified by perfluorinated butanol—a bioactive compound that is biocompatible. The main aim was to functionalize the polyacrylic acid by fluorine. This functionalization could offer a permanent graft by an esterification reaction. The chemical structure of the polymer has been characterized by the hydrogen nuclear magnetic resonance spectrum (1H-NMR) and fourier transform infrared spectrum (FT-IR). Thermal analysis showed that the esterification reaction was successful on the polyacrylic acid. Viscosity testing showed that the viscosity of the sample aqueous solution increases along with esterification. The surface tension of the polymer was also tested. The results show that the surface tension of the polyacrylic acid markedly decreased when modified by fluorine. Finally, the interaction of these materials with macrophages was tested on cell test. Results showed that the modified polyacrylic acid performed better improve the activation effect than pure polyacrylic acid in 1L-1β.

Keywords: Vaccine adjuvant, Polyacrylic acid, Activation

1. Introduction

Vaccines greatly reduce mortality and prolong life [1] and control and prevent the spread of infectious diseases by generating a protective immune response [2]. They have changed modern life [3]. Vaccines against influenza are actively under development [4].

Adjuvants are added to vaccines to improve the immunogenicity of the antigens. The adjuvant can also induce a stronger immune response to the antigens and can reduce the dosage and production costs of vaccines in populations whose response is poor. Adjuvants in use or in development include oil emulsions, aluminum salts, immune-stimulating complexes, liposomes, nonionic block copolymers, polysaccharides, cytokines, and bacterial derivatives [5].

Aluminum salts are the most common vaccine adjuvants [6]. They have good performance for controlled release of antigens, antigen-presenting cell recruitment, local inflammation induction, and increased uptake of antigen [7]. However, aluminum salts cannot enhance cell-mediated type-1 helper T cell (Th1) or cytotoxic T lymphocyte (CTL) response [8]. Aluminum salt adjuvants have failed to stimulate a sufficient antigen immune response, and their accumulation may affect brain tissue and bone tissue including neurological disorders or dialysis-related dementia [9].

Many veterinary vaccines are either live-attenuated vaccines or inactivated organisms formulated by an oil-

based emulsion adjuvant [10]. However, these compounds have problems with local injection site reactions from the oil-based emulsion adjuvant. Although mineral oil-based systems are more effective for veterinary vaccine, they can cause serious side-effects such as abscesses, fevers, or local granulomas [11].

Polymers have many advantages as an adjuvant. They can enhance the immune effect of vaccines leading to non-specific immunity, body-specific immunity, humoral immunity, and cellular immunity. The polymers are intrinsically immune-modulating with low toxicity, good biocompatibility, and high safety [12]. Therefore, polymer adjuvants have attracted much attention in the preparation of vaccines [13] including chitosan, alginate, hyaluronic acid (HA), poly-(lactic-co-glycolic acid) (PLGA), and poly-acrylic acid [14].

Poly-acrylic acid and its derivatives are particularly biocompatible, stable, non-toxic, and affordable. They have been applied widely in pharmaceutical field. Pure poly-acrylic acid has long been used as an adjuvant with good results, but several groups have improved the adjuvanticity via structural modifications. Poly-acrylic acid has been grafted with alkyl-chains by esterifying the carboxylic group with octanol and butanol, and the esterified poly-acrylic achieved better adjuvanticity [15-16]. A copolymer of acrylic acid and divinylbenzene was synthesized via suspension polymerization as an effective drug carrier. This can be applied in the manufacture of animal vaccine adjuvants [17].

* Corresponding author: xiaojj@hebust.edu.cn

More recently, fluorinated polymer gene carriers have been shown to greatly promote cellular uptake with increased endosomal escape of their siRNA cargos [18]. Yuan et al. have modified a disulfide bond-containing hyperbranched poly (amidoamine) using heptafluorobutyric anhydride to prepare fluorinated which was used as a vaccine delivery system for antitumor immunotherapy [19]. Therefore, we concluded that fluorinated polymers might have utility as adjuvants to enhance intracellular uptake and cytoplasmic delivery of antigens. This can induce cross-presentation and cellular immunity with potentially good adjuvanticity. Here, we prepared perfluorinated butanol and show that it combines the advantages of a poly-acrylic system and the fluoride. The physical and chemical properties of the polymer were measured. At the same time, the effect of the fluorinated polymer on macrophages was studied.

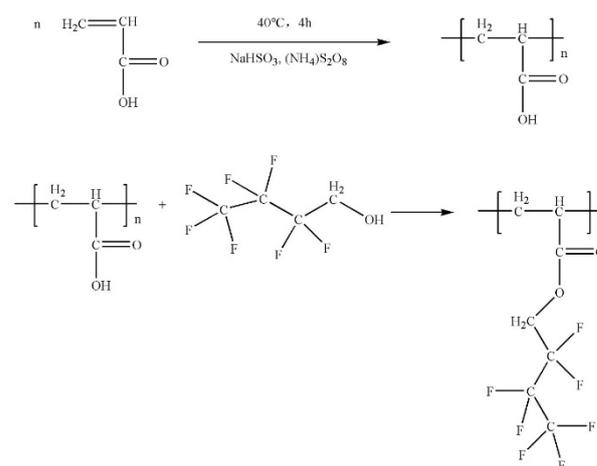


Figure. 1. Synthesis of the fluorinate polyacrylic acid

2. Experimental Details

2.1 Materials

The acrylic acid was purchased from Tianjin Yongda Chemical Reagent Co., Ltd. The sodium hydrogen sulfite and the potassium persulfate were all purchased from Aladdin. The heptafluoro-butanol was purchased from the XiYa reagent company of Shandong. The concentrated sulfuric acid (98%) was purchased from the DaLu Reagent Company of Tianjin. All water was distilled.

2.2 Synthesis of the fluorinate polyacrylic acid

Firstly, the polyacrylic acid was synthesized via solution polymerization. Briefly, polyacrylic acid and distilled water were stirred in a round bottom flask equipped with a stirring paddle, thermometer, reflux condenser, and a constant pressure drip funnel. The mixture was then stirred and heated to 40°C. Next, sodium hydrogen sulfite and potassium persulfate were dissolved in distilled water and dropped into the flask separately. This was stirred at 40°C for 4 h. Finally, the product was dried in a vacuum oven at 40°C (Figure 1).

Secondly, the fluorinated polyacrylic acid was synthesized via an esterification reaction. The polyacrylic acid, distilled water, heptafluoro-butanol, and concentrated sulfuric acid (98%) were added to the flask and stirred at 80°C for 8 h. After cooling the mixture to room temperature, it was neutralized with sodium hydroxide to pH=7. Finally, the product was dried in a vacuum oven at 40°C (Figure 1). Thus, we acquire different esterification rates by changing the dose of the fluorinated butanol. The weight gain of the pure polyacrylic acid was determined to evaluate the esterification rate.

2.3 Characterizations

A nuclear magnetic resonance 1H-NMR spectrometer (BRUKER nuclear magnetic resonance spectrometer, 400 MHz, University of Science and Technology of HeBei) was used to confirm the chemical structures of our samples. An FT-IR spectrometer (Agilent Technologies, Cary 600 Series FT-IR Spectrometer PIKE Technologies GladiATR, Academy of Science of HeBei Province) in ATR mode was used to confirm our esterification.

Thermal stability of pure polyacrylic acid and the fluorinated polyacrylic acid were measured on TGA (TGA instrument Q50 thermal analysis, New Castle, DE, USA) measurement. The viscosity of the unesterified and esterified samples was evaluated by the rotary viscosity tester (Changji Geological Instrument, Shanghai) via aqueous solution testing.

The surface tension of the unesterified and esterified samples was evaluated by surface tension measurements in aqueous solution (Digidrop image analysis software from GBX was used). Each value is the average of five measurements.

The impact of fluorination on macrophage uptake was tested with 1.0×10^6 cells/mL in a 24-well plate. The macrophages were cultured for 24 h, and OVA and the fluorinated polyacrylic acid were then added. After another 24 h, the cells were centrifuged to obtain the supernatant. The concentration of IL-1 β in the supernatant was measured with a commercial kit.

3. Results and Discussion

3.1 ¹H-NMR characterization of the fluoridated polyacrylic acid

The chemical structure of the fluoridated polyacrylic acid was identified by 1H nuclear magnetic resonance. Figure 2 shows that the peaks at a chemical shift of 1.2ppm were due to hydrogens on the methylene of polyacrylic acid. The peaks at 2.6 ppm were attributed to the hydrogens on the methyne of polyacrylic acid, and the peaks at 3.8 ppm were due to hydrogens on the methylene of heptafluoro-

butanol. This verified the preparation of the fluoride polyacrylic acid.

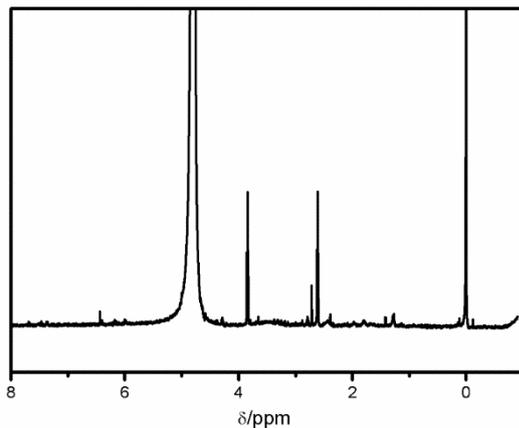


Figure 2. ¹H-NMR characterization of the esterified polyacrylic acid

3.2 FT-IR characterization of the fluoridated polyacrylic acid

Figure 3 shows the FT-IR spectra of both fluoridated and unfluoridated polyacrylic acid samples. They have obviously different bands. The chemical structure formula showed the 2989 cm⁻¹ (CH) and 2910 cm⁻¹ (CH) peaks. The band centered at 1706 cm⁻¹ is the absorption peak of ester carbonyl. And the band at 618 cm⁻¹ and the strong band at 1104 cm⁻¹ are related to the C-F bond. This confirms that the esterification reaction has occurred. Therefore, the comparative analysis of different bands of both spectra (unfluoridated and fluoridated polyacrylic acid) revealed that the polyacrylic acid has been modified by the fluoridated butanol successfully.

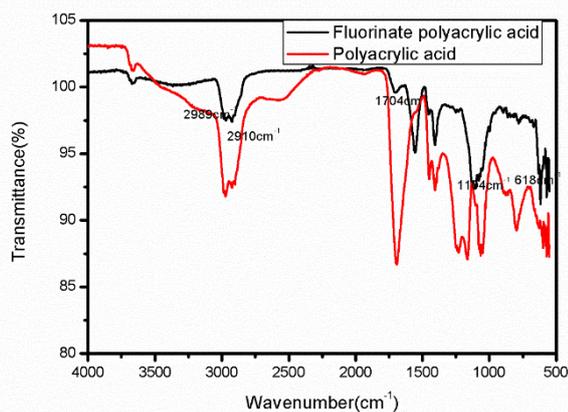


Figure 3. The FT-IR of fluoridated and unfluoridated polyacrylic acid

3.3 TGA analysis of fluoridated and unfluoridated polyacrylic acid

The effectiveness of the esterification rate via the polyesterification reaction was also demonstrated via changes in decomposition behavior and thermal stability via thermogravimetry analysis. The thermograms detail dehydration, denaturation, and degradation of virgin and fluoridated polyacrylic acid. These different phenomena

corresponded to the decrease in weight percentage at characteristic temperatures. As shown in Figure 4, TGA of the unfluoridated samples showed three phenomena: a decrease in weight percentage near 280°C corresponding to dehydration (water loss) and probably the hydroxyl combination with the active hydrogen atom. The second stage is manifested by a big loss of mass corresponding to the degradation of the carbonyl in the samples. The final weight loss is at 430°C corresponding to the degradation of the backbone of the polymer. Here, the fluoridated sample has a strongly reduced dehydration weight. This is likely because of the decrease in the carboxyl content in the sample. The total residue of the fluoridated sample is much higher than the unfluoridated sample because the fluoridated sample is more stable. This confirms that the esterification reaction occurred on the polyacrylic acid.

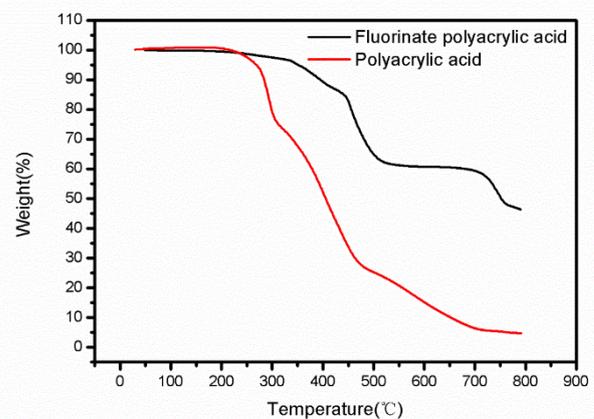


Figure 4. TGA curves of fluorinate and unfluoridated polyacrylic acid

3.4 The viscosity of the aqueous solution of the samples

The viscosity is an important index for samples when it is used as an adjuvant. The adjuvant will be injected in vivo, and a high viscosity adjuvant is not conducive to injection. Therefore, a lower viscosity is necessary for our newly prepared adjuvant. The viscosity of the samples is detailed in Figure 5. Here, all samples were dissolved in aqueous solution, and the concentration of the aqueous solution is 5 mg/mL; this test was conducted at room temperature. The curve shows that the viscosity of the solution seriously increases the esterification rate of the samples. The viscosity of the pure polyacrylic acid solution is 30.6 mPa·s, but the viscosity of the modified sample with an esterification rate of 12.03% is 70.3 mPa·s. Considering the disadvantages of the high viscosity for injection, this study selected an esterification rate of 10.52% to be optimal leading to a viscosity of 64.8 mPa·s.

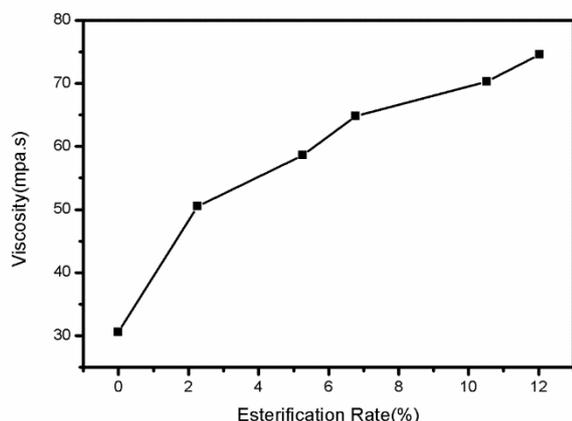


Figure 5. The viscosity of the fluorite polyacrylic acid with different esterification rates

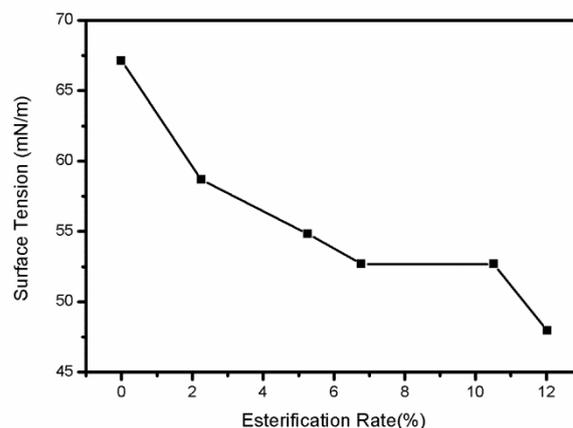


Figure 6. The surface tension of the fluorite polyacrylic acid with different esterification rates.

3.5 The surface tension of the solution of the fluorite polyacrylic acid with different esterification rates

The surface tension of the solution was measured to assess hydrophobicity. A smaller value implies a more hydrophobic material. Many researchers have shown that the antigen-presenting cells tend to take in the materials whose hydrophobicity is high [17]; thus, hydrophobicity of the samples should be measured. Here, all samples were dissolved in aqueous solution (5 mg/mL) at room temperature.

The relationship between the surface tension and the esterification rate is shown in Figure 6. The surface tension obviously decreases with increasing esterification rate. Thus, the hydrophobicity of the samples decreases following an increase in the esterification. This phenomenon conforms to the normal law of the surfactant. The fluorine atom is hydrophobic property, and the polyacrylic acid is hydrophilic; thus, the polyacrylic acid modified by the perfluorinated butanol is both hydrophobic and hydrophilic. Therefore, the samples could act as a surfactant. A higher esterification rate could result in a higher hydrophobicity, and the high hydrophobicity of the materials could induce phagocytosis. However, the higher esterification rate could also result in a high viscosity. A high esterification rate requires significant perfluorinated butanol that is expensive. The optimized sample had a surface tension of 52.67 mN/m when the esterification rate is 10.52%.

3.6 The activation effect of the fluoridated polyacrylic acid on macrophages

The secretion of pro-inflammatory cytokines is one of the activation signs for antigen presenting, and IL-1 β is an important pro-inflammatory cytokine. Thus, we evaluated the activation effect by measuring IL-1 β in tissue culture media (Figure 7). The pure polyacrylic acid has an obvious effect for the activation of the antigen-presenting cells. This confirms the literature on polyacrylic acid. In addition, the fluoridated polyacrylic acid can better improve the activation effect on macrophages than pure polyacrylic acid. Thus, the fluoridated polyacrylic acid has better performance than pure polyacrylic acid, which may potentially used as an adjuvant.

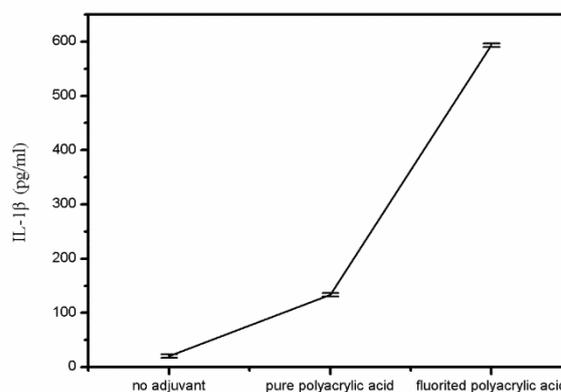


Figure 7. The activation effect of the fluoridated polyacrylic acid on macrophages

4. Conclusion

We prepared a novel polyacrylic acid modified by fluorine to improve the performance of the polyacrylic acid when used as an adjuvant. We characterized the chemical structure of the polyacrylic acid esterified by perfluorinated butanol via $^1\text{H-NMR}$. FT-IR confirmed esterification. The thermal analysis showed enhanced stability with the fluorine groups. The viscosity significantly increased with esterification, which revealed

that the fluoridation has an adverse effect for adjuvants. The hydrophobicity rapidly increased with esterification rate. The activation effect of the fluoridated polyacrylic acid on macrophages showed that content of IL-1 β was increased largely by adding fluoridated polyacrylic acid as adjuvant. Therefore, fluorinated polyacrylic acid polymers are expected to be used in the field of vaccines, and a great deal of research work on their mechanism is waiting for us to carry out in the future.

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