Radiation Physics and Chemistry 91 (2013) 114–119

Contents lists available at SciVerse ScienceDirect

Radiation Physics and Chemistry

journal homepage: www.elsevier.com/locate/radphyschem

Antibacterial activity of starch/acrylamide/allyl triphenyl phosphonium bromide copolymers synthesized by gamma irradiation

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HIGHLIGHTS

- Cationic starch is prepared from AM and TP by Gamma irradiation.
- Cationic starch is characterized by FTIR, NMR, weight method and titration method.
- Grafting ratio and cationic degree depend on absorbed dose and composition.
- Cationic starch shows good antibacterial activity against *Staphylococcus aureus*.

ARTICLE INFO

Article history:
Received 30 November 2012
Accepted 7 June 2013
Available online 15 June 2013

Keywords:
Cationic starch
Allyl triphenyl phosphonium bromide
Acrylamide
Gamma irradiation
Antibacterial activity

ABSTRACT

Starch/acrylamide/allyl triphenyl phosphonium bromide (St/AM/TP) copolymers were synthesized by simultaneous gamma irradiation and characterized by FTIR and ^{1}H NMR techniques, weight measurement and titration method. Moreover, their antibacterial activities against *Staphylococcus aureus* were explored by the viable cell counting method in sterile distilled water. At St/AM/TP 6:8.4:5.6 g, copolymers with higher graft ratio (G) and higher (AM+TP) graft efficiency (E_{AM+TP}) were obtained at 3 and 6 kGy, while cationic degree (CD) and TP graft efficiency (E_{TP}) continuously increased with absorbed dose from 1 to 6 kGy. All of the copolymers were capable of killing $>99.75\%$ of 10^7 CFU/ml *S. aureus* within 30 mins. Moreover, at 3 kGy, G, E_{AM+TP} and E_{TP} increased with AM/TP from 0:14 to 11.2:2.8 g at St/(AM+TP) 6:14 g, while the optimum CD and antibacterial activity were achieved at AM/TP 7:7 and 8.4:5.6 g. In addition, at 3 kGy, G, E_{AM+TP} and CD increased with St/(AM+TP) from 6:3 to 6:18 g at AM/TP 8.4:5.6 g, while the optimum antibacterial activity was achieved at 6:10 to 6:18 g, and the optimum E_{TP} was achieved at 6:14.

1. Introduction

Starch is one of nature's major renewable resources and also a mainstay of our food. As petroleum supplies dwindle and become less reliable, starch takes on new proportions as an abundant source of basic industrial chemicals. However, native starch has limited usage due to its inherent weakness of hydration, swelling and structural organization. In order to remove these limitations and enhance their viscosity, texture and stability, starch and its derivatives are generally modified by chemical, physical and biotechnological methods (Singh et al., 2010). Among them, starch Modifications, by grafting vinyl or acrylic monomers onto itself and then polymerization initiated either by a chemical free radical initiator (e.g. ceric ammonium nitrate or ammonium persulfate) (Chen et al., 1999; Athawale and Lele, 2000) or by high energy irradiation (e.g. gamma ray or electron beam) (Kiatkamjornwong et al., 2002; Song et al., 2007), have largely improved its hydrophilicity, hydrophobicity and polyelectrolyte nature. The grafting is achieved by first generating free radicals on starch backbone and then these radicals serve as macro initiators for vinyl or acrylic monomers. Compared with irradiation method, chemical method has low reproducibility and is not very suitable for commercial scale synthesis. Irradiation-induced grafting is of high efficiency and does not cause any further contamination associated with chemical initiators (Khalek and Mahmoud, 2011).

Cationic starch can be obtained through grafting with cationic monomers (Carr, 1994; Lu et al., 2004; Liu et al., 2011), and the cationic groups are generally amino, immonium, ammonium, sulphonium and phosphonium. Generally, they have been synthesized by grafting quaternary ammonium salt monomers onto starch using gamma irradiation (Peoria, 1984; Lv et al., 2013). However, starch modified by quaternary phosphonium is rear in literature due to the polarizability difference between ammonium

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0969-806X/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.radphyschem.2013.06.003

and phosphonium cationic centers. Phosphonium-based cationic starch can be expected to have different capability comparing to its ammonium counterpart. In the present paper, cationic starches are synthesized by copolymerization of starch (St), acrylamide (AM) and allyl triphenyl phosphonium bromide (TP) by means of simultaneous gamma irradiation, and the effects of absorbed dose and St/AM/TP feed ratio on the grafting parameters are investigated.

Besides these reports, a number of studies have investigated composite materials impregnated with antibacterial agents and the antibacterial effects of most of these materials were due to active ingredients released from the composites themselves. Therefore, their effects were time-dependent, and generally short-lasting. Moreover, antibacterial agents impregnated with such as silver–zeolite, may deteriorate the mechanical properties of the carrier material, and the antibacterial moieties released may be toxic if present at high concentrations (Yoshida et al., 1999; Appendini and Hotchkiss, 2002; Xiao et al., 2008). In order to overcome the disadvantages of agent-releasing composites, antibacterial agents can be chemically bound to polymer carriers via active groups, for example, quaternary ammonium salt monomers, which have been widely used in paint, water treatment, textiles, and the food industry because of their relatively low toxicity and broad antibacterial spectrum. These antibacterial monomers can be grafted onto polymer carriers through radical polymerization initiated by either chemical method or high energy irradiation. As compared with small molecule antibacterial agents, antibacterial polymers decrease associated environmental hazards due to non-volatility and chemical stability (Xiao et al., 2008). However, the polymeric phosphonium salt exhibited a higher activity by two-orders of magnitude than the polymeric quaternary ammonium salt with the same structure except cationic part (Kanazawa et al., 1993), which implied that better antibacterial activity can be achieved with a smaller amount of the polymeric phosphonium salts. A small amount of the polymeric phosphonium salt might reduce the risk of mechanical and chemical deterioration of carrier materials. In the present paper, the antibacterial activities of St/AM/TP copolymers are specially investigated.

2. Experimental

2.1. Materials

Food grade corn starch powder was supplied from Henan Yongchang Starch Sugar Co., Ltd. Allyl triphenyl phosphonium bromide (analytical reagent) was purchased from Shandong Luyue Chemical Co., Ltd. Acrylamide (analytical reagent) was obtained from Tianjin NO.3 Chemical Reagent Factory. All other chemicals were analytical reagent. Double distilled water was used for preparation and measurements. *Staphylococcus aureus* (*S. aureus*) was supplied by Henan Centers for Disease Control and Prevention.

2.2. Irradiation induced copolymerization

A mixture of corn starch powder and distilled water was continuously stirred using a mechanical stirrer under nitrogen atmosphere in water bath at 85 °C for 30 min. The obtained gelatinized corn starch was mixed with AM and TP, and continuously stirred to form a homogenous mixture under nitrogen atmosphere. Thereafter, the homogenous mixture was transferred into a wide-mouth, screw-cap glass bottle and repressured with nitrogen four times. The bottle was tightly closed with a cap, irradiated with cobalt 60 to a required absorbed dose at 40 Gy/min dose rate at 25 °C, and allowed to stand at ambient temperature for 2 h.

2.3. Determination of graft ratio and graft efficiency of binary monomers

The rubbery product obtained was broken up, washed with ethanol, vacuum-dried at 60 °C and ground into fine powder. The powder was extracted continuously for 18 h in a Soxhlet extractor with a mixture of acetone and glacial acetic acid (1:1 v/v), and thus ungrafted poly(AM-co-TP) and unreacted monomers were removed from the powder. The refined copolymer after solvent extraction was dried in a vacuum oven at 60 °C to a constant weight.

Graft performance in terms of percentage graft ratio (G) and graft efficiency (E_{AM+TP}) of binary monomers (AM+TP) was determined by weight measurement. G and E_{AM+TP} were calculated by Eqs. (1) and (2).

$$G = \frac{m}{m_{St}} \times 100\% \tag{1}$$

$$E_{AM+AT} = \frac{m - m_{St}}{m_{AM} + m_{AT}} \times 100\% \tag{2}$$

where, m is the mass of St/AM/TP copolymer. m_{St}, m_{AM} and m_{AT} are the initial masses of starch, AM and TP, respectively.

2.4. Determination of cationic degree and graft efficiency of TP alone

Cationic degree (CD) of the starch graft copolymer was determined by titration (Mohr's method) (Block and Waters, 1967), which depended upon the amount of TP units incorporated into the copolymer. The copolymer was weighed into 250 ml Erlenmeyer flask and dissolved in 100 ml of distilled water, and then the pH value of the solution was adjusted between 6.5 and 7.2. Potassium chromate served as the end point indicator in the titration of chloride ions with a silver nitrate standard solution, and the colour changed from yellow to brick-red which signalled the end point of this titration. CD used in mmol/g was calculated by Eq. (3).

$$CD = \frac{M \times (V - V^0)}{m} \tag{3}$$

where, M is the molarity of the silver nitrate standard solution and V is the volume consumed during titration, V^0 is the volume of the silver nitrate standard solution consumed during blank titration. M, V and V^0 are used in mmol/ml, ml and ml, respectively. In this

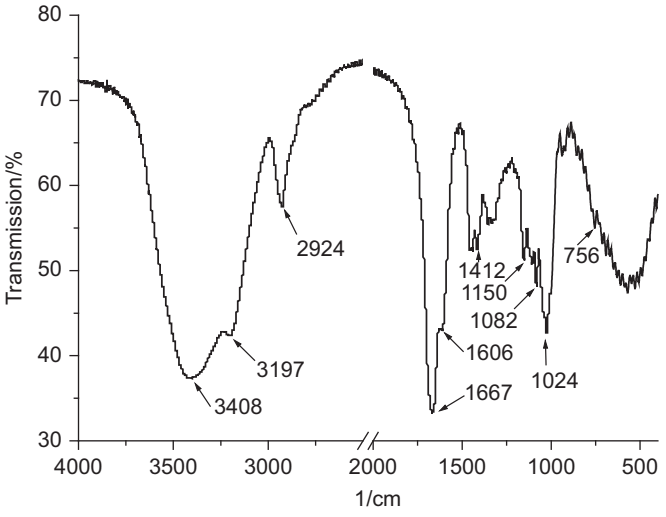

Fig. 1. FTIR spectrum of St/AM/TP copolymer prepared using a 6:8.4:5.6 g ratio of St/AM/TP in 60 ml water at absorbed dose of 5 kGy.