Carbohydrate Polymers 89 (2012) 842–848

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Chitosan-functionalized poly(methyl methacrylate) particles by spinning disk processing for lipase immobilization

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ARTICLE INFO

Article history:
Received 10 January 2012
Received in revised form 7 April 2012
Accepted 7 April 2012
Available online 26 April 2012

Keywords:
Poly(methyl methacrylate)
Chitosan
Core–shell particle
Lipase immobilization
Spinning disk processing

ABSTRACT

Chitosan-functionalized poly(methyl methacrylate) (PMMA-CH) particles were prepared by complexation between the negatively charged PMMA particles and the positively charged chitosan *via* a spinning disk processing. Processing parameters; feed rate and spinning speed, were optimized, which were traced by size distribution profiles of the formed PMMA-CH particles. Their sizes and net surface charges were found to be affected by MWs of chitosan (45, 100, and 230 kDa) used. Microscopic evidences were used to confirm their core–shell morphology. Chemical characteristics and thermal stability of such particles were determined by FTIR and TGA, respectively. Then, their ability to immobilize lipase (EC 3.1.1.3) was conducted and followed through zeta potential measurement. The percentage of lipase adsorption capacity increased with increasing lipase content, but the value decreased when the size of PMMA-CH particles increased. Also, the activity of lipase attached on PMMA-CH particles' surface was preserved and increased with lipase loading.

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1. Introduction

Chitosan is a naturally occurring polymer obtained by partial deacetylation of chitin, a main structural component of cuticles of crustaceans. It is insoluble in water, but becomes soluble and positively charged in acidic media (Marie, Landfester, & Antonietti, 2002). Due to its biodegradability, biocompatibility, and renewability, chitosan has been used in a wide range of applications (Jia et al., 2011). In addition, this polymer has been used successfully to colloidally stabilize polymer nanoparticles, such as poly(methyl methacrylate), or polystyrene nanoparticles (Caruso, Lichtenfeld, Giersig, & Mohwald, 1998; Li, Zhu, Sunintaboon, & Harris, 2002). Nowadays, chitosan-decorated materials attract much attention because they could be used in diverse aspects such as controlled release systems of drugs, antibiotics, or biological active agents (Torrado, Prada, De la Torre, & Torrado, 2004), particularly when chitosan was cross-linked with genipin (Muzzarelli, 2009). Moreover, chitosan has also been used as a matrix for lipase immobilization since it has numerous amine groups, which can interact with enzyme (Chiou & Wu, 2004; Muzzarelli, Francescangeli, Tosi, & Muzzarelli, 2004). This methodology is very simple and improves enzyme stability significantly. Chitosan is also recognized by its excellent properties for lipase support such as biocompatibility, biodegradability, nontoxicity, physiological inertness, and great affinity for proteins (Krajewska, 2004).

Lipases (EC 3.1.1.3) are in a family of enzymes that, in their natural environment, can catalyze the hydrolysis of triacylglyceral to glycerol and fatty acid. However, under appropriate conditions, lipases have shown to be very active catalysts in transesterification, alcoholysis, and esterification (Foresti & Ferreira, 2007). An important application of lipase is in industrial processes and products due to their high specificity, good rate of reaction, non-toxicity, and water solubility, which are major advantages over inorganic catalysts. However, the use of enzymes and other proteins has been limited due to their considerably unstable nature and the resulting requirement of stringent conditions, such as a particular pH and temperature. Immobilization onto matrix has become a widely used technique to overcome these problems. The immobilization may also enhance the operation lifetime, thermal stability, structural rigidity, and recoverability of the biocatalyst. Generally, the efficiency of enzyme immobilization is decided by the support

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http://dx.doi.org/10.1016/j.carbpol.2012.04.019

size or surface area. If lipase is immobilized on nanoparticle or submicron particle supports, which have high specific surface area and low diffusion resistance, this would improve the effectiveness of the catalyst considerably (Wu, Wang, Lou, & Dai, 2009).

Recently, spinning disk processing (SDP) emerges as a technology in process intensification, which provides uniform reaction conditions on a rotating disk, in contrast to traditional batch technology where conditions can vary across the dimensions of reaction vessel. SDP also provides rapid mixing and high contact between mixing components. The centrifugal force produces intense interfering waves, which generates high heat transfer between liquid solution and disk, and high mass transfer between the solution and air above the disk. The waves of starting materials generated on the disk form almost pure plug flow, causing every molecule to experience the same environment. In addition, the residence time is typically in seconds. Due to these reasons, SDP can lead to enormous improvement in reaction selectivity and outputs. Another novel feature of SDP is that it is a continuous flow process. Therefore, it can facilitate large scale production (Anantachoke et al., 2006).

In this work, the successful preparation of chitosan-functionalized PMMA particles by SDP was illustrated. The positively charged chitosan was coated on negatively charged PMMA particles as a result of electrostatic interaction and efficient mixing conditions provided by SDP. Then, the complexes obtained were subjected to various physico-chemical characterizations. The positive charges of outer chitosan layer were then used for lipase immobilization. Adsorption behavior of lipase on PMMA-CH particles was investigated by zeta potential measurements. The specific activity of free and immobilized enzymes was also investigated.

2. Materials and methods

2.1. Materials

Chitosan (from Seafresh Chitosan Lab., Thailand) solutions (1 wt%) of three molecular weights: 45, 100, and 230 kDa (abbreviated as CH45, CH100, and CH230, respectively) were prepared by dissolving the materials in 1 wt% acetic acid aqueous solution. Methyl methacrylate (MMA) monomer was purchased from Sigma–Aldrich and purified by distillation under reduced pressure and was stored at 4 °C before use. Potassium persulfate (KPS) was purchased from Fluka. It is an analytical grade reagent and used without further purification. Lipase AY (from *Candida rugosa*, lyophilized powder) was supplied by Amano Enzyme Co., Nagoya, Japan with the activity and the molecular weight of 32,800 U/g and 60,000 g/mol, respectively (Lot No. LAY E0151016). 1,2,3-Tributyrylglyceral (tributyrin) was purchased from Sigma–Aldrich. Gum arabic (Ga) was purchased from Ajax Finechem, New Zealand. Tris (hydroxymethyl) aminomethane (Tris) was purchased from Fisher Scientific U.K. Limited, United Kingdom. Distilled water was used for all experiments.

2.2. Preparation of negatively charged PMMA particles

Negatively charged PMMA particles were synthesized *via* a soap-free emulsion polymerization. Briefly, 40 g of distilled water and 3 g of MMA monomer were charged into a 100 mL three-necked round-bottom flask with a reflux condenser, and nitrogen inlet. After degassed for 30 min and the temperature of the reactor raised to 80 °C, then 7 g of aqueous KPS solution (9.16 mM) was added into the reactor. After feeding initiator, the polymerization was carried out at the same temperature for 3 h. Then, percentages of monomer conversion and solid content were determined gravimetrically. About 3 g of prepared latex was taken into a pre-weighed aluminum pan. After complete evaporation in a fume-hood, the air-dried latex was further dried in a vacuum oven at 70 °C. The percent monomer conversion and solid content were calculated (Inphonlek, Pimpha, & Sunintaboon, 2010).

2.3. Preparation of PMMA-CH core–shell particles

PMMA particle (1 wt%) dispersion was allowed to interact with 0.1, 0.5, and 1 wt% of chitosan solution on a spinning disk rector (SDR type-102 series, 100 mm diameter of the disk) *via* electrostatic interaction to obtain PMMA-CH core–shell particles. Both components were pumped to mix on the disk with feed rates of 1.0, 1.5, and 2.0 mL/s using 2.1 mm feed injection diameter. The speed levels of spinning disk were varied as 500, 1000, and 1500 rpm. After the optimum feed rate and spinning speed were obtained, the effect of chitosan molecular weights was studied. The complexed particles prepared from chitosan 45, 100, and 230 kDa were abbreviated as PMMA-CH45, PMMA-CH100, and PMMA-CH230 particles, respectively. In each mixing process on SDR, the mixture was collected from the product discharge panel.

2.4. Measurement of the mean particle size and zeta potential

The mean particle size and size distribution of neat PMMA and PMMA-CH particles were measured by dynamic light scattering (DLS) (MALVERN instruments) with the refractive index of 1.476 for neat PMMA and 1.590 for PMMA-CH particles. All DLS measurements were carried out at room temperature and in aqueous medium. A mechanical stirring was applied to enhance dispersibility of the sample. A background measurement was performed using distilled water before sample measurement to avoid background electrical noise. Then, the sample were dropped into the sample chamber until the suitable concentration was attained, which was in a range of 5–10 wt%. All results were the average of triplicate measurement.

Surface charges of PMMA-CH particles were determined on electrophoretic light scattering using zetasizer (zetasizer 3000, Malvern Instruments, UK). Samples were diluted with 1 mM NaCl solution at room temperature and measured in the automatic mode. All measurements were performed in triplicates.

2.5. Morphological characterization

The size and morphology of particles were also observed with transmission electron microscope (TEM, Phillip TECNAI 20). The particle dispersion was diluted fifty times with distilled water. An aliquot of 50 μL was then deposited on a copper grid and stained with 1.5 wt% phosphotungstic acid (PTA). The particle diameter was denoted with arithmetic average diameter. In addition, the information of particle size and morphology was investigated by scanning electron microscope (SEM, S-2500, Hitachi) and atomic force microscopy (AFM). To prepare the samples for SEM, particle dispersion was dropped on a cover glass and dried in a dust-free environment. The dried specimens were attached on the sample holders with a double-coated carbon conductive tape and then were sputter-coated under vacuum with platinum and palladium by using a sputter coater (E102, Hitachi, Japan). The sputter-coated samples were then observed by the microscope at 15 kV. For AFM, the particle dispersion with a suitable dilution in distilled water was dropped on a cover glass, and dried overnight. AFM images were observed in air with a Nanoscope IIIa (model Ns3a) operating in a tapping mode.

2.6. FT-IR spectroscopic analysis

Fourier-transform infrared (FTIR) spectroscopy (Perkin-Elmer, PE2000) was utilized to characterize the functional groups of

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