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Comparison of using formaldehyde and carboxy methyl chitosan in preparation of Fe_3O_4 superparamagnetic nanoparticles-chitosan hydrogel network: Sorption behavior toward bovine serum albumin

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ABSTRACT

A novel and cost effective method of bio-separation developed recently is magnetic separation technology. In this study, super paramagnetic Fe_3O_4 nanoparticles are used for separation of bovine serum albumin (BSA) protein from plasma/serum samples at optimized conditions. The synthesis of chitosan hydrogel networks by two variant approaches that involve (1) crosslinking of chitosan with formaldehyde and (2) formation of carboxy methyl chitosan mediated complex, was investigated and the percent of gelation, swelling ratio and equilibrium water content were calculated. The results revealed the formation of better quality hydrogel from the first approach. In step 1, to quantify the BSA separation using the chitosan gel, the protein yield and purification factor relationships were introduced. In step 2, superparamagnetic Fe_3O_4 nanoparticles were synthesized using co-precipitation method and immobilized on chitosan hydrogel networks which were produced following the first technique and used in magnetic separation approach. Fe_3O_4 nanoparticles were immobilized on chitosan hydrogel network and their size was calculated to be about 9.8 nm. The separation of BSA by hydrogel-nanoparticles network was evaluated comprehensively and the conditions for achieving the highest separation efficiency were determined. The percentage of gelation reached to its maximum value by adding 7 mL formaldehyde. Results from atomic force microscopy indicated that separation efficiency significantly improved from 48% to 70% with less number of steps by using hydrogel-nanoparticles network as compared to chitosan hydrogel network. The results also confirmed that iron oxide nanoparticles maintained their magnetic properties after immobilization on the chitosan hydrogel network. Moreover, the separation process was found to be more convenient and efficient in case of hydrogel-nanoparticles network, due to the application of simple magnet for separation.

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Nomenclature

D	particle size (μm)
K	constant equal to 0.9
DS	degree of swelling
EWC	equilibrium water content
W_d	weight of hydrogel after dying
W_i	weight of hydrogel
W_s	weight of sample after removing excessive surface water

Greek letters

β	excess line broadening (rad)
λ	X-ray wavelength (nm)
θ	Bragg angle (rad)

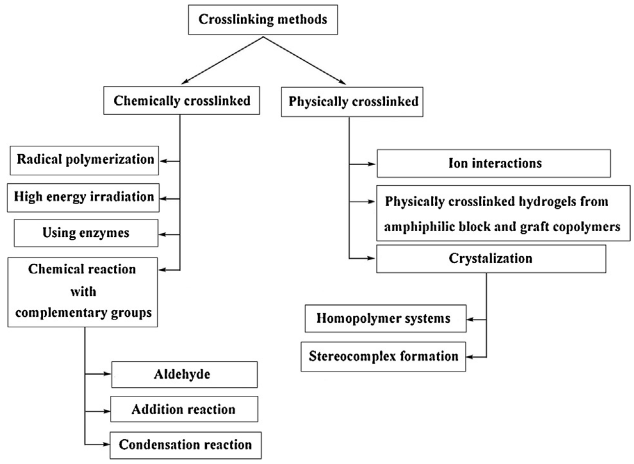

Fig. 1 – Novel cross-linking methods used in hydrogels.

1. Introduction

Magnetic separation is a technology developed recently and is mostly applied to bio-separation. The principle is to utilize magnetic particles for binding targeted biomolecules to form a complex that can be separated from the bulk of a solution by magnetic field gradient. Several studies in the past have focused on the application of the magnetic separation for the cell sorting (Prabaharan and Fau Mano, 2006; George et al., 2006; Sharma et al., 2006), enzyme immobilization (Rubinstein, 1995; Kato et al., 2003), nucleic acid detachment (Shutava et al., 2006; Fan et al., 2006), protein adsorption and purification (van der Lubben and Fau Verhoef, 2001; Bernkop Schnurch and Fau Walker, 2001) and drug delivery (Pandey et al., 2005). This approach showed several advantages as compared to conventional separation techniques. It can be executed directly into crud samples containing suspended solid materials such as fermentation broth or culture medium. Also, limited studies have been found in literature on the application of hydrogel nanoparticles in the separation of proteins. Hydrogel nanoparticles have gained considerable attention in the recent years as one of the most promising nanoparticulate delivery systems that combine the characteristics of a hydrogel system with nanoparticles. For example, such characteristics are hydrophilicity and high water content. Several polymeric hydrogel nanoparticulate systems based on both natural and synthetic polymers, each with its own advantages and disadvantages, have been prepared and characterized lately. Among the natural polymers, chitosan and alginate were studied extensively for preparation of hydrogel nanoparticles (Lehr, 1992; Janes et al., 2001; Artursson et al., 1994; Borchard et al., 1996; Schipper et al., 1996, 1997, 1999; Zhang et al., 2010; Carreño Gómez and Duncan, 1997; Dodane and Vilivalam, 1998; Hamidi et al., 2008).

Chitosan possesses excellent chemical properties, such as chemical stability and compatibility with bioactive compounds. Hence, it can be used as a gel carrier. Chitosan is an N-deactivated derivative of chitin, a cationic polysaccharide, composed of β-D-glucosamine and N-acetyl-β-D-glucosamine residues with a single 1,4 linkage (George et al., 2006). It is a favorable nanoadsorbent, having a large number of hydroxyl and amine groups. Chitosan's unique structure describes its ability to uptake heavy metal ions through different mechanisms, such as ion exchange or chelation (Zhou et al., 2006; Zhang et al., 2007). Several cross-linking methods are used to form the hydrogel matrix structures that can be classified into chemically and physically induced crosslinking. A range of crosslinking methods is developed to synthesize desired hydrogels for each particular application. These crosslinking methods that are most commonly used for hydrogel preparation are listed in Fig. 1.

Particles with at least one dimension being less than 100 nm are referred to as nanoparticles. These nanoparticles offer challenges in several areas such as biomedical (Nishio et al., 2007), polymer (Dutta et al., 2009) and aerosol (Wang et al., 2005; Amouei Torkmahalleh et al., 2012a,b) sciences, etc. In recent years, superparamagnetic nanoparticles (SION) have gained significant attention in material science because of its strong magnetic properties and low toxicity (Lu et al., 2007). Applications of magnetic nanoparticles are not only restricted to traditional electrical, optical, and magnetic areas, but also extended support toward new arenas, including magnetically assisted bioseparation. The utmost advantage of using SION is its rapid separation by applying an external magnetic field. Industrial methods for the synthesis of SION include ball milling (Caruso, 2001), chemical precipitation (Tsukasa Torimoto and Shin Murakami, 2003; Messer et al., 2000; Cosnier et al., 2004), thermal decomposition (Chen et al., 2009) and sonochemical synthesis (Rudakovskaya et al., 2011). Among these methods, chemical precipitation such as co-precipitation of ferric (Fe^{3+}) and ferrous (Fe^{2+}) ions is comparatively the simplest, and the most commonly used technique. In addition, it provides a relatively good control over the size and morphology of the nanoparticles.

Immobilized proteins on nanoparticles have wider applications in drug delivery and high sensitivity immunoassay. To immobilize the proteins on the magnetic nanoparticles, Mehta et al. (1997) proposed a simple method for direct binding of proteins on magnetic nanoparticles via carbodiimide activation. Their results revealed that bovine serum albumin (BSA) protein can be covalently bonded to magnetic particles while maintaining its biological properties. Chen and Chen (1997) used carbodiimide for the immobilization of lysozyme on a polymer, while Dilgimen et al. (2001) utilized carbodiimide to conjugate BSA on a polymer. Carbodiimide can also be used to activate the direct adsorption of BSA and alkaline phosphatase on magnetic particles. As a result, a number of protein conjugated magnetic nanoparticles have been proposed in the field of biotechnology (Koneracká et al., 1999; Peng et al., 2004; Ma et al., 2006; Salehizadeh et al., 2012). Recently, a successful synthesis and characterization of