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A Modified Chitosan Adsorbent for Selective Removal of Low Density Lipoprotein

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Abstract: A modified chitosan adsorbent was synthesized through a simple preparation procedure, and it demonstrated good adsorption performance for selective removal of low density lipoprotein in human plasma. Phase inversion technique was employed to form chitosan beads, to which epoxy groups were then introduced by reacting with ethyleneglycol diglycidylether, and tryptophan was subsequently coupled to the epoxy-activated beads.

Keywords: Chitosan bead, low density lipoprotein, cholesterol, adsorption, tryptophan.

Hypercholesterolemia has been identified as a major risk factor in the development of atherosclerosis and its clinical sequelae like coronary heart disease and myocardial infarction[1]. Among the lipoproteins transporting cholesterol in the blood, low density lipoprotein (LDL) is known to be causative of atherosclerosis, while high density lipoprotein (HDL) is known to serve as an atherosclerosis retarding factor[2]. For the patients with refractory hypercholesterolemia, selective adsorption, known as hemoperfusion, has been proved as a better extracorporeal therapy. Some immunoadsorbents (like Sepharose beads immobilized with anti-LDL antibodies) and affinity adsorbents (such as cellulose beads bearing dextran sulfate) have been brought into clinical application, but they are too costly to be widely used[3]. Considerable efforts have been made to develop other LDL binding adsorbents[4~9], especially with inexpensive small molecules as ligands. We have prepared a new chitosan adsorbent bearing tryptophan (Trp) *via* simple reaction routes, and this adsorbent has been proved to be very effective in selective removal of LDL in plasma. The preparation and the adsorption performance of the adsorbent are reported in this work.

Synthesis of the adsorbent consisted of three steps: preparation of chitosan beads, activating and cross-linking of the beads, and coupling of Trp. The structure of the adsorbent is shown in **Figure 1**, where P denotes the matrixes, *i.e.* chitosan beads.

Phase-inversion technique[10] was employed to form the chitosan beads. We used a powdered chitosan of which the average molecular weight was about 40000 and the

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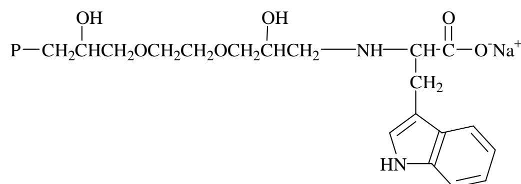
Figure 1 The structure of the adsorbent



degree of deacetylation was 90 %. 1 g chitosan was dissolved in 19 mL 3 wt% aqueous solution of acetic acid. The chitosan solution was sprayed through a spinnerette into a precipitation bath consisting of 8 wt% aqueous NaOH, and spherical porous beads were formed in the bath. The mean diameter of the beads was regulated to about 0.5 mm. The chitosan beads were then washed thoroughly with deionized water.

For activating and cross-linking of the beads, 15 mL of ethyleneglycol diglycidylether (EGDE) and 15 mL of 0.6 mol/L NaOH aqueous solution containing 30 mg NaH_4 were mixed with the above prepared beads and the reaction was continued under gentle stirring at 25℃ for 8 hours. Epoxy groups were introduced to the chitosan beads by the above reaction. The epoxy-activated beads were then washed with deionized water to remove unreacted reagents.

Coupling of Trp was conducted in the following way. 2 g of L-Trp was mixed with 5 mL 2 mol/L NaOH aqueous solution to obtain corresponding sodium salt, which was then dissolved in 20 mL of carbonate buffer, pH 10.5. The solution was added to the epoxy-activated beads and the reaction was allowed to proceed with agitation at 65℃ for 24 hours. The Trp immobilized beads were washed extensively with water, 1 mol/L NaCl solution, and again with water to remove the remaining carbonate and unreacted ligand, then stored in 0.15 mol/L NaCl aqueous solution at 4 ℃ for further use. The resultant adsorbent did not dissolve in 0.5 mol/L acetic acid aqueous solution, characterized by water content 89 % and amount of immobilized Trp 0.6 mmol/g xerogel.

In vitro tests were carried out in a batch system to evaluate the adsorption properties for lipoproteins. 0.5 g of suction-dried adsorbent in each case was incubated with 1.0 mL of plasma taken from the patients with hypercholesterolemia and stirred at 37℃ for 3 hours. Total cholesterol (TC), LDL cholesterol (LDL_C), and HDL cholesterol (HDL_C) in plasma samples before and after adsorption were determined respectively with commercial test kits. The reduction rates for TC and LDL _C were determined as 54.8±0.5 % and 66.2±2.0 % respectively, while HDL_C decreased only by 9.7±5.7 %. The corresponding initial concentrations were 6.04 mmol/L, 4.82 mmol/L and 1.22 mmol/L, respectively.

The adsorption performance of the adsorbent thus obtained is quite satisfactory, whereas the preparation procedure is very simple. Compared with other LDL adsorbents, for example, with those reported in the references[6,7,8], this adsorbent still provides much higher reduction rates for TC and LDL_C, even though twice as much plasma was added to about equal amount of adsorbents in the evaluation tests. This adsorbent also manifests good adsorption selectivity. The reduction rate for HDL_C is less than 10.0 %. This may be ascribed to the macroporous structure derived from the phase inversion method, hydrophilicity of the chitosan beads, and good affinity of Trp for

LDL. While the beads were activated with EGDE, they were also cross-linked at the same time, furthermore, a long hydrophilic spacer arm was introduced due to the molecular structure of EGDE as shown in **Figure 1**. The preparation routes is hence simplified to a great degree in contrast to other routes reported[6~9], which usually consisted of three separate steps for the above process. The adsorbent prepared in this work may be an alternative for clinical application in the future, and further study is in progress.

Acknowledgments

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