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Functional hydrophilic polystyrene beads with uniformly size and high cross-linking degree facilitated rapid separation of exenatide

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ABSTRACT

A high cross-linking polystyrene(PSt)-based anion-exchange material with uniformly size, high ion exchange capacity, and high hydrophilicity was synthesized by a novel surface functionalization approach in this study. Uniformly sized PSt microspheres were prepared by the membrane emulsion polymerization strategy, and then modified by (1) conversing resid ual surface vinyl groups to epoxy groups followed by quaternization, and (2) decorating aromatic ring matrix including nitration, reduction and attachment of glycidyltrimethylammonium chloride. The 3-D morphology and porous features of microspheres were observed by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The surface of the modified PSt became roughness but the particle size remained same. Meanwhile, FT-IR spectra and laser scanning confocal microscope (LCSM) indicated that the modification groups had been successfully covalently coated onto the PSt microspheres. Modified PSt microspheres showed greatly improved hydrophilicity and biocompatibility with 0.387 mmol/mL ion exchange capacity (IEC). In the application evaluation procedure, exenatide can be purified from 42.9% (peptide crudes) to 88.6% by modified PSt column with 97.1% recovery yield. This modified PSt microspheres had a large potential in application for efficient separation of peptides.

1. Introduction

Generally, the chromatographic media used for separation of proteins and peptides are microspheres based on homogeneous cross-linking polysaccharides, such as agarose (Sepharose^{GE}) and dextran (Sephadex). Although these polysaccharide-based media have good capacity, they cannot nearly stand back-pressures up to 1 MPa, thus, the low recommended pressure or flow rate sometimes limit their applications for large-scale operations [1]. High cross-linking polystyrene/divinylbenzene (PSt) microspheres have good mechanical strength and can be prepared with a wide variety of diameters and pore sizes. Therefore, cross-linking polystyrene particles have become the most widely used insoluble polymeric supports for ion-exchange, chromatographic, and biosynthetic applications [2]. The presence of over 50% divinylbenzene cross-linker leads highly cross-linking PSt to swelling stability and mechanical strength. However, PSt can be only directly used in reversed phase chromatography (RPC) due to its strong hydrophobicity. When biomolecules touch the hydrophobic polystyrene surface, they always suffer considerable denaturation and even lose biological activity due to the strong hydrophobic interaction. Therefore, it is necessary for PSt to be hydrophilized in other chromatography modes. The hydrophobicity and non-specific adsorption of the PSt can be decreased by covalently incorporating various functional groups such as hydroxyl, polyethyleneimine, amino or protein onto its surface [3,4]. It is easy to chemically modify the low cross-linking PSt (≤8% cross-linker divinylbenzene) because of its good swelling behavior in organic solvents [5,6]. However, the chemical modification of highly cross-linking PSt is difficult or insufficient because of its stable aromatic structure and tight cross-linking steric framework, which cause a relatively low modification yield.

Commercial companies, such as GE and Life Technologies, choose monosized rigid PSt as matrix, and successfully develop profitable PSt-based functional chromatographic microspheres (GE SOURCE^{TM} series, Life Technologies POROS^{TM} series). For example, based on a unique 30 μm matrix, PSt SOURCE^{TM} 30Q is produced and substituted with quaternary ammonium groups. The ion exchange groups are attached to the matrix via hydrophilic

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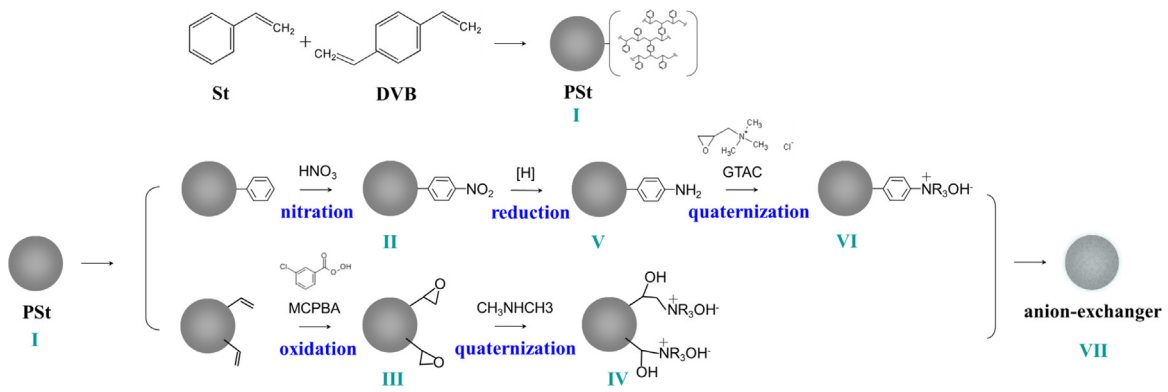

Fig. 1. Scheme of preparation and modification processes of PSt microspheres.

spacer arms after hydrophilization of the polymeric base matrix [7]. Typical flow rates of anion-exchange SOURCE™ 30Q can be up to 1000 cm/h at large-scale, and even higher on the laboratory bench [8]. POROS™ chromatography resins are also mechanically rigid and can be packed in high-pressure stainless steel columns. Surface of POROS™ XQ/HQ resins are functionalized with quaternary amine based on PSt backbone, and their macroporous region are made by interconnected throughpores ($ø_{pore}$ > 500 nm) where intraparticle convection and pore diffusion occur [9].

Exenatide (exendin-4) was a 39 amino acid peptide (53% structural homology to GLP-1), and was first isolated from the salivary secretions of Gila monster lizard [10]. It shared many of the glucoregulatory actions with GLP-1 and had aroused great attention for its potential for the treatment of diabetes [11]. It has several beneficial anti-diabetic actions such as glucose dependent enhancement of insulin secretion, glucosedependent suppression of inappropriately high glucagon secretion, and slowing of gastric emptying [12]. The isoelectric point of exenatide was 5.46. Cai and Yue separated exenatide analogue mono-PEGylated with 40 kDA polyethylene glycol by agarose-based cation exchange chromatography SP Sepharose® [13]. Zhou et al. purified recombinant exenatide using anion exchange resin DEAE–Sephadex® A-25, which synthetically derived from dextran [14]. But the recovery yield by the polysaccharose ion-exchange resins was relatively low.

In a previous work, structure regulation of uniform PSt micro/nano-particles can be systematic controlled by the membrane emulsification coupled polymerization processes [15]. Our ongoing work aimed at constructing functionalized PSt microspheres using Friedel–Crafts (FC) alkylation/acylation reactions for graft substitution to the aromatic ring [16]. However, the modified PSt microspheres exhibited relatively low capacity and high non-specific adsorption. The possible reason was that the hydrophobicity of PSt ascribed to not only the unsubstituted aromatic ring but also the residual vinylic groups of the matrix. Residual vinylic groups can be converted into epoxy functionality on the poly-divinylbenzene surface thereby served purposes

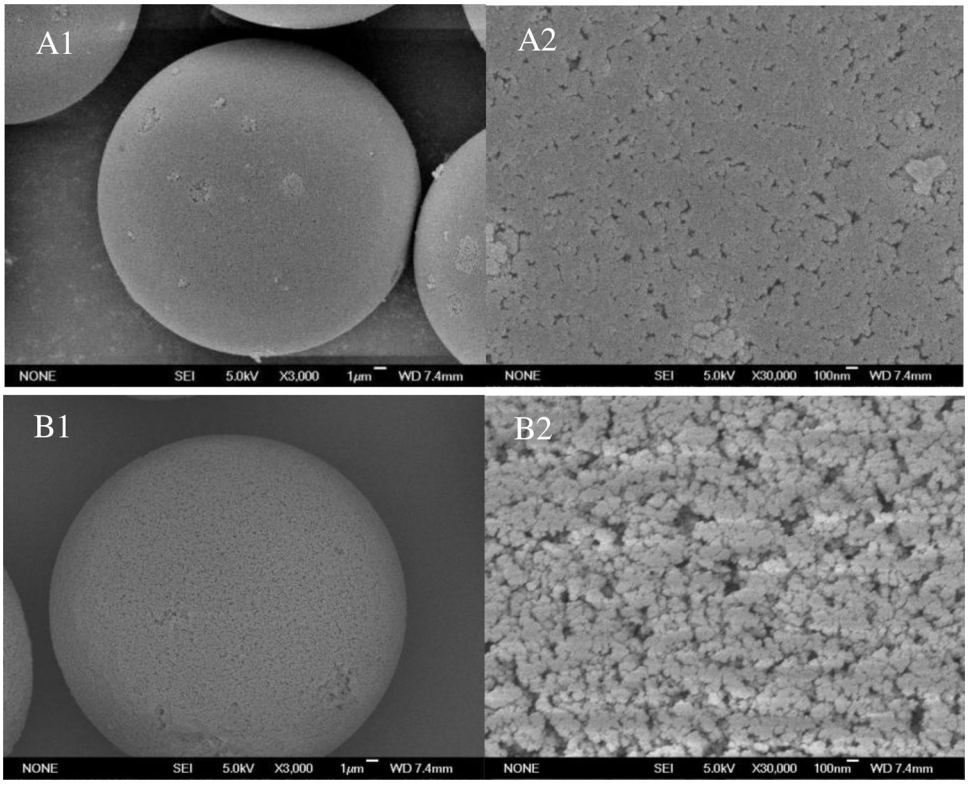

Fig. 2. SEM images of PSt before and after modification (A1, A2 before; B1, B2 after).