PREPARATION AND CHARACTERIZATION OF THE CHIRAL STATIONARY PHASE BASED ON THE CHITOSAN

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ABSTRACT

A chiral stationary phase (CSP) was synthesized by the modification of the chitosan using N-nicotinoyl phenylalanine and 3,5-dimethylphenylisocyanate. The CSP based on the chitosan was then characterized in terms of their chemical structure and physical properties. To test its performance as a CSP, the silica powder with 5 μm of diameter were coated with the CSP to pack a column for High Performance Liquid Chromatograph (HPLC). Using the packed column, several racemates were tried to separate under various separation conditions with different compositions of eluents.

INTRODUCTION

Historically, natural polymers, such as cellulose or starch components, were the first to be used as chromatographic chiral selectors due to their inherent chiral nature and ready availability. The resolving ability of polysaccharides, in particular cellulose, was first observed in paper chromatography when a racemic amino acid gave two spots. This led to further use of cellulose and other polysaccharides, mainly amylose, as the chiral starting material in the preparation of selectors to be used in chiral stationary phases (CSPs).

Some other polysaccharides, such as xylan, dextran, chitin or chitosan, have occasionally been used as a starting material for the preparation of CSPs. However, the trisphenylaminocarbonyl and the 3,5-dimethylphenylaminocarbonyl derivatives of chitosan, and the phenylcarbamate and 3,5-dimethylphenylcarbamate of the closely related chitin are the only derivatives described as chromatographic chiral selectors.

In this study, chitosan, which has good enantioselectivity and is convenient for chemical modifications at the free amino groups, was used as a starting material for the preparation of a CSP. In this paper, the details of the synthesis of the CSP getting through several synthetic steps, and characterization using several instrumental methods such as DSC, TGA and others were elaborated.

RESULTS AND DISCUSSION

Preparation of the chiral stationary phases

Chitosan was used as a starting material for the preparation of the CSP. *L*-phenylalanines were nicotinoylated with an active ester synthesized from nicotinic acid and *N*-hydroxy succinimide. The synthesized N-nicotinoylated phenylalanines were then coupled with chitosan. In the next step, *N*-(*N*-nicotinoyl-*L*-phenylalanyl)-chitosan

bis-(3,5 -dimethylphenylcarbamates) was synthesized by the reaction of the remaining hydroxyl groups of the chitosan derivative with an excess amount of 3,5-dimethylphenylisocyanate as shown in Figure 1.

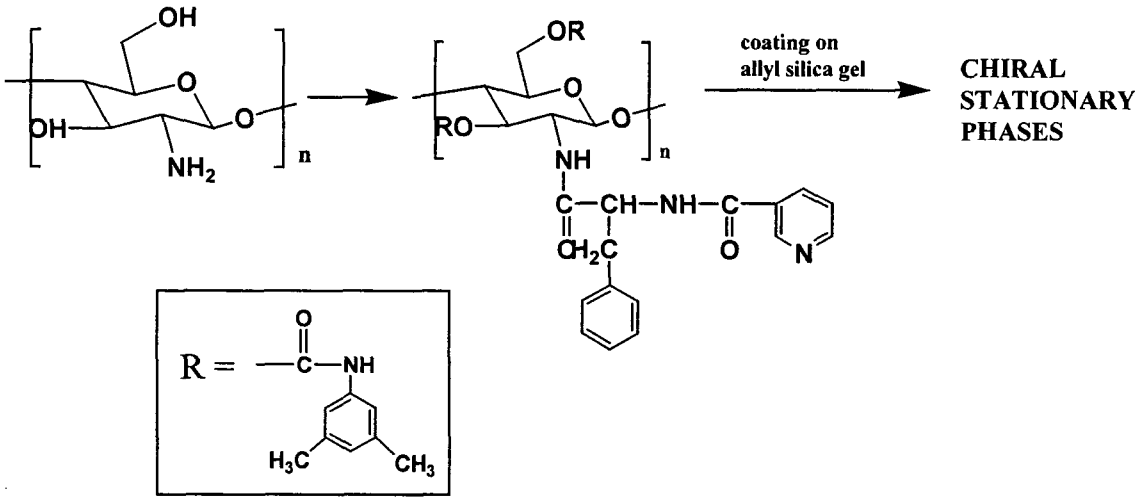

Figure 1. Schematic of synthesis of the chiral stationary phase based on the chitosan

From the chemical structure of the CSP produced (Figure 1) consisting of chitosan main chain, two kinds of such side groups as 3,5-dimethyl phenyl carbamate groups and N-nicotinoyl phenylalanine, it is expected to be possible for the chiral recognition.

Using the CSP produced, silica powder with 5 μm of diameter was coated and used for the packing of a column with 15 cm in length for HPLC tests. For the packing of the column, conventional a slurry packing apparatus was used, pressure used about 5000 psi for half an hour using hexane as a solvent for the packing.

Enantioseparation by the HPLC

The capabilities of the CSP prepared in this study for the application as a CSP for the HPLC chiral column were measured by trying to separate several kinds of racemates generally being used, using the HPLC column prepared as mentioned above. The HPLC operating conditions such as pressure, eluents, and others were controlled to find right separation conditions for the different kinds of racemates. Figure 3 shows some of the results obtained by using the HPLC chiral column prepared in this study. As shown in Figure 3, the CSP appeared to be possible for the separation of racemates including the amino acid racemates.

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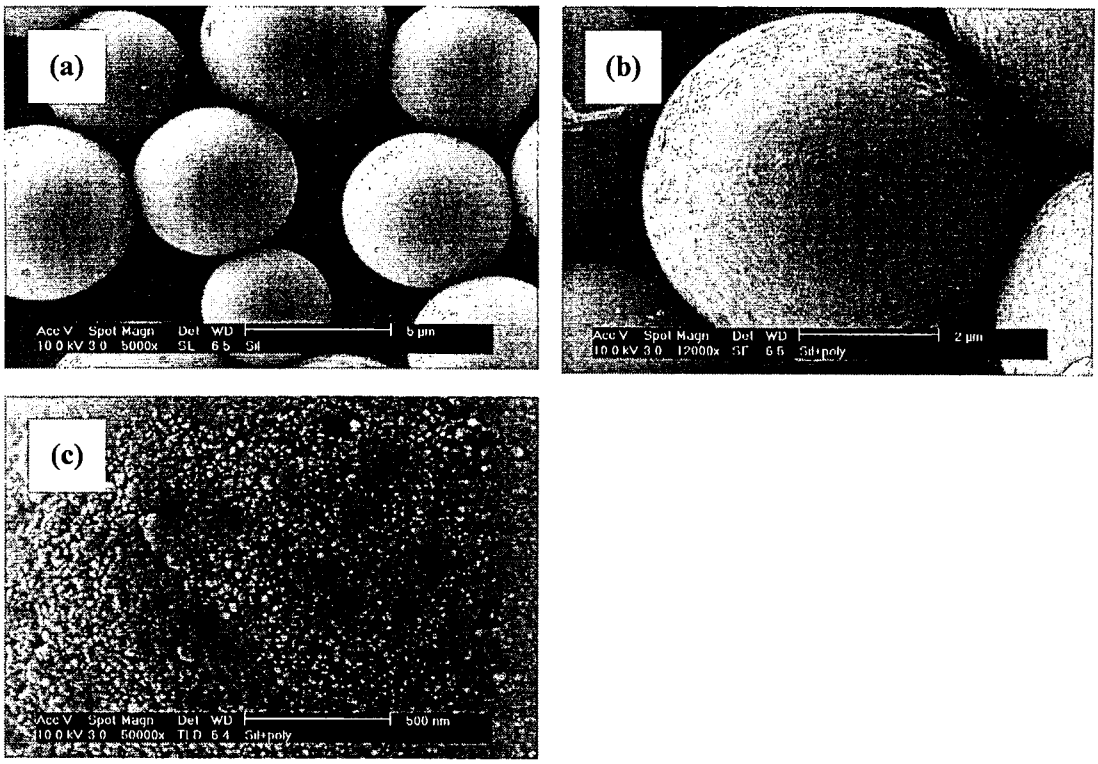

Figure 2. SEM photographs of the silica before and after coating with the CSP based on chitosan prepared in this study: (a) before coating, (b) after coating, and (c) the magnification of the coated surface.

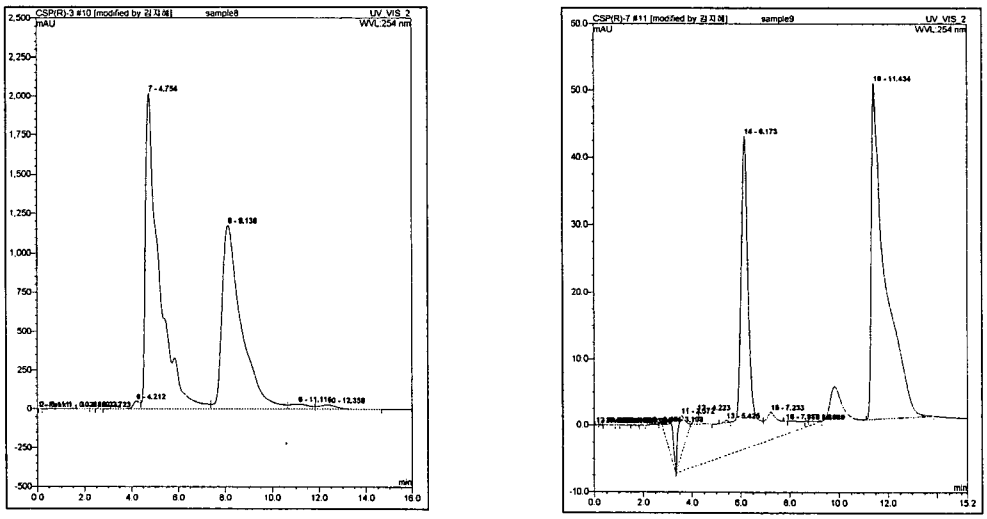

Figure 3. Chromatographic resolution of flavanone(8) [mobile phase, hexane/IPA(75:25)] and trans-cyclopropanedicarboxylic acid dianilide(9), using the column prepared in this study [mobile phase, hexane/chloroform(25:75)].