HPLC 칼럼에 충전된 분자각인 고분자 미세입자를 이용한 키랄 분리

Hamayun Khan, 박중곤*
경북대학교 공과대학 화학공학과
(parkjk@knu.ac.kr*)

Chiral Separation Using Molecularly Imprinted Polymeric Microbeads Packed in HPLC Column

Hamayun Khan, Joong Kon Park*
Department of Chemical Engineering, Kyungpook National University
(parkjk@knu.ac.kr*)

Introduction

Molecular imprinting can easily create recognition sites in polymer matrices. Molecularly imprinted polymers (MIPs) can be prepared by various polymerization methods in the presence of template molecules. Some cavities remain after extraction, which are complementary to the template molecules both in their shape and in the alignment of their functional moieties. Thus, MIPs can selectively bind the imprinted enantiomer from a racemate solution and can able to separate target molecules from substrates of a similar structure. Therefore, MIPs can be considered as artificial affinity media.

MIPs have received much attention and have been extensively studied by many authors in recent years. Currently, polymers imprinted with different templates like drugs, herbicides, sugars, nucleotides, amino acids, and proteins, are more and more applied in analytics, catalysis, and synthetic processes. MIPs have been widely used as solid separation media, in liquid chromatographies, affinity based solid-phase extraction for separations of chiral compounds and amino acid derivatives. Recently, an efficient polymer enzyme mimic has been reported with noncovalent interactions such as ionic or hydrogen bonding between the template molecule and the functional monomers that act as binding sites and as catalytic groups. Similarly, molecularly imprinted hydrogels, which contain catalytically active groups like the active sites of enzymes, have been reported.

Some MIPs are prepared by the conventional bulk polymerization method. However, the conventional bulk polymerization method leads to the preparation of a block polymer which has to be subsequently broken down, crushed, ground, and sieved several times in order to obtain the desired particle size. Thus, this method is not only tedious, laborious, and time consuming, but also the obtained particles are of irregular shape and polydisperse along with huge amount of fine waste. Typically less than 50% of the ground polymer is recovered as usable particles. The grinding process

may also be detrimental to some of the binding sites. Moreover, the irregular particles resulting from bulk polymerization are generally less efficient for column packing in chromatography and often prove troublesome in process scale up.

In order to overcome the difficulties associated with bulk polymerization, an alternative method was desired, namely the generation of spherical MIPs. These MIPs are used directly after production and template removal via extraction. This method is even more advantageous because the spherical particles can be produced in a uniform manner, which is preferable for many applications, particularly those involving chromatography, solid phase extraction, and other flow-through applications. Columns and cartridges packed with uniform spherical beads exhibit better flow characteristics than those packed with irregular particles. The chromatographic results are much easier to reproduce if the molecularly imprinted stationary phase is of a defined structure and reliable quality, which is more difficult to achieve by grinding the MIPs. The chemical and mechanical stabilities of spherical MIP are very good because they are resistant to impact, high temperature, high pressure, acid and alkali conditions, and many kinds of organic solvents.

Spherical polymer beads can be prepared by various polymerization methods, several of which have been applied to the preparation of MIPs including suspension, dispersion, precipitation, miniemulsion, multi-steps swelling polymerizations, grafting (silica or acrylate) the imprinted polymer, and wet phase inversion methods. From the literature cited and our point of view, suspension polymerisation is one of the most well suited methods to obtain MIP microspheres as polymerization of the pre-polymer solution (template, monomer, and cross-linker) is carried out in the presence of porogen dispersed in the aqueous phase in the form of micro-droplets with the help of a dispersing agent or stabilizer. In a continuation to our previous research on the development of MIPs and their applications, the present work focuses on the characterizations of D-Phe imprinted microsphere polymeric beads prepared by suspension polymerization. Prepared D-Phe imprinted MIPMs were evaluated as HPLC stationary phase for the chiral separation of Phe enantiomers.

Materials and methods

D-Phe imprinted microsphere polymeric beads were prepared by suspension polymerization and modified suspension polymerization. Beads were prepared using the following three solutions. Solution-1: D-Phe was mixed with MAA using toluene as porogen, followed by the addition of small amount of acetic acid and TFA in order to dissolve D-Phe by stirring the mixture. Solution-2: AIBN was dissolved in toluene and EDGMA. Solution-3: PVA was dissolved in hot distilled water. All three solutions were mixed in a three-nick double jacket glass vessel, purged with N_2, and then stirred. Polymerization was then carried out at 60 ^oC for 24 h with continuous stirring under an N_2 atmosphere. After polymerization, the micro beads were washed with distilled water containing 5% ethanol to

remove the unreacted monomers, initiator and porogen, followed by washing with distilled water to remove the stabilizer.

Template molecules were removed from the polymer matrix by washing with acetic acid solution and then with distilled water. After template removal, the residual acetic acid was removed from microbeads by washing with an excess of distilled water.

Results

The morphology and particle size of the prepared microbeads were determined using FE-SEM. All resulting particles were spherical. In the magnified FE-SEM photographs, uniformly distributed pores are evident on the surfaces of microbeads.. The average particle size was 40 μm with a major particle distribution (~ 60%, in numbers) of 35-45 μm. Mixing the pre-polymerization solution for 2 h reduced the particle size. The particle shape, size, and porosity are of prime importance because the mass transfer rate, the specific surface area, and the adsorption capacity depend upon these parameters.

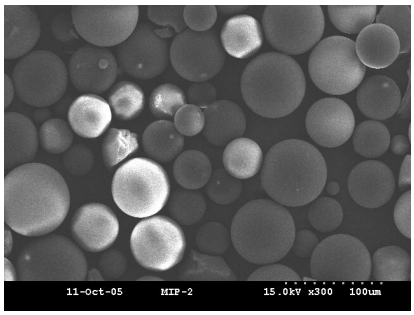

Fig. 1. FE-SEM photographs of D-Phe imprinted P(MAA-*co*-EGDMA) micro beads

The adsorption amount of adsorbate usually increases with the adsorption time until equilibrium is reached. Phe adsorption increased with the adsorption time during the first 1-6 h, and after that reached equilibrium. The amount of Phe adsorbed on microbeads for 6 h was evaluated as a function of its concentration in the range from 25-1000 mg/L at pH 2. The amount of adsorbed Phe increased with the concentration of the Phe racemate solution, but the adsorption selectivity decreased from 1.28 to 1.19. The affinity of the conventional MIPs usually decreases with the template concentration. This can be explained by the model of heterogeneous binding sites on the imprinted polymer surface in which one is selective or high-affinity and the other is non-selective or low-affinity. At the low-concentration range, the adsorption on the selective binding site is stronger than on non-selective binding sites. The adsorption selectivity of microbeads was 1.19 at 1g Phe/L of racemate solution.

The separation factor and resolution were 2.56 and 1.38, respectively when these particles were packed in a conventional HPLC column and used for the chiral separation of a Phe racemate solution.

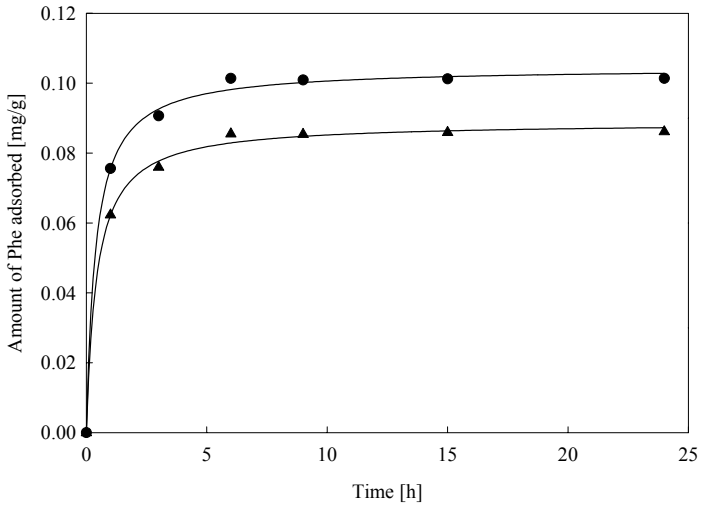

Fig. 2. Adsorption profile; D-Phe and L-Phe. Adsorption conditions: Microbeads: 0.8 g; Phe concentration: 100 mg/L; Volume: 5 mL; pH 2, Time: 1-24 h; Temperature: 25 ^{o}C; Shaking speed: 150 rpm

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