A128 Preparation of Molecularly Imprinted Monolithic Polymers as the Stationary Phase for Liquid Chromatography

Yuanwei Gao, BS, and Seongshin Gwak, BS, Sam Houston State University, 555 Bowers Boulevard, #1528B, Huntsville, TX 77340; and Jorn C.C. Yu, PhD, Sam Houston State University, Box 2525, College of Criminal Justice, Sam Houston State University, Huntsville, TX 77341*

After attending this presentation, attendees will learn the theory of MIP, preparation of MIP, and the practical use of MIP in analytical instrumentation such as high performance liquid chromatography (HPLC).

This presentation will impact the forensic science community by showing how molecularly imprinted monolithic polymers (MIMPs) were prepared in three different types of materials including polyetherether ketone tubing, polymer sheathed fused silca tubing, and empty stainless steel columns by *in situ* polymerization with thermal initiation. Attendees will be able to learn how different materials impact the practical use of MIMPs in an analytical column.

The preparation of MIPs usually consists of three steps. First, the functional monomers and template (target) molecules are mixed and interacted by either non-covalent or covalent bonding. Then, the functional monomer aggregation follows in order to allow the formation of the alignment in the presence of cross-linkers. In other words, imprinting of template molecules in a polymer is achieved by the polymerization of the functional monomers and cross-linkers in the presence of template molecules. Finally, the imprinted templates are removed from the polymer to produce binding sites, which are specific to the template molecules. These binding sites on MIPs have a permanent memory of the template molecules in terms of complementary size, geometry, and orientation of functional groups. These specific binding sites can selectively recognize the target molecules, even in a complex sample solution. It has been demonstrated that MIPs have molecular recognition property as a bio-mimetic recognition layer in enzyme-linked immunosorbent assay (ELISA), chemical sensor systems, selective molecularly imprinted solid phase extraction (MISPE), and in HPLC with a chiral stationary phase.

Molecularly imprinted monolithic polymers (MIMPs) were first studied in the early 1990s. This type of MIPs can be prepared directly in a column or a capillary. Therefore, the process is relatively simple and it can reduce the amount of template molecules consumed during the preparation of MIPs. In this work, MIMPs using (-)-pseudoephedrine as a template were prepared inside of capillaries or columns by *in situ* polymerization. The idea was to prepare a MIP stationary phase for chiral separation of methamphetamines. In order to identify an optimal polymerization condition for the preparation of MIMPs, the back pressure of each polymer was monitored by a liquid chromatography (LC) pump under an isocratic flow condition. The separation of (-)-pseudoephedrine from its stereoisomers including (+)-ephedrine, (-)-ephedrine, and (+)-pseudoephedrine were tested in different mobile phases and flow rate conditions using LC equipped with a tandem mass spectrometer (MS/MS). The final polymerization condition for the preparation of MIMPs was determined using a polymerization mixture with (-)-pseudoephedrine as the template, methacrylic acid as the functional monomer, and ethylene glycol dimethacrylate as the cross-linker. Concentration ratio of template, functional monomer, and cross-linker was 1:3:27. The porogenic solvent mixtures which include toluene and 1-dodecanol, and cyclcohexanol and 1-dodecanol were used with the ratio of 1:9.5 and 1:9.3, respectively. The back pressures of MIMP columns were monitored with flow rates from 0.01 to 0.15 mL/min isocratically. The MIMP column prepared with toluene as porogen showed relatively lower backpressure than the one with cyclohexanol. Although the separation of (-)-pseudoephedrine from its stereoisomers has not yet been achieved, the next step is to maximize the selectivity of MIMP by optimizing the polarities of the mobile phases. Once the optimal condition is determined, MIMPs may be prepared in narrower inner diameter tubings (such as nano-LC columns) to maximize the advantages of using MIMPs for chiral separation in advanced
LC systems.

Forensic Science, Molecularly Imprinted Polymer, Chiral Separation

 ** Presenting Author*