ANVESHA-A Multidisciplinary E-Journal for all Researches Volume 1, Issue 2, December 2020
Bi annual, Bi lingual, Peer reviewed, Referred Journal
Available online on: www.anveshaejournal.com e-ISSN 2582-6719

Fabrication of L-Serine imprinted polymer based microextracting device for its selective separation

Amrita Srivastava*[1] and Amrita Prasad[2]

[1]Archaeological Survey of India, Dehradun, India
[2]Magadh Mahila College, Patna University, India

Abstract

Molecularly imprinted polymers (MIP) is a good matrix that exhibit satisfactory extraction ability when integrated on the microextraction device (Stir-bar). We report the preparation of such material by graft-polymerizing MIP on the surface of stir-bar using MIP as a chiral stationary phase for amino-acid separation. The MIP is selective for L-serine than non-imprinted polymer (NIP) grafted on stir-bar.

Keywords: Molecularly imprinted polymers, stir bar sorptive extraction, L-serine, chiral stationary phases.

Based on the Green Analytical Chemistry principles, sample pretreatment is an important step in chemical analysis. In recent years, environment friendly and consolidated sample preparation approach has been widely used in analytical chemistry. In this context, stir bar sorptive extraction (SBSE) method has shown higher extraction efficiency and better reproducibility than other static microextraction techniques [1]. SBSE, the most widely used stirring integrated technique, paying special attention to the development of new coating focuses on selectivity enhancement by isolation of target analyte [1]. The most attractive one is MIPs to obtain a selective polymer layer on the surface of stir bar. Molecular imprinting is the process of polymerizing selected functional monomers around the template molecules in the presence of a cross-linking agent. After polymerization, the template molecules are extracted to obtain a polymer matrix that is complementary in shape and functionality to the template. Thus, the polymer has ability to attach the analyte selectively [2]. The preparation and characteristics of a sorptive stir bar coated with MIP using L-serine is described here. Raw glass capillary was coated with MIP through chemical bonding. The synthesized stir bar proved to be highly stable in most of the solvents for use. The purpose of this study was to immobilize MIP on the surface of stir bar for selective extraction of L-serine, in order to fabricate a microextraction device using MIP as chiral stationary phases.

Experimental Reagents

Acryloyl chloride (AC), p-Amino phenol (p-AP), glucose were purchased from Loba Chemie, Mumbai, India. Cupric chloride, 2, 2-bi-pyridal, ethylene glycol dimethacrylate (EGDMA), L-serine and 2-Chloro N-(4-hydroxy-phenyl)-acetamide (CHPA) were purchased from Sigma Aldrich (Steinheim, Germany). Solvents, dimethylsulphoxide (DMSO), acetonitrile (ACN), triethylamine (TEA), ethanol (EtOH), acetic acid (AcOH), ammonia hydroxide (about 25% NH3), chloroform, tetrahydrofuran (THF), acetone, methanol, ether and dimethylformamide (DMF), glucose, were of analytical reagent grade and

* Corresponding Authors E mail: amritadgr8@gmail.com

purchased from Spectrochem Pvt. Ltd. (Mumbai, India).

Preparation of monomer 4-(2-chloro-acetylamino)-phenyl Acrylate (CAPA)

The functional monomer CAPA was synthesized on the basis of a known recipe [3]. In brief, the reaction mixture of equimolar THF solutions of CHPA and AC in the presence of TEA were refluxed together for 72 h. After filtration of the TEA acid salt and evaporating THF, the product obtained was washed thoroughly with triple distilled water and methanol. This was finally vacuum dried at 40 ^{0}C for 24 h. The compound was characterized by FT-IR and elemental analyses: CHN analysis (Found: C 54.84; H 4.65; N 5.52%. C11H12NO3Cl requires C 54.88; H 4.57; N 5.82%). FT-IR (KBr), ν_{max}(cm^{-1}): 1665 (-C=O stretch), 1638 (-C=C stretch), 1170 (phenolic C-O stretch), 1490 (aromatic C-C ring stretch), 745 (-C-Cl stretch).

Preparation of molecularly imprinted polymer based stir-bar (micro-extraction device)

Preparation of Stir bars

The stir bars were prepared following a known procedure [4]. In brief, first a glass capillary (1.5 mm diameter, 20 mm length) consisting of 1.00 mm long iron wire inside, was sealed at both end on flame. This resulted in spherical bubbled ends because of inside hot-air expansion. The stir bars were washed with water and methylene chloride. Later, these were treated with 2 *M* NaOH solution for 10 h to obtain Si-O-Na group on its surface. The stir bar was then cleaned with water and dried in air.

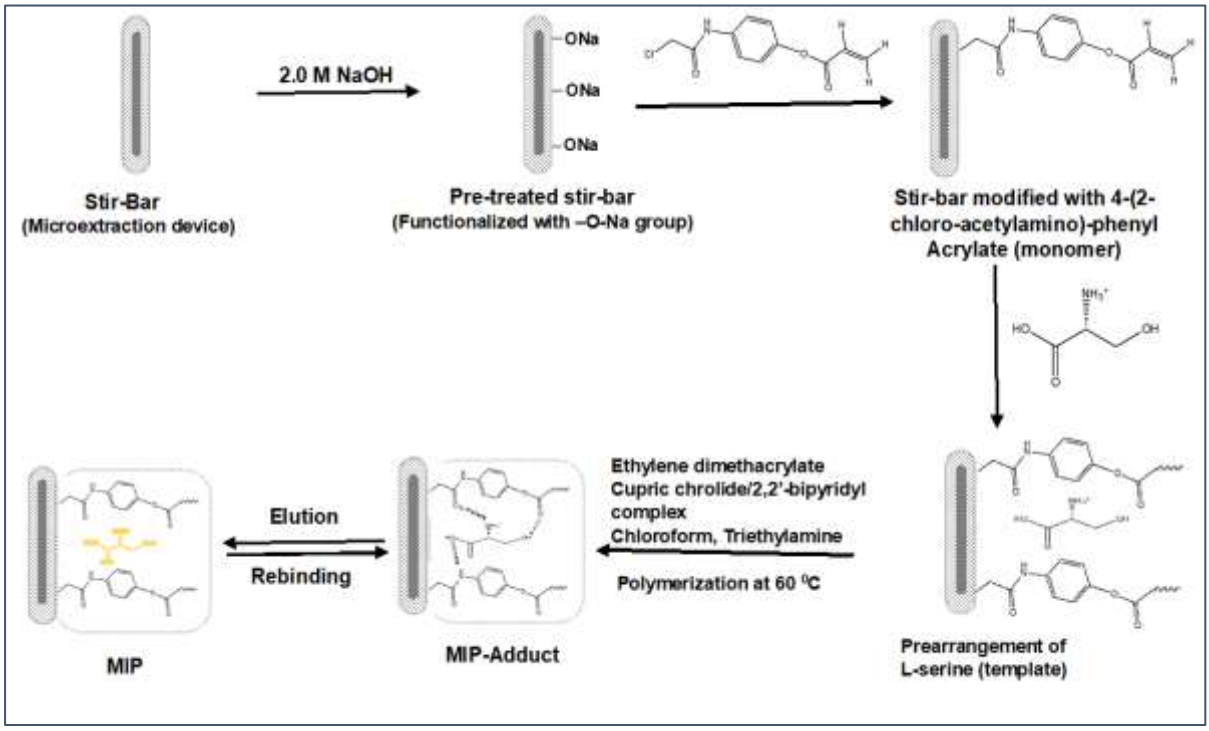
Grafting of MIP on stir bars

The pretreated Si-O-Na stir bar was immersed in an aliquot of monomer solution in DMSO (CAPA, 0.2 mmol/1 mL DMSO), under stirring for 12 h at room temperature, to obtain monomer functionalized stir bar. This was characterized by FT-IR spectra showing an additional peak at 1170 cm^{-1} due to Si-O-C stretch. Synthetic protocol for the preparation of MIP by activator generated by electron transfer for atom transfer radical polymerization (AGET-ATRP) technique is shown in Scheme 1. Accordingly, a pre-polymer solution was prepared by adding template (L-serine, 0.1 mmol/1 mL DMSO) in a beaker having monomer functionalized stir bar which was left for some time so that interaction is established between the template and monomers on the stir bar then the cross-linker (EGDMA, 0.6 mmol)], catalyst [bpy (0.2 mmol) and CuCl2 (0.02 mmol) were mixed in 1.0 mL DMSO to obtain a solution of Cu-II complex], and reducing agent (glucose, 0.2 mmol) were added. This pre-polymer mixture was duly purged with N2 stream for 10 min. To a 2.0 mL of this mixture, the monomer functionalized stir bar was immersed for 15 min and then cured at 60 ^{0}C for 4 h. This yielded an optimized film of MIP- template adduct grafted stir bar (MIP-stir bar, MISB). Template molecule was retrieved from the MIP-template adduct grafted stir-bar by immersing into 150 µL 0.1 M NaOH: 0.1M phosphate buffer (1:1) solution. Water-washing has been done after the extraction of template. Non-imprinted polymers (NIPs)-based stir bar (NISB), using same modification, were also made, but omitting template during the course of polymerization

Result and Discussion

Spectral Characterization

Firstly, FT-IR spectroscopy was performed to characterize the grafting of monomer (CAPA) onto the stir bar. In the FT-IR spectra, Si-O-C stretch band at 1124 cm^{-1} depicting the interaction of monomer on pretreated stir bar with electrostatic interaction (for FTIR analysis coating was scrapped from modified stir bar). Secondly, FT-IR spectra of template, monomer, MIP and MIP-adduct are compared with each other to support the proposed binding mechanism as shown in Scheme 1. All hydrogen bonding involved in monomer-template complexation are suggested on the basis of downward shift of the respective IR band of



Scheme 1: Synthetic protocol for MIP fabrication at the surface of pretreated stir-bar

participating key groups. In this context, FT-IRbands due to hydrophilic –NH bending and carboxylate anion of template (1630 and 1710 cm^{-1}) and –C=O and –NH group of monomer (1680 and 1530 cm^{-1}) were shifted downwards to 1590, 1665, 1648 and 1510 cm^{-1} in MIP-template adduct respectively. Further, a broad band due to –O-H stretching (3640 cm^{-1}) of template and –C=O stretching (1740 cm^{-1}) of monomer are shifted to 3450 and 1720 cm^{-1} suggesting an hydrogen bonding between –O-H (template) and –C=O (monomer). However, all bands corresponding to the template in MIP adduct disappeared in MIP and were found to be restated at the same position with similar magnitude after rebinding in aqueous medium.

Validation of proposed micro-extraction device

The proposed micro-extraction device was validated for selective isolation of serine isomers. Since enantiomers have same physico-chemical properties but their three- dimensional orientation in space is different and this could be measured by the virtue of imprinting effect. Notably, binding affinity of D-serine was negligible when measured with L-ser imprinted stir bar, apparently because of functional constraints. This is confirmed upon D-serine rebinding to L-serine imprinted polymer and the FT-IR studies of MIP-template adduct confirms the bands are found to be similar as shown in MIP (i.e. there is no shifting of IR peaks as in MIP adduct after binding to D-serine. Thus, the chiral imprinted sites in L-ser imprinted MISB are highly matched with L-ser in terms of space, structure, and spatial arrangement of action sites of MIP-adduct.

Conclusion

It is found that higher binding affinity of L-serine imprinted polymer towards L-serine. The negligible results for binding amount of NIP proves that NIP has less binding interactions with L-serine. In addition this MIP grafted stir bar act as chiral stationary phase for microextraction devices.

References

1. Cárdenas S. and Lucena R. (2017): Recent Advances in Extraction and Stirring Integrated Techniques. Separations, 4: 6-21.
2. Tian Y., Deng P., Wu Y., Ding Z., Li G., Liu J., and He Q. (2019): Simple and Effcient Molecularly Imprinted Electrochemical Sensor for the Selective Determination of Tryptophan. Biomolecules, 9: 294-310.
3. Zhao X. D., Fan X. H., Chen X. F., Chai C. P., and Zhao Q. F., (2006): Surface Modification of Multiwalled Carbon Nanotubes via Nitroxide-Mediated Radical Polymerization. J. Polym. Sci. Part A: Polym. Chem., 44: 4656-4667.
4. Yu C., Yao Z., Hu .B., (2009): Preparation of polydimethylsiloxane/β-cyclodextrin/ divinyl - benzene coated “dumbbell-shaped” stir bar and its application to theanalysis of polycyclic aromatic hydrocarbons and polycyclic aromatic sulfur heterocycles compounds in lake water and soil by high performance liquid chromatography. Anal ChimActa, 641: 75-82