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APPLICATIONS OF MOLECULARLY IMPRINTED POLYMER IN SEPARATION AND ANALYTICAL CHEMISTRY

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Abstract

The qualitative and quantitative analysis generally can be performed directly to the sample, but sometimes it needs sample separation from nuisance substances to obtain selective, sensitive and specific measurements. One way to achieve these reliable measurements can be done through the use of Molecularly Imprinted Polymer (MIP) for separation and chemical analysis.

MIP is polymer that synthesized with the presence of a template molecule to produce cavities on the polymer after template extraction. The synthesis of MIP was usually performed by mixing a functional monomer, a cross-linker, an initiator, and a target molecule template in a homogenous solution. The process continued by polymerizing the solution to obtain a polymer with an entrapped template. The template molecule was then removed by extraction method to produce MIP. MIP has high selectivity, affinity and stability and also specific towards molecule target. MIP can be used in variety of chemical separation methods. Generally, the use of MIP in analytical chemistry applied to small molecule in micro-scale measurements like mycotoxin analysis, drug analysis, and pesticides analysis. Recently, it also developed the use of bio-imprinted molecule for proteins and polysaccharides analysis that having high molecular weight. MIP can also widely used as sensor, sorbent and membrane.

Key words: molecularly imprinted polymer, separation, analysis

INTRODUCTION

Chemical analysis generally is divided into two terms, qualitative and quantitative determinations. Qualitative analysis aims to identify the existence of substances, whereas quantitative analysis aims to measure the amount of substances in the sample. Qualitative identification and quantitative measurement of substances required selectivity, sensitivity and specificity of reagents or measuring instruments.

Specification in analytical chemistry can be understood through two approaches, targets and methods. Based on the target, chemical analysis can be divided into bioanalytical chemistry, material analysis, environmental analysis, and forensic analysis. Based on the method, chemical analysis can be divided into spectroscopy, chromatography, electrophoresis and electrochemistry. Selection process and determination of appropriate methods become an important step for the high reliability results of analysis. The requirements of the analysis determine the best method. In choosing a method, several criteria in selecting methods such accuracy, precision, sensitivity,

selectivity, robustness, ruggedness, scale of operation, analysis time, availability of equipment, and cost must be considered (Harvey, 2009). Reliability analysis results can be achieved with proper preparation steps, such as by doing sample separation, especially if the analyte has a complex matrix.

Sample separation does not always have to be done.This step becomes important if the sample contained many nuisance substances that can prevent the successful measurement process.If there are many nuisance substances and make a complex form, the separation phase of analysis becomes the most difficult stage. So, quantitative analysis stage include: the selection and preparation of samples, sample pretreatment, separation of the desired components, measurement of the desired component, analyzing the data, and reporting. If the desired components are together with other components, the measurement results may be biased. This will affect the data analysis process to make conclusions (Soebagio*et al.*, 2005).

Separation procedure can be used for compound purification and qualitative or quantitative componentsdetermination analysis.Compound purification is made during the preparative work,while quantitative identification is donefor chemical analysis. Other components that existed together with the targetcomponents can interfere identification and quantitative determination because the reliability requirement can't be fulfilled. Therefore it needs separation process in the chemical analysis. To meet these objectives in the separation and chemical analysis, a polymer material known as molecularly imprinted polymer (MIP) can be used.

DISCUSSION

MIP Preparation

MIP is a polymer that synthesized in the presence of a template molecule to produce cavities after template extraction. MIP has ability to identify the original molecule (template) and can distinguish the differences of structure from substrate in interactions side, so it can be said that MIP has a high selectivity. The resulting polymer also has high stability. MIP is used for variety purposes, such asformingthe stronger bond with the target analytes in the separation process, adsorbents and sensors.

According to Lok and Son (2009), MIP is the latest technology that allowed us to synthesize materials with the very specific receptors on the target molecule, because the polymer is prepared in the presence of the target molecule as a template. The advantages of this method, MIP is easier to characterize and has a rational design approach (Huang *et al*., 2011). It also because the MIP is having a sensor that comes from the substance to be determined in the sample, technical and chemical stability, low cost and easy preparation.

MIP is formed by the presence of a molecule which is then separated and left the complement cavity behind. These polymers have a certain chemical affinity for the original molecule that can be used as sensors, catalysts, and separation methods in certain analysis. Functional mechanism is similar to antibodies or enzymes. In the presence of template molecule that plays as a substance which will be separated from the MIP, the depiction of the interaction between the polymer and the analyte as well as depictions of the enzyme, i.e. the lock and key system. MIP provides artificial cavity key for the target molecule as a key (Borje, 2001).

The MIP ability to bind specific target molecules are needed in the separation of certain analysis, especially for small molecules in the terms of size and purity. In addition, because of the template in MIP is only the target molecule, its presence also serves as an agent of the target molecule selectors among other substances in a mixture. In other words, MIP is specific and selective.

There are three main steps on MIP preparation (Komiyama et al, 2003 and Sellergen, 2001 in Lok and Son, 2009). First, the interaction of complex formation between molecule template and monomers gave functional groups both of covalent or non-covalent. For non-

covalent interaction, the functional monomer and the template is placed adjacent through hydrogen bonding, electrostatic or hydrophobic with polar or non-covalent interaction. Figure 1 showed the interaction.

Figure 1. The non-covalent imprinting (Yan and Row, 2006)

Second, polymerization is to maintain the optimal arrangement of functional groups to bind template molecule. The structure of conjugate found in 3-D polymer network.Third, releasing the template molecules from the producedpolymer matrix, leaving the binding site for the target analyte. Thus, the space/cavity in the polymer occupied by template molecules and createdcomplement cavity. With appropriate conditions, this cavity is suitable for recording the size, structure and other physic-chemistryproperties of template and bind molecules or structurallyanalogousmolecules to the template molecule in an efficient and selective way. The description of covalent interaction in MIP preparation are presented in figure 2.

Figure 2. The covalent imprinting (Yan and Row, 2006)

Polymerization methods of MIP synthesis

MIP formation can be done through several polymerization methods (Perez-Moral and Mayes, 2004) (Yan and Row, 2006) there are:

1. Bulk Polymerization

This polymerization method is a classical method that is still widely used in synthesizing MIP although it has many disadvantages. The manufacturing process is the simplest process by directlymixing all the needed materials such as monomer, cross - linker, solvent, initiator and templates, then polymerize them simultaneously. The results obtained in the form of a hard polymer blocks that need to be crushed in advance. The crushing process produces polymers with irregular shapes,many cavities are broken, and the polymer mass loss.Approximately 70% of the polymer can be wasted in this stage.

2. Polymerization suspension

This method does not require the smoothing process. The result is a microsphere particle. If the solution is completely soluble, it can produce a uniform micro-size spheres ingeneral conditions of perfluorocarbon solvent and aqueous solution. Unfortunately, the use of this solvent makes MIP very limited in use.

3. Polymerization in precipitation

Micro-particles with a more uniform size can be obtained by precipitation polymerization method. The resulting polymer particles can be enhanced by improving the composition of the

active site. This technique involves the coagulation of nano-gel granules followed by the growth of the particles by trapping oligomer composition of the surrounding solution. In this way, a uniform particle can be obtained, as well as the size and porosity. As a result, the use of MIP were created with this method is potential for chromatographic purposes.

4. Polymerization surface

Surface area of the adsorbent that coated by a very attractive and realistic MIP, is used as a stationary phase for chromatography. MIP thin layer on silica beads are made using radical polymerization technique on the surface of silica particles. This method is used by Sreenivasan *et al.* (Yan and Row 2006) as a separation media, sensor and medical analysis. Until now, this is a quite effective way to make membrane separator.

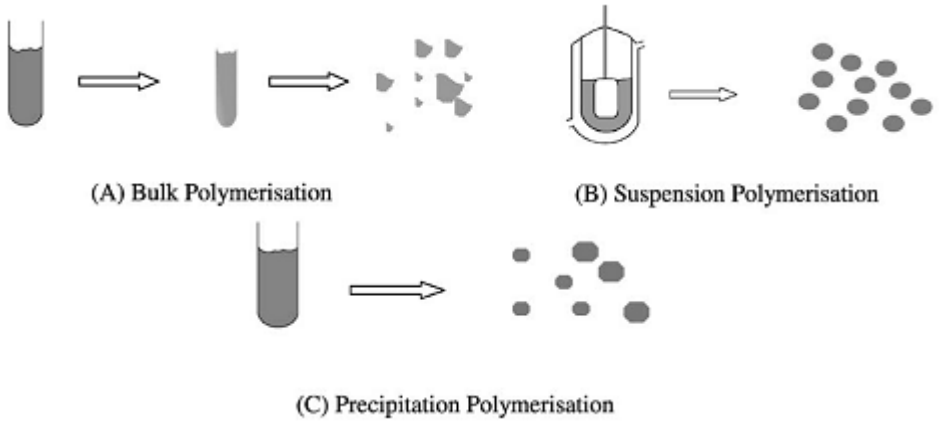

Figure 3. The schematical of different polymerization method (Perez-Moral and Mayes, 2004)

The above polymerization methods have both of advantages and disadvantages. Polymerization method chosen should be met to the needs of what the MIP will be used. Monomers and cross-linkers are used according to the character templates and the expected interaction of the selected polymerization method. Cavities are formed complementary to the template in the MIP. The use of MIP to trap the analyte is an advantage in chemical analysis. It sounds easier to identify. For that reason the MIP can be widely used in the separation and chemical analysis, of whom is as the stationary phase chromatography, as adsorbent in solid phase extraction and electrochemical sensors.

The application of MIP

One of the MIP applications in separation and chemical analysis is the use of MIP-chloramphenicol as separation media packed in HPLC columns. This is done by Zhang and Lei, 2013 that synthesize MIP by suspension polymerization in water system using chloramphenicol as template and ethyl acetate as porogenic solvent. The result, MIP-chloramphenicol (CAP - MIPs), has high selectivity and adsorption capacity to chloramphenicol in the sample. In column, chloramphenicol can be separated, even from the erythromycin and tetracycline which has an analogue structure.

Selection of carbamazepine in pharmaceutical waste leachate conducted by Schweiger *et al*.., 2009 by using carbamazepin as template for creating MIP-carbamazepin. Waste leachate was also contained caffeine and salicylic acid. The recovery of MIP-carbamazepine is up to 93%, and the non-imprinted polymer reached 75%, while the recovery reached 73% if it used activated carbon. Drug analysis is also performed by Hung *et al*., 2006 using ibuprofen as a template in the MIP. Methods of free radical polymerization of functional monomers used to form intramolecular hydrogen bonds in the polymer produced. The resulting polymer granules used as the stationary phase in HPLC to separate ibuprofen and ketoprofen that was a drug compounds

with a similar structure. The result is quite efficient as a separation media with high accuracy and precision. Meanwhile, Pan *et al*., 2007 used N-acetylneuraminic molecules (Neu5Ac) as a template to detect Neu5Ac and other structural analogues in good results.

Improving the performance analysis of caffeine is one of the goals of Lin *et al.* (2003a) research. They developed a random copolymer of poly{(methacrylamide)-co-(vinyl trimetoksisilan)} and {poly(methacrylic acid)-co-(vinyl trimetoksisilan)} that synthesized by free radicalspolymerization reaction using caffeine as template. The results revealed that the use of caffeine poly -{(methacrylamide)-co-(vinyl trimetoksisilan)} and {poly(methacrylic acid)-co-(vinyl trimetoksisilan)} have selectivity factor (β) less than one, which means has high selectivity for caffeine and other structural analogues. The resulting polymer was analyzed using HPLC. Lin et al (2003b) subsequently developed a caffeine {poly(methacrylic acid)-co-(ethylene glycol dimethacrilat)} with other polymerization methods, that is suspension polymerization. They obtained granules with a uniform size in average 96 mm with adsorption capacity of 0.32 mol/g. The resulting polymers can be used to decaffeination a beverage product without reducing the aroma and taste of the drink.

Piacham *et al.*, 2009 setting out the procedures for analytical separation of tocopherols using MIP-tocopherol. This polymer is capable to adsorb tocopherol up to 2 mg/g of polymer in ethanol-water solvent system. Other molecule of tocopherols can be separated in this way because of having ananalogue structure oftocopherol or tocopherol derivatives, such as tocopherol acetate. Besides tocopherol, the development of food analysissuch as carbohydrate conducted by Striegler, 2001 in the synthesis of MIP-glucose. High selectivity of glucose by the polymer receptorat physiological pH is higher than mannose and galactose.

MIP can be created as sensor in chemical analysis application. The sensor is a practical and comfortable monitoring tool in routine analysis. For this purpose, it is necessary that the sensor has a sensitivity, reproducibility and stability analysis. Polymer-based sensors developed by Yong-xing*et al.*, 2011 for quantitative determination of parathion in pesticide analysis made on the chitosan matrix via electrochemical deposition constant. Sensitive response obtained with the detection limit of 1.0 × 10-7 mol/L and with a good memory capacity of the sensor. Reproducibility and stability can be accepted. Yarman and Scheller, 2014 also synthesized electrochemical sensors for tamoxifen (TAM) based MIP. Electropolymerization synthesis performed on the electrode surface reducingferrycyanidesignal as interference substance. Decreased peak ferry cyanide of MIP electrode, increased TAM from 1 to 100 nM. The advantage of this measure significantly reduces the electrochemical interference. Wang and Wang, 2008 also reviewed the importance of the use of sensors in routine analysis for mycotoxins determination.

Food contamination from natural or anthropogenic sources posed a serious risk to human health. Exposure in continuous low doses of toxic chemicals can be associated with several chronic diseases, including some types of cancer and serious hormonal dysfunction. Classical analysis method is quite sensitive for the detection and quantification of contaminant, but the direct application of this method in food samples is difficult. In fact, the complex matrix can make serious interfere in determination, while the new modern analysis can be done only after clean-up and preconcentration step have done. Recently, sample preparation methods are mostly based on solid phase extraction technique that very fast and cheap procedure but less selective. This method is based on highly selective immune-affinity extraction,but it is so expensive. Thus, it needs cheaper, fast and selective method for the clean-up stage. Baggiani *et al.*, 2007, revealed that the Molecularly Imprinted Solid Phase Extraction (MISPE) is an intelligent solution for food contaminant analysis techniques. This technique is very suitable for applications with high selectivity even for analytes with very complex matrix. Another example of the MISPE application revealed by Rahiminejad *et al.*, 2009 for detecting diazinon. Generally, the

determination of diazinon in drinking water using HPLC separation process is quite complicated. The existence of the MIP as SPE sorbent diazion (MISPE-diazinon) obtained recovery up to 90% for drinking water samples with different levels of diazinon.

Isolating substance, such as melamine, can also take as one of MIP technologyadvantages by making a combination of molecular form as sorbent for solid phase extraction (MISPE). By a precipitation polymerization method, Yusof *et al*.,2013 polymerize 9 - vinylcarbazole as a functional monomer, ethylene glycol dimethacrylate as cross-linker and benzoyl peroxide as an initiator together melamine template. MIP showed a better affinity for melamine compared to non- imprinted polymer (NIP) with a maximum binding capacity of 53.01 mg/g MIP. Based on the correlation coefficient, the adsorption kinetics of MIP-melamine fit with pseudo-second model. From isotherm data, adsorption of MIP-melamine increases when the concentration of melamine increases. So this phenomenon follow Freundlich isotherm model, which shows multilayer adsorption. This proves that the MIP has a better binding capacity of melamine compared with NIP.

CONCLUSION AND SUGGESTION

Molecularly Imprinted Polymer (MIP) is used in several analysis applications such as drug, pesticides, food analysis as sorbents, sensor or isolating particles. The results showed sensitivity, selectivity and specificity measurements. So, it must be useful particle for future modern analysis applications.

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