Physical Selectivity of Molecularly Imprinted polymers evaluated through free volume size distributions derived from Positron Lifetime Spectroscopy

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Abstract. The technique of imprinting molecules of various sizes in a stable structure of polymer matrix has derived multitudes of applications. Once the template molecule is extracted from the polymer matrix, it leaves behind a cavity which is physically (size and shape) and chemically (functional binding site) compatible to the particular template molecule. Positron Annihilation Lifetime Spectroscopy (PALS) is a well known technique to measure cavity sizes precisely in the nanoscale and is not being used in the field of MIPs effectively. This method is capable of measuring nanopores and hence suitable to understand the physical selectivity of the MIPs better. With this idea in mind, we have prepared molecular imprinted polymers (MIPs) with methacrylic acid (MAA) as monomer and EGDMA as cross linker in different molar ratio for three different size template molecules, viz. 4-Chlorophenol (4CP) (2.29 Å), 2-Nepthol (2NP) (3.36 Å) and Phenolphthalein (PP) (4.47Å). FTIR and the dye chemical reactions are used to confirm the complete extraction of the template molecules from the polymer matrix. The free volume size and its distribution have been derived from the measured o-Ps lifetime spectra. Based on the free volume distribution analysis, the percentage of functional cavities for the three template molecules are determined. Percentage of functional binding cavities for 4-CP molecules has been found out to be 70.2% and the rest are native cavities. Similarly for 2NP it is 81.5% and nearly 100% for PP. Therefore, PALS method proves to be very precise and accurate for determining the physical selectivity of MIPs.

1. Introduction

The Molecular Imprinted Polymers (MIPs) are man tailored polymers with specific recognition cavities complementary in shape, size and functionality to the target molecule [1]. Research in MIPs has emerged as an attractive field owing to their wide variety of applications like chemical and biosensors, solid-phase extraction (SPE), drug delivery systems etc.

Selectivity of an MIP is the prime character from the view point of its numerous applications particularly as sensors. However, in such designs there is a probability of non-selective binding sites in the presence of foreign molecule with similar functional group as that of the template molecule or with the similar size of the template. Therefore, to achieve high selectivity for a given molecule by the MIP, both functional group selectivity (chemical) and pore-size selectivity (physical) must be properly understood and estimated. With this view point, MIPs with MAA-co-EGDMA as a matrix polymer...
and three different molecules (viz 2-Nephthol, 2-naphthol and Phenolphthalein) as the templates have been synthesized by non covalent binding method and characterized with Positron lifetime spectroscopy with a special emphasis on physical selectivity of the MIPs.

2. Experiment

2.1. Materials

The chemicals for the synthesis of MIPs namely methacrylic acid (MAA), ethylene glycol dimethacrylate (EGDMA) and 4-Chlorophenol (4-CP) were procured from Sigma Aldrich, Bangalore, India. 2-Naphthol (2NP) and Phenolphthalein (PP) were procured from Loba Chemie, India. The radical initiator 2,2’-azobisisobutyronitrile (AIBN) and solvent acetonitrile (ACN) were obtained from Merck (Germany). All the chemicals were of lab grade and used as received.

2.2. Synthesis of MIP

MIPs were synthesized by employing thermal bulk polymerization [1]. The pre-polymerization complex comprises of the mixture of monomer MAA and the template taken in a 25ml thick walled glass tube. The mixture was dissolved in the solvent (ACN). After proper mixing of pre-polymerization complex, cross linker (EGDMA) of known molar ratio to monomer and radical initiator (AIBN) were added to the solution. The solution was kept in an ice bath for cooling and nitrogen gas was purged for removal of oxygen. Then the solution was placed in a water bath at a constant temperature of 60°C. The thermal polymerization was allowed to proceed for nearly 19 hours [1,2] to get hard monolith product. Later the template molecules were chemically extracted from this using a soxhlet extractor following the standard procedure. Then the hard sample was sliced to make positron measurement samples.

The control polymer without template molecule was prepared using same method and is labeled as NIP5 for monomer cross linker ratio 1:5. When the template is incorporated, it will be addressed as NIP5+template; on extraction of template from the polymer matrix, it is called MIP5 and finally when the template is reloaded to the polymer matrix, it is labeled as RMIP5.

2.3. Positron Lifetime Spectroscopy.

In polymers, the positron captures an electron with parallel spin to form ortho-Positronium (o-Ps) and localized in free volume or voids is the process of interest. o-Ps annihilation [3] process is a microprobe for free volume cavities since its lifetime and intensity are characteristics of free-volume size and their relative concentration in polymers. In the present work positron lifetime measurements were made using a standard fast–fast coincidence system with time resolution of 220 ps. All measurements were made at room temperature. More details of the experimental procedure and analysis can be found in the earlier works [2, 4].

The lifetime spectra so acquired were analyzed into three average lifetime components using PATFIT-88 [5], with proper source correction applied. The reproducibility of the PALS results was found to be satisfactory within the experimental error limits. To get the distribution of free volume cavity sizes from the same measured positron lifetime spectrum, we used another computer program CONTIN-PALS2 [6]. CONTIN - PALS2 generates annihilation rate probabilities that produce distribution peaks from which free volume cavity radius and size (volume) distributions can be generated.

2.4. FTIR

The Fourier transform infrared spectra were recorded using FTIR, JASCO-460 PLUS, Japan, with a resolution of 4 cm$^{-1}$.

3. Results and Discussion

The formation of co-polymerization of MAA-co-EGDMA have been verified using FTIR. FTIR scans also confirm the incorporation, extraction and rebinding of the template molecules [2,4]. Using the Bondi’s method [7], the radius of the template molecules 4-CP, 2NP and PP were found to be 2.99, 3.36 and 4.47 Å respectively. The results obtained from both PATFIT-88 and CONTIN-PALS2 are
found to be consistent. It is known that the free volume cavities ($\omega$-Ps lifetime) come with a distribution rather than a single value and maximum probability corresponds to the peak of this distribution as can be seen in the graphs below.

Figure 1: Pore radius distribution curve of different stages in 4-CP imprinting polymer

Figure 2: Free volume cavity radius distribution of MIP5 with cutoff radius line for 4-CP

As we can see from figure 1 for 4-CP imprinted polymer, pore size radius distribution in each stage of the imprinting gets slightly displaced. NIP5 will have only the pores which are due to chain folding and cross-linking of the polymer chains. When the monomer is polymerized in the presence of template molecules, the template molecules get into the polymer matrix thus affecting the microstructure of the polymer. These changes can be clearly seen in the distribution of radius distribution ($\omega$-Ps lifetimes). It is to be noted that some of the template molecules may get in to the preexisting pores big enough to accommodate them and the rest will be embedded to polymer matrix when the monomer is cross-linked around these templates. The pores present in the NIP5+template sample are taken as “native pores”.

When the template molecules in the matrix are extracted chemically, it is expected that they leave cavities behind which are complementary to their size, shape and functionally. Thus, the MIP samples will have natives pore in addition to the cavities left behind by the template molecules. In the process of template rebinding, the templates will go in to the cavities created by the template and few to the native pores with similar size. Thus, the RMIP will have all the pores in MIP minus pores filled by template molecules [4].

Table 1: Percentage area accessible and non-accessible to the 4-CP template molecules in 1:5 ratio samples

| Sample        | Area under the radius distribution PDF curve (%) | Area under the curve above cut-off accessible to 4-CP template (%) | Area under the curve below the cut-off line non-accessible to 4-CP template (%) |
|---------------|-----------------------------------------------|---------------------------------------------------------------|--------------------------------------------------------------------------------|
| NIP5          | 9.1                                           | 4.79                                                          | 4.31                                                                           |
| NIP5+template | 8.12                                          | 4.43(100)                                                     | 3.69                                                                           |
| MIP5          | 9.45                                          | 5.28(119.2)                                                   | 4.17                                                                           |
| RMIP5         | 7.15                                          | 4.07(91.9)                                                    | 3.08                                                                           |

Physical selectivity according to equation 1 is 70%

Now, with the understanding that the template molecules can be accommodated by a pore with radius greater or equal to its size, the free volume cavities/pores with respect to imprinting, extracted and rebinding, we use the following formulation to compute physical selectivity of the MIPs as.

$$\text{Physical selectivity} = \frac{X-Y}{X-Z} \times 100\%$$
Where, \( X = \text{Area under the Gaussian curve above the cutoff radius in MIP5} \); \( Y = \text{Area under the Gaussian curve above the cutoff radius in NIP5+template} \); \( Z = \text{Area under the Gaussian curve above the cutoff radius in RMIP5 sample} \). The data in Table 1 are computed by calculating the total area under free volume radius distribution curve and the area above the cutoff line (radius of template) for different samples.

By considering the available native pores in NIP5+template sample accessible to 4-CP as 100%, the available accessible cavities in MIP5 (after template extraction) has increased to 119.2% which is 19.2% higher. When cavities in MIP5 are rebound with template molecules (RMIP5 is produced) the available accessible pores decreases to 91.9%. This is less by 27.3% as compared to MIP5. This is also less compared to NIP5+template by 8.1%. From these we understand that the difference of 8.1% native pores which may have been occupied by the template molecules are non-intentional or we can label them as non-functional binding sites for the template molecules. This is very interesting and found for the first time. Therefore, the present MIP5 is characterized to have \((19.2/27.2)\times100=70.2\%\) physical selectivity for 4-CP molecules and this is tabulated in Table 1.

![Figure 3: Pore Radius distribution curve of different stages in 2NP imprinting polymer](image1.png)

![Figure 4: Pore Radius distribution curve of different stages in PP imprinting polymer](image2.png)

Similarly, we have evaluated the physical selectivity for 2NP and PP imprinted polymers. The radius distribution of different stages of 2NP and PP imprinted polymers are shown in figure 3 and figure 4 respectively. We found the physical selectivity for 2NP and PP imprinted polymers according the simple equation 1 to be 81.5% and close to 100% respectively.

4. Conclusion

Based on the free volume PDF analysis and results presented here, we propose that not all pores in the MIP are accessible to template molecules when reloaded. This work portrays that PALS is a useful technique to evaluate the physical selectivity of MIPs. We also see from the results that the physical selectivity clearly depends on the size of template molecule used in imprinting. This method could be further tried for other MIPs with different base polymer for determining the selectivity.

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