Journal of Chromatography B, 909 (2012) 61–64

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

Short communication

Improving the imprinting effect by optimizing template:monomer:cross-linker ratios in a molecularly imprinted polymer for sulfadimethoxine

Lou Ann Tom*, Nicole A. Schneck, Carla Walter

Susquehanna University, 514 University Avenue, Selinsgrove, PA 17870, USA

ARTICLE INFO

Article history:
Received 24 July 2012
Accepted 15 October 2012
Available online 23 October 2012

Keywords:
Molecularly imprinted polymer
Sulfadimethoxine

ABSTRACT

Four non-covalently prepared molecularly imprinted polymers (MIPs) for sulfadimethoxine (SDM) were prepared using different ratios of SDM template, methacrylic acid monomer, and ethylene glycol dimethacrylate cross-linker. The imprinting factor (IF) was calculated by comparing the retention of SDM on the imprinted polymer with a comparable non-imprinted polymer. The template:monomer:cross-linker ratio of 1:6:20 resulted in an IF of 3.94 which is higher than found in previous studies. A significant decrease in IF to 0.89 when template:cross-linker ratio was 1:40 contradicts most literature where higher cross-linker concentration improves selectivity. IF was 4.36 when 20% water was added to the acetonitrile HPLC mobile phase during evaluation. Retention of SDM increased as water concentration changed as: 20, 40, 0, 60 and 70%, indicating a combination of shape recognition, hydrogen bonding and hydrophobic interactions contributing to retention of analyte. The MIP has the potential for use in SPE for purification and concentration of SDM and with further optimization, possibly direct HPLC analysis.

1. Introduction

Sulfonamides are broad-spectrum synthetic antibiotics often used in veterinary medicine for the prevention and treatment of diseases in livestock. This particular class of antibiotics is effective, inexpensive and easily available to the community, allowing their excessive use in animal husbandry [1,2]. Residues of these drugs can remain in animal tissues and biofluids, which is a public health concern due to the risk of developing drug resistance [3]. The widespread use of these drugs also leads to potential contamination in the environment, which is a concern because some are suspected to be carcinogenic [4].

Sulfadimethoxine (Fig. 1) is one of the sulfonamides that is being released into the environment and has the potential to induce adverse effects in terrestrial or aquatic organisms [5]. Because this molecule has numerous locations with the potential to hydrogen bond, it has been studied as a potential template in the preparation of molecularly imprinted polymers (MIPs) for the sulfonamides as a means for analysis of these compounds in environmental samples.

MIPs have been shown to be an attractive analytical tool for the isolation and detection of low concentration analytes. They have unique characteristics that are beneficial in analytical applications. They provide an analytically powerful and inexpensive alternative to conventional technologies by enabling the identification of a target molecule ("template") in the presence of interfering species. They exhibit good specificity for various compounds of interest, and in some cases, the selectivities and binding affinities achieved from the imprinting process approach those demonstrated by antigen–antibody systems [6,7].

MIPs can be synthesized either covalently or non-covalently, the difference being the method of binding the analyte during polymerization. Covalently prepared MIPs use covalent bonds to bind the analyte to the monomer prior to polymerization, and the bond must be cleaved before use of the MIP. Non-covalent polymers rely primarily on hydrogen bonding to bind the analyte to the monomer during polymerization, which allows for easy removal of the template and reversible binding during later use. Other non-covalent forces could include ionic bonding, hydrophilic/hydrophobic interactions, and biological interactions depending on the application [8]. MIPs are currently being studied for applications such as separations [9], solid phase extraction [10], sensing [11], catalysis [12], and drug delivery [13] among others.

In this study, an improvement in the imprinting factor of an MIP for sulfadimethoxine (SDM) over previous studies [14] was attempted by altering the monomer:template:cross-linker ratio during preparation. Because SDM has more locations of potential non-covalent interactions than most other sulfanomides, it was hypothesized that using SDM as the template instead of a sulfonamide with fewer functional groups, and increasing the monomer concentration used during preparation should increase the number of non-covalent interactions during polymerization, improving the imprinting factor. Using a higher template:cross-linker ratio

* Corresponding author. Tel.: +1 570 372 4540; fax: +1 570 372 2752.
E-mail address: toml@susqu.edu (L.A. Tom).

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http://dx.doi.org/10.1016/j.jchromb.2012.10.020

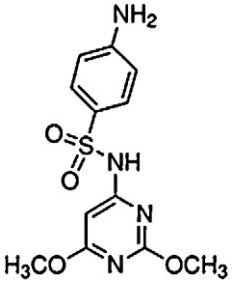

Fig. 1. The chemical structure of sulfadimethoxine.

Table 1
Preparation of MIPs (imprinted) and NIPs (non-imprinted, no template) with different ratios of template (SDM):monomer (MAA):cross-linker (EGDMA).

Polymer	Template:monomer:cross-linker
MIP1	1:4:20
NIP1	0:4:20
MIP2	1:6:20
NIP2	0:6:20
MIP3	1:15:20
NIP3	0:15:20
MIP4	1:15:40
NIP4	0:15:40

than typical (1:20 instead of 1:40) was also evaluated. The imprinting factor of each was determined by comparing each imprinted "test" polymer with a non-imprinted "control" polymer prepared in a similar manner but with the absence of template.

2. Experimental

2.1. Chemicals

Methacrylic acid (MAA) inhibited with 100–250 ppm hydroquinone, ethylene glycol dimethacrylate (EGDMA) inhibited with 100 ppm monomethyl ether hydroquinone, 2,2″-azobisisobutyronitrile (AIBN), and SDM were obtained from Aldrich and used as received. Acetonitrile, acetone, and all other solvents and reagents were obtained from Fisher Scientific or Acros.

2.2. Synthesis of MIPs

Four MIPs were synthesized using SDM as the template, MAA as functional monomer, EGDMA as cross-linker, and acetonitrile as the solvent. The SDM:MAA:EGDMA ratio used for each polymer is shown in Table 1 (1 mmol SDM is 0.31 g, 15 mmol MAA is 1.27 mL, 40 mmol EGDMA is 7.54 mL) 10 mg of 2,2′-azobisisobutyronitrile photo-initiator was added to the reactants in a 25 mL scintillation vial with 12 mL acetonitrile. Vials were mixed by vigorous shaking, purged with nitrogen for 5 min, and placed under UV light (365 nm) at 4 °C for 24 h, and then placed into a 75 °C oven for several hours to ensure complete polymerization. Polymers were removed from vials by breaking the glass away from the polymer. A non-imprinted polymer (NIP) was made for each MIP by eliminating the template.

2.3. Packing of polymers into columns for analysis

Dry polymers were crushed using a mechanical grinder, and washed with 3 aliquots of 100 mL of 10 vol% acetic acid in acetonitrile, followed by 2 aliquots of 50 mL acetonitrile to remove the template molecule (SDM). To determine percent recovery of the template from the imprinted polymer, washes were analyzed for SDM by HPLC using a 4.6 × 250 mm Waters Symmetry C18 column and acetonitrile mobile phase at a flow rate of 1.0 mL min^{-1}. Evaluation of the washes confirmed ~99% removal of the SDM added during polymerization, indicating that the template molecule remained intact during polymerization and that essentially all of the template was recovered, leaving a majority of the cavities formed during polymerization available for rebinding SDM during use.

Washed polymers were sieved to collect ~1.5 g of particles between 25 and 38 μm in size. A slurry of the collected particles was prepared in acetonitrile and packed into an empty 0.46 (I.D.) × 10 cm stainless steel HPLC column using a custom-made adapter and a Waters Delta 600 HPLC pump, using acetonitrile as the packing solvent. The flow rate was gradually increased from 0.20 to 6.00 mL min^{-1} for 15–30 min to provide sufficient pressure to ensure efficient packing of the column. After packing, the column was removed from the adapter and the inlet endfitting was attached.

2.4. Analyses of polymers by HPLC

The analytical system consisted of a Waters 1525 Binary HPLC pump and a Waters 2487 dual wavelength absorbance detector. Analysis was performed by manually injecting a 100 ppm solution of sulfadimethoxine diluted in acetonitrile, using mobile phase of acetonitrile containing various concentrations of aqueous 50 mM phosphoric acid, at a flow rate of 1.00 mL min^{-1} and absorbance at 270 nm. Acetone was used to determine the retention time of an unretained compound.

3. Results and discussion

The selectivity of each MIP was evaluated by comparing capacity factor (k') of sulfadimethoxine on the imprinted polymer (MIP) compared with the non-imprinted (NIP) polymer. Capacity factors were calculated from adjusted retention times as follows [14]:

t_0 = retention time of an unretained compound
t_r = retention time of SDM
t'_r = adjusted retention time of SDM = $t_r - t_0$
k' = capacity factor for SDM = t'_r/t_0
IF = imprinting factor = k' (MIP)/k' (NIP)

Adjusted retention times were used in calculations because differences in retention time can result from differences in packing efficiencies. The IF is a measure of the imprinting effectiveness for the MIP column compared to its corresponding NIP.

Previously reported MIPs for SDM indicated an IF value of 2.29 [14], obtained with a template:monomer:cross-linker ratio of 1:4:40. In another study, a lower ratio of monomer was used for the preparation of MIPs for sulfapyridine and sulfamethazine which also belong to the sulfonamide class but contain fewer functional groups for hydrogen bonding with MAA [15,16]. The structure of SDM (Fig. 1) suggests that a higher ratio of monomer:template may improve the imprinting effect due to the numerous locations on this molecule that could interact non-covalently with MAA. The current research evaluated polymers prepared using both higher monomer:template ratios and lower cross-linker:monomer ratios to determine the effect on the IF.

Several imprinted polymers were prepared using different template:monomer:cross-linker ratios (Table 1). These polymers were evaluated by HPLC to compare the IF of each using acetonitrile as the mobile phase. Acetone was injected to determine t_0 and 100 ppm SDM in acetonitrile was injected to determine t_r. Capacity factors for the MIPs and the corresponding NIPs are shown in Table 2. MIP1 is similar to a previously reported MIP for SDM

Table 2
Comparison of chromatographic analysis of SDM compared with acetone in four different imprinted polymers compared to their control NIPs. The mobile phase was acetonitrile. In chromatograms with no separation of acetone and SDM, acetone was injected separately to obtain a t_0 value to calculate the k'.

Polymer	k'_{MIP}	k'_{NIP}	IF
MIP1	0.263	0.100	2.63
MIP2	0.425	0.109	3.94
MIP3	0.145	0.127	1.14
MIP4	0.259	0.265	0.89

that resulted in an IF of 2.29 but MIP1 has a lower concentration of cross-linker in the polymerization mixture (1:4:20 instead of 1:4:40, Table 1) [14]. The IF value of 2.63 for MIP1 indicates that less cross-linker in the mixture may have improved the imprinting slightly. It is possible that less cross-linker allowed a greater percentage of SDA–MAA interactions, forming more SDA "pockets" within the polymer, therefore improving the imprinting effect during polymerization.

Of the four polymers, MIP2 resulted in the highest IF value (3.92), which is 1.7 times higher than that seen in previous studies [14]. This polymer had a higher ratio of monomer:template (6:1) with a cross-linker ratio of 20. These results indicate that the increased amount of monomer with a decreased amount of cross-linker in the polymerization mixture improves the efficiency of imprinting of MIP2. This is not surprising because there are at least six locations of potential hydrogen bonding on the SDM molecule, and adding sufficient MAA to interact with all of those locations should maximize the interaction of SDA with MAA and improve the quality of the imprinted sites in the polymer. To determine if a large excess of monomer would further enhance the imprinting effect, MIP3 was prepared with a monomer:template ratio of 15:1. Results show that this excess of monomer reduced the IF to 1.14, signifying very little specific retention of SDA over a non-retained compound compared with NIP3. It is likely that the excess monomer in this MIP increased the number of EGDMA–MAA or MAA–MAA reactions during polymerization. This would reduce the number of specific SDM–MAA interactions, which did not allow sufficient SDM "pockets" to form in the polymer, resulting in the limited ability of the polymer to retain SDM during use. The template rebinding selectivity is affected by the shape and rigidity of the template assemblies which form in solution prior to polymerization [17]. Having a slight excess of MAA available in solution during polymerization may optimize the number of interaction between the template and the MAA functional groups which ultimately result in binding sites; however, having too much excess reduces the complementarity between the template and the cavity, either due to a reduction in the number of binding sites in the cavity, or to a change in the rigidity of the polymer and therefore, the cavities left behind by the template molecules.

The effectiveness of the cavities are also affected by the template:cross-linker ratio in the polymerization mixture. Typically a template:cross-linker ratio of 1:40 is used when preparing non-covalent MIPs. This provides rigidity in the polymer network that helps to ensure a cavity that is complementary in shape as well as functionality to the template. When using EGDMA, typically the greatest selectivity occurs at about 40–60 vol% cross-linker [8]. The amount of EGDMA used in these studies was lower than typical (template:cross-linker 1:20) which is equivalent to 18 vol% EGDMA. This lower volume of cross-linker was used to increase the concentration of template:monomer interaction in the polymer network, resulting in a polymer with maximum number of binding sites, while maintaining sufficient rigidity to conserve the integrity of the imprinted sites. To determine if increasing the concentration of cross-linker in the polymer when using excess MAA would improve IF, the typical template:monomer:cross-linker ratio

Table 3
Comparison of imprinted factor (IF) for MIP2 using different amounts of aqueous 50 mM phosphoric acid in the HPLC mobile phase (100% aqueous 50 mM phosphoric acid data is not included because elution of SDM could not be detected after 1 h).

Mobile phase (% 50 mM phosphoric acid in acetonitrile)	k'_{MIP}	k'_{NIP}	IF
0	0.425	0.109	3.94
20	0.061	0.014	4.36
40	0.171	0.098	1.74
60	0.701	0.443	1.58
70	2.080	1.301	1.60
80	10.60	5.721	1.85

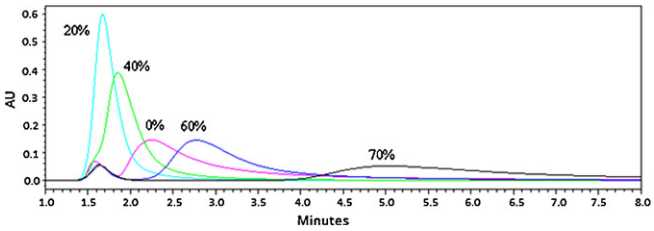

Fig. 2. Overlaid chromatograms of a sample containing acetone and SDM on MIP2 in mobile phases containing 0, 20, 40, 60 and 70% aqueous 50 mM phosphoric acid in acetonitrile. Chromatograms for 80 and 100% 50 mM phosphoric acid are not shown because elution times were greater than 8 min.

of 1:15:40 for MIP4. This is 36 vol% cross-linker which is closer to the previously reported optimum of 40–60% [8]. The IF of this polymer was lower (0.89) than MIP3 which was prepared using a ratio of 1:15:20 (18 vol% EGDMA) (Table 2). These results indicate that the higher volume of EDGMA essentially eliminated the imprinting effect, signifying no specific retention of SDA over a non-retained compound. This is contrary to results typically found using EDGMA as a cross-linker where the stability of the cavities increases as the degree of cross-linking increases, which in turn increases the specificity of the polymer [18]. It is possible that the excess EGDMA interacted non-covalently with the MAA as well as with the template which contains several amine groups which could hydrogen bond with EGDMA. This could reduce the number of interactions between the template and monomer which could generate fewer effective cavities in the polymer and reduce the number of binding sites. It is also possible that the excess EGDMA formed a polymer that was too rigid to allow the relatively large SDM molecule to squeeze into the cavity in the proper position and orientation and to be retained efficiently. Although past studies have found improvement in selectivity as cross-linker was increased [19], MIP4 demonstrate a decrease in the imprinting effect when cross-linker was increased from 18 to 36%, which is in interesting unusual finding.

An ideal application for this MIP would be the direct analysis of SDM in aqueous samples such as waste treatment plant effluent or environmental waters using an HPLC column packed with an MIP for SDM. This would eliminate a sample preparation step such a SPE generally required to isolate the analyte from the complex matrices in which low concentrations of sulfonamides are generally found. To evaluate this possibility, MIP2 was used in further studies to evaluate the IF and retention of SDM in an aqueous environment. Chromatograms of the evaluation of a sample containing acetone and SDM on MIP2 in acetonitrile mobile phase containing increasing amounts of water (Table 3) are shown in Fig. 2. The water contained 50 mM phosphoric acid to maintain an acidic pH. Retention time of acetone is at ~1.6 min in all chromatograms. As the amount of water is increased from 0 to 20%, the retention time of the SDM decreases, signifying a loss of ability of the MIP to selectively recognize the SDM. Although the water in the mobile phase most likely interferes with hydrogen bonding between the SDM

and the polymer, retention of SDM increases as the water is further increased above 20%, indicating that hydrophobic interactions may become the dominant interaction as the portion of water increases. To determine if this effect is specific to the imprinting of SDM or simply increases the retention of the SDM on the polymer material itself, the same study was performed on the control polymer NIP2. The retention time of SDM also increases steadily in the control polymer, but not as rapidly as in the MIP because the NIP has only the hydrophobic interactions to retain the SDM whereas the MIP has shape recognition as well, allowing the retention to increase more on the MIP. Although the highest IF of 4.36 was observed at 20% water in acetonitrile, elution time of SDM in this mobile phase was very fast and separation from acetone was not effective. Retention times for 60 and 70% water provided the greatest separation from the non-retained acetone. However, compared with the NIP, the IFs for these two mobile phases were much lower than the IF for 0 and 20%. The best combination of IF and separation from acetone occurs in 100% acetonitrile mobile phase. Results suggest that because the SDM completely separated from a non-retained compound, direct analysis of SDM on a column packed with MIP might be feasible.

When using HPLC to analyze non-covalently prepared MIPs, one common problem is tailing of the broad analyte peaks. This may be due to site heterogeneity created during non-covalent molecular imprinting due to the random arrangement of the template and monomer molecules, resulting in a distribution of binding sites with different affinities for the sample molecule [20]. Slow mass transfer and binding kinetics may also contribute to the poor peak shape [6]. Template molecules that contain two or three sites for hydrogen bonding result in MIPs with higher selectivity and stronger bonding, resulting in broader peaks compared to MIPs prepared with template molecules with fewer interaction sites [17]. It is not surprising that an MIP prepared with SDM, which contains many potential interaction sites would also display broad peaks when used in chromatography. Not only can tailing considerably increase the time needed to complete one analysis, but bad peak symmetry also makes it difficult to measure HPLC chromatogram parameters accurately. As a result, although a polymer prepared as MIP2 has the potential to be used directly in a chromatographic assay, it would require further optimization, as well as better column packing, and optimized column length and chromatographic conditions to be useful directly in a chromatographic application. Results suggest this study is worth pursuing. However, based on the highest reported IF for SDM, it does have the potential to provide better selective recognition of SDM when used in a SPE method coupled with HPLC–UV than MIPs for SDM previously reported [14].

4. Conclusion

Synthesis and evaluation of several MIPs for SDM using a variety of monomer:template ratios indicated that the higher concentration of monomer to template (6:1) results in a higher IF than a lower ratio (4:1), most likely due to the increased number of non-covalent interactions between the SDM and MAA. However, a significantly higher ratio of monomer:template (15:1) essentially eliminated the imprinting effect of SDM. Reducing the amount of cross-linker was also found to improve the IF, which is not typical of non-covalently prepared MIPs. Further studies to optimize template:monomer:cross-linker ratio to maximizing the imprinting effect and to optimize the selectivity of the MIP may result in a polymer that can be used directly in HPLC analysis, or at minimum, a better stationary phase for SPE prior to HPLC analysis.

Acknowledgment

We thank Susquehanna University for the financial support of this work.

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