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Synthesis and evaluation of tetracycline imprinted xerogels: Comparison of experiment and computational modeling

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ABSTRACT

A series of silica-based tetracycline (TC)-imprinted xerogel sorbents were prepared by sol–gel processing and were characterized for TC binding. Molecularly imprinted xerogels (MIXs) formed from allyltriethoxysilane (AtEOS) and tetraethoxysilane (TEOS) and end capped with trimethylchlorosilane exhibited the best analytical performance (imprinting factor, IF, of 7.46 ± 0.13). Computational modeling was used to estimate the interaction energy (IE) between TC and each type of silane to evaluate our ability to predict the analytical performance of a given MIX. Rankings from the computations agreed with the experimental data showing the AtEOS having the highest IE in comparison to the other formulations. Together, these results demonstrate the potential and limitations of using theoretical calculations to guide the development of analyte selective MIXs in comparison to arbitrary trial and error approaches traditionally used to produce MIXs as sorbents for solid phase extraction.

1. Introduction

Molecular imprinting generates synthetic materials that mimic the action of antibodies possessing selective cavities for the template molecule used in the synthesis. The imprinted material can therefore serve as host system that can be used for chemical sensors [1], chromatographic separations [2], and sample pre-concentration [3], among many other applications. Typically, development of molecularly imprinted polymers (MIP) uses the traditional trial and error approach which can be tedious, time intensive, and expensive in terms of reagent use. In addition, several combinatorial approaches creating MIP libraries and screening those materials from within the library that exhibit high selectivity and binding capacity are reported [4–6]. This approach is time and cost effective and provides new insight into the influence and interaction of the main factors that affect MIP performance [5].

Recently, there have been reports on the use of computational approaches to guide MIP development [7,8]. Virtual libraries can be designed [9] to screen *in silico* the best possible functional monomers for forming a target analyte-binding MIP by calculating interaction energies between each monomer within the library and the target analyte [8,10]. Different computational methods (e.g., semi-empirical (PM3) [11], *ab initio* calculations [10] and methods employing molecular dynamics [12]) have been used. Most of these studies focus on binding within a single cavity wherein a scoring function approach based on the interaction energy of the prepolymerization step is used as the basis of optimization [13]. Fundamental computational studies or development of models on the process of MIP formation have been reported elsewhere [13–15].

In this study, new molecularly imprinted xerogels (MIXs) were synthesized for the selective binding of tetracycline (TC) antibiotics (Fig. 1). Because TCs are amongst the most widely used antibiotics in animals and humans, TC residues are sometimes found in meat [16], milk [17], eggs [18], cheese [19] and honey [18]. TCs are also poorly metabolized in animals [20], hence, significant quantities are excreted and remain biologically active in animal wastes. Further, TC-containing manure are land-applied to fertilize croplands [21]. Therefore, the presence of TC residues in terrestrial environments [22] and aquatic systems [23] has become an ecological concern because persistent antibiotics can contribute to increased occurrences of antibiotic resistance among pathogenic bacteria [24].

Analysis of TC residues in environmental samples is most commonly performed by using liquid chromatography/mass spectrometry (LC/MS). However, despite the relatively low detection limits offered by LC/MS, proper sample clean-up is critical to achieving good accuracy and precision. Analysis of TC residues by LC/MS is very challenging because the ionization process in electrospray MS is highly sensitive to matrix effects [25]. Therefore, solid phase extraction (SPE) [26] or liquid–liquid extraction [27] are commonly used to clean up and pre-concentrate environmen-

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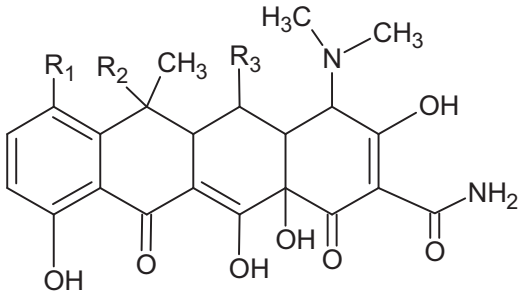

Fig. 1. General structure of tetracycline.

tal samples prior to LC/MS analysis. Unfortunately, conventional extraction techniques are not selective and result in co-extraction of undesirable matrix components that can interfere in the analysis. For example, the use of HLB™ (hydrophilic–lyphophilic balance) SPE cartridge, the most commonly used SPE sorbent for concentrating TCs, resulted in 24–49% signal suppression for TCs in chlorinated drinking water samples [28]. Similarly, the use of a mixed-mode strong cation exchange cartridge [29] showed 80% signal suppression for doxycycline in surface water samples. In contrast, a significant signal enhancement was observed in the LC–MS analysis of surface water samples for TCs [30]. The use of mixed-mode anion-exchange SPE cartridges [31] resulted in a large baseline drift in the LC/MS chromatogram and other interfering peaks were observed in the determination of TCs in wastewater effluents.

To improve the accuracy of TC analysis by LC/MS, a more selective SPE sorbent is needed. One strategy is to develop molecularly imprinted sorbents [32] that are selective for TCs. Toward this end, several research groups have reported TC-selective MIPs based on organic acrylate or acrylic type polymers [33–37] (Table 1) and some of these MIPs have been used in the analysis of food samples [38–40]. Molecularly imprinted materials can also be created by using organically modified silicas (ormosils) [41] and sol–gel processing [41–44]. In comparison to acrylic-based imprinted polymers, xerogel based materials can be more specific towards the target analyte, and they exhibit faster analyte diffusion within the imprinted material [45,46].

The objectives of this study are to: (i) create molecularly imprinted xerogels (MIXs) for TCs and (ii) compare the experimental results (imprint factor, IF) with molecular modeling (interaction energy, IE) to determine the effectiveness of computational approaches for rationally designing TC-imprinted MIX-based sorbents. Recent studies [47,48] used a related strategy to help design molecularly imprinted silica for binding β-damascenone and neurotransmitters. In the present work, a larger number of experimental results (which includes the effect of end capping and the use of different solvents in rebinding studies) are compared with theoretical results. Unlike the previous studies which made use of stable species, TC is inherently unstable with time, pH, and temperature [49–51] hence the current research reflects an extremely complex system. TC also possesses many more functional groups in comparison to β-damascenone and neurotransmitters reported in previous studies [47,48].

2. Experimental

2.1. Reagents

Water was purified by using a Nanopure Diamond™ water purifier. The following reagents were used: TC (Fisher Scientific); acetone (EMD Chemicals); methanol (HPLC grade), acetonitrile (LC/MS grade) and tetrahydrofuran (THF) (Burdick & Jackson); tetramethoxysilane (TMOS), tetraethoxysilane (TEOS), 2-cyanoethyltrimethoxysilane (CNEtMOS), allyltriethoxysilane (AtEOS), ethyltrimethoxysilane (C2tMOS), *n*-butyltrimethoxysilane (C4tMOS), *n*-pentyltriethoxysilane (C5tEOS), *n*-octyltriethoxysilane (C8tEOS), phenyltriethoxysilane (PhetEOS) and trimethylchlorosilane (TMCS) (Gelest); ethanol (EtOH) (200 proof ACS/USP grade) (Pharmco); hydrochloric acid (ACS Grade) and dimethyl sulfoxide (DMSO) (Fisher Scientific); ^{3}H-labeled TC [7-^{3}H(N)], ^{3}H-labeled erythromycin, ^{14}C-labeled sulfamethazine (American Radiolabeled Chemicals, Inc.); and

Table 1
Reported imprinting factors (IF) for TC-imprinted MIPs.

Number	Composition/description[a]	Solvent	IF[b]	References
MIP 1	EGDMA and MAA	Water	1.74	[39][c]
	Multiple-TCs MIP	Acetonitrile	1.92	
MIP 2	EDMA and MAA	Water	4.13	[39]
	Single-TC MIP	Acetonitrile	4.80	
MIP 3	MAA and TRIM different ratio of	Methanol	2.708	[36][d]
MIP 4	monomer and template ranging from	Methanol	2.549	[36]
MIP 5	2:1 in MIP 3 to 10:1 in MIP 7 TC-MIP	Methanol	2.149	[36]
MIP 6		Methanol	4.949	[36]
MIP 7		Methanol	2.075	[36]
MIP 8	EGDMA/MAA OTC-MIP	Acetonitrile	4.0	[38][d]
MIP 9	EGDMA and MAA	Water	nr	[33][c]
MIP 10	P(AA-coAN) and AA	Water	nr	[37][d]
MIP 11	MIP coated fiber; TRIM and acrylamide	Benzene	3.9	[34][d]
MIP 12	EGDMA and MAA		nr	[40][c]
MIP 13	MAA and TRIM; MIP 13 to MIP 16 deals	Methanol	2.0	[35][d]
MIP 14	on the effect of volume ratio of	Methanol	1.2	[35]
MIP 15	porogen; MIP 17 used EGDMA instead	Methanol	1.9	[35]
MIP 16	of TRIM[e]; MIP 18 to MIP 20 deals on	Methanol	4.9	[35]
MIP 17	effect of crosslinker (TRIM) amount	Methanol	1.7	[35]
MIP 18		Methanol	2.8	[35]
MIP 19		Methanol	7.0	[35]
MIP 20		Methanol	2.3	[35]

[a] EGDMA – ethylene glycol dimethacrylate; MAA – methacrylic acid; p(AA-co-AN) – polyacrylonitrile-co-acrylic acid; AA – acrylic acid; TRIM – 2,2-bis(hydroxymethyl)butanol trimethacrylate; OTC – oxytetracycline.
[b] Capacity factor ratio of MIP to NIP (non-imprinted polymer); (nr – not reported).
[c] IF value obtained under equilibrium condition.
[d] IF value obtained under chromatographic (non-equilibrium) condition.
[e] EGDMA was used as crosslinker instead of TRIM in MIP 17.