Materials Letters 159 (2015) 317–320

Contents lists available at ScienceDirect

Materials Letters

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

Harnessing non-covalent interactions in molecular traps for probable human carcinogen butylated hydroxyanisole

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ARTICLE INFO

Article history:
Received 18 May 2015
Received in revised form
5 June 2015
Accepted 6 July 2015
Available online 8 July 2015

Keywords:
Molecularly imprinted polymer
Butylated hydroxyanisole
Batch-binding
DFT

ABSTRACT

We report the synthesis, characterization and performance evaluation of nine molecularly imprinted polymers (MIPs) for butylated hydroxyanisole (BHA), a common antioxidant used in the food industry but is carcinogenic at high concentrations. Vinyl-functionalized imidazole and pyridine monomers were thermally cured with BHA as template, ethylene glycol dimethacrylate as cross-linker, and 1,1′-azobis (cyanohexane carbonitrile) as initiator. The molar ratio of two functional monomer combinations, which can be 1-vinylimidazole (1-VI), 2-vinylpyridine (2-VP) or 4-vinylpyridine (4-VP), was varied and the polymer was cured at 60 °C for 36 h. Spectrophotometric batch-binding analyses of BHA showed that the optimum uptake was observed for 1-VI- and 4-VP-based MIPs, with a maximum binding capacity of 6.078 mg BHA per gram MIP. The observed performance was rationalized by the non-covalent interactions between the template and the functional monomers.

1. Introduction

Molecularly imprinted polymers (MIPs) are a group of highly cross-linked polymers with high selectivity and affinity to a predetermined molecule known as the template. Molecular imprinting involves the integration into a polymer matrix of small quantities of the template molecule, which is subsequently removed leaving cavities complementary in shape, size, and chemical functionality with that of the template molecule [1], affording unique recognition sites [2–4]. MIPs have found extensive application in various fields of chemistry and biology as affinity material for sensors [5], adsorbent for solid phase extraction [6], artificial antibodies [7], and chromatographic stationary phases [4]. The molecular imprinting technique was first demonstrated by Wulff et al. [8] who designed polymeric receptors by exploiting the reversible covalent interaction between a template and a monomer. Since then, this technology has continually gained attention primarily due to the ease of preparation and sensitive molecular detection. In recent years, molecular recognition of ciprofloxacin [1], flavin [9], cinchonidine [10], endocrine disrupting compounds, EDCs [11], polycyclic aromatic hydrocarbons, PAHs [12] and perfluorooctane sulfonate, PFOS [13] have been demonstrated.

Synthetic antioxidants are food additives that retard deterioration and rancidity due to lipid degradation [14]. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been widely used for many years as antioxidants to preserve and stabilize the freshness, nutritive value, flavor and color of food and animal feed products [15]. BHA is harmful if swallowed, inhaled, or absorbed through skin. The target organs for toxicity are liver, lungs, and forestomach [16]. However, BHA is classified as Generally Recognized as Safe by the US FDA, when the total content of antioxidants is less than 0.02 wt% of the total fat or oil content of the food [15]; above this limit, BHA is considered carcinogenic [16]. As such, constant monitoring of BHA content in food products is essential.

We report herein the synthesis of nine MIPs based on 1-vinylimidazole (1-VI), 2-vinylpyridine (2-VI) and 4-vinylpyridine (4-VP) through thermal free-radical polymerization. The binding capacities of each MIPs were evaluated via a spectrophotometric monitoring of the absorbance of an aqueous solution of BHA after a 24 h incubation period. The observed high affinity of 1-VI and 4-VP-based MIPs was further supported by molecular simulation

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http://dx.doi.org/10.1016/j.matlet.2015.07.026
0167-577X/

Table. 1
The molar amount of the template, functional monomers, cross-linker and initiator used for the preparation of MIPs 1–9.

Sample	Template, BHA (mmol)	Monomer (mmol) 1-VI	2-VP	4-VP	Crosslinker EGDMA (mmol)	Initiator ABCHC (mmol)
MIP1	0.25	0.25	0.75	–	28	0.40
MIP2	0.25	0.50	0.50	–	28	0.40
MIP3	0.25	0.75	0.25	–	28	0.40
MIP4	0.25	0.25	–	0.75	28	0.40
MIP5	0.25	0.50	–	0.50	28	0.40
MIP6	0.25	0.75	–	0.25	28	0.40
MIP7	0.25	–	0.25	0.75	28	0.40
MIP8	0.25	–	0.50	0.50	28	0.40
MIP9	0.25	–	0.75	0.25	28	0.40

studies.

2. Experimental details

2.1. Materials

The template molecule, BHA (Fluka); the monomers, 1-VI (Fluka), 2-VP (Fluka), and 4-VP (Fluka); the cross-linker, ethylene glycol dimethacrylate (EGDMA, Fluka); and the initiator, 1,1′-azobis (cyanohexane carbonitrile) (ABCHC, Fluka) were all used without further purification. Chloroform (Univar) was used as solvent during polymerization, while ethanol (EtOH, Merck), acetic acid (HOAc, JT Baker) and distilled water were used to remove the template.

2.2. MIP synthesis

Table 1 shows the molar amounts of the template, functional monomers, cross-linker and initiator used for the preparation of MIPs 1 to 9. All MIPs were prepared by a one-step precipitation polymerization. In a typical setup, BHA and the corresponding amounts of functional monomers were dissolved in chloroform. The solution was allowed to stand for 30 min to maximize molecular interaction among components. EGDMA and ABCHC were then added, followed by sonication and nitrogen purging. The solution was cured at 60 °C for 36 h. The resulting monolithic polymer was ground to a fine powder. Template removal was accomplished by repeated washing with 9:1 (v/v) EtOH/HOAc solution until no trace of BHA was detected. MIP5, with or without the template, was subjected to DRIFTS (diffuse reflectance infrared Fourier transform spectroscopy) using Shimadzu IR Prestige-21. A blank was also included as a reference. The blank was synthesized by mixing the monomers, crosslinker and initiator in the same ratio as MIP5 except the template was not included.

2.3. Batch-binding analysis

Each of the MIPs (0.50 g) was placed in a vial containing 5 ml of 0.1250 mM of the analyte (BHA) and was incubated for 24 h. After incubation, an aliquot of the liquid was assayed for BHA using a Shimadzu 2101 PC UV-Vis Spectrophotometer. The difference in the corrected concentration before and after the incubation provides a measure for the binding capacity of the polymers.

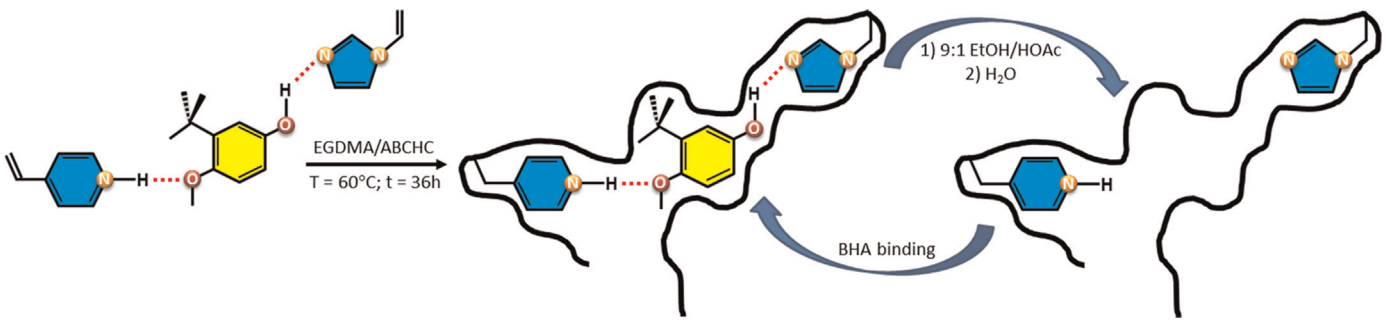
2.4. Conformational studies

The search for possible interactions of the functional monomers with the template was carried in a Spartan '08 platform following a protocol involving molecular mechanics simulation using the MMFF94 force field, followed by geometry optimization at DFT/B3LYP/6-31G(d) level of theory.

3. Results and discussion

3.1. Preparation of MIPs through thermal free radical polymerization

Free radical thermal polymerization was employed to produce nine MIPs used in this study. The mechanism of formation of the molecular recognition site from the pre-arrangement of the monomers and the template prior to polymerization, along with the mechanism of template extraction and rebinding during the batch-binding analyses is shown in Scheme 1. These mechanisms prevail in all of the MIP preparations of interest but with slight variations in the extent of the interaction between the template and the monomers. Thermal curing was chosen over conventional photopolymerization due to the sensitivity of BHA to UV radiation. As such, the formation of pre-polymer complex through pre-arrangement of the template and the functional monomers was maximized by allowing the reaction mixture to stand for 30 min prior to the addition of the initiator and the cross-linker. The basic monomers used (1-VI, 2-VP, and 4-VP) were chosen on the basis of the complementarity of their functional groups with those of the template. BHA includes both H-bond donor and acceptor moieties, and its ring structure can play an important role in the formation of non-covalent interactions through ring stacking. These monomers contain vinyl moiety which undergoes well established free radical polymerization mechanism. Nitrogen purging was done to remove oxygen from the system, which can slow down or terminate the polymerization by scavenging free radicals generated from the decomposition of the initiator. Chloroform, a weakly polar porogenic solvent was employed because the use of solvents with low polarity has been reported [1] to enhance the selectivity of MIPs. The fast polymerization process observed in thermally synthesized MIPs results in the formation of monoliths [10]. These monoliths were crushed and ground to produce microparticles needed for batch-binding analysis. The removal of the template



Scheme. 1. Formation of molecular recognition site within the polymer matrix.