Removal of patulin from aqueous solutions by propylthiol functionalized SBA-15

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A B S T R A C T
Propylthiol functionalized SBA-15 silica was investigated to detoxify aqueous solutions contaminated with the regulated mycotoxin patulin. Micelle templated silicones with a specific pore size were synthetically modified to possess propylthiol groups, a functional group known to form Michael reaction products with the conjugated double bond system of patulin. BET surface area analysis indicated the propylthiol functionalized SBA-15 possesses channels with the pore size of 5.4 nm and a surface area of 345 m² g⁻¹. Elemental analysis indicates the silicon/sulfur ratio to be 10:1, inferring one propylthiol substituent for every ten silica residues. The propylthiol modified SBA-15 was effective at significantly reducing high levels of patulin from aqueous solutions (pH 7.0) in batch sorption assays at room temperature. The material was less effective at lower pH; however heating low pH solutions and apple juice to 60 °C in the presence of propylthiol functionalized SBA-15 significantly reduced the levels of patulin in contaminated samples. Composite molecular models developed by semi-empirical PM3 and empirical force field methods support patulin permeation through the mesoporous channels of propylthiol functionalized SBA-15. Density functional study at the B3LYP/6-31G(d,p) level predicts the proposed patulin adducts formed with reaction with the thiol residues exhibit less electrophilic properties than patulin. It is demonstrated the use of propylthiol functionalized SBA-15 is a viable approach to reduce patulin levels in aqueous solutions, including contaminated apple juice.
aquuous solutions. Herein, we report the development of a functionalized material capable of removing patulin from apple juice and other aqueous systems through covalent sequestration.

The toxic effects of patulin are due to the deleterious formation of adducts with important biomolecules, such as glutathione, cysteine residues of proteins, and DNA [3,14]. In fact, the electrophilic properties related to the conjugated double bond system of patulin have been utilized to reduce patulin levels by reaction with free thiols, in solution. For example, thiol containing reducing agents increased the rate of patulin removal from contaminated alfalfa in rumen fluid [15]. In addition, cysteine has been investigated as an additive to detoxify patulin contaminated commodities [16]. However, these approaches leave patulin–thiol adducts free in solution with unknown properties or stabilities.

A promising field for the development of catalysts and other types of synthetic receptors is templated functionalized silicas tuned with specific pore sizes [17]. The mesoporous molecular sieve SBA-15 possesses a unique pore topology with hexagonally arranged adjacent mesoporous channels connected by micropore tunnels [18]. Specific applications of these types of nanoporous materials include catalyst supports, adsorbents, materials for size selective separations of small organics and proteins, and scaffolds for biosensors [17–20]. In this report, a SBA-15 silica is synthetically modified by reaction with 3-mercaptopropyltrimethoxysilane (MPTMS) to produce a thiol-modified material possessing mesoporous cavities suitable for patulin interaction. It is shown that the material is capable of removing high levels of patulin from aqueous solutions and the mechanism of action is probed by molecular modeling and quantum mechanical methods.

2. Experimental

2.1. Reagents and materials

Methanol, ethanol, aceton (HPLC grade), ethyl acetate (HPLC grade), hexane (HPLC grade), acetonitrile (HPLC grade), sodium phosphate, disodium phosphate, sodium acetate, acetic acid, patulin, 1, tetraethylylthiosilicate (TEOS), 3-mercaptopropyltrimethoxysilane (MPTMS) and the templating molecule Pluronic P123 were obtained from Sigma–Aldrich, Inc. (Milwaukee, WI, USA).

2.2. Synthesis of SBA-15 functionalized silica

The sorbents were prepared by modifications of the methods described in the literature [21]. For SBA-15-PSH synthesis, 36 mmol tetraethylylthiosilicate was dissolved in 125 ml of a 2 N HCl solution containing 3 wt.% Pluronic P123. This was stirred at 50 °C for 1 h. To the resulting suspension, 3.6 mmol 3-mercaptopropyltrimethoxysilane is added. This was stirred for an additional 90 min and was then aged at 90 °C for 16 h. The product was collected by filtration and washed with water, ethanol, and methanol to remove excess template. The product was then dried in vacuo at 90 °C prior to analysis.

2.3. Materials characterization analytical methods

Sulfur analysis was performed on a Thermo Flash EA1112. Textural properties of the sorbents were calculated from nitrogen isotherms collected at 77 K using a Quantachrome Autosorb-1. Approximately 50 mg samples of the sorbents were degassed at 100 °C for 18 h for analysis. Surface areas were calculated using the multipoint BET method and pore sizes calculated using the BJH method on the adsorption branch. The solid phase CPMA$^{13}$CNMR spectrum was recorded on a Bruker Avance 300 with a rotor speed of 3200 rpm.

2.4. LC-analysis

Patulin levels were determined by LC-analysis. The HPLC system consisted of a Shimadzu LC-20AT pump, a manual HPLC injector, a CBM-20A communications bus module, and a Phenomenex Luna 5 μC18 100 A column. Instrumentation was controlled by Shimadzu EZstart software. The LC-mobile phase consisted of 10% acetonitrile in water. Unless otherwise noted, patulin concentrations were calculated based on a standard curve using peak areas recorded at 276 nm. Patulin standard was stored in 1:1 acetonitrile/water (1 mg ml$^{-1}$). Patulin levels in spiked apple juice were evaluated following a previously published procedure [6] with the following modifications: Sep-Pak Vac 3cc (500 mg) C18 solid phase extraction columns were applied for sample clean-up, elution solvent was reduced by rotary evaporation, and injection was through a 20 μl injection loop (0.05–5 μg ml$^{-1}$, r$^2$ = 0.975).

2.5. Binding assays

SBA-15 and SBA-15-PSH were evaluated by measuring the capability to remove patulin from aqueous solutions in equilibrium binding assays. Assays were conducted in 1.5 ml silanized screw cap vials with a specified amount of SBA-15 or SBA-15-PSH in 1 ml solutions of patulin at various concentrations in 10 mM sodium phosphate buffer or 100% pure apple juice. Mesoporous silica were evaluated at concentrations of 5 mg ml$^{-1}$ unless otherwise noted. For the room temperature experiments, vials were shaken on a Lab-line Multiwrist shaker set on speed 8 for the specified amount of time. Higher temperature assays were conducted in a Magni Whirl constant temperature bath with the shaker control set at 11. Experiments were performed in triplicate. Following incubation, the vials were centrifuged and the collected supernatant was filtered through Millex syringe driven PTFE filters (0.2 μm). Removal efficiency from the equilibrium binding assays was determined by comparing the patulin peak area of samples run in the presence of the mesoporous silica with the patulin peak area of samples run without mesoporous silica through the following relationship:

\[
\% \text{Removal efficiency} = \left( \frac{C_{\text{Standard}} - C_{\text{SBA-15}}}{C_{\text{Standard}}} \right) \times 100
\]

where $C_{\text{SBA-15}}$ is concentration of the patulin recovered in the supernatant from the sample run with silica, and $C_{\text{Standard}}$ is the concentration of the patulin standard without silica (0.05–50 μg ml$^{-1}$, pH 7.0, r$^2$ = 0.998; pH 4.0, r$^2$ = 0.997) (0.05–5 μg ml$^{-1}$, pH 9.0, r$^2$ = 0.995; pH 8.0, r$^2$ = 0.999; pH 6.0, r$^2$ = 0.998; pH 5.0, r$^2$ = 0.997; pH 3.0, r$^2$ = 0.999).
2.6. Molecular modeling

Molecular modeling was carried out using density functional theory, PM3 semi-empirical, and MM+ empirical methods. Density functional theory geometry optimization calculations were carried out using Parallel Quantum Solutions (Fayetteville, AR, U.S.A.) hardware and software v3.2 [22]. Molecular orbitals were modeled using the HyperChem v8.08 program (Gainesville, FL, U.S.A.) [23].

Composite models of the SBA-15 and SBA-15-PSH synthesized in this study were built based on previously published SBA-15 models [17,18] using the Hyperchem program v8.08 [23]. A layer of silica with the specified pore size was built using the PM3 semi-empirical method and MM+ force field, as implemented in Hyperchem. Components were initially optimized using the PM3 semi-empirical method. The model was built using the PM3 charges, the MM+ force field, and geometry optimization with the Polak–Ribiere conjugate gradient method. The depth of pore in the initial model was 9.1 Å, and the hexagonal pores were connected with layers of silica. Successive layers were added to develop a model with the depth of approximately 40 Å.

B3LYP density functional methods have provided insight into the structures patulin and related mycotoxins, and we applied this hybrid functional for the single molecule studies of patulin and proposed patulin–propylthiol adducts at the 6-31G(d,p) level [24–26]. Geometry optimization was performed in the gas phase using B3LYP/6-31G(d,p) level of theory on delocalized internal coordinates using the Eigenvector Following Algorithm with the convergence criteria set at $1 \times 10^{-6}$ Hartree and a gradient of less than $3 \times 10^{-4}$ a.u. Gauge Including Atomic Orbital (GIAO) methods were applied for shielding tensor calculations on geometry optimized structures of the propylthiol residue [27]. Results were evaluated as chemical shifts relative to the reference standard, tetramethylsilane. Frontier molecular orbitals were calculated and modeled using Hyperchem v8.08 with the default settings. The quantum chemical descriptors (orbital energy, gap energy, electronegativity, hardness, softness, and electrophilicity index) were calculated based on structures optimized at the B3LYP/6-31G(d,p) level as previously defined [28–30].

3. Results and discussion

3.1. Characterization of the propylthiol functionalized SBA-15

Elemental analysis studies indicate that the total sulfur content of the propylthiol functionalized SBA-15 (from here on SBA-15-PSH) to be 3.1%, which calculates to one propylthiol residue per 11 silica (SiO$_2$) residues (see Table 1). The nitrogen adsorption–desorption isotherms for the sorbents in this study are shown in Fig. 2. The transition in volume is sharp and the pore diameter peaks are relatively narrow indicating a moderately consistent population of pores with a narrow range of pore volumes and pore diameters. The median pore diameter is significantly reduced for the SBA-15-PSH (5.4 nm) compared to SBA-15 (9.0 nm).

Modification of the SBA-15 with propylthiol substituents reduces the pore volume and surface area determined by BET analysis by approximately four-fold. The solid state carbon-13 chemical shifts of SBA-15-PSH matched the calculated values predicted by GIAO-B3LYP/6-31G(d,p) NMR analysis. Rotating the propylthiol chain had little effect on the calculated carbon chemical shifts compared to calculated chemical shifts for the straight chain propylthiol moiety. The properties of the SBA-15–PSH reported in this study compare favorably with related templated functionalized silicas [17]. Published TEM and HTREM micrographs of propylthiol functionalized SBA-15 with reagent ratios similar to the SBA-15–PSH reported here maintain the ordered morphology characteristic of mesoporous silicas synthesized in the presence of the Pluronic P123 template [17,31].

3.2. Modeling of SBA-15 and propylthiol functionalized SBA-15

Models of SBA-15 and SBA-15-PSH were built to gain insight into the influence of propylthiol substituents on the SBA-15 pores at the molecular level. A composite model was built based on physical parameters provided by the material characterization of the SBA-15 and SBA-15-PSH and previously published models of SBA-15 materials [17,18]. Specifically, we built the mesoporous channels using the experimentally determined pore diameters and previously published templated silica channel structures [18]. To simulate the imperfect nature of the silica channels, silicon-oxygen bonds were broken on the surface of the channels which provided hydrophilic silanol groups. For efficiency, atomistic scaffolds between the channels were built with 10 Å walls of silica and a length of 20 Å. The resulting model of SBA-15 is shown in Fig. 3(a), with a mesoporous honeycomb structure of ordered hexagonal channels. The unifor-

![Fig. 2. ](image-url) Nitrogen adsorption–desorption isotherms at 77 K and resulting BJH mean pore diameters of SBA-15 and SBA-15-PSH. Solid symbols are the adsorption branches and open symbols are the desorption branches.
Fig. 3. (a) Cut-out view of a representative model of mesoporous SBA-15 with ordered hexagonal channels. Block size is 100 Å × 70 Å × 40 Å. (b) Molecular 3D model of patulin molecules permeating a mesoporous silica channel of SBA-15-PSH.


Initially, we investigated the ability of SBA-15 and SBA-15-PSH to act as a filter to remove patulin from contaminated aqueous solutions, such as apple juice or water. However, solid phase extraction columns filled with 25 mg of SBA-15 or SBA-15-PSH were unsuccessful at reducing patulin concentrations in buffered solutions (10 mM sodium phosphate, pH 7.0). The SBA-15-PSH was more effective as a solid sorbent additive to detoxify patulin contaminated solutions. Batch sorption results for assays carried out in 10 mM sodium phosphate solution are shown in Figs. 4–7. Fig. 4(a) provides the influence of time on a 5 µg ml⁻¹ (5 ppm) solution of patulin in the presence of SBA-15 and SBA-15-PSH (5 mg) at pH 7.0. At 8 h, patulin is almost completely removed from solution in the presence of SBA-15-PSH. All other binding assay experiments reported here were carried out with 24-h incubation durations, which provided reproducible results over the range of concentrations studied.

The capacities of SBA-15 and SBA-15-PSH for high concentrations of patulin (20 µg ml⁻¹) at pH 7.0 are given in Fig. 4(b). Remarkably, 3 mg ml⁻¹ of SBA-15-PSH is capable of removing 94% of patulin from high concentration samples. One generality observed in Fig. 4(a and b) is unmodified SBA-15 does not remove significant levels of patulin in the batch sorption assays. In contrast, the propylthiol functionalized SBA-15 exhibits a very large capacity for patulin, indicating the propylthiol residue is critical for removing patulin from solution in the batch sorption assay. Efforts to recover patulin from the used sorbent (20 µg ml⁻¹ patulin/5 mg SBA-15-PSH) with extraction (1 ml) by sonication for 15 min (acetonitrile 3× and methanol 3×) recovered less than 0.1% of the calculated bound patulin. These results suggest the SBA-15-PSH covalently binds patulin and is capable of removing the toxin from solutions without leaching of bound patulin.

The binding properties of SBA-15-PSH over a range of patulin concentrations were determined by batch sorption experiments carried out in aqueous solutions (pH 7.0, 10 mM sodium phosphate buffer) at room temperature with 24-h incubation (see Fig. 5). SBA-15-PSH is capable of removing over 90% of patulin from aqueous solutions at pH 7.0 over concentrations 0.05–50 µg ml⁻¹. Fig. 6 provides the relationship between pH and patulin removal by SBA-15-PSH. Patulin removal by SBA-15-PSH decreases at lower pH values. However, we found heating the low pH solutions (50 µg ml⁻¹ patulin, pH 4.0) to 60 °C increased patulin removal by 30% compared to experiments carried out at room temperature. The patulin binding properties of SBA-15-PSH at pH 4.0 were suitable for non-equilibrium Freundlich isotherm analysis for levels between 0.05 and 50 µg ml⁻¹ and the sorption isotherm is pro-
Patulin sorption isotherm of SBA-15-PSH obtained in sodium phosphate buffer (10 mM). Experiments were conducted at room temperature and 24-h incubation. The values are mean ± standard deviation.

Fig. 5. Sorption of patulin by SBA-15-PSH in sodium phosphate buffer (10 mM, pH 7.0). Experiments were conducted at room temperature and 24-h incubation. The values are mean ± standard deviation.

Fig. 6. Influence of pH on patulin removal by SBA-15-PSH in sodium phosphate buffer (10 mM). Experiments were conducted at room temperature and 24-h incubation. The values are mean ± standard deviation.

Patulin removal yield (%)

![Graph showing Patulin removal yield (%)](image)

Table 2
Reduction of patulin in apple juice by SBA-15-PSH.

<table>
<thead>
<tr>
<th>[Patulin]_{initial} (µg ml⁻¹)</th>
<th>% Patulin removed</th>
<th>[Patulin]_{bound} (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>34%</td>
<td>1.71 ± 0.07</td>
</tr>
<tr>
<td>1</td>
<td>37%</td>
<td>0.37 ± 0.06</td>
</tr>
<tr>
<td>0.5</td>
<td>42%</td>
<td>0.21 ± 0.01</td>
</tr>
<tr>
<td>0.1</td>
<td>72%</td>
<td>0.072 ± 0.009</td>
</tr>
<tr>
<td>0.05</td>
<td>48%</td>
<td>0.024 ± 0.002</td>
</tr>
</tbody>
</table>

*Apple juice samples spiked with patulin were incubated for 24h at 60°C. Patulin levels were determined following an established procedure with solid phase extraction clean-up and HPLC–UV detection at 276 nm [6].

**Mean ± standard deviation.

Fig. 7. Patulin sorption isotherm of SBA-15-PSH obtained in sodium phosphate buffer (10 mM, pH 4.0) at 60°C. Incubation time was 24 h. C is concentration of free patulin, and q is the bound patulin per gram of SBA-15-PSH. q = KfC^{1/n}, Kf = 4330 (µmol g⁻¹)(mol⁻¹)¹/n, 1/n = 0.72 ± 0.04; r² = 0.995. The values are mean ± standard deviation.

3.4. Toxicological potential of patulin adducts

The toxicological potential of the adducts formed through the sequestration of patulin by the SBA-15-PSH was investigated using computational chemistry. Adducts formed by patulin and thiols have been extensively investigated, and a complex set of over 15 adducts have been isolated and characterized [35,36]. Based on published reports, two fundamental propylthiol–patulin adducts were studied using B3LYP/6-31(d,p) density functional methods to gain insight into the electrophilic properties important for reactivity of these species. It should be noted QSAR studies have predicted correlation between frontier orbital descriptors (LUMO) of soft electrophilic compounds and toxicity [37,38]. The LUMO and HOMO frontier orbitals of patulin and patulin adducts are given in Fig. 8, and the molecular orbital and electrophilicity properties are given Table 3. Adduct 2 is based on the reaction of propylthiol with patulin at the C6 carbon. Proposed adduct 3 is a Michael addition-like intermediate at the C3 carbon of patulin. The HOMO and LUMO orbitals of patulin, 1, are primarily associated with the conjugated double bond system. In contrast the HOMO and LUMO frontier orbitals of adducts 2 and 3 include the thioether functional group with noticeable disruption of the conjugated system. The thiol adducts formed through disruption of the conjugated double bond system of patulin possess significantly dissimilar electronic properties compared to patulin. The proposed adducts possess a significantly lower global electrophilicity index, ω, compared to patulin, suggesting the proposed adducts are less reactive with biologically important soft nucleophiles. Similar patulin adducts...
formed from free cysteine residues have been shown to be less toxic than patulin [15,16]. An advantage SBA-15-PSH has over the use of free thiols to detoxify patulin contaminated solutions is the SBA-15-PSH removes patulin from solution or juice and sequesters the patulin adducts within the mesoporous silica.

4. Summary and conclusion

A propylthiol modified SBA-15 is developed as an approach to detoxify aqueous commodities contaminated with patulin. The effectiveness of the SBA-15-PSH to reduce patulin levels is sensitive to pH and time, and large quantities of material (5 mg ml$^{-1}$) are able to detoxify large concentrations of patulin. In addition, the material was suitable to reduce patulin from acidic solutions and apple juice with 24 h incubation at 60 °C. A composite model was built based on the characterization of the SBA-15-PSH material, and SBA-15 modeling studies carried out by other groups. Molecular modeling provided insight into the effects of synthetic modification on the pore size of mesoporous SBA-15. The SBA-15-PSH is useful to reduce levels of patulin in contaminated apple juice and other aqueous solutions.

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References


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