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ADSORPTION OF PROTEINS ON MESOPOROUS MOLECULAR SIEVES

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Abstract. Mesoporous molecular sieves such as MCM-41 and SBA-15 have many desirable properties for applications as separations media. Their high surface area (~ 1000 $m^2 \cdot g^{-1}$) and tuneable uniform pore size of 1.8 – 40 nm make them ideal for size exclusion separations of proteins and other biological molecules of importance in the food and pharmaceutical industries. However, the stability of MCM-41 in aqueous solution is limited. Therefore, in this work a hydrophobic coating has been applied to siliceous MCM-41 using hexamethyldisilazane to reduce degradation of the structure by hydrolysis and so increase its stability in water. This coating was covalently bound to the pore and particle surfaces and was stable in the presence of water for at least 12 days. The protein adsorption properties of the coated material compare favourably to the uncoated material, with up to 100 % more lysozyme adsorbed on the coated material than untreated MCM-41. The increased capacity and stability of this material make it promising for protein separation based on both size exclusion and chemical selectivity.

1. INTRODUCTION

Mesoporous molecular sieves (MMS), such as the M41S family of materials, have attracted substantial research attention since they were first reported by Mobil Corporation in 1991 [1, 2]. Their remarkable properties make them promising as catalysts or adsorbents for industrial separation processes, as well as being of fundamental interest [3-6]. In particular, their high surface area (≥ 1000 $m^2 \cdot g^{-1}$) and tuneable pore size with narrow pore size distributions in the mesoporous region (2 – 50 nm), give them substantial potential for size exclusion separations of large molecules such as proteins, which are important in the food and pharmaceutical industries [7]. In addition, their surfaces may be functionalised by binding organic ligands to the silanol groups on the surfaces of the pores or incorporating such ligands into the porous structure through co-condensation [8], to enhance their selective adsorption of a target molecule.

M41S materials are synthesized using a method termed liquid crystal templating, in which the silica structure is formed around surfactant aggregates which are subsequently removed by calcination or solvent extraction. One member of this group of materials, MCM-41, has a hexagonal array of uniform cylindrical pores, the size of which can be tuned in the range 1.5 – 10 nm. Many other types of MMS materials have been developed in the last decade with a range of structures and pore sizes up to about 40 nm.

In several recent studies organosilanes with thiol functional groups were bound to MMS or incorporated by co-condensation during synthesis of microporous molecular sieves to produce efficient adsorbents for heavy metal removal from aqueous solutions [5, 9-11]. Their capacity for Hg^{2+} was more than an order of magnitude greater than that of an amorphous silica sample of similar average pore size and surface area functionalised in the same way [5], due primarily to the uniform pore structure which provides much greater access to the pores without the limitations of blockages which occur in disordered porous media.

Indications of the size selectivity of MCM-41 were first shown in an enzyme immobilisation study [12],

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in which the protein loading into MCM-41 in a limited contact time decreased with increasing protein molecular weight as the protein size approached the mesopore size, as would be expected. The amounts of the protein cytochrome c adsorbed onto M41S materials were comparable to those adsorbed onto amorphous silica xerogel, and the protein has been shown to retain its activity after adsorption [13, 14]. Takahashi *et al.* [15] studied enzyme immobilisation on three types of mesoporous silica: MCM-41, SBA-15 and FSM-16. They found that greater amounts of the enzymes were adsorbed onto larger pore materials, with up to 150 $mg \cdot g^{-1}$ adsorbed on MCM-41 with 6.8 nm pores and up to 200 $mg \cdot g^{-1}$ adsorbed on FSM-16 with 8.9 nm pores. Much less was adsorbed on SBA-15 and a commercial silica gel with a wide pore size distribution. These findings were attributed to different surface characteristics between the different material samples, which depend on the synthesis methods used. Larger pore mesoporous materials (SBA-15 and MCF) have also been recently demonstrated to adsorb proteins after surface derivatization with silylamine groups [16]. Smaller proteins were noted to adsorb to a greater extent than larger ones.

We have demonstrated [7, 17] that the amount of a solute adsorbed on MCM-41 in a certain contact time depends strongly on the molecular size relative to the pore size for a range of solute sizes and contact times. Furthermore, the times for the amount of solute adsorbed to reach equilibrium were found to depend strongly on the size of the adsorbing molecule relative to the pore size. The fastest equilibration of three solutes (riboflavin, lysozyme and trypsin) was observed for the smallest, riboflavin (~2 hr), whereas up to four days was required to achieve equilibrium for the larger proteins. Thus, the outlook for application of these materials in size-based separations of biological molecules appears favourable.

However, the stability of many silica MMS materials, including MCM-41, in contact with water or water vapour is limited. Recent work has indicated that the structure of M41S materials can be significantly modified by both high compressive forces and by prolonged exposure to water or water vapour [18-22]. The stability of these materials can be somewhat improved by altering the synthesis conditions [22, 23]. Silylation also improves their stability by forming a hydrophobic coating on the surfaces to limit hydrolytic attack. Trimethylsilylated samples have been shown to have improved stability upon the exposure to moisture [18, 21, 24]. The stability of a vinyl-functionalised MCM-41 sample in boiling water for 24 h was also found to be greater than siliceous MCM-41 alone [8], although the structure of the functionalised material was modified to a degree. These results show the promise of hydrophobic coatings for protecting the MCM-41 structure in the presence of water. However, the choice of coating reagents and methodology is important to ensure long term stability of these materials in aqueous solutions of relevance for industrial separations applications.

Trimethylchlorosilane (TMCS) and trichloromethylsilane (TCMS) are commonly used as silylating reagents for silica surfaces and have been applied to MMS materials [25-27]. These silanes react with molecular water, if any is present either adsorbed on the surface or dissolved in solution. Therefore they tend to physically adsorb onto the silica surface, causing perturbation and hydrogen bonding of the surface hydroxyls but not reaction, unless a two-step reaction using an amine or high temperature conditions are employed [28]. For example, TMCS in organic solution forms a polymeric layer, physisorbed onto the surface, which comes off in water with time. In contrast, hexamethyldisilazane (HMDS) forms a covalently bound hydrophobic layer on a silica surface under mild reaction conditions with a low rate of surface reaction that results in uniform coating of mesoporous material surfaces [29-31]. The HMDS has been shown by infrared spectroscopy to react with the surface silanol groups of silica in both a gas phase reaction on flat silica disks and in a liquid phase reaction with siliceous MCM-41 [29, 30].

2. EXPERIMENTAL

Siliceous MCM-41 materials were synthesized using standard hydrothermal synthesis and calcination methods [26, 27, 32]. They were characterised by x-ray powder diffraction (XRD) (Philips PW1800 diffractometer, Cu$K\alpha$ radiation of wavelength 1.54056Å) and nitrogen adsorption at 77K (Micromeritics ASAP 2000). A hydrophobic surface coating was applied to calcined MCM-41 by gas phase reaction with HMDS (Merck 98 %), which was freeze-dried under vacuum prior to reaction. The MCM-41 sample was heated gradually to 250 °C and held for at least a further eight hours at this temperature under vacuum to remove water that may have been adsorbed on the surface. HMDS vapour was then introduced into the reaction chamber and allowed to react with the MCM-41 for at least 16 h as the chamber cooled to room temperature.

Stability tests on coated and uncoated MCM-41 were conducted by contacting 350 mg of MCM-41

with 250 ml of solution, either distilled water or a pH 6 potassium phosphate buffer (50 mmol·L^{-1}). These tests were conducted at room temperature (25 °C) with slow stirring.

The proteins lysozyme and trypsin were obtained from Sigma. Batch adsorption tests were performed with lysozyme for contact times of 96 h on the coated and uncoated materials. In each experiment 50 mg of MCM-41 was mixed with 10 mL of a lysozyme solution in 50 mmol·L^{-1} potassium phosphate buffer at pH 6 at 20±1 °C. Kinetic experiments to determine the amount of lysozyme or trypsin adsorbed as a function of contact time were conducted by contacting 250 mL of 10 µmol·L^{-1} protein solution with 0.5 g of MCM-41 with stirring at 20 °C in a vessel covered to prevent evaporation. Samples were withdrawn periodically for immediate analysis and then returned to the mixture. Adsorbed amounts were determined by solution depletion analysis using UV-visible spectroscopy (Varian Cary 1E UV-Visible Spectrophotometer), after centrifugation to avoid potential interference in the spectra from suspended scattering particles.

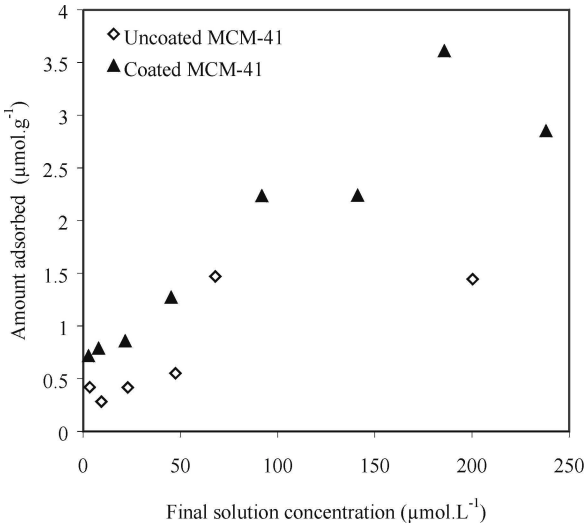

Fig. 1. Amount of lysozyme adsorbed onto MCM-41 with and without HMDS coating in 96 h from a pH 6 buffer solution.

3. RESULTS AND DISCUSSION

The XRD patterns of some samples of uncoated MCM-41 showed signs of structural degradation after immersion in water or buffer solution for as little as 24 h at 25 °C. After 12 days' immersion the XRD patterns of these samples had reduced intensity in the d_{100} peak and loss of higher angle peaks indicating significant loss of structural order. While some other samples retained the XRD patterns typical of MCM-41, all the samples tested showed significant changes in their nitrogen adsorption isotherms and hence decreases in BJH pore diameter and pore volume after 12 days' immersion in either water or buffer solution. The BET surface area of the MCM-41 was not significantly altered by immersion in water alone, but dropped by about 40% after 12 days in the buffer solution.

Reaction of MCM-41 with HMDS resulted in a decrease in the pore diameter and hence the surface area and pore volume of the samples, as expected if the mesopore surfaces were coated. In a typical sample the pore volume determined from nitrogen adsorption at a relative pressure of 0.99 dropped by 24% and the BET surface area dropped by 16%. The coated MCM-41 was much more stable upon immersion in water or buffer solution for up to 12 days. Its XRD pattern did not change significantly after either solution treatment and its nitrogen adsorption isotherm was less affected than that of the uncoated material. The total pore volume and BET surface area after treatment were within 10% of their original values for the coated material. It is likely that small changes in the properties of the coated material on exposure to water resulted from incomplete coverage of the sample surface area. Details of the properties and stability of the coated materials will be published elsewhere [33].

Lysozyme was adsorbed onto uncoated MCM-41, with increasing amounts adsorbed as the solution concentration increased, as expected (Fig. 1). The isoelectric point of lysozyme is 11.4 [34], making it is positively charged at pH 6, whereas the silica surface of MCM-41 has an isoelectric point of around 2.0 and so is negatively charged at this pH [31]. Thus there is an electrostatic attraction between lysozyme and the MCM-41 surface, favouring adsorption from solution. The spherical molecular diameter of lysozyme (3.2 nm based on its partial specific volume) [34] is similar to the BJH pore diameter of the MCM-41 samples used in this work (around 2.9 nm before coating). However, this model is known to underestimate the pore size of MCM-41 [35]. Additionally, lysozyme is ellipsoidal in shape, so it is possible that lysozyme may adsorb into the pores of MCM-41, although steric hindrance would make its uptake slow and limit the capacity of the MCM-41 to adsorb this molecule. The rate of adsorption of lysozyme was found to be greater than that of the larger protein trypsin, with a spherical molecular diameter of 3.8 nm [34], which was also adsorbed onto MCM-41 at pH 6 (Fig. 2). These data

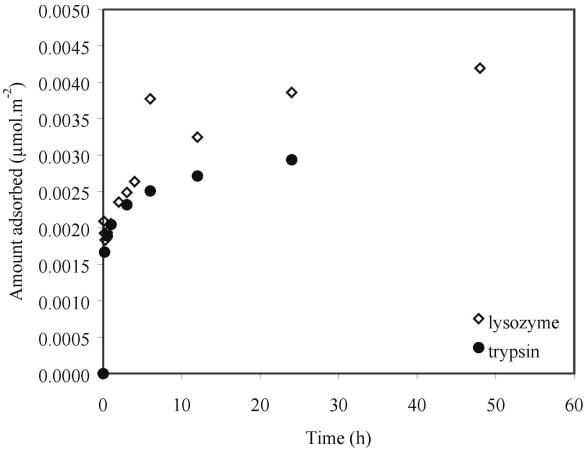

Fig. 2. Adsorption of lysozyme and trpysin onto MCM-41 as a function of contact time from a pH=6 buffer solution.

are normalised with respect to the BET surface areas of the MCM-41 samples used, to eliminate any differences in adsorbed amounts due to variations in their specific surface area. The isoelectric point of trypsin is 10.5, so it is also positively charged at pH 6 and thus expected to be electrostatically attracted to the silica surface. The difference in adsorbed amount may be due to greater ability of lysozyme to penetrate the mesopores.

HMDS-coated MCM-41 adsorbed significantly more lysozyme than the same MCM-41 sample without coating, as shown in Fig. 1. The amount adsorbed was about twice as much as on the uncoated sample, despite the coating causing a reduction in the material's pore size and surface area. Thus adsorption of lysozyme to the hydrophobic HMDS coating is favoured over the negatively charged silica surface of MCM-41 under the solution conditions tested in this work. The combination of increased protein binding capacity and improved stability upon extended contact with aqueous solutions makes HMDS-coated MCM-41 promising for application to separation of proteins by adsorption.

4. CONCLUSIONS

MCM-41 adsorbed more lysozyme, with dimensions close to its pore size, than the larger protein trypsin, supporting its potential for size selective separations. The application of a hydrophobic coating to the mesopore surfaces of MCM-41 using hexamethyldisilazane greatly improves its stability in aqueous solutions for periods of at least 12 days. The presence of the coating also enhances the adsorption capacity of MCM-41 for lysozyme, increasing its potential benefits for protein separation applications. Further work is underway to optimise the coverage of the surface coating and test its stability over longer exposure times to aqueous solutions.

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