Adsorption of Cytochrome C on New Mesoporous Carbon Molecular Sieves

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Carbon mesoporous molecular sieves have been prepared from SBA-15 materials synthesized at different temperatures. This allows the synthesis of hexagonally arranged mesoporous carbons with pore diameters between 3.0 nm (CMK-3–100) and 6.5 nm (CMK-3–150). The novel materials have been studied in the adsorption of horse heart cytochrome *c* from solutions with different pH. A maximal adsorption capacity of 18.5 μ mol/g was found for CMK-3–130 ($d_p = 4.5$ nm) at pH 9.6, which is near the isoelectric point of cytochrome *c* (pI = 9.8).

In recent years, porous carbon materials have been used for many applications such as catalysts, battery electrodes, capacitors, gas storage, biomedical engineering, and adsorbents.¹ For such applications, the carbon materials should possess a high mesoporosity allowing the adsorption of molecules and ions that are too large to enter micropores. Ordered mesoporous carbons prepared from mesoporous silica templates possess large specific surface areas, large specific pore volumes, and electrical conductivity and are of general interest owing to their potential applications, especially as adsorbents for relative bulky molecules.^{2–5} Synthesis of large pore mesoporous carbons is extremely important for the envisaged applications in the separation and adsorption of bulky molecules such as proteins and enzymes.

Adsorption of cytochrome c (cyt c) over porous glass and sol-gels has been extensively studied due to potential applications in the fields of bio-sensing and enzymatic catalysis.^{6,7} However, porous glass materials are expensive and sol-gel materials have a broad pore size distribution (PSD), and, hence, cannot be used to selectively adsorb proteins with specific molecular size. Some studies are dealing with the adsorption of cyt c over mesoporous silica molecular sieves such as MCM-41, MCM-48, and SBA-15.8-10 However, for these materials strong electrostatic interactions are observed between the surface silanol groups and the surface charge on the amino acid residues on the surface of cyt c,⁸ which affect the structure and orientation of the active (heme) site of cvt c. Recently, we have reported the synthesis of SBA-15 materials with different pore diameters prepared by changing the synthesis temperature.¹¹ We used these materials as templates for the preparation of mesoporous carbons with large pore diameter. Here we report the adsorption of cyt c on (hydrophobic) mesoporous carbon molecular sieves.

Three different SBA-15 materials with pore diameters in the range of 9 to 12 nm were prepared according to the method reported by Hartmann and Vinu,¹¹ and designated as SBA-15–X, where X indicates the synthesis temperature (X = 100, 130, and 150 °C). Mesoporous carbons with different pore diameters were prepared by using the SBA-15–X materials as templates

and sucrose as the carbon source. The obtained materials were designated CMK-3-100,² CMK-3-130, and CMK-3-150, where the number indicates the synthesis temperature of the SBA-15 template. In a typical synthesis of mesoporous carbon, 1 g of template was added to a solution obtained by dissolving 1.25 g of sucrose and 0.14 g of H₂SO₄ in 5 g of water, and keeping the mixture in an oven for 6 h at 373 K. Subsequently, the oven temperature was raised to 433 K for another 6 h. To obtain fully polymerized and carbonized sucrose inside the pores of the silica template, 0.8 g of sucrose, 0.09 g of H₂SO₄, and 5 g of water were again added to the pretreated sample and the mixture was subjected to the thermal treatment described above. The template-polymer composites were then pyrolyzed in a nitrogen flow at a heating rate of 5 K min⁻¹ up to 1150 K and kept under these conditions for 6 h to carbonize the polymer. The mesoporous carbon was recovered by filtration after dissolution of the silica framework in 5 wt % hydrofluoric acid, washed several times with ethanol and dried at 393 K.

For cyt *c* adsorption, a series of standard cyt *c* solutions with concentrations ranging from 0.25 g/L to 4.0 g/L were prepared by dissolving cyt *c* in different 25 mM buffer solutions (pH = 6.5 potassium phosphate buffer and pH = 9.6 and 10.5 sodium bicarbonate buffer). Horse heart cyt *c* (90% pure) was obtained from Acros and used without further purification. In each adsorption experiment, 20 mg of the mesoporous adsorbent were suspended in 4 mL of cyt *c* solution. The resulting mixture was continuously shaken at 293 K for 96 h to reach the adsorption equilibrium. The amount of cyt *c* adsorbed was determined by UV absorption at 409 nm.

Figure 1 shows the powder X-ray diffraction patterns (XRD) of CMK-3–100, CMK-3–130, and CMK-3–150. These materials are hexagonally ordered mesostructures, as is evident from the presence of at least three XRD lines that can be indexed as (100), (110), and (200) reflections. Consequently, the obtained materials are a replica of the parent material SBA-15.^{2,6} The low intensity of the higher order reflections in the case of CMK-3–150 indicates that some structural changes occurred during silica template removal. This reduced stability is probably a consequence of the changes in the number and size of the carbon rods (formed from the micropores of SBA-15) that connect the carbon rods formed from the main pores of SBA-15. The

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Figure 1. XRD powder patterns of the mesoporous carbon materials CMK-3–150, CMK-3–130, and CMK-3–100.



Figure 2. Nitrogen adsorption isotherms at 77 K (a) and BJH pore size distributions (b) of mesoporous carbon materials (closed symbols: adsorption; open symbols: desorption). The isotherms are shifted by 300 cm³/g for clarity.

number of micropores is drastically reduced in the case of SBA-15-150, which was used as a template for CMK-3-150.^{11,12}

Figure 2a shows the nitrogen adsorption isotherms at 77 K of CMK-3–130 and CMK-3–150 along with the BJH–PSD (Figure 2b) in comparison to CMK-3–100. All isotherms are of type IV, according to the IUPAC classification, and exhibit a H1 hysteresis loop. The materials obtained from SBA-15 prepared at 130 and 150 °C possess pores with diameters of about 4.3 and 6.5 nm, high BET surface areas of 1250 and 1350 m²/g, and large pore volumes of 1.3 and 1.6 cm³/g, respectively (Table 1). Pore diameter and specific pore volume are significantly larger than those of CMK-3–100 ($d_p = 3.0$ nm, $A_{BET} = 1260 \text{ m}^2/\text{g}$, and $V_p = 1.1 \text{ cm}^3/\text{g}$) and facilitate the adsorption of cyt *c* (size: 2.6 × 3.2 × 3.3 nm).

Adsorption isotherms of cyt *c* adsorbed onto CMK-3-130 with different solution pH ranging from 6.5 to 10.5 are shown in Figure 3. As cyt *c* is denatured at pH above 11, we precluded the adsorption study outside the pH range 3-11. Each isotherm

 TABLE 1: Textural Properties of the Mesoporous Carbon

 Supports and the SBA-15 Parent Materials

sample	unit cell size a ₀ (nm)	specific surface area (m ² /g)	specific pore volume ^{<i>a</i>} (cm ³ /g)	pore diameter $d_{p,BJH}^{b}$ (nm)	pore diameter $d_{p,NLDFT}^{c}$ (nm)
SBA-15-100	10.22	1100	1.60	9.0	
SBA-15-130	10.54	546	1.19	8.7	
SBA-15-150	10.83	393	1.10	11.2	
CMK-3-100	10.07	1260	1.1 (0.41)	3.0	3.3
CMK-3-130	10.45	1250	1.3 (0.46)	4.3	4.3
CMK-3-150	10.75	1350	1.6 (0.49)	6.5	5.4

^{*a*} Total pore volume calculated at $p/p_0 = 0.88$. Values in parentheses are micropore volumes ($d_p < 1.4$ nm). ^{*b*} Calculated from the adsorption branch of the isotherm. ^{*c*} Pore sizes obtained from the N₂ isotherm following NLDFT (nonlocal density functional theory) analysis.¹³



Figure 3. Adsorption of cyt c on CMK-3-130 at various solution pH. The solid lines are fits using the Langmuir isotherm.

shows a sharp initial rise (type L isotherm), suggesting a high affinity between cyt c and the adsorbent surface. Finally, the saturation level is reached at concentrations above 2 g/L. The adsorption of cyt c is typically determined by electrostatic and hydrophobic interactions (a kind of van der Waals attraction) and the cohesive attraction and repulsion of the amino acids present in the cyt c molecule. The adsorption maximum is also influenced by the expansion and contraction of the molecular diameter of the cyt c molecule at different pH. It is evident from Figure 3 that the amount of adsorption is significantly affected by the pH of the cyt c solution and increases from pH 6.5 to 9.6 and decreases at higher pH (10.5). The amount of cyt c adsorbed is maximal (18.5 μ mol/g; determined using the monolayer capacity of the Langmuir fit) at pH 9.6, which is near the isoelectric point (pI) of cyt c (pI = 9.8). Near the isoelectric point, the net charge of cyt c is around zero. Thus the hydrophobic interactions between adsorbate and adsorbent (carbon rods are hydrophobic¹⁴) are maximal. These hydrophobic interactions may either originate from attraction of the nonpolar side chains of the amino acids residues on the surface of the cyt c molecule by carbon species (chains, aromatic rings, etc.) at the adsorbent surface or from cyt c-cyt c interaction between the hydrophobic side chains of neighboring cyt cmolecules adsorbed on the surface of the mesoporous carbon. As there is no repulsion between the amino acid residues on the surface of adsorbed cyt c molecules near the pI, the cyt cmolecules are more densely packed on the adsorbent surface. As a result, the amount of adsorption increases near the pI of cyt c. The adsorption of cyt c is reduced below and above the isoelectric point. This behavior is ascribed to the expansion of the effective molecular diameter of cyt c since strong repulsive forces between the amino acid residues of the molecule are

present below and above the pI and more space is needed for the expanded cyt c molecule.

Among all the mesoporous carbon materials studied, CMK-3-130 shows maximum adsorption of cvt c (18.5 μ mol/g) whereas CMK-3-100 and CMK-3-150 exhibit adsorption capacities of 10.9 and 14.5 μ mol/g, respectively. The low cyt c adsorption observed for CMK-3-100 is probably due to its smaller pore diameter and lower pore volume. CMK-3-100 possesses a broad pore size distribution (Figure 2b) centered at 3 nm (BJH) or 3.3 nm (NLDFT) (Table 1), showing that CMK-3-100 has pores which are a few angstroms smaller or bigger than the size of the cyt c molecule (ca. $2.6 \times 3.2 \times 3.3$ nm). Pores which are a few angstroms smaller than the cyt *c* molecule might not be accessible (molecular sieving). Thus, CMK-3-100 shows a lower adsorption capacity as compared to CMK-3-130 and CMK-3-150. Moreover, the adsorption capacity of CMK-3-150 is lower as compared to CMK-3-130, which is probably a consequence of its slightly disordered structure (see XRD) and/or the presence of micropores. Assuming a cyt c volume of 14.3 nm,^{3,9} the extent of pore filling with cyt c can be calculated provided that the accessible volume is the difference between the total pore volume and the micropore volume (Table 1). The cyt c molecules thus occupy only 13, 19, and 11% of the accessible pore volume in CMK-3-100, CMK-3-130, and CMK-3-150, respectively. The reasons for the low pore filling in particular for CMK-3-150 are at present not clear and are subject to further studies.

Our results indicate that cyt *c* can be adsorbed in the pores of mesoporous carbon molecular sieves and that the adsorption capacity can be controlled by using different mesoporous carbon molecular sieves. The adsorption of cytochrome *c* on mesoporous carbons is higher compared to mesoporous silica, where the adsorption capacity amounts to ca. 1.7 μ mol/g for MCM-41 and 6.8 μ mol/g for SBA-15.⁹

Using X-band ESR spectroscopy,¹⁵ cyt *c* adsorbed on CMK-3–130 was observed to occur both in high-spin and low-spin states of Fe(III) in contrast to solution, where the Fe(III) is predominately in the low-spin state. Similar observations have been made for cyt *c* adsorbed on mesoporous silica, viz., MCM-41, where also significantly higher levels of high-spin Fe(III) have been observed using resonance Raman spectroscopy.⁹ In this study it was concluded that the higher level of high-spin Fe(III) is responsible for the observed increased peroxidative activity of cyt *c*. Studies on the peroxidative activity of cyt *c* adsorbed on the different CMK-3 materials are currently underway.

In summary, mesoporous carbons with tunable pore diameters from 3 to 6.5 nm can be prepared by using SBA-15 silica with various pore diameters as template. These materials allow the adsorption and separation of bulky molecules such as proteins and vitamins. A high adsorption capacity of 18.5 μ mol/g has been observed for CMK-3–130 which is significantly higher than that reported for mesoporous silicas such as MCM-41 and SBA-15.

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