NANOSTRUCTURED CARBONS OBTAINED BY TEMPLATE METHOD FOR SELECTIVE ADSORPTION OF BIOMOLECULES

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Introduction

Adsorption of proteins on solid adsorbents holds significance in medicine, biology, biotechnology and food processing [1]. Activated carbons are used as oral adsorbents or as hemosorbents to treat cases of poisoning. The discovery of ordered porous carbons has attracted considerable interest due to well-ordered porous structure with adjustable pore size in nanoscale range, high surface area and large pore volume.

Experimental

Nanostructured carbons were obtained by templating technique [2] using sucrose or furfuryl alcohol as carbon source and cheap commercial inorganic templates (zeolite NaY, silica gels SG60, SGAO, ZK or colloidal silica Ludox AS40) as structure directing material. After carbonization inorganic framework was dissolved in 40% HF.

Carbons were characterized by nitrogen adsorption isotherms measured at 77 K using Autosorb-6 gas adsorption analyzer (Quantachrome, USA). Pore size distribution was calculated using NLDFT equilibrium model with slit/cylinder pore shape.

Adsorption of two proteins, albumin from egg white and fibrinogen (Sigma), on nanostructured carbons was investigated. Weighed amount of carbon was added to protein solution in phosphate buffered saline with pH=7.4 and shacked at room temperature for 3 hours. After adsorption the solution was centrifuged at 12000 rpm for 10 min and supernatants were collected for measuring concentration of remaining protein. Protein concentration was measured spectrophotometrically at 280 nm.

Results and Discussion

Nanostructured carbons show well developed porous structure with relatively uniform pores (Fig. 1). Depending on inorganic template average pore size may be from 1.0 to 11.3 nm (Fig 1, Table 1). The lowest pore size 1.0 nm was obtained for carbon templated by zeolite NaY. The largest pore size 11 nm was obtained using colloidal silica Ludox AS40. When commercial silica gels were used as structure directing materials nanostructured carbons with intermediate pore size in the range 6-9 nm were obtained. All nanostructured carbons have high surface area (1200-1900 m^2/g) and large pore volume (2.1-4.1 cm^3/g). Nanostructured carbons retained the shape of inorganic template used for synthesis. Carbons templated by zeolite NaY and colloidal silica Ludox AS40 have very fine particles. Carbons obtained using silica gels possess much larger granules in millimeter range (Table 1). Having a series of carbons with varied pore size it was expected to reveal the impact of porous structure on adsorption of large molecules.

Table 1. BET Surface Area, Pore Volume, Pore Size and Size of Granule on Nanostructured Carbons

Carbon	BET surface area [m^2/g]	Pore volume [cm^3/g]	Pore width [nm]	Granule size [μm]
C(NaY)	1885	1.34	1.0	0.4-0.6
C(SG60)	1374	1.88	5.9	50-300
C(SGAO)	1232	2.07	6.6	80-200
C(ZK)	1186	2.50	8.8	700-1200
C(AS40)	1345	4.09	11.3	4-6

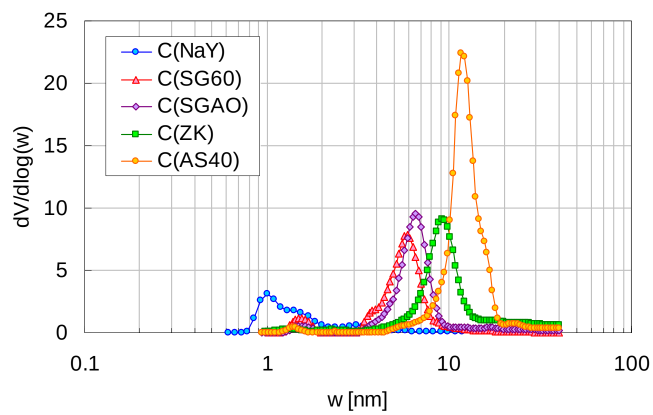

Fig. 1 Pore size distributions for nanostructured carbons.

Ovalbumin is the main protein found in egg white, its molecular mass 45 kDa and isoelectric point 4.6 [3]. The molecule is ellipsoidal in shape with 4 x 6.8 nm in size. Fibrinogen is the protein of the blood plasma, its molecular mass 340 kDa and isoelectric point 5.6-5.8. The molecule is oblong ellipsoid 23 x 4.7 nm in size. Testing adsorption of so large molecules it was expected molecular sieve effect – no adsorption on carbons with pore size less then dimension of adsorbed molecule and high adsorption on carbons with pores that match the size of the adsorbate molecule. Molecular sieve effect was observed for adsorption of cytochrome c using a series of CMK-3 carbons with different pore size. The largest adsorption was observed for carbon with 4.3 nm, while carbons with lower and larger pore sizes adsorbed less amount of cytochrome c [4]. Increasing adsorption of lysozyme on

CMK-3 carbons [5] and mesoporous silica KIT-6 [6] was observed. The same tendency was reported for myoglobin adsorption on mesoporous silica molecular sieves MCM-41 and SBA-15 [7].

However, experimental observation (Fig. 2) shows different trend for both investigated proteins. The highest adsorption was observed for carbon C(NaY) with lowest pore size of 1 mn. The protein adsorption decreases and as pore size of carbon increased up to 6.6 nm with increasing uptake on further increasing pore size. The trend holds over an order of magnitude of protein concentration (Fig. 3) indicating its stability. The unexpected dependence of protein adsorption on pore size of carbon suggests another factor is responsible for protein uptake. Factors related to porous structure can not explain observed trend in protein adsorption. Indeed, BET surface area decreases with increasing pore size as pore size increases with slight increase for carbon C(AS40) having the largest pore size. Nevertheless, the variation of BET surface area is much less than that of protein adsorption. Pore volume constantly increases with increasing pore size and thus can not explain observed trend in protein adsorption.

Another factor that may explain the observed tendency in protein adsorption is deformation of the protein molecule near rigid surface [8]. However, conformational changes may explain increasing adsorption on carbons with small pore size but can not explain decreasing protein uptake with increasing pore size.

Observed experimental trend may be attributed to kinetics of protein adsorption. 3 hours adsorption experiment was used in present research based on modeling contact time in real systems such as oral administration or hemoperfusion on carbon adsorbent. Obviously this time is not enough to reach equilibrium. Consequently the highest adsorption was observed on carbons C(NaY) and C(AS40) having the lowest particle size (Table 1).

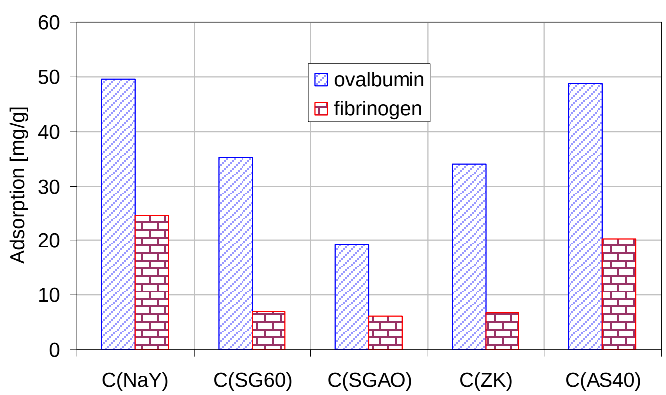

Fig. 2 Adsorption of ovalbumin and fibrinogen on nanostructured carbons.

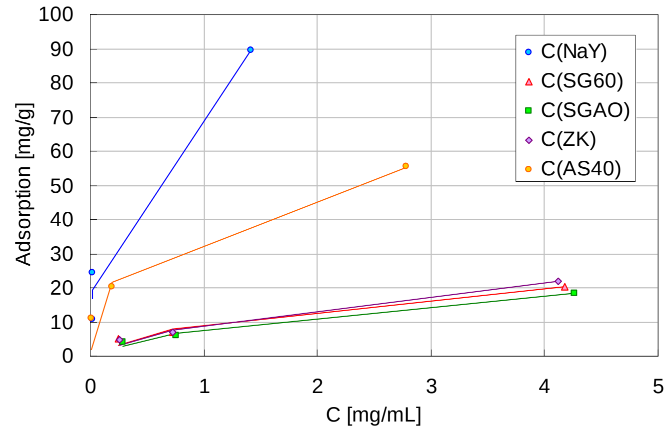

Fig. 3 Adsorption isotherms of fibrinogen (Fg) on nanostructured carbons. Points – experimental data, lines – Langmuir equation fit.

Conclusions

For short contact time the highest protein adsorption is observed on carbons with very fine particles.

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