EFFECTS OF SURFACE MODIFICATION OF ACTIVATED CARBON ON ADSORPTION AND DESORPTION OF DOXORUBICIN

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Introduction

It has been known for a long time that activated carbons can be used to completely or partially reverse drug-induced toxicities in humans by drug adsorption (1-3). The adsorption and desorption characteristics of activated carbons are known for many molecules including peptides, proteins and drugs (2-4). Activated carbon as a carrier for anticancer drugs has been reported to improve the chemotherapy of several types of human malignancies. Novel dosage forms based on activated carbons with sustained drug release profile for such anticancer drugs as mitomycin-C, 5-fluorouracil, aclarubicin, doxorubicin, and pepleomycin have been developed (5-12). In a limited clinical trial in gastric cancer, an activated carbon-Mitomycin-c complex increased the number of one-year survivors four-fold (5).

Recently, Rudge *et al* reported a novel site-specific drug delivery technology based on magnetically targeted carriers (MTCs) (13). MTCs are iron/carbon microparticles of approximately one micron in diameter, formed by a high energy milling technique. Metallic iron comprises approximately 75% of the microparticle mass and results in a carrier with high magnetic susceptibility which can efficiently deliver drugs to the target site (14). The activated carbon component of the MTCs adsorbs and transports the therapeutically relevant bioactive molecules. MTCs have undergone toxicity testing in swine and canines, and are currently in Phase I/II clinical trials in the U.S (15).

A variety of commercially available activated carbons have been investigated for adsorption of different organic molecules. However, the complex physicochemical interactions taking place between an adsorbed molecule and the carbon surface are not well understood. Although various carbons have been tested for drug binding, not much work has been done to correlate the surface characteristics of activated carbons to the drug adsorption and, especially, desorption profile. In principle, the surface area, pore structure and surface chemistry of activated carbons determine the adsorption and desorption characteristics of various drug molecules. Optimization of those characteristics of activated carbons might improve their performance for drug adsorption and desorption. Recently, Radovic et al have demonstrated the role of carbon surface chemistry in optimizing the performance of carbon-supported catalysts (16) and maximizing the uptakes of organics from aqueous systems (17). It has also been shown that chemical modifications can improve the adsorption capabilities of activated carbons for various molecules (18-23).

In this abstract, we report on liquid phase oxidative treatments of activated carbon. Various oxidants and reaction conditions were investigated. The structural characteristics of carbons were determined using sorption of nitrogen. The functional groups on different modified activated carbon were characterized with titration and Infrared analysis. Characterization of the adsorption and desorption of doxorubicin with oxidized activated carbons was performed.

Experimental

Activated carbons were obtained from Norit Americas (Norcros, GA) and Scientific Carbons. Sulfuric acid, Nitric acid, and Ammonium persulfate were purchased from Aldrich. Hydrogen peroxide (29-32%, ACS reagent grade) was obtained from Rocky Mountain Reagent, Inc. and MilliQ^{UF} water was generated in house.

Oxidation of Carbons

Activated carbons were oxidized with nitric acid, ammonium persulfate, and hydrogen peroxide/H_2SO_4 following a procedure described by Salame and Bandosz (24). Activated carbons were also oxidized with hydrogen peroxide alone. Twenty grams of activated carbon was placed into a flask, followed by adding 100 ml of water to wet the surface. The mixture was stirred while vacuum was applied. After degassing for 30 min, the temperature was raised to 50 ^{o}C. Then 200 ml of H_2O_2 was added to the flask. The reaction was continued for another 30 min, 2 h, 5 h and 22 h.

After reaction was stopped, the carbon was filtered and washed with MilliQ^{UF} water, and extracted with acetone overnight using a Soxhlet extractor. The product was dried under vacuum at 100 ^{o}C for 48 h.

Sorption of Nitrogen

Surface area and porosity characteristics were determined using physical adsorption of N_2 with an Autosorb SA-1 (Quantachrome Corp) and an SA3100 (Beckmenn Coulter). Before each experiment, the samples were outgassed at 200 ^{o}C for 16 h. The isotherms were used to calculate the surface area, pore volume and pore size distribution (24).

FTIR Characterization

IR spectra were collected using a Nicolet Impact 410 FT-IR spectrometer equipped with a diffuse reflectance unit. The instrument's resolution was set at 4 cm^{-1}. Carbon powder was pressed to a pellet with KBr. Before each measurement, the instrument was run to collect the background, which was then automatically subtracted from the sample spectrum.

Surface Functional Group Titration

The Boehm titration method was used to determine the number of oxygenated surface groups (23-24). One gram of carbon sample was placed in 50 ml of the following 0.05 N solutions: hydrochloric acid, sodium hydroxide, sodium carbonate, and sodium bicarbonate. The vials were sealed and stirred for 24 h and filtered. Then 5 ml of each filtrate was titrated with HCl and NaOH depending on the original titrant. The number of acidic sites of various types was calculated using the assumption that NaOH neutralizes carboxylic, phenolic, and lactonic; Na_2CO_3 neutralizes carboxylic and lactonic groups; and $NaHCO_3$ neutralizes only carboxylic groups. The number of basic sites was calculated from the amount of HCl that reacted with the carbon.

Adsorption and desorption of doxorubicin onto/from activated carbons

Activated carbons were characterized by their ability to adsorb and desorb doxorubicin. Adsorption was measured by incubating activated carbons with varying concentrations of doxorubicin, from 0.83 mg/mL to 1.33 mg/mL. Doxorubicin (Fujisawa) was used out of its commercial formulation, and was diluted with 0.9% saline in water, pH 3.0. The incubation takes 30 minutes to reach equilibrium at 25°C. Test samples are rotated during the incubation. The amount of doxorubicin not adsorbed was determined by UV spectrometer. The absorbance of the supernatants was measured at 480 nm after centrifugation of the sample. The amount of doxorubicin adsorbed onto activated carbons is determined by mass balance.

Desorption was measured in human plasma. Activated carbons were mixed with doxorubicin at 8% by weight, centrifuged, and the supernatants discarded. Activated carbons were then combined with human plasma at a ratio of 50 mg particles per 10 mL plasma, and incubated for various times at 37 °C. At the specified time, a test sample was centrifuged and a supernatant aliquot was combined with a 2.5% HCl, 50% isopropanol solution to precipitate the plasma proteins. The cleared supernatant was assayed by fluorometry (excitation 490 nm, detection 570 nm). After establishing the equilibrium time for this solid liquid ratio, particles were extracted with 10 mL aliquots of plasma until the amount of doxorubicin found in the supernatant at each extraction reached a constant.

Results and Discussion

Seven activated carbons with different surface properties were studies for doxorubicin binding. The surface acidic functional groups were determined by Boehm titration. Table 1 shows the structural parameters, surface acidic groups, and doxorubicin binding capacities of various activated carbons. Norit Darco KB is chemically activated, while other carbons are steam activated. KB carbon has a high pore volume, indicative of an "open" pore structure. Norit A Supra also has a high pore volume with about 25% of the pore in the micropore range, while the other carbons have a small volume with more 50% of the pores in the micropore range and thus a much more closed pore structure.

The oxidation changed the characteristics of carbon surface by the introduction of oxygen groups and removal of some carbon atoms from the matrix (23, 26). The type and quantity of the oxygen groups were determined using the Boehm titration method. The initial KB carbon has a variety of species on the surface due to its origin and the chemical activation method. As expected, nitric acid oxidation introduced a significant number of oxygen containing groups in almost each category classified by Boehm. Hydrogen peroxide oxidations introduced similar functional groups as nitric acid oxidation. The combination of the hydrogen peroxide and sulfuric acid generated more acidic groups on the surface than hydrogen peroxide alone. The results also showed that the acidic groups on the surface increased with the reaction time. Ammonium persulfate treatment increased the number of carboxylic groups, but also created many phenols and no lactonic groups. As the results of oxidation, the total number of surface

chemical functional groups increased up to more than 6-fold.

The FTIR results obtained for the oxidized activated carbons are consistent with Boehm titration analysis (data not shown). The spectra of oxidized sample show absorption bands at 1710 cm^{-1} due to C=O stretching vibrations together with the C-O vibration at 1275 cm^{-1} and C-O vibration at 1604 cm^{-1} which are characteristics of carboxylic groups (27-28). The intensity of the peaks increases with increasing oxidation strength. In the case of nitric acid oxidized carbon, the spectrum shows two additional peaks, one at 1547 cm^{-1} and another at 1321 cm^{-1}. These peaks are characteristic of nitro groups, which is consistent with the oxidation method used.

Langmuir isotherms can be used to describe the adsorption of doxorubicin onto both modified and unmodified carbons (Figure 1). The Langmuir binding curve suggests monolayer coverage of doxorubicin to the carbon surface. The modification of KB carbon with ammonium persulfate reduced the binding capacity of doxorubicin (Table 2). Theoretically, the introduction of acidic groups on carbon surface should increase the binding affinity of the positively charged molecule, doxorubicin. However, the modification process reduced the pore volume and surface area. This may partially account for the decrease in doxorubicin binding. It seemed that the modification with ammonium persulfate did not increase the overall binding affinity of doxorubicin under the condition tested. When hydrogen peroxide was used to modify the carbon, the harsher the oxidation condition, the smaller the resulting surface area, and the lower the doxorubicin binding. When the reaction time was stopped at 30 min, the hydrogen peroxide modification increased the doxorubicin binding by 14%.

The doxorubicin initially adsorbed to the particles may be desorbed in human plasma, as shown in Figure 2. Both KB carbon and hydrogen peroxide modified carbon first adsorbed the capacity of doxorubicin binding, i.e., 398 and 445 μg/mg respectively. The rate of doxorubicin desorption from both carbons was very similar. Within the first two and half hours, both release curves are linear.

Conclusion

The surfaces of KB and A carbons were oxidized with various oxidation reagents. A significant amount of acidic groups were introduced on the carbon surfaces. During this process, the porous structure of the carbons was altered. The pore volume and surface area were dramatically decreased. Although many acidic functional groups were introduced with harsher oxidizing conditions, a significant decrease in binding of the drugs was observed. However, when the modification did not reduce the surface area but increased the concentration of surface functional groups, an increase in binding and release of doxorubicin was observed. This indicates that the surface modification of activated carbon could significantly enhance the strength of adsorption on an adsorbate of opposite surface polarity if the original porous structure could be maintained. Ongoing research continues to optimize the oxidation conditions and to characterize the modified activated carbons.

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Table 1. The structural parameters and doxorubicin binding capacities of various carbons

Carbon	Base Material	Surface Area (m^2/g)	Pore Volume (cc/g)	Micropore Volume (cc/g)	Acidic Groups (mmol/g)	Dox binding (μg/mg)
Norit Darco KB	Oak Wood	1437	1.582	0.1143	0.484	400
Norit A Supra	Coconut	1861.3	1.058	0.2526	0.182	345
Norit E Supra	Peat	1042.4	0.678	0.2659	0.115	310
Norit B USP	Coconut/Peat	1451.7	1.102	0.2455	ND*	333
SXRO	Peat	1209.9	0.5348	0.3710	ND	206
PWC162	Wood	1034.7	0.5275	0.2663	ND	260

* ND: not determined

Table 2. The maximum amount of doxorubicin adsorbed onto modified KB carbons

Sample	Surface Area (m^2/g)	Volume (ml/g)	Acidic groups (mmol/g)	C_{total} (μg Dox/mg AC)
A	1437	1.595	0.56	400
B	1462	1.585	1.02	455
C	1197	1.238	2.53	357
D	1089	1.047	2.45	323

Note: A – unoxidized carbon; B – H_2O_2 oxidized for 30 min at 50 ^oC; C - H_2O_2/H_2SO_4 oxidized for 60 min at 50 ^oC; D – $(NH_4)S_2O_8$ oxidized for 18 h at room temperature.

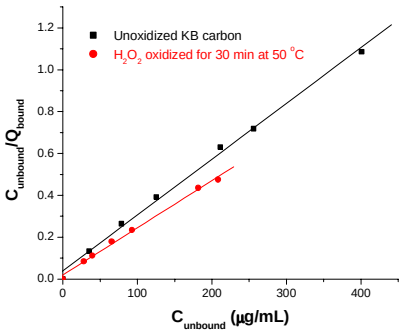

Figure 1. Langmuir isotherms of doxorubicin binding onto activated carbons

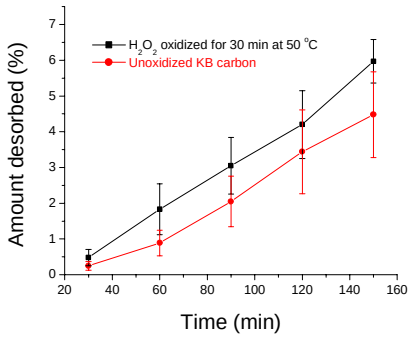

Figure 2. Desorption of the adsorbed doxorubicin from initial acivated carbons