# BIOSELECTIVE MEDICAL ACTIVATED CARBONS – ARE THEY REAL?

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## Introduction

The most fundamental distinction of extracorporeal methods such as adsorption, filtration and dialysis from drug therapy is that the former do not introduce any drugs into the human organism. This is particularly important for chronic patients who need regular treatment. Adsorption methods are most advantageous because they are accompanied with only minor losses of blood or plasma and do not require high volumes of expensive replacement fluids. Another important advantage of adsorption over filtration or dialysis is the potential ability of adsorbents to remove large molecules such as proteins, lipoproteins and circulating immune complexes. The use of adsorbent-based technologies has been limited by poor bio- (haemo)compatibility in the past. However, in 1990s, selective adsorption treatment of patients with autoimmune diseases has proved to be a successful and efficient alternative to conventional methods. Commercial bioselective or immunosorbent (IS) columns for medical use are currently manufactured in the USA, Germany and Japan. Recently FDA approved this treatment for patients with moderate and severe rheumatoid arthritis who are resistant to any drug therapy (ProSorba® column, Cypress and Fresenius). The use of LDL-apheresis methods such as dextrane sulphate adsorption has also been widely reported for the treatment of familial hypercholesterolaemia in Germany. These adsorbents not only selectively remove low-density lipoproteins (LDL) from blood plasma but also reduce levels of plasma fibrinogen [1-3].

Presently bioselective adsorbents are made by covalent attachment of a bioligand to a solid support used in analytical and preparative chromatography. Most carriers used in apheresis are hydrogel-like materials such as agarose, dextran or cellulose derivatives [2,3]. Such carriers have not been engineered for medical use and as such are soft, compressible and have small pore sizes making most of the surface inaccessible to large protein molecules.

A stereotypical image of activated carbons is that they are non-selective adsorbents. In medicine they are successfully used for treatment of poisoning, particularly of unknown aetiology. Selectivity of their action can be created using one or a combination of the following approaches: (i) regulation of pore size distribution; (ii) modification of surface chemical functional groups; (iii) attachment of a bioligand with a specific affinity towards a target biomolecule; (iv) catalytic activity of carbons.

The most selective adsorbents can be made using the affinity chromatography approach by covalent attachment of a bioligand (BL) to the carbon surface. Mesoporous carbons are the matrice of choice as they can accomodate large amounts of BL with high molecular mass such as proteins. Contrary to a common view that carbons are chemically inert materials, various surface functional groups can be created using polymer-analogous transformations. A bioligand is covalently attached to the carbon surface via a suitable coupling agent, such as water-soluble carbodiimide (CDI). Here we report the results of our attempts to design carbon based bioselective adsorbents for medical applications.

## Experimental

Activated and carbonised carbon SCN was produced by pyrolysis of cross-linked styrene-vinyl-pyridine copolymer. Oxidised SCN was prepared by carbon treatment with dilute nitric acid. Carbon felt was obtained by pyrolysis of cellulose. Covalent immobilisation of proteins on carbon surface was carried out using water-soluble CDI as a coupling agent on the assumption that surface carboxylic groups have properties similar to those in organic acids. In the experiment, 100 mg of carbon was shaken with 0.1 M solution of CDI to activate carboxylic groups. Protein immobilisation was carried out from 0.2% solution. Alternatively, BL was immobilised by physical adsorption. After immobilisation, adsorbents were thoroughly washed with aqueous buffer solutions of different pH and distilled water. Several biospecific carbon sorbents were synthesised: (i) with immobilised protein - antigen (Ag) to remove specific Ab; (ii) with immobilised anti-LDL antibodies to remove LDL; (iii) with immobilised low molecular mass BL (adenosine mono-, di-, or tri-phosphate, AMP, ADP or ATP) to remove heat shock protein; (iv) with immobilised cells - hepatocytes to remove bilirubin. Ligands (i) to (iii) were immobilised on the granulated carbon SCN, whereas hepatocytes were immobilised on carbon felt. Adsorption measurements with sorbents (i) and (ii) were carried out in batch, and adsorption of the heat shock protein HSP-70 was studied in a column experiment. After loading sorbent (iii) with a solution of HSP-70, the unbound and weakly adsorbed heat protein was washed out using phosphate buffer solution, pH 7.2, containing 0.1% of Twin X-100. The strongly bound HSP-70 was desorbed from the sorbent column using 20

mM solution of ATP – a bioligand with high affinity towards the heat shock proteins. Cells were cultivated on carbon felt from hepatocyte suspension, so that initially $(7.0\pm0.5)\cdot10^4$ hepatocytes per 1 mg of matrice were immobilised. Preliminary experiments showed that sufficient attachment of hepatocytes to the matrice could be achieved if the latter was soaked in 0.02% collagen solution. Carbon felt with immobilised hepatocytes was implanted in rats with induced mechanical jaundice and blood level of total bilirubin was monitored.

## Results and discussion

Results presented in Table 1 show that covalent immobilisation is more efficient than physical adsorption in terms of the amount of bioligand fixed on the surface as well as by the capacity of the adsorbent towards the target solute (anti-BL antibodies, Ab). Although the carbonised SCN has lower surface area than the activated SCN (600 $m^2/g$ vs. 1,600 $m^2/g$) it was found that in the course of activation mainly micropores inaccessible to large protein molecules are developed. Micropores are responsible for non-specific adsorption. The carbonised material is therefore better than the activated carbon as a matrice for IS.

**Table 1**. Protein binding by carbonised SCN.

| Bioligand (antigen) | Bound BL, mg/g sorbent | | Adsorbed anti-BL Ab, mg/g sorbent | |
|---|---|---|---|---|
| | CDI | Physical adsorption | CDI | Physical adsorption |
| IgG (rabbit), 150 kD | 0.84 | 0.010 | 1.3 | ND* |
| Cytochrome C (14 kD) | 1.6 | 0.020 | 1.8 | ND* |
| Protein S-100 | 0.70 | 0.010 | 0.75 | ND* |

ND* - not detectable.

Carbon based immunosorbent with immobilised Ab against LDL adsorbs cholesterol less efficiently than the commercial Sepharose based IS, it has similar capacity towards LDL (Table 2).

**Table 2**. Sorption of cholesterol and LDL from the blood plasma by IS with immobilised anti-LDL Ab.

| Matrice | % Cholesterol adsorbed | % LDL adsorbed |
|---|---|---|
| Sepharose | 98 | 38 |
| SCN (activated) | 34 | 39 |

Selectivity of carbon based IS with immobilised low molecular mass ligand was tested in the system "IS – heat shock protein HSP-70". As in the case of high molecular mass ligand (Table 1), IS with covalently attached BL demonstrated higher adsorption capacity and selectivity towards HSP-70 (Table 3). Activated carbon is used in bioartificial liver as a non-specific adsorbent. Hepatocytes suspended in solution or immobilised on a solid matrice, are used in such a system to replace the liver function, most notably, to metabolise bilirubin. We evaluated the ability of hepatocytes immobilised on carbon felt to metabolise bilirubin (Table 4).

**Table 3**. Sorption of HSP-70 by activated SCN with immobilised low molecular mass bioligand.

| Bioligand | HSP-70 bound, % | |
|---|---|---|
| | CDI | Physical adsorption |
| None | ND* | ND* |
| AMP | 27 | 1.0 |
| ADP | 11 | 0.2 |
| ATP | 22 | 3.5 |

**Table 4**. Some parameters of liver function in rats with mechanical jaundice 18 days after implantation of carbon felt.

| Sorbent implanted | Total bilirubin, mM/L | Liver mass/body mass, %* |
|---|---|---|
| None (control) (n = 6)** | 3.97 ± 1.06 | 5.2 |
| Carbon felt (n = 5)** | 3.65 ± 1.02 | 4.7 |
| Carbon felt + hepatocytes (n = 6)** | 1.33 ± 0.90 | 3.6 |

* Liver-to-body mass ratio is 2.9 in healthy animals.
** Number of animals.

## Conclusions

It has been shown that carbon materials can be used as a solid support in the design of biospecific and immunosorbents.

## References


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