

BILIRUBIN ADSORPTION AND CATALYTIC OXIDATION ON ACTIVATED CARBONS

E. A. Fesenko, J.D.S. Gaylor, E. M. Smith, O. P. Kozynchenko*, J. M. Courtney, and S. V. Mikhalovsky**

University of Strathclyde, 106 Rottenrow, Glasgow G4 0NW, UK

* Institute of Sorption and Problems of Endoecology, Kiev, Ukraine

** University of Brighton, Brighton BN2 4GJ, UK

Introduction

Direct haemoperfusion, DHP, over activated carbons, AC, is an efficient method of blood purification from circulating toxins mainly of exogenous origin. Coating of AC improves their haemo-compatibility but restricts their use to removal of low molecular weight, MW, substances only due to diffusion limitations. Uncoated polymer-pyrolysed AC are highly biocompatible and capable of removing high MW metabolites. Hence, DHP over uncoated AC could be a method of choice in case of severe hyperbilirubinaemia caused by liver malfunction. Here results of *in vitro* study of bilirubin adsorption on uncoated adsorbents are presented.

Experimental

Mesoporous AC were made by step pyrolysis followed by steam activation of styrene-divinylbenzene, S-DVB copolymer (SUCS) and vinylpyridine-DVB copolymer (SCN). Oxidised carbon samples, SCN-ox and SUCS-ox, were produced from AC by liquid phase oxidation with 20% HNO₃. Commercially manufactured adsorbents of different chemical nature such as Aerosil, a finely dispersed silica, and Cholestyramine, CA, a quaternary ammonium S-DVB anion exchange resin were also used. CA contains 2% of DVB and about 43% of water.

Solution containing 0.55 mg/ml of Bilirubin, Bil, and 45 mg/ml bovine serum albumin, BSA (molar ratio Bil/BSA = 2:1), in 0.01 M phosphate buffer, pH 7.4 was used as a model of unconjugated or indirect bilirubin. Bilirubin concentration was measured using a standard diagnostic kit 605-C (Sigma) and a UV-Vis spectrophotometer UV-2101PC (Shimadzu). Direct/ indirect Bil ratio in the initial solution was 1:6. All experiments were carried out at 37°C in sealed flasks protected from sunlight, for 24 h.

Results and Discussion

Under the experimental conditions, an equilibrium is established in the solution between free Bil and Bil bound with BSA: $2 \text{ Bil} + \text{BSA} = 2 \text{ Bil} \dots \text{BSA}$

Concentration of free Bil and total Bil can be determined separately and concentration of bound Bil has been calculated as difference between total and free Bil. The adsorption isotherms of Bil at 37°C, pH 7.4 are of Freundlich type (Fig. 1). A model based on a 'multi-

Freundlich' equation for a two-component system has been used to describe the experimental data (Table 2).

$$a_d = K_d C_d (C_d - C_{in} \cdot L_{in})^{n_d}, \text{ and}$$

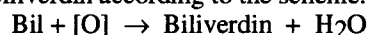
$$a_{in} = K_{in} C_{in} (C_{in} - C_d \cdot L_d)^{n_{in}}$$

where a - amount of bilirubin adsorbed, mg/g; C - equilibrium concentration, mg/ml; K and n - coefficients in the Freundlich equation, 'd' and 'in' refer to direct or indirect Bil, respectively; and L - impact factor of the first component on the other one. L is an empirical coefficient that can be determined numerically.

L_d/L_{in} ratio for all AC samples is similar suggesting that adsorption of a large Bil-BSA complex is a slow diffusion controlled process compared to adsorption of Bil. For CA, this ratio is close to unity which confirms specific affinity between positively charged surface groups and negatively charged Bil molecules regardless of being free or bound with BSA. Although polar, OH-groups on SiO₂ surface do not bear electric charge, and high value of L_d/L_{in} ratio indicates that Bil bound with BSA interacts weakly with the Aerosil surface compared to the free Bil (Table 2). Direct/indirect Bil ratio in the solution after adsorption reflects L_d/L_{in} ratio.

Pretreatment of adsorbent surface with BSA changes the ratio of adsorbed direct/indirect Bil. It indicates the complexity of the mechanism of Bil adsorption which may involve dissociation of the adsorbed Bil-BSA complex and also formation of the secondary adsorption sites by adsorbed BSA molecules.

It has been found that AC and Aerosil catalyse oxidation of Bil into biliverdin according to the scheme:



Both SCN samples are the most active catalysts for this reaction due to the presence of nitrogen atoms in the lattice (Fig. 2). As expected, silica shows the lowest catalytic activity in this red-ox reaction.

Conclusions

Adsorption of bilirubin on AC is a complex process comprising adsorption of free Bil, adsorption and dissociation of Bil-BSA complex and oxidation of Bil to biliverdin. Adsorption of Bil occurs on primary sites of carbon surface and on secondary sites formed by adsorbed albumin molecules.

Table 1. Some characteristics of adsorbents

Adsorbent	Particle Shape and Size	NaOH Uptake (meq/g)	Bulk Density (g/cm ³)	BET surface area (m ² /g)
SCN	spherical, 0.5-0.6 mm	0.64	0.55	990
SCN-ox	spherical, 0.5-0.6 mm	1.38	0.56	950
SUCS	spherical, 0.15-0.2 mm	0.38	0.51	1120
SUCS-oxi	spherical, 0.15-0.2 mm	1.40	0.53	1100
Aerosil	irregular, 15-20 nm	not measured	0.05	290
CA	irregular*	not measured	0.48	not measured

* At least 95% particles < 100 μm and at least 40% < 50 μm.

Table 2. Parameters of 'multi-Freundlich' equation for bilirubin adsorption

Adsorbent	Free		Bilirubin			L _d /L _{in}	
	K	n	L _{in}	K	n		
SCN	15.4	0.71	4.0	18.7	0.03	23.1	5.8
SCN-ox	21.3	0.72	7.2	29.8	0.02	37.7	5.3
SUCS	36.8	0.88	7.4	51.2	0.24	32.5	4.4
SUCS-ox	59.4	0.85	11.9	94.9	0.02	40.9	3.4
Aerosil	4.03	0.68	1.4	8.97	0.81	68.6	49
CA	171	0.69	64	4,000	0.65	74.4	1.2

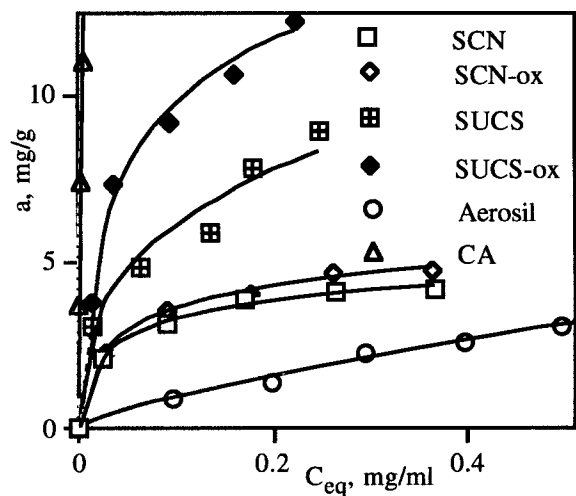


Figure 1. Adsorption isotherms of total bilirubin. Experimental conditions: 20 ml of solution, 1.0 g catalyst.

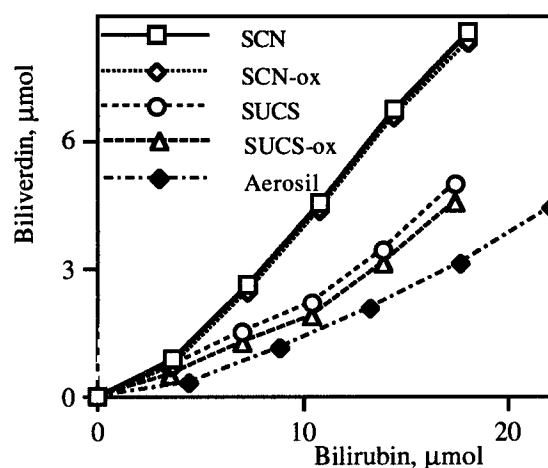


Figure 2. Oxidation of bilirubin into biliverdin. Experimental conditions: 20 ml of solution, 1.0 g catalyst.

References

1. Sheindorf C, Rebhun M, Sheintuch M. Prediction of breakthrough curves from fixed-bed adsorbents with Freundlich-type multisolite isotherm. *Chem Eng Sci* 1983;38(2):335-342.

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