SYNTHESIS AND CHARACTERISATION OF NOVEL CARBON MATRICES WITH SPECIFIC BIOAFFINITY FOR USE IN EXTRACORPOREAL SYSTEMS

S.T. Meikle[1], G. Gallacher[1], S.V. Mikhalovsky[1] and S.R. Tennison[2]
[1] School of Pharmacy and Biomolecular Sciences, University of Brighton, Cockcroft Building, Lewes Road, Brighton, East Sussex, England BN2 4GJ, UK.
[2] MAST Carbon Ltd., Henley Science Park, Guildford, Surrey, UK.

Corresponding author e-mail address: s.t.meikle@bton.ac.uk

Introduction

Activated carbons possess many of the properties desirable for use as a sorbent material in extracorporeal systems. They are physically and chemically stable, may be modified in terms of porosity or surface chemistry, thus allowing a wide range of sorbent materials to be produced.

One drawback of activated carbons is their inherent hydrophobicity. Unless they are used as hydrophobic matrices, the surfaces have to be modified to produce a more hydrophilic surface, to increase interaction with the biological solution of interest. Modification of the surface is usually performed by oxidation of the surface groups, using liquid or gaseous treatments, such as nitric acid, hydrogen peroxide or oxygen respectively, to produce surfaces with different distributions of functional groups.

As the first step to producing activated carbons which have specific bioaffinity for species in the plasma or blood, the conjugation of amines to the surface of non-oxidised activated carbons was undertaken using a water-soluble carbodiimide crosslinker.

To determine the distribution of surface functional groups, the activated carbons were examined using of a variation of the titration method proposed by Boehm [1].

Experimental

The carbons examined were NOVACARB^{TM} activated carbons supplied by MAST Carbon Limited, Henley Science Park, Guildford, Surrey, UK. All chemicals were purchased from Sigma Aldrich Company. Ltd, except for 1-ethyl-3- (3-dimethyl aminopropyl) carbodiimide (EDC) and N- hydroxysuccinimide (NHS) purchased from Acros Organics, acetonitrile (Fisher Scientific) and nitric acid (Sure Chem Products Limited).

HNO_3 Oxidation

10 g of NOVACARB were placed in 100 cm^3 20% ($^w/_v$) HNO_3 at 50, 70 and 90^oC for a total contact time of 2 hours. The carbons were then collected by filtration and washed

with hot water until the pH of the washings was above 6. The samples were then dried at 120^{o}C in an oven until constant weight and stored in a dessicator.

Butylamine Conjugation

1.5 g of each NOVACARB was placed into a 50-cm^3 flask to which 20 cm^3 of dried acetonitrile was added, then a 10-fold molar excess of EDC and NHS with respect to the number of carboxyl groups was added. The flask was fitted with a drying tube and placed on an orbital mixer for 18 hours at room temperature, with gentle agitation.

A 20 fold molar excess of butylamine, with respect to the number of carboxyl groups, was added to the flask and allowed to react for a further 18 hours at room temperature on an orbital mixer. After 18 hours the samples were filtered and washed with water, 0.1 M NaOH and methanol then dried in an oven at 30 ^{o}C until constant weight.

Blank reactions were performed as outlined above with 10 fold molar excess of NHS and 20 fold molar excess of butylamine (Blank 1) and 10-fold molar excess of EDC and NHS (Blank 2).

Determination of Surface Functional Groups

NOVACARB's were examined using a variation on the titration method proposed by Boehm [1]. 0.5 g of each NOVACARB and oxidised NOVACARB, were incubated with 20 cm^3 0.1 M HCl, NaOH, Na_2CO_3 and $NaHCO_3$ in 1 M NaCl for 48 hours at 25^{o}C at 100 rpm. 5 cm^3 aliquots were removed and titrated with 0.1 M NaOH or HCl respectively. Solutions without carbon were used as controls.

The amount absorbed was calculated using Eq. (1)

$$a = (c_0\text{-}c_1)\ V/m \qquad (1)$$

where a = number of groups neutralised (meq/g); c_0 = initial concentration of solution (meq/L); c_1 = concentration of solution after incubation with carbon (meq/L); V = volume of solution (L); m = mass of carbon (g).

Results and Discussion

Table 1. Sample codes for nitric acid oxidised NOVACARB's.

NOVACARB	*Oxidation Temperature (^{o}C)*	*Code*
S-50-9/100/25-00	50	N-100-50-2
	70	N-100-70-2
	90	N-100-90-2
S-50-9/125-00	50	N-125-50-2
	70	N-125-70-2
	90	N-125-90-2

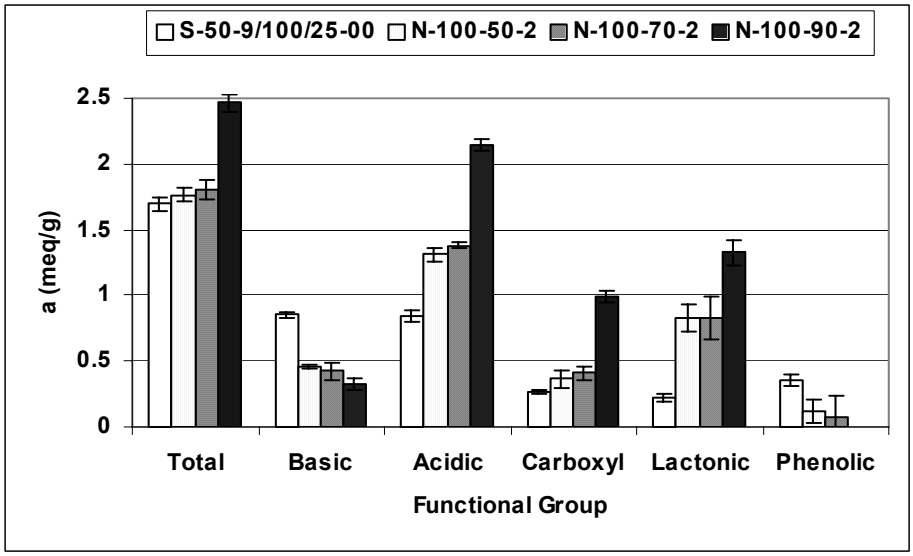

Figure 1. Distribution of surface functional groups on S-50-9/100/25-00 and nitric acid oxidised S-50-9/100/25-00. Mean (n=3) ± Standard Deviation

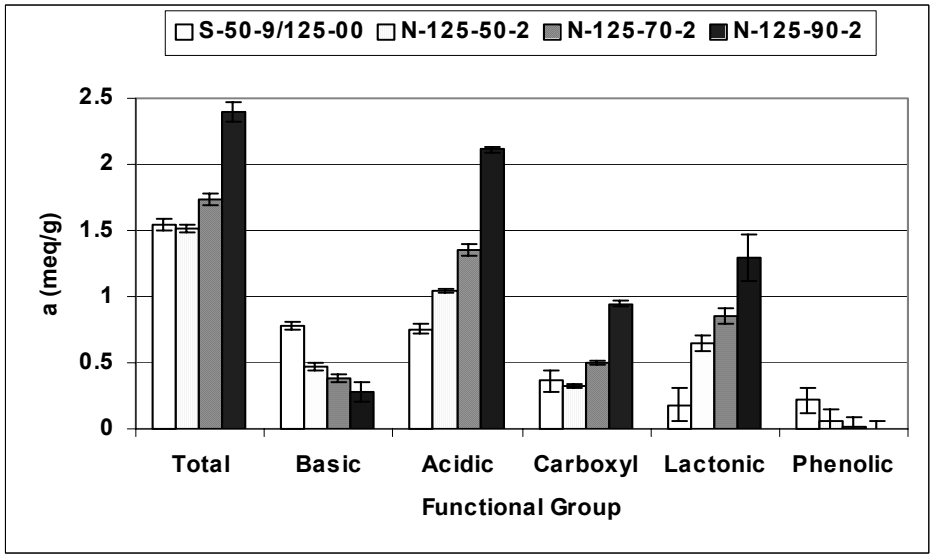

Figure 2. Distribution of surface functional groups on S-50-9/125-00 and nitric acid oxidised S-50-9/125-00. Mean (n=3) ± Standard Deviation

As the temperature at which nitric acid oxidation is increased (Figures 1 and 2), the number of carboxyl and lactonic groups increase, however this is accompanied by a decrease in the number of basic and elimination of the phenolic groups. This method of oxidation allows for the generation of surfaces with a predominance of carboxyl type moieties, thereby minimising any possible side reactions on subsequent surface modification.

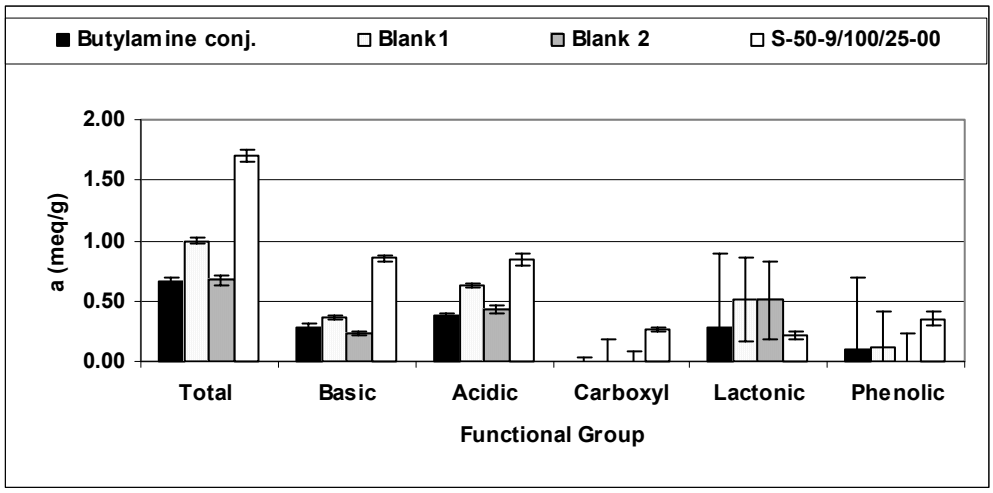

Figure 3. Distribution of surface functional groups on the surface of S-50-9/100/25-00 conjugated with n-butylamine.
Mean (n=3) ± Standard Deviation

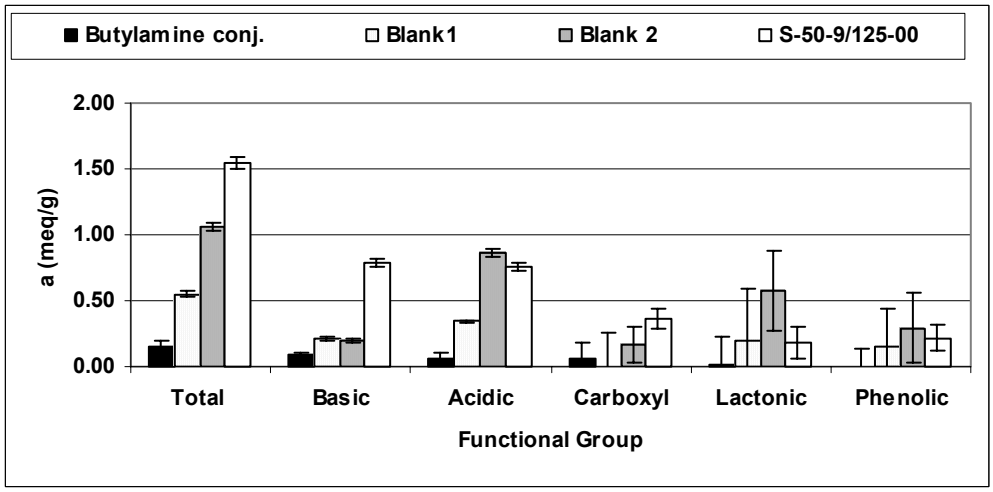

Figure 4. Distribution of surface functional groups on the surface of S-50-9/125-00 conjugated with n-butylamine.
Mean (n=3) ± Standard Deviation

From the results of conjugation of butylamine to the surface of the two non-oxidised carbons, it appears that coupling is achieved as may be seen from the decrease in the number of carboxyl type groups. However, these results should be viewed with some skepticism due to the size of the error bars for the lactonic and phenolic type groups. Preliminary results from the incubation of the carbon with acetonitrile and butylamine only, suggests the formation of unexpected groups such as isontiriles from the reaction of NaOH and acetonitrile which may interfere with the titrations.

Conclusions

As the first step to producing activated carbons which have specific bioaffinity for species in the plasma or blood, the conjugation of amines to the surface of non-oxidised activated carbons was undertaken using a water-soluble carbodiimide crosslinker.

Conjugation of butylamine to non-oxidised activated carbons appears to show the formation of a peptide bond, which is observed as a decrease in the number of carboxyl groups, however the use of acetonitrile as a solvent to carry out the conjugation reaction, produced some unexpected results. The use of a more appropriate solvent which will minimize the formation of byproducts such as isontiriles, should give a better indication of whether the conjugation has been successful.

The oxidation of NOVACARB's S-50-9/100/25-00 and S-50-9/125-00 using 20% nitric acid for 2 hours at 50, 70 or 90^{o}C, allows the generation of a series of matrices with different functional group distributions. These differences in surface chemistry may be exploited for subsequent surface modification or bioligand attachment.

The use of oxidised activated carbons (N-100-90-2 or N-125-90-2) with a greater density of carboxyl groups should provide an opportunity to synthesize activated carbon with a higher surface density of amine groups and to minimize side reactions. Activated carbon with surface amine groups can further be used for bioligand immobilisation and synthesis of carbon adsorbents with specific bioaffinity.

References

[1] Boehm HP, Diehl E, Heck W, Sappok R. Surface oxides of carbon. Angewandte Chemie: International Edition 1964;3(10):669-677.

Acknowledgments: This work was supported by an EPSRC (UK) CASE Award No 01300437 and MAST Carbon Limited.