Modification of nanocellulose for blood purification

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Abstract

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This report treats chemical modification of nanocellulose with aim to employ it in dialysis. The modification steps consist of oxidizing and sulfonation reactions. Infra-red spectroscopy, BET surface area analysis and zeta potential was used to characterize the modified nanocellulose. The results show that the modified nanocellulose achieve the desired properties.
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1 Populärvetenskaplig sammanfattning av projektet på svenska


2 Introduction

Cellulose is an polymer that exists in different forms and in various lifeforms in nature, it can be found in plants, animals and in bacteria for example. One of the forms is nanocellulose which can be extracted from Cladophora green algae, figure 1, and this the raw material used in this project. Nanocellulose is described as products or extracts from native cellulose composed of nanoscaled structure material[6]. The nanocellulose is the subject for an experimental project at the Department of Engineering Sciences, Nanotechnology and Functional Materials at Uppsala University. The project involves manipulation of the material to give it desired properties that can be used for blood purification in dialysis. Dialysis is often used for patients with kidney failure. The technique consists of passing blood through an extra corporeal device filled with a porous material containing antibodies that retain specific toxins. During the dialysis, the blood is activated and coagulate because of the material inside the dialysis machine. In order to avoid activation and coagulation of the blood, heparin is administrated to patients. Heparin is a sulfonated polysaccharide that cause many side effects such as intense bleeding and osteoporosis. Therefore the goal is to obtain a material for blood purification with improved anticoagulant properties so that a decrease in heparin intake for the patients would be possible.
2.1 Aim
This project aims to understand and correlate the properties of modified nanocellulose with various analytical methods. The analysis methods in this project are zeta potential, IR spectroscopy and BET surface area analysis.

3 Experimental modification
This section explain the required methods to modify the cellulose so it achieves the desired properties. The modification steps consist of oxidation and sulfonation, figure 2. The oxidation process modifies the hydroxyl group ($R - OH$) to an aldehyde group ($R = O$), the oxidizing agent in this case is sodium periodate $NaIO_4$. This process makes the hydroxyl group lose the hydrogen atom and the remaining oxygen atom forms a double bond with the carbon chain. The main goal with the oxidation is to form the aldehyde group which is sufficient for further modifications. In this project, the oxidation was carried out using 1800 ml buffer (0.98 ml $HOAc$, 13.35 mg $NaOAc$, 1800 ml $H_2O$), 79 g of $NaIO_4$, 12 g of Cladophora cellulose and 180 ml of 1-propanol that were put in a round bottom flask covered by aluminum foil for light protection, together with a magnetic stirrer for 240 hours. Further in this report, oxidized Cladophora is reffered to DAC beads, DAC is a shortening for DiAldehyde Cellulose.

One of the main goals of this project is to make a material with anticoagulant properties similar to heparin. Figure 1 represent the chemical structure of the nanocellulose Cladophora and figure 3 shows the chemical structure of heparin. The functional groups that gives heparin the anticoagulant properties are the sulphite groups ($SO_3$) in figure 3. The sulfonation reaction substitutes
the aldehyde group with a sulphite group \((SO_3^-)\). Herein, the sulfonation was carried out using two using two different concentrations of sodium bisulfite \((Na_2SO_3)\). In the first reaction, 0.55 g of DAC beads was mixed with 0.77 ml of \(Na_2SO_3\), this reaction represent high concentration sulfonated DAC beads. In the second reaction, 0.32 g of DAC beads was mixed with with 0.44 ml of \(Na_2SO_3\), this reaction represent low concentration sulfonated DAC beads. For both reactions, the DAC beads and \(Na_2SO_3\) were put in a container with 150 ml of destillated water and a magnetic stirrer for 72 hours. The aim was to obtain two materials with different concentration of sulphite groups present on the surface. However, even if the sulfonation reaction have been successful, the whole surface will not consist entirely of sulphite functional groups. It will be shared by the remaining aldehyde groups that not have been effected by the reaction. [9]

![Figure 2: Illustration of the oxidation and sulfonation reactions.][9]

![Figure 3: Chemical structure of Heparin.](image)
4 Material characterization

In order to verify that a reaction have taken place, it is necessary to have a various of analytical methods to study the product. The methods that were used in this project are infra-red spectroscopy (IR), Brunauer/Emmet/Teller (BET) surface analysis and zeta potential.

4.1 IR spectroscopy

One way to analyse the bonds within a molecule is using IR spectroscopy. When a matter is exposed to electromagnetic radiation such as IR, it causes the chemical bonds inside to vibrate and rotate three dimensionally with certain frequencies that can be translated to wavelength. The vibrational intensity is measured at different time points and thereafter the Fourier transformation is applied to transform the time data to frequencies data. This information is required to characterize the bonds within the substance. In this project, IR is used to verify the oxidation reaction.[3] The x axis in the IR plot is the wavenumber of the spectrum. To convert to the corresponding wavelength, equation 1 is used.

\[ v = \frac{1}{\lambda} \quad (1) \]

where \( v \) is the wavenumber and \( \lambda \) is the wavelength.[7]

4.2 BET surface area analysis

Since the aim with the modified material are to adsorb toxins in the dialysis process, it is necessary to evaluate the surface area (BET surface area). The way to measure it is to expose the sample of interest to a non-reactive gas and see how much the material adsorbs, this gives the material isotherm from which the BET area can be determined. The BET equation is described by equation 2

\[ \frac{1}{v \left( \frac{P}{P_0} - 1 \right)} = \frac{c - 1}{v_m} \left( \frac{P}{P_0} \right) + \frac{1}{v_m c} \quad (2) \]

where \( P \) is the varying pressure, \( P_0 \) the saturation pressure, \( v \) the molar volume of the adsorbed gas, \( v_m \) the monolayer molar volume adsorbed gas and \( c \) the BET constant. Plotting the left hand side of equation 1 against \( \frac{P}{P_0} \) holds a linear equation where the slope and intersect at y-axis determines the BET constant and adsorbed amount of gas. The linear relationship holds
for the region $0.05 < \frac{P}{P_0} < 0.35$.\cite{3} The value of $v_m$ is used to determine the specific area $S_{BET}$. Equation 3 describes the relationship.

$$S_{BET} = v_m N \psi$$  \hspace{1cm} (3)

where $N$ is Avogadro's number for the adsorbed gas and $\psi$ is the average area occupied by each molecule in the monolayer. To determine $\psi$, Emmett and Brunauer postulate that it can be calculated by the formula

$$\psi = f \left( \frac{M}{\rho N} \right)^{\frac{2}{3}}$$  \hspace{1cm} (4)

Where $f$ is the packing factor, $M$ the molar mass of the adsorptive and $\rho$ the absolute density of the liquid adsorptive at the operational temperature. For the special case of nitrogen adsorption at 77 K, $\psi$ is usually by convention 0.162 $nm^2$. \cite{8}
4.3 Zeta potential

As we can see from figure 1, the aldehyde group is neutral in charge and the sulphite groups are negatively charged. Therefore can zeta potential be used to study the sulfonation reaction. In order to explain this technique one can assume a particle with arbitrary surface charge that is dispersed in a solvent. At the surface, ions of opposite charge tend to stick and form a ionic layer around the surface. This layer is fairly strong and contribute most to the particles mobility in the solvent. The second layer contains ions of opposite charge to the first layer and these are more mobile than the first layer. The outer boundary of the mobile ions in both layers is called the radius of shear. The potential at the radius of shear relative to the distant value in the bulk medium is called the zeta potential. To measure the zeta potential, particles are put in a dispersion with a solvent in a container with two electrodes on each side of the container. The electrodes will create an external electromagnetic field that affects the particle with a forces which accelerate it to one side depending on the particles charge. Viscous forces is going to act on the particle to oppose this movement. When equilibrium is reached between those forces, the acceleration is zero and the particle moves with constant speed. The velocity is used in the Henry equation (eq 5) to determine the zeta potential.

\[ V_e = \frac{2\epsilon z f(ka)}{3\eta} \]  

(5)

where \( \epsilon \) is the dielectric constant, \( V_e \) the velocity of the particle, \( z \) the zeta potential, \( \eta \) the viscosity and \( f(ka) \) the Henry function. In this case, \( \eta \) is 0.89 mPa\(\times\)s, \( \epsilon \) is \( 7.12 \times 10^{-10} \) F/m. To evaluate the value of the Henry function, Smoluchowski approximation is used and adresses the value 1.5 to the Henry function.[3][1]
5 Result

5.1 Results from IR

As described in section 1.3.1, the IR analysis was used to verify the oxidation. Figure 4 shows the result from the analysis.

![Figure 4: Result from IR of the oxidation process.](image)

Figure 4: Result from IR of the oxidation process.
5.2 Results from BET surface analysis

Table 1 shows the result from the BET surface analysis.\(^1\) \(A\) is the BET surface area, \(c\) is the BET constant and \(v_m\) the monolayer molar volume adsorbed gas. From equation 2, a linear model is used between \(0.05 < \frac{P}{P_0} < 0.35\), \(k\) is the slope of the line and \(m\) is the intercept value of \(\frac{1}{v(P/P_0-1)}\).

<table>
<thead>
<tr>
<th></th>
<th>(A \text{ [m}^2\text{g}^{-1})</th>
<th>(k)</th>
<th>(m)</th>
<th>(v_m)</th>
<th>(c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DAC beads</td>
<td>56.91 (11)</td>
<td>0.07542 (15)</td>
<td>0.001067 (28)</td>
<td>13.07</td>
<td>71.68</td>
</tr>
<tr>
<td>HC Sulf. DAC beads</td>
<td>106.76 (63)</td>
<td>0.04051 (24)</td>
<td>0.000254 (44)</td>
<td>24.53</td>
<td>160.53</td>
</tr>
</tbody>
</table>

5.3 Results from zeta potential analysis

Table 2 shows the result from the zeta potential measurements\(^2\). Three zeta potential measurements was performed and \(\zeta_i\) is the zeta potential measurements with number index \(i\), \(\zeta_{mean}\) the mean value and \(\zeta_{std}\) is the standard deviation of the measurements.

<table>
<thead>
<tr>
<th></th>
<th>(\zeta_1 \text{ [mV]})</th>
<th>(\zeta_2 \text{ [mV]})</th>
<th>(\zeta_3 \text{ [mV]})</th>
<th>(\zeta_{mean} \text{ [mV]})</th>
<th>(\zeta_{std} \text{ [mV]})</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC-sulf-DAC-beads</td>
<td>-20.8</td>
<td>-23.1</td>
<td>-22.4</td>
<td>-22.1</td>
<td>1.18</td>
</tr>
<tr>
<td>HC-sulf-DAC-beads</td>
<td>-36.1</td>
<td>-35.7</td>
<td>-36.3</td>
<td>-36.0</td>
<td>0.306</td>
</tr>
</tbody>
</table>

6 Discussion

From figure 4 it is clear that after 240 hours of oxidation, new peaks have been formed at \(1730 \text{ cm}^{-1}\) \((\lambda_1 = 5.78 \text{ cm})\) and at \(885 \text{ cm}^{-1}\) \((\lambda_2 = 11.29 \text{ cm})\) in comparison with the initial state of non oxidized Cladophora cellulose. This shows that the oxidation reaction has been succesful since \(1730 \text{ cm}^{-1}\) and \(885 \text{ cm}^{-1}\) correspond to the aldehyde group.[5]

From the BET analysis, table 2, it can be seen that the sulfonated DAC beads have a larger surface area than the non-sulfonated DAC beads. This imply that the surface reaction capacity will be improved for the sulfonated DAC beads since there exist more area for a reaction to occur. However,

\(^1\)It should have been measurements on low concentration DAC beads. Unfortunately the sample disappeared and the project time is limited so a remeasuring was not possible within the time frame for this project.

\(^2\)There should have been measurements on the the DAC beads but unfortunately did the oxidation process fail because the dried DAC beads were unable to disperse. The pH value for the different dispersed solvents was forgotten to be measured as well.
the surface of the sulfonated DAC beads will not entirely consist of the sulphite groups, it will be shared by the remaining aldehyde groups that is left after the sulfonation reaction. Another benefit with a larger surface area is that the amount of sulphite and aldehyde functional group increases. The purification process works so that the antibodies attach specific toxins, the identification between them is possible through the antigen mechanism. This mechanism means that if a specific toxin/virus or other particles that are harmful to the human body enters inside the body, the antibodies creates an antigen that works as a identification key to the specific toxin/virus so the antibodies can bond with this harmful object.[2] Further on, the antibodies is attached to aldehyde groups on the materials surface. This means that the non purified blood runs through dialysis machine, enters a chamber with the anticoagulent material which is acting like a filter so that the toxins attaches to the antibodies on the materials surface and stay there.[4] The increment of surface area should increase the amount of retained toxins due to more aldehyde groups, however this was not measured and therefore not verified in this project.

The zeta potential analysis was used to verify the sulfonation reaction. Since the aldehyde groups in the DAC beads is neutrally charged and sulphite groups in the sulfonated DAC beads is negative charged, the materials overall charge is going to be different before and after the sulfonation reaction. Therefore the sulfonation reaction could be studied using the zeta potential. The results shows that for the zeta potential mean value, the low concentration sulfonated DAC beads got -22.1 mV and the high concentration DAC beads got -36 mV. The higher the concentration of sulfonation agent, the more negatively zeta potential. Regarding the absolute value of the zeta potential, it becomes higher with higher sulfonation concentrations, the sign of the potential is dependent on how the reference direction is defined. As described in section 1.3.3, the zeta potential gives information about the dispersed particles stability in a solvent. From the result of the analysis, the increase of sulfonation agent entails the zeta potential and the kinetic stability increases since the radius of shear decreases to keep the potential energy that comes from repulsion and attraction in balance against each other. The potential energy from repulsion is defined as the energy that repulsive interactions between charges in the electric double layer produces. The attractive potential energy comes from the van der Waals interactions between the molecules in the particle. However, the reliability of the results is effected negative since the pH value was not measured and it play a role since high and low pH have different affects on the samples charge. [3]

It was observed that the sulfonated DAC beads, regardless of the concentration, were repelled by a metallic spoon when dried. The unsulfonated DAC
beads did not show the same repelling properties. This can be understood since the sulfonated DAC beads got negatively charged sulfonated functional groups on the surface and metallic object is generally surrounded by free electrons.

7 Conclusions

The oxidizing reaction of the Cladophora cellulose were successful since the IR spectrum showed that new functional group with new bonds have been formed. From the BET analysis, it was clear that using more sulfonation agent resulted in a higher surface area. This is of importance since one of the main functions is to purify blood from toxins and a larger surface area may increase the amount of retained toxins. This project is a part of making the nanocellulose Cladophora to a functional material which purpose is to purify blood and act anticouagulent in the dialysis process, other part such as analysing the toxicity, testing the anticoagulant properties was not involved in this project. With this results, it could be verified that oxidizing and sulfonation reactions was successful and future studies will determine if it posses anticoagulant and purification properties, suitable for dialysis.

References


