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USE OF HYDROPHILIC POLYMER COATINGS FOR CONTROL OF ELECTROOSMOSIS AND PROTEIN ADSORPTION

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ABSTRACT

The purpose of this project was to examine the utility of polyethylene glycol (PEG) and dextran coatings for control of electroosmosis and protein adsorption; electroosmosis is an important, deleterious process affecting electrophoretic separations, and protein adsorption is a factor which needs to be controlled during protein crystal growth to avoid multiple nucleation sites. Performance of the project required use of X-ray photoelectron spectroscopy to refine previously developed synthetic methods. The results of this spectroscopic examination are reported. Measurements of electroosmotic mobility of charged particles in appropriately coated capillaries reveals that a new, one-step route to coating capillaries gives a surface in which electroosmosis is dramatically reduced. Similarly, both PEG and dextran coatings were shown by protein adsorption measurements to be highly effective at reducing protein adsorption on solid surfaces. These results should have impact on future low-g electrophoretic and protein crystal growth experiments.
INTRODUCTION

This work involves use of organic polymer coatings to control electroosmosis and protein adsorption. Before giving details of the experiments that were performed it is useful to provide a brief review of some background concepts.

Electroosmosis can be described as movement of a conducting liquid relative to a stationary charged surface, which results when an electric field is applied to the liquid. Generally, clean surfaces will be negatively charged, so that application of an electric field will produce movement of the positive counterions toward the negative electrode. This electroosmotic flow reduces the effectiveness of electrophoretic processes and thus tends to eliminate the benefits achieved by performing electrophoresis in low g where thermal and concentration driven convection is eliminated.

We have previously shown that polyethylene glycol (PEG) coatings are effective at reducing electroosmosis (1). Apparently this reduction results from the hydrophilic polymer coating acting as a wetted viscous layer (a "kelp forest") over the charged surface. The goal of the present work is to simplify the coating chemistry by preparing a PEG-silane that can be directly applied to glass surfaces. The previous technique required three steps: preparation of an activated PEG, amination of the glass surface, and coupling of the activated PEG to the aminated surface.

The second part of this project is concerned with examining the ability of hydrophilic polymer coatings (PEG and dextran) for their ability to reject proteins (i.e., their biocompatibility). The coatings are tethered to the surface by a single covalent linkage (the kelp forest analogy) and would be expected to exclude proteins from the surface simply by waving around. This phenomenon has been used to explain the ability of covalently-linked PEG to render proteins nonimmunogenic. In this work we have attached PEG and dextran to glass slides and measured the extent of fibrinogen 1-125 adsorption. Controlling protein adsorption is important in protein electrophoresis and in protein crystal growth experiments where it is desirable to avoid multiple nucleation sites.

An important aspect of all this work is utilization of X-ray photoelectron spectroscopy (XPS) to characterize our surfaces. This technique provides a direct elemental analysis of surfaces. By using it we should have a more quantitative and direct means of following chemistry on the surfaces. Previously, we have used wet chemical techniques that give averages of surface chemistry. With XPS we can study surface homogeneity and we can work with small slides having too little surface area to use the wet chemical methods previously applied.
OBJECTIVES

The objectives of this work are as follows:

(1) to prepare a PEG silane and couple it to glass

(2) to measure the electroosmotic mobility of a polystyrene microsphere in a PEG-silane coated glass capillary

(3) to improve our glass amination procedures by use of X-ray photoelectron spectroscopy

(4) to measure the extent of protein (fibrinogen) adsorption on dextran- and PEG-coated glass using improved amination procedures to coat the glass
Results of X-Ray Photoelectron Spectroscopic (XPS) Surface Analysis

Our first goal was to use XPS to examine coating chemistry. The first step in the standard coating procedure is to aminate the glass surface using trimethoxyaminopropylsilane, 1. Previously we examined the efficiency of this process by conducting the reaction on porous glass (with a large surface area) and titrating the amine groups with acid. With XPS we can directly determine the percentage of nitrogen on the surface of slides actually used in the protein adsorption experiments. Nitrogen has a relatively small cross section, so we have fluorinated the surface by reacting the aminated surface with pentafluorobenzaldehyde. Thus we can check the nitrogen results by measuring the percentage of fluorine, an element having a much larger cross section.

In a typical amination procedure the glass substrate is immersed in a 5% solution of 1 in water for 24 hours at 80 degrees, and then cured dry at 110 degrees for four hours; after curing, the glass is exhaustively washed with distilled water. Surfaces prepared in four different ways were examined. The four routes are: (1) treatment with 1 followed by washing with water before curing; (2) as in example 1, but done twice; (3) treatment with 1 followed by curing without washing; (4) as in example 3, but done twice. The results of these experiments, using two different XPS instruments (a Perkin-Elmer at UAH and a Surface Science at University of Washington), are given in Table 1.

Table 1. Measurement of percent nitrogen and fluorine on aminated glass slides (prepared four ways as described in text) treated with pentafluorobenzaldehyde on an Perkin-Elmer XPS and a Surface Science XPS.

<table>
<thead>
<tr>
<th>Method</th>
<th>#1</th>
<th>#2</th>
<th>#3</th>
<th>#4</th>
</tr>
</thead>
<tbody>
<tr>
<td>%N (Perkin-Elmer)</td>
<td>0.98</td>
<td>1.98</td>
<td>2.30</td>
<td>2.37</td>
</tr>
<tr>
<td>%N (Surface-Sci.)</td>
<td>1.66</td>
<td>1.95</td>
<td>2.75</td>
<td>5.09</td>
</tr>
<tr>
<td>%F (Surface-Sci.)</td>
<td>4.49</td>
<td>5.45</td>
<td>4.17</td>
<td>7.17</td>
</tr>
</tbody>
</table>

The data show that higher percentages of nitrogen are obtained if the surface is not washed before curing. Also a higher nitrogen coverage is obtained if the process is repeated. It is noteworthy that significant different values for percent nitrogen were measured with the two instruments. The Perkin-Elmer machine is new and uncalibrated, so it is likely that the Surface Science data are more trustworthy. Further comparison of the two machines with known substrates will be done. Also, the %F does not parallel the trends in %N. This result has two possible explanations. First, it is known that the XPS probes the first 5 nm of the surface, and the fluorines are applied directly onto the surface; so the nitrogen and fluorine measurements are actually made on different layers of surface. A second possibility is that the pentafluorobenzaldehyde liquid alters the surface in some way. We are now exploring a vapor-phase
fluorination procedure with trifluoroacetic anhydride to avoid this second possibility.

RESULTS OF ELECTROOSMOSIS EXPERIMENTS

As noted above, the goal of this segment of the project is to attach PEG to glass capillaries in a single step by reaction with a PEG-silane. The PEG-silane was synthesized by reacting PEG with trimethoxysilylpropyl isocyanate in methylene chloride. The reaction could be followed on IR by monitoring the isocyanate absorbance. Reaction of the PEG-silane with quartz electrophoresis capillaries was performed as described above. Electroosmotic mobilities were then determined for uncoated, standard two-step coated, and silane coated capillaries; standard coating involves the two-step process with amination and reaction with activated PEG described above. In both cases, the PEG used was the monomethyl ether of PEG 5000. Details on electroosmosis measurements are available in reference 1.

Measured electroosmotic mobilities (in um/s cm/V at pH 5.78, 7.5 mM NaCl) were: uncoated, 1.7; standard coated, 0.3; and silane coated, 0.7. This result shows that the single-step, silane coating is effective at reducing electroosmosis, although it is not as effective as the standard two-step procedure. It will be very interesting to follow up on these results by doing XPS experiments to determine the extent of PEG coverage using the two methods. Also it will be interesting to determine the pH dependence of the two different coatings, since we earlier concluded that the two-step procedure left some uncoupled amine groups which could show a large response to pH variation.

RESULTS ON PROTEIN ADSORPTION

The final set of experiments were to measure the extent of protein adsorption on PEG- and dextran-coated surfaces. The actual measurement was extent of fibrinogen I-125 adsorbed onto the materials. The results are presented in Table 2.

Table 2. Fibrinogen I-125 adsorption of PEG coated glass slides

<table>
<thead>
<tr>
<th>substrate</th>
<th>protein adsorbed (ug/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glass</td>
<td>0.420</td>
</tr>
<tr>
<td>glass-amine</td>
<td>0.683</td>
</tr>
<tr>
<td>glass -PEG200</td>
<td>0.540</td>
</tr>
<tr>
<td>-PEG400</td>
<td>0.468</td>
</tr>
<tr>
<td>-PEG1000</td>
<td>0.382</td>
</tr>
<tr>
<td>-PEG3000</td>
<td>0.206</td>
</tr>
<tr>
<td>-PEG8000</td>
<td>0.104</td>
</tr>
<tr>
<td>-PEG20000</td>
<td>0.072</td>
</tr>
</tbody>
</table>
As can be seen from Table 2, there is a dramatic drop in protein adsorption with the PEG MW. Presumably the larger PEGs have a larger exclusion volume and are thus better able to "sweep" the protein from the surface. We have also examined dextran coatings. Although the experiments are preliminary, we do have qualitative data showing that dextran is even more effective than PEG at reducing protein adsorption. The dextran was attached by reductive amination of aminated glass by the reducing end of the polysaccharide.

CONCLUSIONS

The goals of the proposed work have been met. Polymer coating chemistry was improved by use of XPS, and the PEG-silane was synthesized. PEG coatings attached to electrophoresis capillaries by means of the PEG-silane are effective at reducing electroosmosis, although they are not as effective as those coatings applied by the standard two-step procedure. Finally, PEG and dextran coatings are very effective at reducing protein adsorption onto glass, and thus these coatings offer potential for controlling nucleation sites in protein crystal growth. Future work will involve refinement of coating chemistry using fully calibrated XPS spectrometers and examination of dextran coatings for control of protein adsorption and electroosmosis.

REFERENCES


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