Application Note: 31070

Characterization of Chemical Gradients and Antibody Immobilization Using XPS and ARXPS

Key Words

- Surface Analysis
- Angle Resolved XPS
- Chemical Gradients

Introduction

Chemical gradients are regions of change in surface chemistry between two points on a substrate. These may be useful in a number of experimental situations, where it is desirable to probe the effect of differing surface chemistries in a single experiment.

In this study, angle resolved XPS was used to characterize materials having an ultra-thin co-polymer layer in which there was a chemical gradient along the surface of the co-polymer.

The chemical gradients produced for this study were also used to immobilize an antibody, bovine IgG, at their surface. XPS was used to characterize the resulting surfaces.

The XPS measurements were made using the Thermo Scientific Theta Probe.

Sample Preparation

A silicon substrate, having a native oxide at its surface, is placed in a box with a slot in the lid. The substrate is attached to a precision, computer-controlled drive so that its surface can be made to pass beneath the slot at a fixed rate. The whole arrangement is placed inside a plasma chamber, Figure 1. The feed of two volatile monomers into the plasma is also computer-controlled and so the proportion of each can be varied as the substrate is passing beneath the slot.

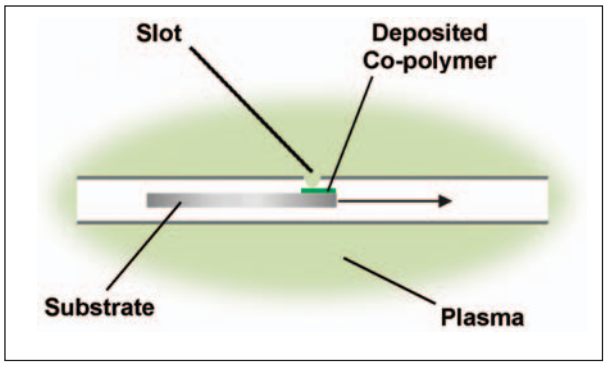

Figure 1: Schematic diagram of the method of sample preparation

In this example, when one end of the substrate is beneath the slot the monomer 1,7-octadiene is present in the plasma. As the substrate is moved, the quantity of octadiene is reduced and the quantity of acrylic acid monomer is increased until there is only acrylic acid in the plasma.

Using this method, a plasma polymer (pp) layer is deposited onto the surface of the substrate, with an area of pp-octadiene at one end of the substrate, an area of pp-acrylic acid at the other and a plasma co-polymer of these materials in between.

Figure 2 represents the intended structure of the material.

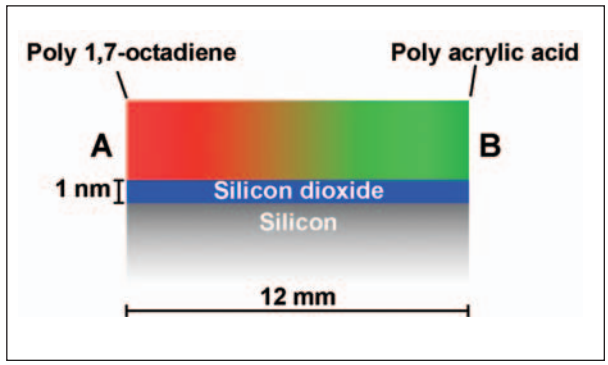

Figure 2: The intended structure of the sample

XPS Analysis

An XPS spectrum line scan was acquired along the gradient, from point A on Figure 2 (pp-1,7-octadiene) to point B (pp-acrylic acid), illustrated in Figure 3.

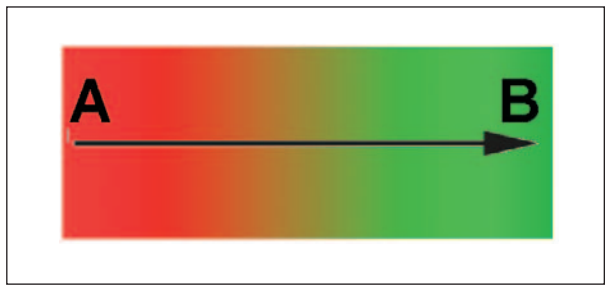

Figure 3: Plan of sample showing the direction of the linescan

The Si 2p spectrum, Figure 4, is independent of the position on the sample from which it was acquired.

The presence of both elemental and oxidized silicon in the spectrum indicates that the plasma polymer layer is very thin.

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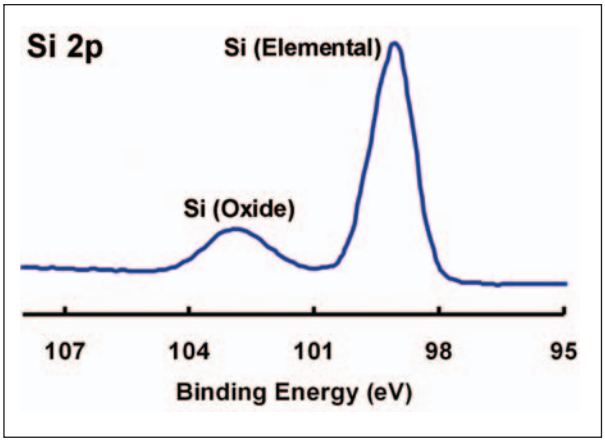

Figure 4: A typical Si 2p spectrum from the sample. This shows little or no variation as a function of position on the sample.

The C 1s spectra, however, change as a function of the analysis position. Spectra, one from each end of the substrate, are shown in Figure 5.This confirms that the surface layer is a hydrocarbon at the pp-octadiene end of the sample and contains C-O and O=C-O bonds at the other end. This is entirely consistent with the model shown in Figure 2.

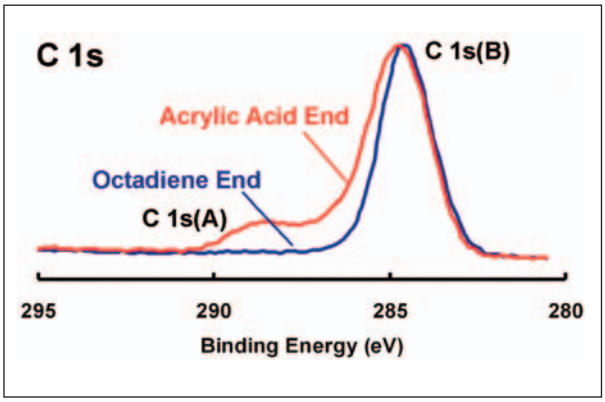

Figure 5: The C 1s spectrum from each end of the sample

The atomic concentration profile along the sample is shown in Figure 6. This shows clearly that the hydrocarbon component of the layer decreases as the oxygen-containing organic material increases. At each end of the profile, there is a region in which the concentrations remain constant.

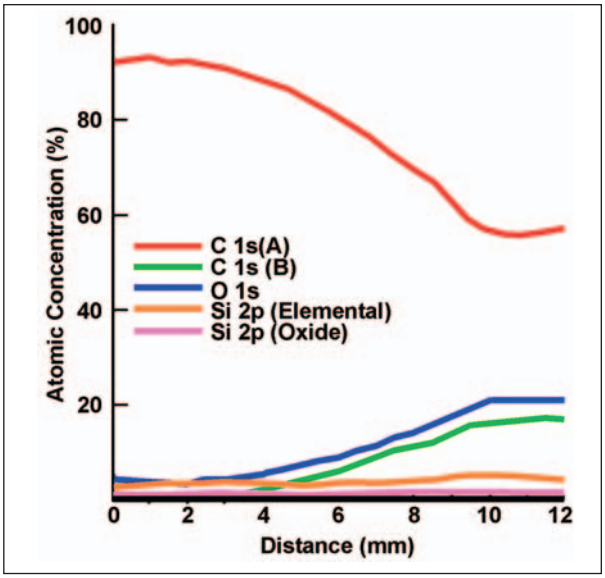

Figure 6: Atomic concentration as a function of distance along the sample

The behavior shown in Figure 6 is reflected in Figure 7, which has three 2-dimensional maps showing the atomic concentration of carbon in each of two chemical states and oxidized silicon. The C 1s maps clearly show the chemical gradient along one axis and that the distribution of carbon is uniform along the other axis. There appears to be a gradient in the oxidized Si distribution but this is because the co-polymer layer does not have uniform thickness (see later) and the signal from silicon is stronger where it is beneath a thinner part of the layer.

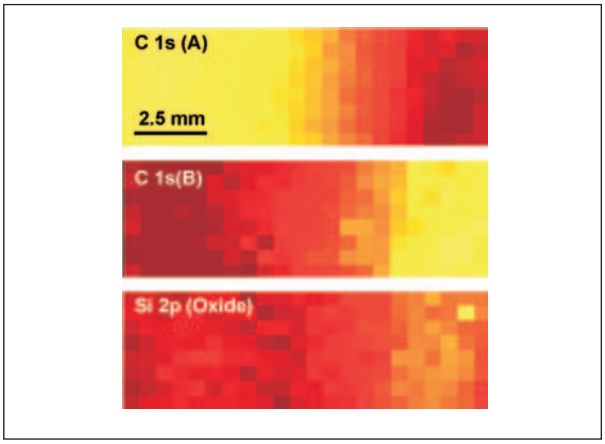

Figure 7: Atomic concentration maps of the co-polymer chemical gradient

Angle Resolved XPS

Using Theta Probe, angle resolved spectra over a 60° range can be collected in parallel from each point on the linescan without tilting the sample. From these data the thickness of the co-polymer layer can be determined at each point on the linescan and a non-destructive depth profile can be produced.

Figure 8 shows the thickness of the co-polymer and the SiO_2 layer as a function of distance along the line scan. These were calculated using the Multi-overlayer Thickness Calculator which is an integral part of the *Avantage* data system. It can be seen from Figure 8 that the thickness of the co-polymer layer decreases by about 2 nm with increasing acrylic acid content and there is a region of constant thickness at each end of the sample. The measured SiO_2 thickness is independent of the position on the sample.

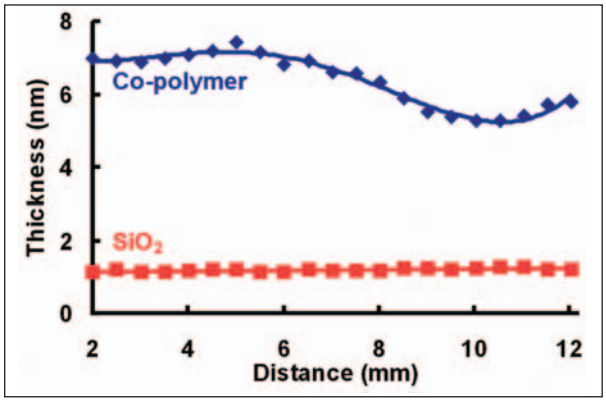

Figure 8: The thickness of the co-polymer and the native SiO_2 as a function of distance along the sample

At each point on the line, data was collected from 16 angles. This is sufficient to allow the construction of a non-destructive depth profile. Figure 9 is an example of such a profile taken from the data acquired 7.5 mm along the linescan, a point were the proportions of 1,7-octadiene and acrylic acid in the plasma feed are similar.

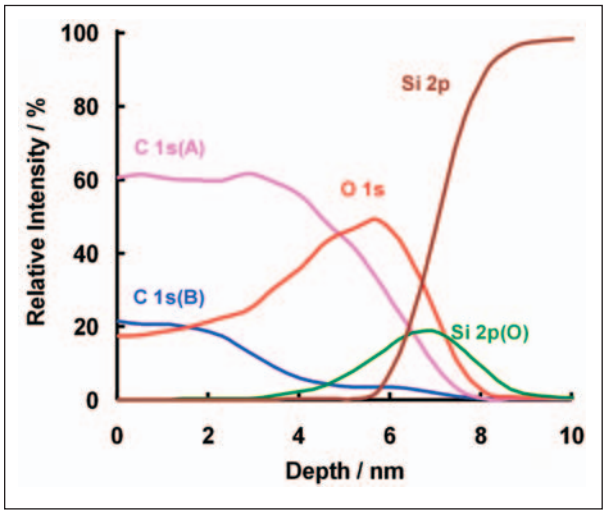

Figure 9: Non-destructive depth profile through the co-polymer layer at a point where both octadiene and acrylic acid species are present in the plasma in roughly equal proportions.

It is clear from this profile that neither of the monomers segregates to the surface because the proportion of carbon in each of the chemical states is almost independent of depth. The Si 2p (oxidized) forms a discrete layer at the interface between the plasma co-polymerized layer and the silicon substrate. This is indicative that the copolymer layer is continuous.

Immobilization of Antibodies on the Chemical Gradient

The aim of this work is to produce an antibody concentration gradient across the surface, controlled by the chemical gradient. Once the gradient has been prepared, immobilization of the antibody is a two step process. First, the surface is treated with EDC (N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride) and NHS (N-hydroxysuccinimide). This produces a succinimide layer, the concentration of which is dependent upon the acid group concentration at the surface of the co-polymer. The next step is to treat the surface with the antibody, in this case bovine IgG. The antibody is expected to covalently couple to the EDC/NHS but not to the polymer surface. In that way it should be possible to prepare a concentration gradient of immobilized antibody material.

Control measurements were performed on the chemical gradients. First, measurements were made on the EDC/NHS treated surface. The second control was to treat the gradient with bovine IgG only.

Control 1

The EDC/NHS treated surface was analyzed using XPS. The plasma co-polymer in the chemical gradient does not contain nitrogen while the EDC/HNS does contain nitrogen. This means that the intensity of the N 1s signal in the XPS spectrum is related to the concentration of EDC/NHS.

Figure 10 shows 2-dimensional maps of the N 1s and C 1s atomic concentration from a chemical gradient sample treated with EDC/NHS. The C 1s distribution is very similar to that shown in Figure 7. The N 1s concentration can be seen to be greater at the end of the sample having the greatest concentration of acrylic acid, as expected.

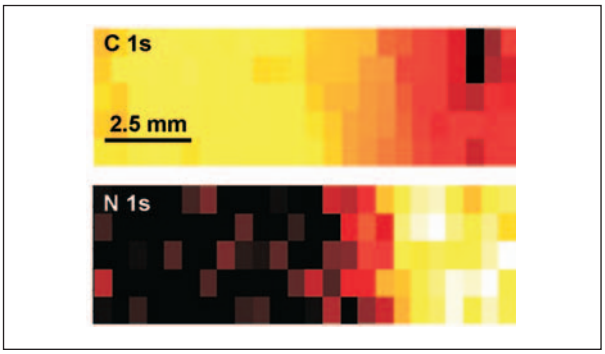

Figure 10: C 1s and N 1s atomic concentration maps

Control 2

In this experiment, the chemical gradient was treated with bovine IgG. Again, the N 1s XPS peak provides a useful marker for the antibody. A map of the nitrogen atomic concentration is shown in Figure 11.

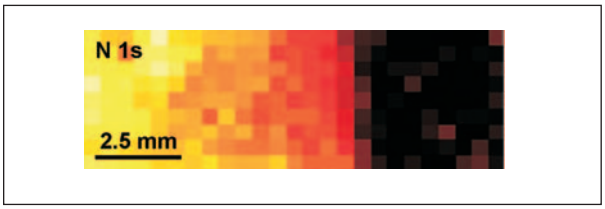

Figure 11: N 1s atomic concentration map following the treatment with bovine IgG

Here, the nitrogen signal is greatest at the part of the surface that contains the highest concentration of pp-octadiene, suggesting non-specific adsorption of the antibody. The reason for this may be that the non-specifically adsorbed antibodies are more easily removed from an acidic surface.

Immobilization of Antibodies

To prepare this sample, the antibody was added to the EDC/NHS treated surface. The N 1s XPS signal was then mapped. The result is shown in Figure 12.

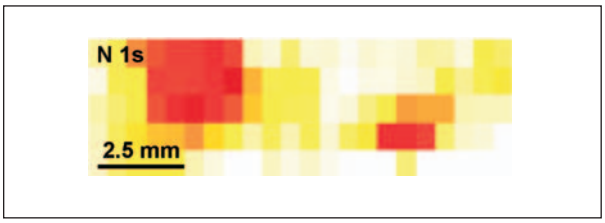

Figure 12: N 1s atomic concentration map following the treatment of the ECD/NHS surface with antibody

There is no clear gradient visible in Figure 12. This suggests that there is a mixture of specific adsorption at the pp-acrylic acid end of the chemical gradient where the EDC/NHS concentration is greatest and non-specific adsorption at the other end of the gradient.

These results suggest that the coupling reaction does take place in the desired manner but that some means must be found to remove the non-specifically adsorbed antibody from the hydrocarbon surface.

Conclusions

XPS and ARXPS are powerful tools for the characterization of both chemical gradients and immobilized antibodies. The use of XPS confirmed that the chemical gradient was present. As well as chemical state information, the techniques provide information on the distribution of the materials within these layers. The uniformity of coverage can be measured and, if present, the segregation of material to the surface can be detected from the non-destructive depth profiles.

An ARXPS linescan has been used to measure the thickness of the layer. In this case, it was found that the thickness depends upon the composition of the co-polymer.

For antibody immobilization, XPS has shown that EDC/NHS coupling correlates with increasing acid group concentration and that non-specific antibody adsorption has the opposite correlation.

Attempts to immobilize antibodies on the gradients were hampered by the non-specific effects. Additional work is required to minimize the non-specific binding.

Acknowledgement

Thermo Fisher Scientific would like to thank Kristina Parry and Jason Whittle of Plasso Technology Ltd., UK (www.plasso.com) for supplying the chemical gradient surfaces analyzed in this work.

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