

Analysis of chemical interactions on customised surfaces

Customised surface chemistry is of interest in a lot of applications. Therefore many possibilities for modifying the surface chemistry were developed. For example this can be done by passive adsorption or covalent binding of molecules to the surface.

Also surface plasma modification, corona treatment or specific chemical treatments are possible methods to influence the surface chemical composition of materials to archive specific properties.



Introduction

For the following it is essential how we define “surface”. Since the top layer of the surface atoms are those which are the immediate interface with the other phases (atoms or molecules in gas, liquid or solid phase) impinging on it, this could be regarded as the surface. However, the structure and chemistry of that top layer of atoms will be significantly determined by the atoms immediately below. In a very real sense the surface can therefore be defined as the uppermost 2-10 atomic layers (about 0.5 – 3 nm) on the surface.

To understand the wanted (and also unwanted) properties and chemical reactivity of a surface, we require information about the physical topography, the chemical composition and structure of the surface.

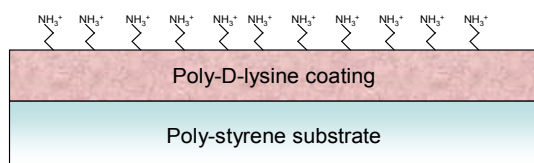


Fig 1: Poly-D-lysine

Here we present some results from a surface analysis of well-plates made of polystyrene, coated by poly-D-lysine. Poly-D-lysine is a non-specific positively charged amino acid polymer used to promote the attachment of biological cells to inert substrates.

Surface chemistry

The typical survey spectrum of a coated polystyrene surface is shown in Fig. 2:

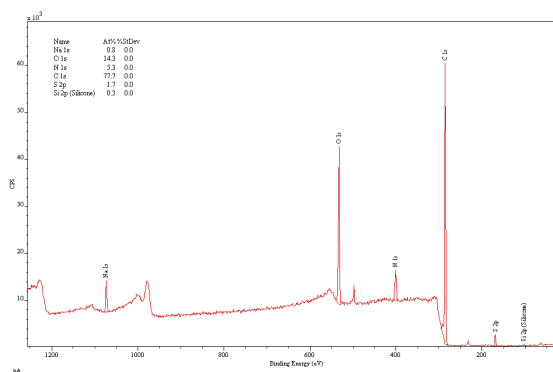


Fig 2: XPS analysis of poly-D-lysine coated Well surface.

XPS element analysis of the coating can be compared with the predicted composition from the molecular structure (excluding the anionic species). This comparison is made in Table 1 and the difference between the two compositions (experimental and theoretical) as is included:

Sample	Carbon	Nitrogen	Oxygen	Sulfur	Silicon	Sodium
Coating	77.7	5.3	14.3	1.7	0.3	0.8
PDL	66.7	22.2	11.1	--	--	--
Difference	11	-16.9	3.2	1.7	0.3	0.8

Table 1: Coating and poly-D-lysine Compositions and Differences
The coating contains not only poly-D-lysine but also at least one other component containing carbon, oxygen, sulfur and sodium (silicon is due to a low level of silicone). The most likely species containing these elements is sodium alkyl sulphates (SAS) which are common anionic surfactants used in coatings.

If all the nitrogen detected by XPS is due to poly-D-lysine then the carbon and oxygen from poly-D-lysine can be calculated from the molecular structure and these concentrations can be subtracted from the well coat composition as shown in Table 2:

Sample	Carbon	Nitrogen	Oxygen	Sulfur	Silicon	Sodium
Coating	77.7	5.3	14.3	1.7	0.3	0.8
PDL	15.9	5.3	2.6	--	--	--
Residue	61.8	0	11.7	1.7	0.3	0.8

Table 2: Coating, poly-D-lysine Element Components and Residues

The residue is similar to the expected composition of SAS of $\text{CH}_3(\text{CH}_2)_n(\text{SO}_4)\text{-Na}$ with a low level of silicone included. However the ratio of sulfur to sodium would be 1:1 in pure SAS compounds and the ratio in the coating is closer to 2:1. This can be explained by the quaternary amine group of the poly-D-lysine displacing the sodium from the SAS so that an ion pair is formed

The presence of this bonding is confirmed by a third peak, due to a quaternary amine state identified as $(-\text{N}(\text{H})_3^{+*})$ (at 403.6 eV) as shown in Fig. 3

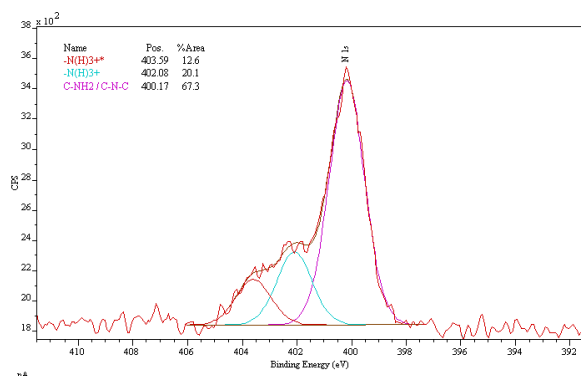


Fig.3: Nitrogen Chemical States in Well Coating

The high binding energy of the quaternary nitrogen in the ion pair state is most likely to be due to the high level of electron withdrawal from the nitrogen by the electronegative sulphate group.

Not all quaternary nitrogen side chains in the poly-D-lysine are ion paired to SAS and so the unpaired quaternary nitrogen groups (about 60% of all quaternary nitrogen groups) are available for attachment to cells and other negatively charged functionalities.

Depth distributions of elements in the near surface can be obtained by changing the electron takeoff angle in the XPS analysis. Analysis depth can range from 1-2

nm (at 75 degrees angle to the surface normal to about 10 nm at the surface normal. Analysis at three angles has been undertaken in this coating and these are equivalent to about 6.4 nm, 9.1 nm and 10.0 nm depth from the outermost surface. The element concentrations are presented in Fig. 4

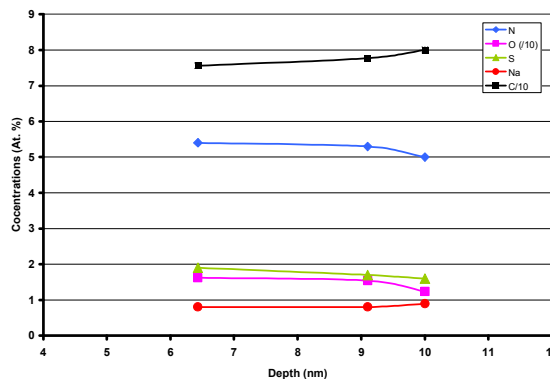


Fig.4: Angle Dependent XPS Depth Profile of Coating on Wells

The gradual trends in element concentrations indicated in Fig. 4, from the outer surface on the left to the bulk on the right, are expected from a well prepared structure and the following observations are made on these trends:

The outer surface is richer in nitrogen, sulphur and oxygen which are the characteristic elements of the ion pair.

The bulk is richer in carbon and sodium which would be expected from SAS not present in ion pairs.

Silicon (as silicone) is not indicated in Fig.4 but is depleted at the surface.

From the XPS analysis the coating can be described in the following model (Fig. 5)

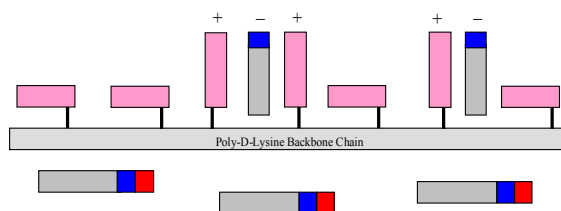


Fig.5: Reactive quaternary amine groups on the poly-D-Lysine (+) are pulled to the outer surface by ion pairs between some adjacent quaternary amine chains and alkyl sulfate surfactant species (+).

The alkyl sulphate ion acts as a "buoyancy aid" to increase the exposure of the unreacted quaternary groups. Excess SAS (+) is present below the surface to maintain an equilibrium with the anions on the outer ion pair surface.

Lateral homogeneity of the coating

The bottom of one of the wells was carefully removed and mounted on a SIMS sample stub. Positive and negative SIMS spectra were collected (not shown here).

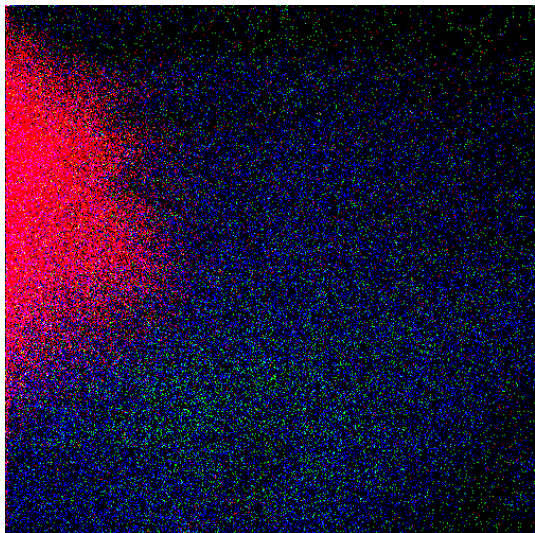


Fig.6: SIMS image of the well surface (area 2 x2 mm²)

Ionic species dominate the SIMS spectra from this well:

- Sodium is the main positive ion but mapping (red) shows that its distribution is non-uniform and concentrated in circular areas. Several negative ion species are detected.
- Chlorine distribution (blue) does not coincide with sodium species which indicates that the main sodium species is not sodium chloride.
- Sulphur oxide species (SO_x⁻, green) are detected and are distributed uniformly across the surface.

These ionic species are not part of the wanted poly-D-lysine coating composition. These are mostly sodium-containing species and may be caused by washing residues or drying stains left after the coating has been deposited. Further washing procedures are likely to be able to remove these ionic species.

Effect of washing the surface

After washing the surface with water, the surface composition is changed, which can be detected for example by XPS:

Dataset	C	N	Na	O	S	Si
unwashed	77.7	5.3	0.8	14.3	17	0.3
washed	86	3.4	0.1	10.2	0.3	0

Table 3: Poly-D-lysine surface before and after washing with water

Nearly all of the sodium, sulphur and silicone was removed by the washing procedure. This means that the detected SAS drying stains were more or less removed completely.

About 60% of the nitrogen signal is left. The nitrogen signal is derived from the poly-D-lysine coating, so the reduction of this signal means a reduction of the coating thickness, which is also supported by an increase in pi-pi* signal (derived from the polystyrene substrate) of the C1s-peak.

An interesting point is, that the value of 60% correlates with the amount of unpaired quaternary nitrogen groups (not saturated by SAS) which were available before washing for attachment to cells and other negatively charged functionalities (see above). This is supported further by the fact that the “3rd state” of the nitrogen peak vanishes after washing:

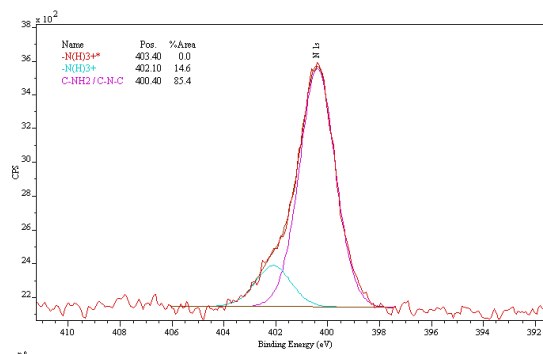


Fig.7: Nitrogen chemical States in well coating after washing