ESRF	**Experiment title:** Determination of the vertical distribution of antimicrobials in biofilms with High Energy Photoelectron Spectroscopy	**Experiment number**: LS 2741
Beamline: BM25B	**Date of experiment**: from: 13.06.2018 to: 18.06.2018	**Date of report**:
Shifts: 15	**Local contact(s)**: Dr. Juan Rubio-Zuazo	*Received at ESRF:*

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Report:

Introduction

In the EMPIR (European Metrology Program for Innovation and Research) project 15HLT01 MetVBadBugs (http://empir.npl.co.uk/metvbadbugs/), methods for the "quantitative measurement and imaging of druguptake by bacteria with antimicrobial resistance" are being developed. This will ultimately give new insights into the interaction between antimicrobials and bacteria. As a first step, investigations of suitable model systems are performed. The simplest model system is films made of agarose- a seaweed derived polysaccharide which resembles the exopolysaccharide matrix in biofilms. The antibacterial agent povidone-iodine (PVP-I) was added to the film, with iodine as a trace element suitable for HAXPES-measurements. The chemical structure of agarose and PVP-I is shown in figure 1. By varying the excitation energy between 7.5 to 12 keV, the vertical concentration profile of iodine down to 17 nm could be traced. Moving closer to a real biological system, we were also able to determine the elemental composition of fibroblasts (human skin cells), and compare it to results obtained by lab-XPS with an Aluminium Kα excitation source.

Results

For calibration measurements, two ionic liquids were measured: 1-butyl-3-methylimidazolium iodide (known as $[C_4C_1im]I$) and 1-methyl-3-propylimidazolium bis(trifluoromethlysulfonyl)imide (known as $[C_3C_1im]NTf_2$). Ionic liquids are molten salts exclusively consisting of ions, and the set stoichiometric composition together with lateral and vertical homogeneity, makes it a suitable calibration sample for quantitative XPS. Due to a malfunction in the detector, electrons with higher kinetic energy than ca 8,5 keV could not be detected, which limited the detection of carbon, nitrogen and oxygen. Thus, a complete atomic composition of the the samples could not be obtained.

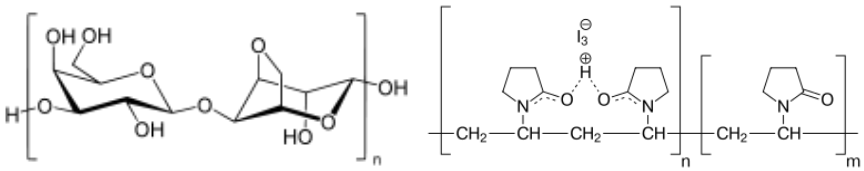

Figure 1: The chemical structure of agarose and povidone-iodine (PVP-I)

Further, three artificial biofilms were measured. Povidone-iodine (PVP-I) was added to the samples in varying concentrations: one sample 1% (sample a), two samples 10% (sample b and c). Carbon, oxygen and nitrogen could still not be detected due to the detector malfunction, but the iodine 2p core level, which lies higher in binding energies, could be recorded. To be able to make a semi-quantitative analysis of the depth-dependent amount of iodine, the Au $3p_{3/2}$ peak from a gold foil was measured as a reference. This was repeated for each sample- and energy change. The peak area was corrected for Scofield´s ionisation cross section, the electron attenuation length [1], the transmission function and photon flux. The corrected areas of the I $2p_{3/2}$ normalised to the corrected area of Au $3p_{3/2}$ are plotted in Figure 2. Error bars reflecting 20% uncertainty are added to each data point.

Figure 2 shows the vertical concentration profile of iodine in an agarose-film for electron kinetic energies of I $2p_{3/2}$ randing from 2957.9 to 7492.9 eV- with a corresponding information depth from 9.9 to 17.7 nm. The information depth z_{95} is defined as three times the electron attenuation length. The intensity from iodine was overall higher for sample a, which had the least amount of added povidione-iodine. These surprising findings were also confirmed by lab-XPS measurements, and imply that the agarose-PVP-I solution reaches saturation at 1% added PVP-I or less. Adding more does not increase the amount of iodine on the sample surface. Within the uncertainty of the measurements, the iodine concentration is either homogeneous (sample b) or slightly increasing (sample a and c) with increasing information depth.

The spectra were in general aquired from low to high excitation energy. Potential radiation damages were invenstigated for sample a by repeating the measurement at an kinetic energy of I $2p_{3/2}$ of 2957.9 eV after the sample had been measured at all other energies. Only the half of the normalised intensity could be observed for this measurement. A similar trend was seen for sample b. This imply that radiation damage cannot be ignored for the sample system. The measurements at higher excitation energy where done last, and it is therefore possible that the measured intensity would be even higher if these spectra were aquired at the beginning. Considering this assumption, the observed increasing iodine amount with increasing information depth seems to be reasonable.

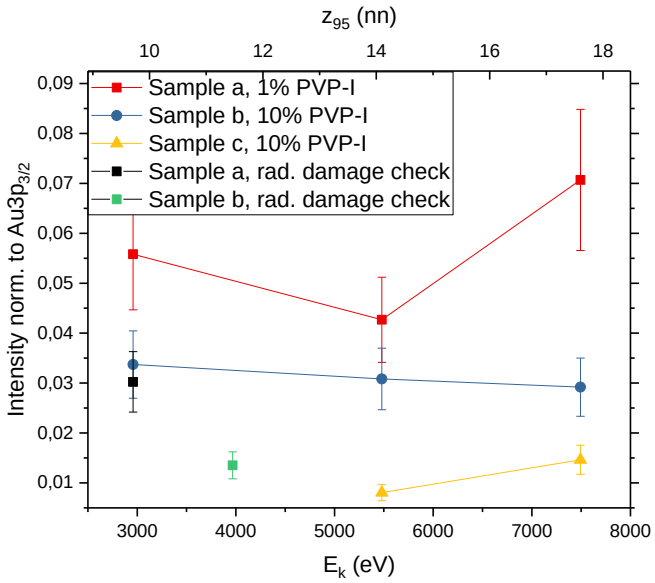

Figure 2: Intensity profile of I2p$_{3/2}$ for the three agarose-PVP-I samples measured.

To move closer to real biological systems, a sample consisting of a uniform cell sheet of fibroblasts (human skin cells) was measured. Prior to the beam time, the sample had been characterised by lab-XPS and the homogeneity and purity of the sample had been confrmed. For this sample, it was possible to obtain the spectra of the light elements C, O, and N, thus, a full quantitative investigation was possible. To assess the effect of radiation damages, the series of spectra (C 1s, O 1s, N 1s, P 1s) were aquired twice. The atomic composition is listed in table 1, compared with the atomic composition obtained with lab-XPS at lower excitation energy.

Most notably, the carbon content was lower for the HAXPES-measurements compared to the lab-XPS measurement, whereas the amounts of the other elements were higher or the same.The increasing carbon content at lower information depth could be explained by adventitious carbon, which contribute to a higher amount of the signal at lower information depth.

Table 1: The atomic composition (%) of a sample of fibroblast-cells on a Si-wafer. Measurements with 1486.6 eV excitation energy where perfomed with lab-XPS prior to the beamtime.

Excitation energy (eV)	**Z_{95} (nm)**	**C 1s**	**O 1s**	**N 1s**	**P 1s**	**P 2p**
1486.6 eV	6.1-6.6	78.9	13.2	7.0	-	0.9
7515 eV #1	14.4-17.4	68.3	20.7	10.0	1.0	-
7515 eV #2	14.4-17.4	70.5	19.8	8.9	0.8	-

Conclusions

It could be shown, that HAXPES is a promising tool for the investigation of the interaction between antimicrobials and biofilms. For the iodine containing samples it was possible to obtain further information about the vertical distribution, but the beam damages must be takeen in account. The results for the real biological systems like cell sheets motivate to further investigations at such systems. An analysator working with an electron kinetic energy up to 15 keV would be desirable for our systems due to the larger information depth which is for such biological systems an important issue.

Acknowledgements

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References

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