Chapter 6

Conclusions

The work described in this thesis has been undertaken in order to allow a greater understanding of the use of surface analytical techniques for the characterisation of rapidly evolving medical devices and treatments. The use of polymers in medicine has become more widespread over the decades and surface analytical techniques have developed to a stage where a great wealth of chemical and structural information can be gleamed in a short period of time. Hence the optimisation of the application of surface analytical techniques and understanding of the formulation are of critical importance. It is this relationship which has been explored in this thesis. Whilst the surface is critical to the performance of specific pharmaceutical delivery systems, the study of the complementary bulk properties is also a focus of this work. Through an analysis of the bulk properties of the systems presented in this thesis and equipped with knowledge of the fabrication process, results based inferences can be made to improve the performance of such formulation for their eventual use.

It is an established fact that the complementary use of multiple surface analytical techniques is capable of providing a deep understanding of the chemical and molecular structure of the sample[1-3]. As described in Chapter 2, the quality of the data obtained is largely reliant on the accuracy, sensitivity, specificity and resolution attainable with the techniques used. In addition the limitations of each technique must be fully understood in order to obtain the most from the analysis. ToF-SIMS has been the main technique used in the work presented as it has high surface specificity, can be operated at nm spatial resolution and is highly sensitive to the surface chemistries encountered in the organics analysed in this work.

The aim at the start of this thesis was to expand the knowledge of the capabilities of ToF-SIMS for the analysis of biomedically relevant systems in terms of depth profiling and improving lateral resolution in imaging. Through the use of surface analytical techniques applied to three formulations of increasing complexity, ToF-

SIMS has proven to be capable of analysing increasingly complex biomaterials and interfaces with varying degrees of success discussed throughout this thesis.

A new capability of XPS analysis, depth profiling of organic material, was applied to a simple binary blend of polymer and drug for the first time. The rationale was to test the feasibility of the technique on a relatively simple thin film before gradually applying it to more complex systems. As such, a blend of the polyester PLA and the small molecule drug codeine was produced and spun cast to produce a ~100 nm thick film and depth profiled using both quantitative XPS depth profiling and qualitative ToF-SIMS depth profiling. These two techniques being complimentary in nature were ideal for comparison. As the C_{60}^{+} sputter source for ToF-SIMS organic depth profiling had only recently been installed this provided an opportunity to assess its performance. Both the reference spectra for ToF-SIMS and XPS of PLA and codeine were in general agreement with the literature and the elemental composition expected was observed for codeine. The uniformity of the sample thickness, composition and surface topography were confirmed using ellipsometry and AFM respectively. This is crucial as if a comparison is to be made between the data obtained from each technique the uniformity of the composition of the nominally created films must be maintained. AFM showed a dewetting phenomena presenting as pores in the resulting film when cast on a solvent cleaned silicon wafer. The presence of pores would be expected to affect the repeatability of any depth profiling especially if they extend through to the silicon interface. A systematic investigation lead to the use of piranha solution for cleaning the silicon wafers which allowed for flat uniform films to be produced suitable for depth profiling. Within each drug loading, the thickness of the films was maintained within a narrow range with the greatest standard deviation being 2.59 nm for the 28.6% codeine/PLA (w/w) loading. The EMA model used to approximate the concentration of drug within each polymer film showed a remarkable linearity indicating the step wise increase in drug concentration within the PLA films confirming the samples suitability for comparison between the various drug loadings. XPS analysis showed a surface depletion of codeine that was mirrored by the ToF-SIMS analysis, however this depletion could be quantified for each formulation for the first time. Due to the adherence to cycles of sputtering and analysis for XPS and continuous sputtering for ToF-SIMS the data points presented are spread more for XPS. The surface depletion was found

to extend ~18 nm into the film for the 28.6% drug loading, ToF-SIMS generally indicated sharper interfaces, reasons for this were discussed in Chapter 3. These reasons include the surface sensitivity of SIMS being up to ten fold greater than that of XPS. As mentioned the cycles of sputtering and analysis could affect the apparent depth of the zone of depletion. Finally, the experimental coronene ion source used for XPS depth profiling compared to the established C_{60}^{+} for ToF-SIMS could contribute to the variation in the extent of the surface depletion observed. Quantitatively it was evident the bulk enrichment offset the depletion observed at the air/polymer and polymer/silicon interfaces observed showing how the ellipsometric modelling provided an accurate guideline for composition in such model systems. SIMS suggested some codeine enrichment at the silicon interface however this is likely due in part to an increase in secondary ion yield on encountering the inorganic conductive silicon interface highlighting a matrix affect described in Chapter 2. This is a limitation of the SIMS technique where any actual enrichment (suggested for lower drug loadings) would be amplified by an unknown factor making quantification of such data difficult. XPS shows the decline in nitrogen intensity to occur gradually without the enrichment observed with SIMS. This may suggest especially for the lower drug loadings XPS is not a sensitive enough technique for the analysis of this model where the key differentiator is one nitrogen atom per codeine molecule. Finally it was shown that XPS depth profiling is able to quantitatively show how the PLA was fragmenting under sputtering by chain scission of ester bonds, supported by the intense ester fragments in the ToF-SIMS depth profiles. This was shown by tracking the deviation from the model calculated for unsputtered codeine/PLA blends. ToF-SIMS and XPS are shown to be complementary in the data produced from depth profiling in addition to the established complementary data obtained from surface scans. Importantly XPS depth profiling of organic material is shown to be a feasible technique for the analysis of biomaterials warranting further research with samples of increasing complexity. The use of codeine as a model drug in this work shows highly consistent ion intensities with the extensive ToF-SIMS analysis of films and uniformity of solution loading shown by ellipsometry, ToF-SIMS and XPS respectively. However, for XPS depth profiling a drug molecule with more distinctive nitrogen atoms would have provided an improvement to the drug signal intensity that may have provided greater depth resolution obtained in XPS depth profiling.

In Chapter 4, ToF-SIMS depth profiling has been employed to further scrutinise its capabilities to depth profile polymers with biomedical relevance. This built on the work of Chapter 3 adding a drug free layer of HPMC either above or below a film of 28.6% codeine/PLA (w/w) for which reference data from the work of Chapter 3 was available. The binary film of PLA cast on top of the HPMC showed the corresponding ions for the polymers used were found to increase and decrease as expected at the interface between the two polymer layers which was displayed as a relatively sharp interface. Codeine however had diffused and uniformly distributed throughout the underlying HPMC layer. This was again observed in the trilayer films further supporting the inference that during the spin casting process the chloroform had solubilised the underlying HPMC layer allowing for small codeine molecules to penetrate the HPMC matrix whilst the PLA remained at the surface. This work shows how the use of ToF-SIMS is capable of characterising such multilayer systems, able to demonstrate diffusion of drug from one layer to another, the integrity of the interfaces between the layers and allowing for inferences to be made of the quality of the formulation that can be applied in industry.

Sodium ion intensity was linked to that of HPMC, this contaminant was shown to increase within the HPMC layer and had the greatest intensity of all characteristic peaks. This is due to the ease at which sodium is ionised and extracted. The data indicated that sodium would preferentially segregate to the substrate interface whether that is polymer or silicon. The matrix effect is clearly apparent at the inorganic silicon interface whereby any sodium enrichment is amplified. This limitation however still provides qualitative information indicating ToF-SIMS depth profiling is a technique of merit for the analysis of samples produced by the medical industry.

Addressing further the aims of this thesis, interface broadening was observed and attributed to the effect of HPMC casting atop a codeine/PLA layer. This was discussed in Chapter 4 with possible causes of this attributed to mixing of adjacent layers either in the casting process or by the ion beams used. The depositing of a layer on a rough layer can also act as a template transferring the roughness to the top layer but as the uniformity of film topography had already been established in Chapter 3 for the codeine/PLA layers this can be neglected for

samples where HPMC is cast on a PLA substrate. It is also plausible ion-induced topography formation may have occurred when depth profiling through the HPMC. This is as it is prone to cross linking on exposure to the ion beams leading to sputtering at less uniform rates causing topography capable of broadening the interface slightly as shown in Chapter 4. It is also generally acknowledged that roughness increases through thickness when depth profiling with C_{60} as there is a decline in the sputtering yield[4]. It is difficult to elucidate which if any of these possibilities is occurring the data generated, still the data has proved informative to the aims of this work. Knowledge of such limitations allows for informed decisions to be made based on such analysis and alterations in formulation i.e. casting the codeine/PLA layer from a less volatile solvent to be made.

Improvements such as large argon cluster gas sources have been developed to improve interfacial widths recorded. These argon clusters are currently gaining in recognition and availability and have been heralded by members of the surface analytical community to be capable of replacing C_{60}^{+}. As such it is an exciting time for cluster depth profiling, the merits of which have been shown in Chapters 3 and 4.

Chapter 5 while still focussed on expanding the knowledge of ToF-SIMS operation analysed a complex biomedically relevant controlled release formulation of microspheres in order to show the wealth of information that can be gathered from the complementary use of surface analytical techniques. SEM showed the macrostructure and indicated the presence of pores at the surface. Scrutiny of the images produced by SEM showed the surface of the microspheres surrounding the pore had been stretched suggesting a water droplet from the dynamic equilibrium stage of the double emulsion production method had begun to break the surfactant stabilised surface of the microsphere.

ToF-SIMS imaging analysis of the surface showed for the first time high lateral resolution imaging of biomedically relevant microspheres allowing for the precise surface chemical characterisation of a drug loaded microsphere. Of immediate significance was the evidence that the PVA surfactant which is often assumed to form a uniform coating at the surface of such microspheres is shown to be laterally incomplete at the surface. PVA appears to present a fractal drying pattern

that is likely due to film contraction once extracted and freeze dried. PVA was often found at the lip of pores observed supporting the SEM inference that these pores are due to surfactant stabilised water droplets breaking the surface.

The surfaces of the microspheres were sputtered in order to observe whether the surface PVA was an overlayer or a feature of the PLGA. With a relatively low primary ion dose of C_{60}^{2+} it was found this PVA layer could be removed. AFM was applied to the top of the microsphere surface before and after sputtering and showed a consistent ~4.5 nm thick smooth layer at the surface of the microspheres at nm resolution before sputtering. Once sputtered, analysis of the same microspheres showed a complete removal of the thin overlayer. This complementary use of ToF-SIMS and AFM confirming the overlayer found in AFM to be the ~4.5 nm thick PVA overlayer shown with both techniques before sputtering. The largely uniform PVA layer thickness observed supporting the formation of this film by film contraction on drying. The complete characterisation of the surface PVA at high resolution has provided valuable information for understanding the surface morphology of such microspheres.

The model protein drug lysozyme at the surface where concentrated is highly localised and observed at greatest concentration surrounding surface pores. This has been resolved down to ~200 nm lateral resolution (measured at the lip of a pore using an 84-16 regime) fulfilling an aim set out in Chapter 1. Where diffuse lysozyme is found at the surface it is shown to be linked to the distribution of PVA at the surface. This suggests the two constituents may be associated and it is likely this occurs when drying. This observation and relationship would be harder to discern without burst alignment operation of the ToF-SIMS bismuth beam, in addition the majority of less sensitive techniques would be unable to localise the distribution of the diffuse lysozyme. Lysozyme is the most commonly used model protein and while its results are not necessarily applicable to all proteins[5] it has some similar properties to BMP2 which would be used in a therapeutic formulation. For example the isoelectric point is 9 which suggests attractions to electronegativity[6] which is shared by lysozyme, this characteristic may contribute to aggregation within the microsphere to the carboxyl groups in the PLGA substrate.

Bulk characterisation used both invasive ToF-SIMS of an ultra-microtomed section of microspheres and non-invasive confocal Raman microscopy. The ToF-SIMS sectioning indicates pores ranging in size from 2 - 18 μm in diameter from the plane shown in the ion images. They also suggest the pores visible contain lysozyme, PVA or are vacant. The confocal Raman spectroscopy however shows the larger pores actually contain a coating of lysozyme and only the 2 μm pores contain solid protein. The pore sizes are consistent between the two techniques (although resolved better in ToF-SIMS) and ToF-SIMS is capable of showing the low concentration of PVA surfactant within pores whereas the Raman has more difficulty showing this. The high resolution ion images in this case are highly complementary to the plane specific Raman mapping. This work has fully characterised the controlled release formulation allowing inferences to be made as to why each constituent is distributed as they are. Using this information the formulation can be tailored to provide the best clinical performance. A precedent for future analysis of such advanced pharmaceutical formulations with the array of techniques has been presented in this work.

The goals set out in Chapter 1 were achieved however it has become clear through the work of this thesis that significant improvements are needed to optimise the application of ToF-SIMS to the characterisation of organic materials. As alluded to earlier the rapid development of next generation sputter sources is the most promising development for ToF-SIMS organic depth profiling as these sources are capable of improving the depth resolution attainable with conventional C_{60} primary ions and reducing the observation of ion beam induced mixing/ topography generation[7]. This improvement is due to the use of gas clusters ranging up to Ar_{1000} spreading the impact energy thus sputtering a shallower crater in organic films increasing the depth resolution attainable without causing excessive damage due to the spread of impact energy and gentler ionisation regime. C_{60}^{+} however has recently been shown to improve in its sputter rate over time and hence reduction in sputter induced topography by stage cooling and sample stage rotation[4]. The work on the multilayer model could have been improved by attempting sample stage cooling or rotation however the data obtained suggests biomedically relevant systems are amenable to analysis with ToF-SIMS and XPS depth profiling.

In this thesis the complexity of the samples analysed was advanced in successive chapters in order to test the limits of ToF-SIMS while discerning the application of such techniques to polymers with biomedical relevance. As such the new technique of XPS depth profiling of organic material was shown to quantitatively show the distribution of drug throughout the depth of a binary blend model of a cardiac stent coating. The distribution of drug within a polymer multilayer was shown to be intimately linked to the fabrication process with ratiometric comparisons providing a quasi-quantitative appreciation of drug distribution through the thickness of the multilayer. Finally a range of surface sensitive techniques were used in the characterisation of a formulation produced for the delivery of protein drug from within a tissue engineering scaffold. The wealth of chemical and structural information provided by these techniques has highlighted the use of surface analysis for improving the understanding of advanced drug delivery devices and formulations in the interest of improving their eventual use. The techniques and analysis used in this thesis are extensive and have built on the literature to improve both the application of the techniques and the formulation of the microspheres shown in Chapter 5.

The work outlined in this thesis is encouraging for the continued proliferation of surface analysis for the characterisation of medical devices and controlled release formulations, which are ever increasing in complexity. The detailed analysis of these complex formulations are a necessity due to the benefits that the final formulation can gain from the analysis outlined throughout. Whilst the application of XPS depth profiling was on a relatively simple model film, the scope is there for advances such as those seen in recent years for ToF-SIMS depth profiling with the advent of cluster ion sources and improved organic depth profiling to be made in the field of XPS depth profiling of organic material. This is a field which would be expected to vastly increase as the availability of such ion sources becomes more widespread.

The analysis and identification of new biomedically relevant polymers with favourable characteristics in a high throughput (HT) manner is now possible as shown in Chapter 1 in the form of microarrays of polymer spots. Advances such as these which rely heavily on the characterisation capabilities outlined here for the improvement of the formulation composition of biomedically relevant

polymers are one of the ways the field of surface analysis is expected to expand. While the techniques outlined in this work are capable of rapid initial identification of candidates for medical application the versatility of such techniques allows for them to be applied to scale up models of favourable polymers. This is important in determining whether the characteristics of each polymer are maintained when made into a form which is of comparable thickness to those used in the final application. Advances in ambient mass spectroscopy techniques such as in desorption electrospray ionisation (DESI)[8] show promise for extending surface analysis from the lab to having a more general use in industry and the public sector for HT quality assurance[9] or the detection of explosive residue/narcotics[10, 11]. However as shown in this thesis the application of a combination of techniques is a necessity in understanding the influence of the formulation and fabrication process on the resultant properties of the biomedically relevant systems. With further development of the techniques described in this work the future of polymer use for biomedical applications is sure to expand becoming the gold standard in patient treatment for increasing pathologies.

6.1 References

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