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Quantitative XPS depth profiling of codeine loaded poly(L-lactic acid) films using a coronene ion sputter source

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Abstract

The controlled release of active pharmaceutical ingredients from polymers over prolonged periods of time is vital for the function of drug eluting stents and other drug loaded delivery devices. Characterisation of the drug distribution in polymers allows the *in vitro* and *in vivo* performance to be rationalised. We present the first X-ray photoelectron spectroscopy (XPS) depth profiling study of such a drug eluting stent system for which we employ a novel coronene ion sputter source. The rationale for this is to ascertain quantitative atomic concentration data through the thickness of flat films containing codeine and poly(L-lactic acid) (PLA) as a model of a drug loaded polymer device. A range of films of thickness of up to 96 nm are spun cast from chloroform onto Piranha cleaned silicon wafers. Ellipsometry of the films is undertaken prior to depth profiling to determine the total film thickness and provide a measure of the relative loading of drug within the PLA matrix through spectroscopic analysis. Progressive XPS analysis of the bottom of the sputter crater with sputter time indicated codeine to be depleted from the surface and segregated to the bulk of the polymer films by comparison with a uniform distribution calculated from the bulk loading. This serves to illustrate that surface depletion of drug occurs, which poses important implications for drug loaded polymer delivery systems.

Keywords

XPS; quantitative; depth profiling; drug eluting polymer; PLA

Introduction

Polymeric delivery systems have proven to be an effective means for the controlled delivery of drugs both in terms of the ability to programme the kinetics of release and the ability to target the location of delivery. One such polymeric delivery system that has received significant attention over the last decade is the drug eluting coronary stent, where the drug is dispersed within a thin biodegradable polymer film on the metal stent [1]. As the polymer degrades the drug is intended to be released in a controlled and predictable way to the local tissues. However, such thin polymer films provide considerable challenges both in terms of fabrication and characterisation. One of the key factors that controls the kinetics of release is the distribution of drug throughout the film and hence, fabrication procedures attempt to

ensure uniform drug distribution. A range of techniques have been applied to elucidate drug distribution within polymer films with varying levels of success [2–4]. Very recently, time of flight secondary ion mass spectrometry (ToF-SIMS) using coronene primary ions, a planar aromatic hydrocarbon $C_{24}H_{12}^+$ shown in Fig. 1, has been shown to exhibit similar performance to the well established organic sputtering source C_{60}^+ [5] and as such, may be applicable for the depth profiling of drugs in polymers. Recently, XPS depth profiling of thin organic films has been reported using a C_{60} sputter source showing that cluster ion sources are useful within the field of biomedical polymer analysis but it has not yet been employed to investigate drug loaded polymer systems [6–9]. Previously, the damage build up in organic films when etched using monatomic argon ions, found on all surface analysis instruments and intended for sputtering metal and inorganic samples, prevented such experiments [10, 11]. In this paper, the first use of XPS depth profiling to quantitatively characterise the drug distribution in thin polymer films is described illustrating its utility in this type of material system.

Experimental Section

Silicon wafers measuring 1 cm^2 were treated with piranha solution (consisting of a 3:1 mixture of 98% H_2SO_4 and 30% H_2O_2 in water) for 30 mins, followed by thorough rinsing with deionised water and drying with gaseous nitrogen before being used as the substrate for spin casting. Solutions containing 10 mg of PLA (Polysciences, Warrington, PA) per ml of chloroform with 0%, 2.4%, 4.7%, 9.1%, 16.7% and 28.6% codeine (Sigma Aldrich) were produced thereby yielding a range of drug loadings. The solutions were spun cast at 4000 rpm for 60 s using a Cordell spin coater Mk. 7 (Cordell Group, Stockton-on-Tees). Five separate drug loadings (2.4%, 4.7%, 9.1%, 16.7% and 28.6% w/w codeine:PLA) were produced in addition to a pure PLA reference sample. Ellipsometry was undertaken using an M – 2000DI spectroscopic ellipsometer (J. A. Woollam Co., Inc., Lincoln, NE). A triplicate of uncoated, cleaned silicon wafers measuring 1 cm^2 were used to ascertain the thickness of the SiO_2 layer.

For two samples (produced from solutions containing pure PLA and 28.6% w/w codeine:PLA) the ellipsometric data at high wavelengths ($>500\text{ nm}$) was used to obtain the film thickness, assuming the films were transparent in this region (extinction coefficient, $k=0$) and the dispersion in refractive index (n) could be approximated with a two parameter Cauchy function. The wavelength-dependent optical constants n and k were then calculated across the whole wavelength range (195 nm to 1700 nm) through a direct inversion of the ellipsometric data. The extracted values were then fitted with a Kramers-Kronig consistent dispersion function using Gaussian oscillators to provide wavelength-dependent values of $n(\text{PLA})$, $n(28.6\% \text{ codeine})$ and $k(\text{PLA})$, $k(28.6\% \text{ codeine})$.

It was assumed that the optical constants for films with an intermediate codeine drug loading are a linear combination of those of the pure PLA and the highest codeine film. Thus, $n = a.n(\text{PLA}) + (1-a).n(28.6\% \text{ codeine})$ at every wavelength; k is derived analogously. The value 'a' is a constant for each sample which represents the volume fraction of codeine normalised to the 28.6% codeine sample. All samples were fitted using 'a' and the thickness as variable parameters for the overlayer film, excellent fits were obtained in all cases.

The XPS spectra were acquired using an Axis Ultra DLD spectrometer (Kratos Analytical, UK) with a monochromated Al K α source producing a 450 W energy. The data was converted to VAMAS format and processed using CasaXPS, version 2.3.14. High resolution C1s, N1s, O1s and Si2p spectra were collected at a pass energy of 80 eV and a step size of 0.1 eV and quantified using empirically derived relative sensitivity factors provided by Kratos Analytical. The pressure in the analysis chamber was maintained below 2×10^{-8} mbar for data acquisition. The coronene primary ion source was mounted at 45 ° to the sample surface which itself was normal to the analyser. The coronene beam was operated at 12 keV energy and only C₂₄H₁₂⁺ (singly charged polyatomic ions) were used for depth profiling. This selection was made using a Wein mass filter. The raster size was fixed at 2.5 × 2.5 mm. XPS spectra were collected using a 110 μ m aperture.

Results and Discussion

Spectroscopic Ellipsometry

The consistency of drug loading and the thickness of doped PLA films were examined using spectroscopic ellipsometry. This was applied to three replicates of each of the six drug loadings. The films were optically homogenous and isotropic, satisfying the criteria for modelling the spectra obtained using ellipsometry. There was a good correlation ($R^2 = 0.9979$) between the solution composition, or drug loading, and the relative composition of the films generated from spectroscopic ellipsometry (Fig. 2). This confirms that the fraction of codeine in the films scales linearly with the fraction in solution. It is noted that the film thickness was highly consistent when cast from solutions with identical drug loadings, exhibiting a maximum value for the coefficient of variation of 2.9%. With the compositional uniformity across a range of samples confirmed, it was judged that the films were appropriate for quantitative assessment of coronene sputtering for a range of drug loadings.

XPS of Polymer Film Surfaces

The elements contained in PLA that are detected by XPS are carbon and oxygen, whereas codeine has one nitrogen atom per molecule within its structure. The chemical structures for PLA and codeine are shown in Fig. 3a & b respectively. Thus, using the nitrogen concentration, the concentration of codeine through the XPS analysis depth of the film may be calculated.

The elemental composition determined by XPS analysis of six films with different codeine loading was determined and the nitrogen composition is presented against drug loading in Fig. 4. The nitrogen concentration was found to be linearly proportional to the codeine loading ($R^2 = 0.9966$), calculated from the concentration of the solution used to spin cast the film. It was apparent that the measured nitrogen concentration was significantly lower than the nitrogen concentration calculated assuming uniform distribution of the codeine for all loadings (dashed line) indicating depletion of codeine at the surface (Fig. 4). The surface depletion inferred from the measured nitrogen concentration was proportional to the codeine loading (Fig. 4) suggesting that as the drug loading increased, a constant proportion of codeine segregated away from the surface to the bulk of the film. An alternative explanation of this observation is an overlayer of constant thickness of PLA is formed on all samples that

attenuates the bulk codeine signal by a constant factor for all drug loadings, our data is unable to confirm which of these scenarios occurs.

XPS Depth Profiling

To ascertain the depth scale, a uniform rate of sputtering through the sample film by the coronene source is assumed. This is an approach supported by other polyatomic sputtering investigations of PLA [12]. The time taken to reach the half maximum of the silicon substrate concentration was used with the depth of the sample film determined by ellipsometry to convert sputtering time to film thickness. The uniformity of the sputter rate was confirmed by plotting the time sputtered against the thickness which exhibited a linear relationship ($R^2 = 0.87$, not shown). The XPS derived elemental composition determined after each coronene etching cycle is presented as a function of depth in Fig. 5 for the sample with 28.6% (w/w codeine:PLA).

Again, surface depletion of codeine is suggested by the lower nitrogen concentration observed for the first point taken before coronene etching. Following further etching, the nitrogen concentration was observed to gradually increase and remain roughly constant within in the bulk followed by a steep decline upon reaching the interface with the silicon wafer. The increase in the nitrogen signal observed with coronene etching is consistent with segregation of the codeine away from the surface to the bulk of the sample. This trend, relative to each calculated solution drug loading, was observed for all other films analysed. This is in agreement with the systematically lower nitrogen concentrations seen in the analysis of films across the range of codeine loadings presented in Fig. 4. From the nitrogen profile in Fig 5, the zone of depletion at the surface may be up to 20 nm thick, since a steady state is attained after this depth. Similar results are found for all other concentrations.

The enrichment of PLA at the surface suggests segregation of PLA to the surface and codeine to the bulk, this would act to reduce the surface free energy of the film and this could be the driving force. Alternatively, rapid evaporation of highly volatile chloroform in the spin coating procedure may encourage surface segregation of PLA if the polymer chains do not have time to reach equilibrium and as such may be “frozen” at the air/film interface as noted previously for a polymer blend containing polystyrene and poly(methyl-methacrylate) [13]. Our data does not provide us with conclusive evidence on which, if either, mechanism is behind the surface enrichment of PLA/depletion of codeine.

The relatively gradual rise in silicon intensity with depth seen in Fig. 5 implies that there is a broad interface between the polymer and the substrate wafer, which we know not to be the case from analysis of the wafer which is very smooth (2.46 nm roughness). Various factors are known to affect the vertical depth resolution as a function of sputtering depth including the depth of analysis, intermixing in the crater and roughening, with the latter dominant when performing ToF-SIMS depth profiling, but the first may be significant in XPS analysis [14].

The component peaks were chosen based upon the shape of the core level envelope. Binding energy shifts from the C-C environment from the pure PLA and pure codeine samples were consistent with literature values [15]. They were applied with fixed relative intensities based

on the pure compounds to the core levels shown in Fig 6a. The C1s core levels acquired from the surface were fitted well by the set of component curves from PLA and codeine. Upon sputtering it was observed that the actual concentration of the C-O environment is reduced to a level lower than can be accommodated by the core level model using the individual pure components illustrated in Fig 6b. This small decrease in the relative intensity of the C-O environment of the sputtered surface is assigned to chain scission of PLA at the ester linkage forming a carboxyl group under coronene sputtering. No damage to the codeine could be discerned from the C1s fits, although the relatively low intensity of the codeine components in the C1s core level would make any such changes difficult to detect. The functional composition of the PLA-codeine surface was determined from curve fitting of the C1s region and is plotted versus depth in Fig 6c.

Two components were noted for the N1s region of the XPS spectra across all samples. These correspond to nitrogen within the codeine molecule and a protonated nitrogen environment. It is proposed that a hydrogen atom from carboxylic acid PLA end groups protonate a constant proportion of the codeine nitrogen atoms to produce this signal. This proposal is supported by the observation that the amount of protonated amine remains constant with increased codeine loading. The components of the N1s region for the 28.6% codeine loading remain at a constant ratio throughout the film thickness of 1:1.8 ($R_3NH^+:NR$) (not shown) however at the surface the ratio is closer to 1:1.2 suggesting that the PLA rich surface is responsible for greater protonation of surface codeine which is a trend found for all analysed drug loadings. The use of monatomic argon for traditional XPS depth profiling has been shown to degrade organic films significantly [7, 16], however the effect of damage on the functional composition using coronene is shown to be minimal and allows for accurate quantitative analysis of organic material through film thickness.

The formulation process assumes that drug loading is constant throughout the film and since the kinetics of drug release will be dependent on the concentration profile within the film this information is extremely important in rationalising and predicting the performance of drug loaded polymer devices. We have shown that it is possible to use XPS to determine a significant drug depletion or enrichment from the surface. This could be used to understand anomalous drug release behaviour such as the observed burst effect found where the rate of drug elution from the polymer film is far higher than expected within the first hours following implantation [17–19].

Previously PLA films have been shown to be sputtered at a constant rate when using C_{60}^+ primary ions, indicating that elucidation of a depth scale over a range of film thicknesses greater than those used in this study is possible using cluster ion sources [11]. The technique is capable of using an aperture to allow acquisition from large areas simultaneously, these experiments used an aperture measuring $110 \times 110 \mu m$, however areas of up to $300 \times 700 \mu m$ can be analysed. These results lay the foundations for future work monitoring the distribution of drug in simulated conditions thereby enabling elution profile interpretation using XPS depth profiling.

Conclusions

Through the combined use of ellipsometry and XPS of spun cast films, both before and after sputtering with coronene ions, we have been able to quantitatively conclude that surface depletion of drug occurs in the polymer films. For the first time we have been able to directly quantify the variation of drug levels within such films with depth profiling XPS analysis. The ability to interrogate the drug distribution levels within such polymer films will provide a greater insight into the influence of fabrication processes on film formation and also on the subsequent drug release kinetics from polymeric drug eluting stents.

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Fig. 1.
Molecular structure of coronene.

Fig. 2.
Concentration of codeine in PLA calculated from ellipsometry versus the solution composition for three replicates of each drug loaded film.

Fig. 3.
Molecular structure of a) PLA and b) codeine.

Fig. 4. Observed XPS surface nitrogen atomic concentration for all six samples (○). The solution concentration based on uniform distribution of the bulk loading (---) is also plotted.

Fig. 5.

XPS depth profile from coronene etched 28.6% drug loaded film showing C1s ($\square$), O1s ($\circ$), N1s $\times 10$ ($\blacktriangle$), Si2p ($\blacklozenge$) and the calculated nitrogen concentration assuming uniform drug distribution $\times 10$ (—) through film thickness. Dashed line indicates calculated Si substrate surface.

Fig. 6.

a) XPS spectra of C 1s region of the 28.6% drug loading at depth = 0 nm b) and depth = 8 nm charge corrected to the C-C at 285 eV. The model fit (—), the XPS trace (●), PLA components (---) and codeine components (....) are plotted. c) Functional composition of the C 1s region of the 28.6% drug loading plotted. Codeine component C-C (◇), C-O (□) and C-N (○). All PLA components are denoted by (△). Within the bulk of the film the actual codeine loading ranges between 30 and 35% (w/w codeine:PLA).