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Surface analysis for compositional, chemical and structural imaging in pharmaceutics with mass spectrometry: A ToF-SIMS perspective

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ABSTRACT

We review the application of time-of-flight secondary-ion mass spectrometry (ToF-SIMS) for the surface chemical identification and distribution analysis (mapping) of pharmaceutically relevant materials. Specifically we explore the characterization of both solid state pharmaceuticals and bio-pharmaceuticals by ToF-SIMS; highlighting specific case studies concerning the distribution and stability of pharmaceutical actives within solid matrices, the face-specific properties of pharmaceutical crystals and elucidation of the structure/conformation of adsorbed proteins. Finally, potential future applications of ToF-SIMS in pharmaceutics are detailed.

1. Introduction

Time of flight secondary ion mass spectrometry (ToF-SIMS) is a highly sensitive surface analytical technique which enables determination of surface chemistry and surface chemical mapping. Furthermore, the extremely short analysis depth (~1 nm) of ToF-SIMS enables molecular orientation to be elucidated. The history, theory, instrumentation, applications and sample preparation considerations of ToF-SIMS have been thoroughly described, and these books are recommended for further details (Grams, 2007; Vickerman and Briggs, 2001). Initially, ToF-SIMS application was dominated by inorganic materials, especially within the semiconductor and coatings industries and later, in minerals processing with regards to surface chemistry, organic contaminants and adsorbates. In many instances the surface of a sample in terms of texture and composition is most critical in controlling chemical, physical and biological interactions. It is here where ToF-SIMS offers one of its greatest advantages in the ability to study the surface composition. These features have made ToF-SIMS appealing for characterising the solid state behaviour of pharmaceutical powders and dosage forms (Aaltonen et al., 2009; Mahoney et al., 2005; Prestidge et al., 2007; Sakamoto et al., 2008; Zeitler and Gladden, 2009). More recent advancements in ToF-SIMS instrumentation, particularly with respect to the sample handling and analyser design, in combination with the availability of advanced ion sources (e.g., SF_5^+, Bi_n^+, C_{60}^+) has seen the technique expand its repertoire to analysing polymeric and biological samples, e.g., biomolecules, tissue samples and cells, including oocytes (Fletcher et al., 2007), HeLa cells (Brison et al., 2011) and other cells (Lanekoff et al., 2011). Boxer et al. (2009) and Vickerman (2009) have recently published informative reviews on the use of SIMS to image biological samples.

In this article we overview ToF-SIMS instrumentation and methodologies applicable to both solid-state pharmaceutical delivery and biopharmaceutical (soft) systems. We then consider three specific pharmaceutical application areas for ToF-SIMS, namely: drug and excipient distribution within pharmaceutical matrices, face specific properties of pharmaceutical crystals and conformation/orientation of peptides and proteins.

2. ToF-SIMS methods

2.1. Instrumentation

ToF-SIMS is a variant of so-called static SIMS, in which sub-monolayer surface sensitivity is attained without significant damage to the sample surface, providing both elemental and molecular information from highly complex surfaces. ToF-SIMS utilises a

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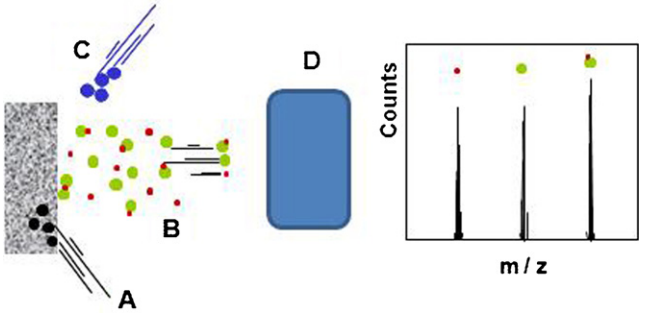

Fig. 1. Schematic diagram of ToF-SIMS system, including; primary ion beam (A), ejected secondary ions (B), electron flood gun for charge compensation (C) and mass analyser (D).

primary ion pulse that is accelerated and focused onto the surface of a sample held under ultra-high vacuum (Fig. 1). The bombarding particle subsequently impacts the surface resulting in a sputter plume containing secondary particles from the sample surface. These sputtered particles consist of atoms, whole molecules and fragments of molecules. A certain portion of these will posses a charge described by their species' ionisation efficiency. The charge on the secondary ion allows it to be accelerated, along with all other ions of the same polarity, to an equal kinetic energy. At this point, the ions enter a time-of-flight (drift) region where they become separated according to their mass-to-charge ratio (typically, the ions are only singly charged). Since each ion has equal kinetic energy, their drift velocity will be governed by the relation of kinetic energy to mass. Thus by measuring the time it takes for the secondary ions to reach the spatially resolved detector, the mass-to-charge ratio can be deduced and a mass spectrum ensues for every detector pixel. Thus a spectrum can be extracted for any region of an image and similarly, an image can be generated from any mass fragment. As one can expect, the positive and negative ions require separate acquisition and in addition, their expulsion from the sample surface can lead to a charge build up. Thus the sample needs to be held at an optimised potential and is ideally conducting, or easily neutralised with an electron flood gun. Most ToF-SIMS instruments available today are capable of sub-micron ion image resolution with simultaneous high mass resolution.

Initially, mono-atomic primary ion beams such as Ga^+, Cs^+, In^+ and Au^+ were used; for example, a Ga primary ion source provides a finely focused beam suitable for high resolution imaging down to 40 nm (Sakamoto et al., 2008). However, more recently other primary ion sources including reactive (e.g., SF_5^+) and cluster (e.g., Bi_n^+, C_{60}^+) beams are receiving increased interest, particularly for enhanced molecular sensitivity in organic materials' analysis (Mahoney et al., 2004; McDonnell et al., 2006; Nagy et al., 2008; Touboul et al., 2005) and biological samples.

Of particular interest in biological examination with ToF-SIMS are the large polyatomic primary ions which give rise to larger secondary ion fragments. The relevance of the C_{60}^+ development for biological analysis has been reviewed elsewhere (Delcorte et al., 2010; Fletcher et al., 2006). They highlight the improved secondary ion yields, lower damage rates and the ability to retain molecular information while depth profiling. For example, it has been reported that the yields obtained with C_{60}^+ are typically ~2 orders of magnitude higher than those measured with isoenergetic Ga^+ for bulk organic samples (Delcorte et al., 2007; Poleunis et al., 2006) and a significant improvement for biological media analysis over Au and Au cluster ion beams (Jones et al., 2006). Similarly, Cheng et al. (2007) reported that C_{60}^+ can be used to sputter for depth profiling with less damage to organic structures than Au_3^+.

A significant advantage of the ion detection systems utilised by ToF-SIMS is the acquisition of full mass spectra. This enables a user to study any fragment within the mass spectrum, or to extract a spectrum from any region of interest from an image. This ability to retrospectively reanalyse data is very powerful for complicated systems in which the fragments or regions of interest are difficult to predict.

Sample preparation for ToF-SIMS is typically simple. For hard, non-hydrated materials, sample preparation can be achieved by adhering the sample to the analysis stage or by sectioning for analysis of internal distributions (Kempson et al., 2002). The advent of more sophisticated sample handling systems, e.g., incorporating cryo-stages for the analysis of hydrated specimens requires greater consideration in sample preparation in terms of freezing and sectioning (if required).

2.2. Modes and data analysis

ToF-SIMS provides an incredibly versatile analytical method capable of detecting and analysing individual elements from H to U and their isotopes, molecular species, as well as gaining insight into structure, binding phenomena and molecular orientations. ToF-SIMS can be used in a range of surface analysis modes, including surface spectroscopy, 2D surface imaging and 3D depth profiling. The surface sensitivity and types of information gleaned through static SIMS, and ToF-SIMS in particular, is derived from the extremely low effective primary ion fluxes impinging on the sample surface. During the course of a ToF-SIMS experiment, less than one in a thousand surface sites may be disrupted by the beam.

Detection of hundreds of mg/kg can very comfortably be measured for metals (i.e., Cr, Mn, Ni, Cu, Zn and Pb) in a carbon based matrix (Denman et al., 2008). It is worthy to emphasise the sensitivity here, in that ppm concentrations can be detected by bombarding less than 1% of the first monolayer of an area of ~150 μm × 150 μm. In contrast, detection of organic fragments typically will not reach the same detection limits, especially for high molecular weights. In these instances it is beneficial to study a small fragment which can be confirmed to be indicative of the larger molecule of interest, but is acquired with a greater yield. Depth profiling can offer very sensitive (approaching 1 nm depth resolution) compositional and molecular information as a function of depth as well as provide full 3-dimensional distributions within the analysed volume (Wucher et al., 2009).

The matrix, information and depth sensitivity acquired in depth profiling is highly dependent upon the primary ion used. For instance, Ga^+, Cs^+ or O_2^+ primary ions will severely destroy organic matrices and are more useful for inorganic distributions, while C_{60}^+ or Ar clusters (Nieuwjaer et al., 2009; Ninomiya et al., 2009) enable preservation of the organic matrix in the sputtering process. The use of less destructive cluster primary ion sources, such as C_{60}^+, can provide the ability to conduct depth profiles yielding full mass-spectral information with depth resolution down to a few nanometers. An application of this has been shown for 3D reconstruction of lipid distributions in an individual freeze dried oocyte cell (Fletcher et al., 2007). Some caution needs to be taken regarding possible effects and implantation of the sputtering ions in spectral interpretation (Brison et al., 2011).

The complexity of ToF-SIMS analysis is greatly assisted with multivariate statistical methods capable of incorporating large amounts of data, such as with principal component analysis. A useful review of multivariate techniques, and their application can be found elsewhere (Wagner et al., 2003b). The power of such approaches has been demonstrated in the differentiation and imaging of tissues in a mouse embryo (Wu et al., 2007).

2.3. Challenges

ToF-SIMS presents numerous challenges as a technique, particularly with respect to sample preparation as well as the reliable quantification of data generated. When preparing samples for ToF-SIMS analysis, great care should be taken with the method used to mount the sample. This is of particular importance if the sample is to be dried down from a solution, careful consideration is required of the likelihood of drying-induced changes to the sample adsorbed conformation, particularly when working with soft (e.g., biological molecules) samples that may deform or change conformation upon drying.

It is worth noting that ToF-SIMS as a 'surface' analysis technique is also meant in a literal sense, in that theoretically a surface can only exist *in vacuo*. In the reality of application the 'surface' is in actual fact an interface and can exhibit properties that the ultra-high vacuum perverts. With this in mind much effort is being directed at using cryo techniques with the growing interest in the analysis of biological samples to retain features of the solid–liquid interface (Piwowar et al., 2011). Cryo-preparation for ToF-SIMS is not trivial since condensation and icing of the surface can occur and is disastrous for analysis.

The quantification of ToF-SIMS spectra is difficult both in a practical sense as well as a conceptual problem. The former is due to orders of magnitude changes in ionisation efficiency and yields of ions depending on species as well as the local chemical environment. The latter problem is due to the concept of the volume of analysis. ToF-SIMS is literally a surface analysis technique in that it is almost exclusively sensitive to the first monolayer of a sample within the static operational regime. In this sense, a typical unit of analyte mass per sample mass (or volume) becomes blurred. For instance (in loose terms), a substrate covered with a monolayer of an analyte will result in an apparent composition of somewhere approaching 100% with ToF-SIMS. X-ray photoelectron spectroscopy (XPS) however, sensitive to the 2–3 nm of a sample, could 'see' a composition of just a few percent, while SEM–EDX, Raman or LA-ICPMS could see on the order of 1%, and bulk analysis (digestive ICP-MS for example) a tiny fraction of a percent. Considering a surface with concentrations in the ppm regime, then the ability of other techniques to detect such concentrations would be highly questionable.

Depth profiling is an attractive analysis method for ascertaining different levels of a species at the surface or in the bulk of a material. However, depth profiling in ToF-SIMS is typically limited to hundreds of nanometres and can be challenging if ion beam etching causes chemical changes, i.e., damage during depth profiling. In addition, highly mobile ions can redistribute with charging of the sample surface and during depth profiling.

3. Pharmaceutical applications of ToF-SIMS

3.1. Spatial analysis of pharmaceutical carrier systems: drug/excipient distributions and molecular associations

The use of solid state dosage forms remains the preferred option for the delivery of a diverse range of pharmaceutical actives, for sustained release to control plasma drug concentrations as well as to reduce adverse side-effects and increase patient compliance. Drug and excipient distributions (and specific molecular associations) within solid state dosage forms are critical in controlling their pharmaceutical performance, e.g., shelf life and delivery characteristics. For both traditional core–shell powders (Edge et al., 2002; Prestidge et al., 2007) and inert porous matrices (Jarvis et al., 2010; Kempson et al., 2010a; Prestidge et al., 2008) ToF-SIMS has been used for chemical and structural analysis. Typical core–shell formulations consist of a core containing the active compound and a polymeric coating, which may contain numerous polymeric binder layers to achieve the required drug release profile. In contrast, for inert porous matrices such as silica, silicon or anodic aluminium oxide ToF-SIMS can be used to probe specific information such as the extent of surface modification across a porous membrane (Britcher et al., 2008; Jani et al., 2010).

Dissolution testing is routinely used to characterise drug release from solid dosage forms, however, it provides only limited information regarding the spatial distribution of the active pharmaceutical ingredient (API) within the matrix, typically inferred from the observed release profile. *In situ* surface chemical analysis techniques such as infrared and Raman microscopy have been used to map API distributions within a formulation matrix (Andrews et al., 2009; Belu et al., 2008; Bugay, 2001; Fisher et al., 2009; Gotter et al., 2010), but often lack dimensional resolution and molecular specificity. *Ex situ* techniques such as X-ray photoelectron spectroscopy (XPS) (Morales et al., 2007; Rafati et al., 2009) and scanning electron microscopy (SEM) (Barkay et al., 2009) provide significantly more detailed surface chemical information, however they give only elemental rather than molecular analysis information and have only specific applications. For example, XPS was used to probe the nitrogen to carbon surface chemical distribution in different morphine sulphate powders and this was related to different crytalline forms (Prestidge and Tsatouhas, 2000). This approach is sound in a pure API study, but would be compromised in studying solid dosage forms where excipients interfere. ToF-SIMS has the advantages of being highly surface sensitive, with a small analysis spot size and it can probe molecular ions (e.g., of drugs) or specific fragment ions, hence is highly specific and not compromised by interference from excipients.

ToF-SIMS can provide significant insight into the distribution of a therapeutic molecule within a delivery matrix, by sputtering/depth profiling of the sample surface. For example, a drug-enriched surface region of rapamycin within a poly(lactic-co-glycolic acid) (PLGA) stent coating was observed by depth profiling using SF_5^+ primary ions and was in good agreement with Raman imaging of the stent cross-section (Belu et al., 2008; Mahoney and Fahey, 2008). In addition, the drug distribution within the stent was obtained by imaging of the specific parent molecular ion of each drug, with observations ranging from distinct micrometer sized regions to completely homogeneous distributions, highlighting the significant potential for inter-batch variability in such dosage forms.

Several studies have used ToF-SIMS to map the distribution of drugs within solid state drug delivery systems by direct analysis of sample cross-sections (Belu et al., 2000; Edge et al., 2002; Prestidge et al., 2007). Cross-sections of three different controlled release pellet formulations (paracetamol, theophylline and prednisone) were studied with ToF-SIMS and the distribution of drug, excipients and coating characterised (Belu et al., 2000). Prednisolone was observed to be located within the core of the particles in clusters ~20 μm in diameter, as shown in Fig. 2. The authors were also able to clearly distinguish between the outer (ethylcellulose) coating and the core (prednisolone and lactose), as shown in Fig. 3. This methodology has also been successfully used to determine the distribution of different drug forms (hydrated and non-hydrated) within a controlled release pellet (Prestidge et al., 2007), see Fig. 4.

More recently, ToF-SIMS has been used to map the distribution of model drugs and proteins (lysozyme, papain, human serum albumin) within porous silicon matrices, using a Ga^+ primary ion source (Jarvis et al., 2010; Kempson et al., 2010a; Prestidge et al., 2008). Although the lateral resolution of ToF-SIMS was not sufficient to resolve protein distribution within individual pores of the silicon carrier (<20 nm), it was able to map the distribution of pro-

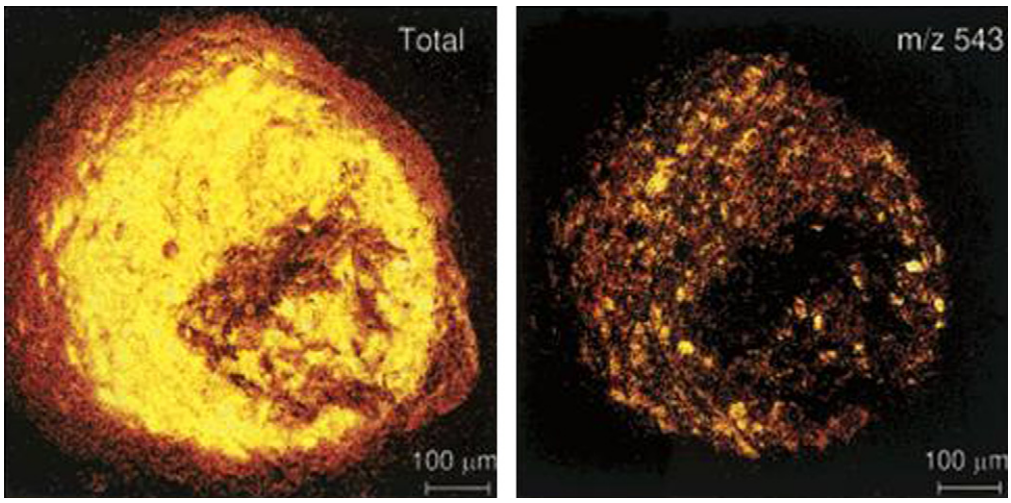

Fig. 2. ToF-SIMS cross section image (800 μm × 800 μm, negative ion mode). (A) Total ion image, the outer shell can be visually distinguished from the inner layer. (B) Prednisolone drug distribution, (prednisolone parent ion m/z 543) was observed only in the inner layer. Distance scale bar = 100 μm (Belu et al., 2000). Used with permission.

tein across the 60 μm porous layer. That is, the C_3H_8N fragment at 58.07 m/z from isoleucine (Suzuki et al., 2007) was used to map lysozyme distribution, as shown in Fig. 5. The C_3H_8N ion fragment was observed to be uniformly distributed across the porous silicon layer, indicating complete pore penetration for unoxidised porous silicon. In contrast, a significant reduction in protein penetration into the pore was observed upon thermal oxidation of the porous silicon, i.e., the C_3H_8N fragment corresponding to lysozyme loaded was only observed at the silicon surface, indicating that protein had not penetrated the pores.

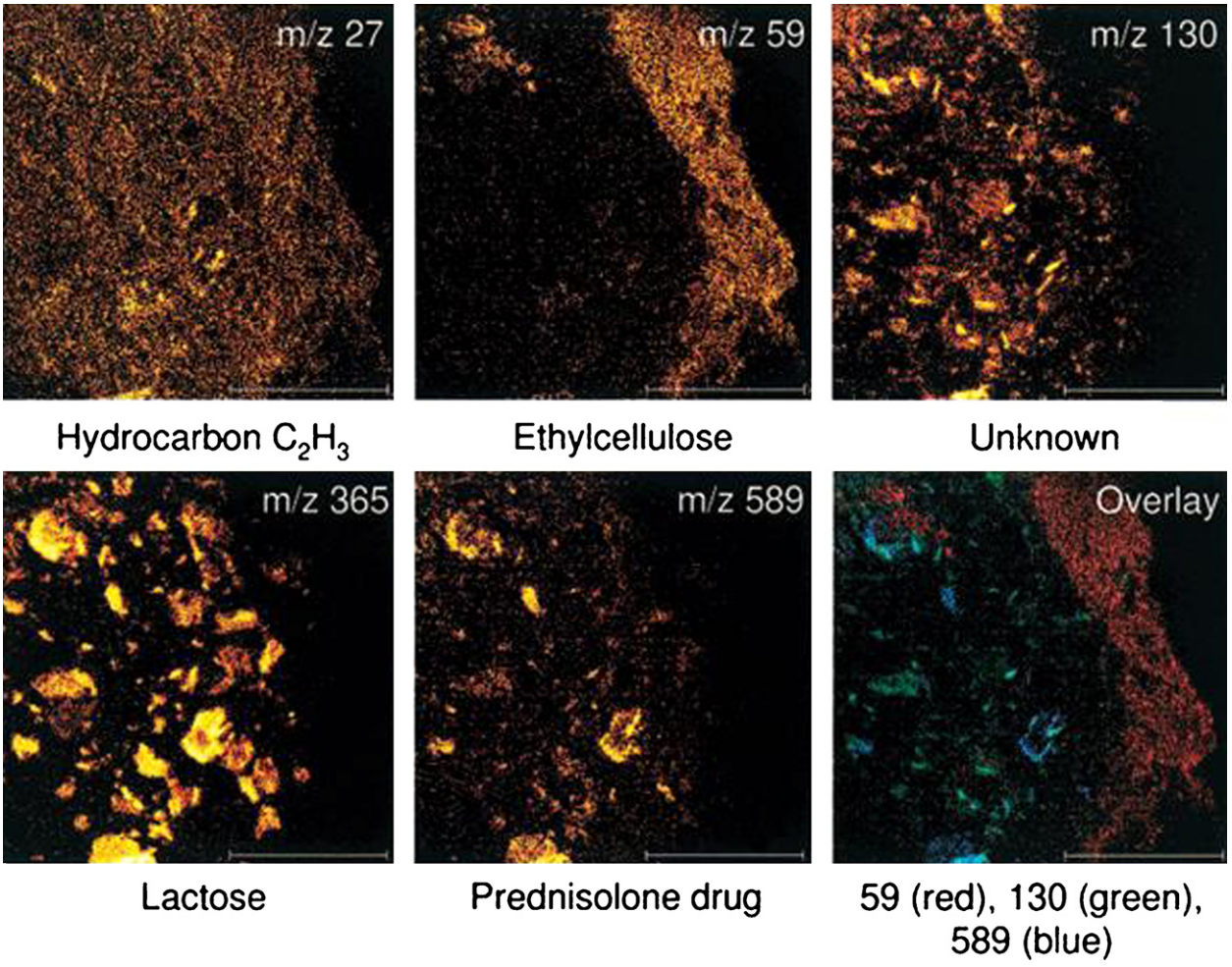

Fig. 3. ToF-SIMS cross section image (250 μm × 250 μm, negative ion mode), indicating spatial distribution of drug prednisolone (m/z = 589) and other formulation excipients including ethylcellulose (m/z = 59), lactose (m/z = 365) and other unidentified components. Overlay of individual signals also provided (Belu et al., 2000). Used with permission.

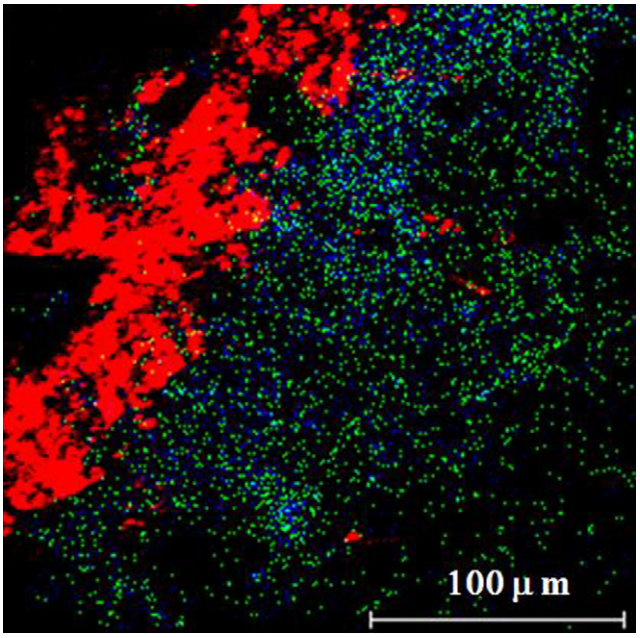

Fig. 4. ToF-SIMS cross section image of a pharmaceutical controlled release pellet: excipient (red), drug form 1 (blue) and drug form 2 (green). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

In addition to its ability to map the spatial distribution of individual ions across a 2D surface, ToF-SIMS provides the ability to discretely map an individual surface monolayer followed by a sputtering step to remove a thin layer of the surface. By repeating this process and obtaining an image after each sputtering stage, the sequential images can be combined to produce a three-dimensional reconstructed volume. The sputtering process can be delicate enough to penetrate a protein monolayer to infer distributions of functional groups as a function of depth at a surface (Fisher et al., 2009; Fletcher and Vickerman, 2010; Mahoney et al., 2006; Mouhib et al., 2010; Piwowar et al., 2011; Reichenbach et al., 2011; Wucher et al., 2009). For example, the 3D distribution of the immunosuppressant drug sirolimus in a stent coating (PLGA matrix) was imaged as a function of elution time using an Au^+ ion beam for analysis in conjunction with a $C_{60}{}^+$ ion beam for sputter depth profiling, as shown in Fig. 6. From the 3D image, it is possible to observe large areas of the surface as well as subsurface channels that are composed primarily of the drug, as well as drug-depleted regions within the PLGA matrix. In this study the effectiveness of a $C_{60}{}^+$ ion beam for use in 3D characterization of organic systems was demonstrated compared to a $SF_5{}^+$ polyatomic ion source, hence highlighting the importance of ion source selection when analysing organic materials using ToF-SIMS (Fisher et al., 2009).

Of further note, with the emerging fields of pharmacogenomics and metabolomics leading to personalised medicine, there is considerable interest in directly monitoring the distribution of drugs after they have been delivered into biological systems. This is currently achieved using radio-labelled compounds, however, this presents numerous challenges with respect to both synthesis and handling/transport/storage of the radioactive compounds. Due to its high elemental sensitivity, spatial and depth resolution, ToF-SIMS can monitor the presence of exogenous compounds directly in a biological sample (without the need for labelling) (Clerc et al., 1997). Several challenges remain however, including the specific methodology required to cryogenically prepare the biological sample for imaging (a flat substrate is required) as well as the so called "sample matrix effects" where changes in the local sample environment can significantly alter the observed mass distribution (Arlinghaus, 2008; Börner et al., 2006; Brison et al., 2011; Clerc et al., 1997; Fletcher et al., 2011; Fletcher and Vickerman, 2010; McDermott et al., 2010; Piwowar et al., 2011; Volandri et al., 2010).

3.2. Characterising pharmaceutical crystal faces

There is considerable interest in the relationship between drug crystal form and its corresponding behaviour both *in vitro* (e.g., during processing) and *in vivo* (dissolution kinetics versus absorption pathway). This behaviour arises due to changes in the specific molecular orientation at each crystal face, and can have a significant influence on key physicochemical parameters, e.g., the wettability of the various crystal faces (Kiang et al., 2009; Muster and Prestidge, 2002a; Prestidge and Tsatouhas, 2000; Puri et al., 2010). The high level of surface sensitivity in ToF-SIMS analysis enables molecular orientation to be probed and hence the face dependent chemical properties of pharmaceutical crystal to be elucidated. The surface chemistry of specific crystal faces of two model drugs, N,N-octyl-D-gluconamide (OGA) and sulfathiazole (STZ) has been characterised using ToF-SIMS (Muster and Prestidge, 2002b). Ion distribution patterns in the ToF-SIMS spectra correlated with the specific molecular orientation at each crystal face as determined by molecular modelling, and also concurred with the sessile drop contact angles and surface roughness as determined by AFM of individual crystal faces.

Specifically, the surface ion concentrations for each mass fraction were estimated by normalising the integrated peak area of interest against both the total ion count (TIC) and the sum of the parent ion and parent ion fragments, i.e., the total molecular count (TMC) (Walls, 1989). Good correlation was observed between the ion fragmentation pattern and the crystal face properties, e.g., the hydrophilic [0 1 0] and hydrophobic [0 $\bar{1}$ 0] faces of OGA, which exhibited distinctly different face specific contact angles (sessile drop) of $\theta = 46^\circ$ and 76°, respectively. This change reflects a difference in the physical arrangement of OGA molecules at the respective crystal faces, i.e., the alkyl chain is exposed at the [0 $\bar{1}$ 0] surface.

For STZ crystals (see Fig. 7), characteristic ion fragments of the STZ molecule with masses 92 amu, 140 amu and 156 amu were emitted at a greater relative concentration from the hydrophilic [1 0 0] crystal face than from the more hydrophobic [1 0 2] face. This confirms that at the [1 0 0] face, the orientation of the STZ molecule is such that the parent molecule is held more tightly within the bulk of the crystal and fragment ions are released in preference to the molecular ion. Complimentary colloid probe AFM adhesion studies confirmed the face specific surface energetics (indicative of wettability) of STZ crystals that relate to molecular orientation. For STZ, the molecular ion is more prevalent in the SIMS spectrum from the [1 0 2] crystal face, whereas fragment-ions are more prevalent from the [1 0 0] face. The STZ molecule are oriented parallel to the [1 0 0] face, hence they are more exposed to the ion beam and more strongly associated with adjacent molecular layers in the crystal, as indicated by the relatively low total ion count observed (Muster and Prestidge, 2005).

These studies further our understanding of the face-specific properties of pharmaceutical crystals and have implications when considering such processes as tablet formation, crystallisation, excipient interaction, dissolution, etc., and hence influence formulation and delivery characteristics. For example, crystal face-dependent adsorption behaviour has also been observed for ethyl(hydroxyethyl) cellulose (EHEC) onto OGA crystals, with preferential EHEC adsorption observed at the hydrophobic [0 $\bar{1}$ 0] OGA face (Muster, 2001) and this correlates with the molecular orientation determined by ToF-SIMS.

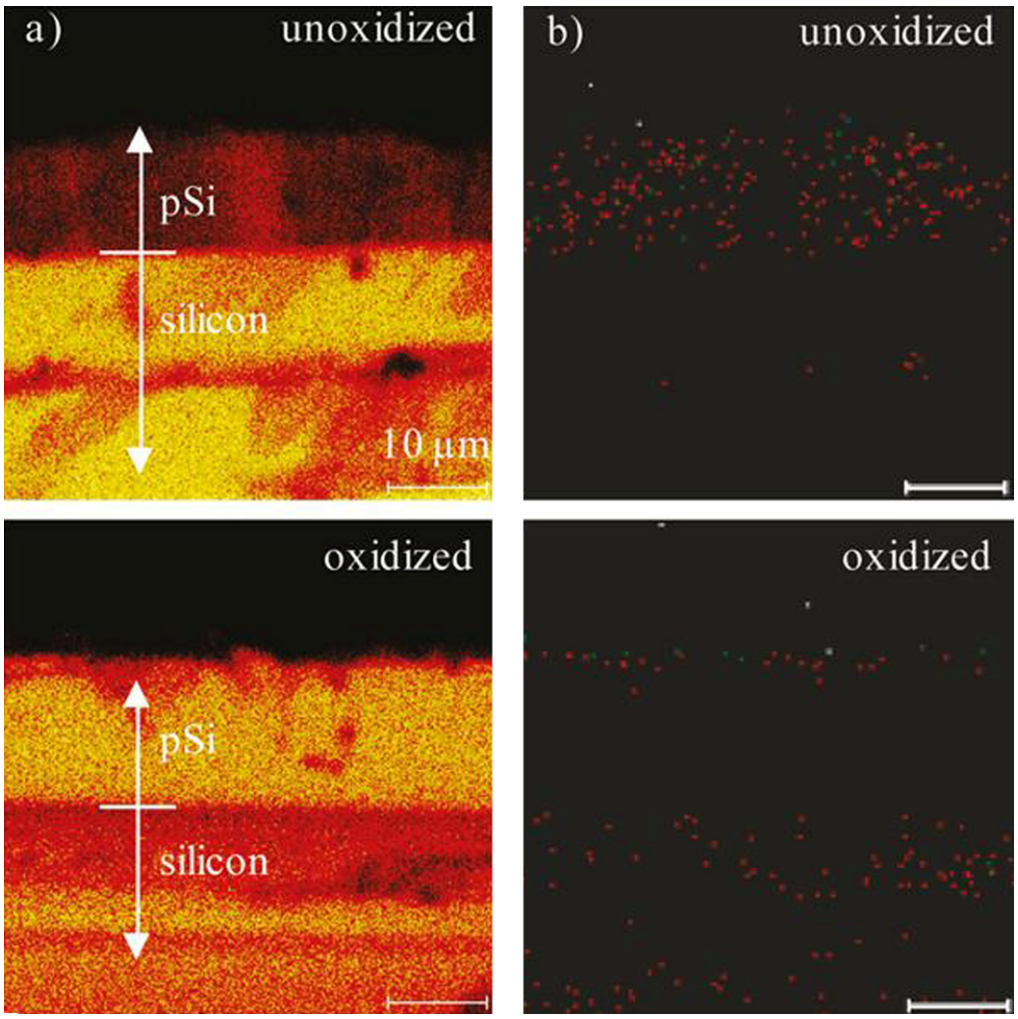

Fig. 5. ToF-SIMS images (100 μm × 100 μm) of unoxidized and oxidized lysozyme loaded pSi wafer cross sections: (a) total positive ion map and (b) C_3H_8N ion map (Jarvis et al., 2010). Used with permission.

3.3. *Protein conformations, interactions and purity: characterising adsorbed monolayers*

The emergence of proteomics and pharmacogenomics over the last decade has led to a significantly increased interest in biopharmaceuticals and their delivery. Accompanying this is the requirement for further understanding of the behaviour of proteins adsorbed at interfaces; this has implications for both optimising protein delivery systems and also protein sensing devices. Numerous techniques (e.g., quartz crystal microbalance (QCM), surface plasmon resonance (SPR) and IR/Raman spectroscopy) are used to characterise *in situ* protein adsorption at an interface (Liu et al., 2010; Sener et al., 2010; Yamamoto et al., 2010). However, these approaches provide limited information concerning the conformation and orientation of adsorbed or encapsulated proteins; this information is critical in the search for delivery systems that retain a protein's bioactivity.

The very high surface sensitivity of ToF-SIMS (analysis depth < 20 Å) facilitates protein distribution and conformation analysis (small changes in protein conformation results in significant changes to the ToF-SIMS spectra), which is not available through other lab based methods. Such structural information is key to pharmaceutical applications, but also numerous other areas, e.g., membranes (Aoyagi et al., 2004a,b; Burns and Gardella, 2008; Mahoney et al., 2006) and biosensors (Aoyagi and Kudo, 2005; Aoyagi et al., 2004c; Ogaki et al., 2010). The sensitivity of ToF-SIMS to the structural and conformational arrangement of proteins also enables the characterisation of protein mixtures as well as denatured proteins (Kempson et al., 2010b; Mouhib et al., 2010; Wagner et al., 2003a; Xia et al., 2002). Mixtures of proteins are easily discriminated by interrogating the complex spectra with multivariate statistical analysis. Spatial distributions of proteins are also readily observed by the imaging modes of ToF-SIMS (see earlier).

ToF-SIMS provides unique mechanistic information into protein adsorption processes, since the mass fragments observed may arise due to interactions of the protein with neighbouring proteins or with the underlying substrate. In the case of a single protein, the orientation may be ascribed by measuring residue components dominating distinct parts of the molecule. This is not trivial however, since protein conformation is highly sensitive to its local environment and hence such processes as drying can dramatically and unpredictably alter protein structure. This has been evidenced by an increase in the hydrophobic residues at the surface as the internally contained residues are revealed upon drying to be exposed outwards. In this sense, the ultra-high vacuum environment of ToF-SIMS is far from ideal, however, using sugars such as trehalose the protein adsorbed conformation can be maintained even upon drying (Kim et al., 2007).

Recently, we have demonstrated the sensitivity of ToF-SIMS when analysing adsorbed layers of human serum albumin (HSA) composed of native HSA, very subtly denatured HSA and their mixtures (Kempson et al., 2010b). N.B. in this study the thermal

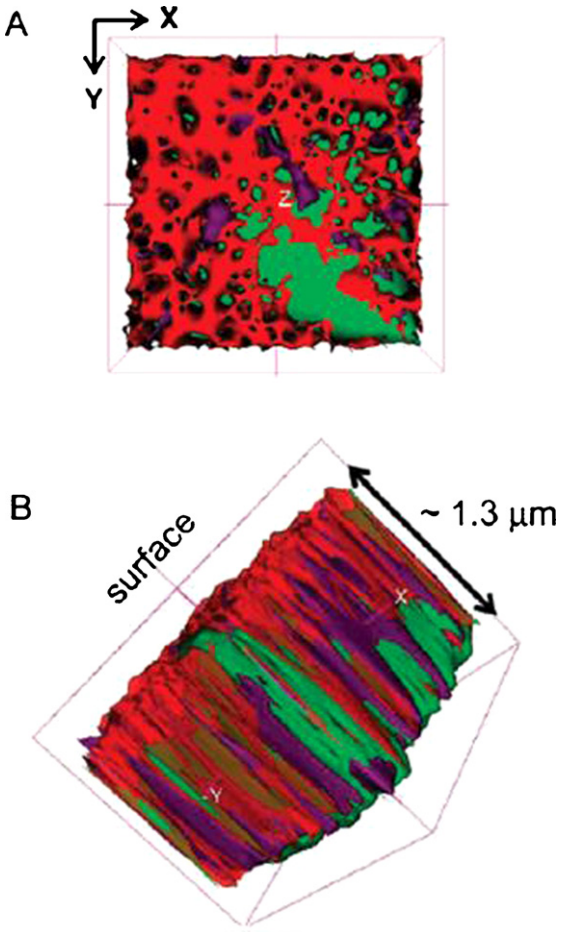

Fig. 6. Red: $C_5H_{10}N^+$ (*sirolimus*, an immunosuppressant drug used to prevent rejection in organ transplantation). Green: $C_3H_4O^+$ (*PLGA*, biocompatible copolymer). Purple: Na^+ (from elution medium) (Fisher et al., 2009). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)
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denaturing process was not sufficient to lead to aggregation of the protein (determined by dynamic light scattering) or to produce a large change in secondary structure (determined by circular dichroism), however these changes were readily observed by ToF-SIMS. Submitting the mass peaks arising due to specific amino acid residues to principle component analysis (Mantus et al., 1993), the adsorbed denatured HSA could be readily differentiated from the native HSA molecule, with a typical factor plot presented in Fig. 8.

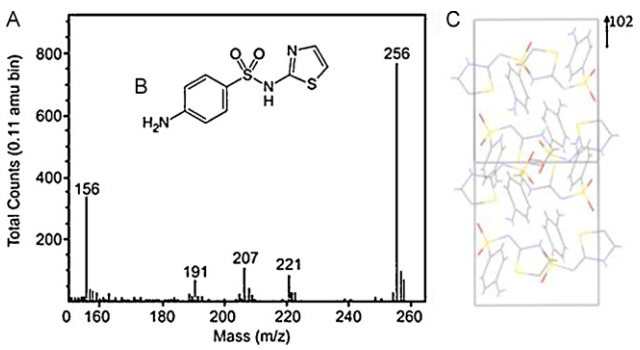

Fig. 7. Time-of-flight secondary-ion mass spectrometry (ToF-SIMS) positive ion mass spectra (A), chemical structure of sulphathiazole (B) and molecular model (C) for hydrophobic sulfathiazole [1 0 2] crystal face.

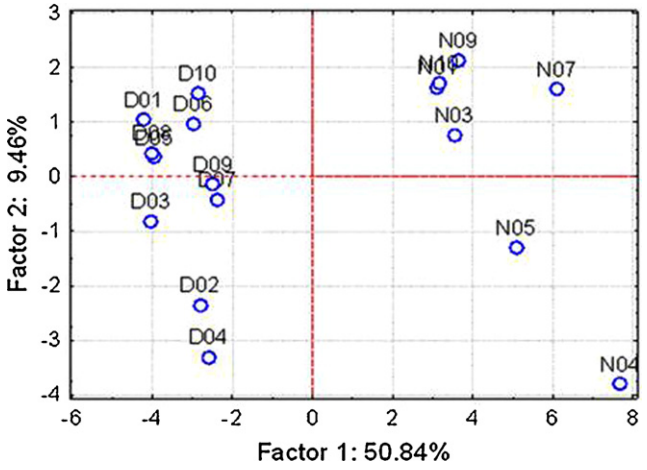

Fig. 8. Denatured (D) HSA has obvious mass spectral differences compared to native HSA (N) based on the first factor scores in PCA of ToF-SIMS spectra. Each data point reflects one spectrum from either of the two samples.
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The observed differences in the mass spectrum were attributed to changes in HSA orientation or conformation. This suggests that subtle changes made in solution significantly impact on the adsorbed conformation of the native and denatured HSA, and, interestingly, these changes are retained during upon drying of the sample and subsequent ToF-SIMS analysis (i.e., exposure to ultra high vacuum). Furthermore, the ability to quantitatively determine the relative amount of the denatured HSA in mixtures with HSA was demonstrated.

4. Future of ToF-SIMS in pharmaceutics and biopharmaceutics

The application of ToF-SIMS in pharmaceutics is in its infancy. Areas that show future promise include:

- 3 dimensional chemical mapping of solid state dosage forms with less than 100 nm resolution, e.g., the distribution of amorphous forms within dosage forms.
- Quality control for solid state dosage forms, e.g., the ability to analyse the effects of trace moisture on the surface properties of a control release pellet or the detection of low levels of polymorphic form variations during processing of API powders.
- Drug distribution within biological tissue without the need for staining or labelling. The improved application of cryo-frozen samples and cold stage methods will enhance this area.
- Distribution analysis and biological state differentiation of therapeutic proteins within carrier systems.
- The ability to identify biological markers based on subtle changes in conformation or structure.

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