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New tensiographic approach to surface studies of protein kinetics showing possible structural rearrangement of protein layers on polymer substrates

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ABSTRACT

An application of optical tensiography is described for studying protein adsorption/desorption/crystallisation processes. This paper concentrates on investigating the physics of the surface kinetics and associated crystallisation processes of the serum protein BSA. The measurements show changes in optical transmission caused by the development of protein layers on surfaces inside a liquid drop. Various absorption mechanisms are evaluated and a physical explanation suggested for the results involving changes in optical scattering as the structure of the protein layers develops. It is shown that tensiography provides a powerful real-time method for monitoring protein adsorption processes on a solid substrate that gives new insights into protein kinetics on these substrates. The study reveals results that are in agreement with earlier work showing multilayer structures and perhaps rearrangement of complex protein layer structures. Finally, consideration is briefly given to potential applications and most importantly the experimental development of this new tensiographic approach for improved real-time absorption measurements of proteins on solid substrates.

1. Introduction

The study of protein adsorption at a solid-liquid interface is important in a number of application areas and it is relevant to note that biosensing is a rapidly expanding field of instrumentation that has been reviewed comprehensively in recent times by Wolfbeis [1]. In this present paper, consideration is given to the new technique of tensiography for monitoring protein adsorption on surfaces. Such a chemical sensor as the tensiograph, that does not involve a biological component, but is merely a sensor placed in a biological matrix, is *not a biosensor*. Biosensors, by definition, make use of a biological component in order to sense a species of interest. Ligler and Rowe-Taitt have edited a book on optical biosensors [2] which contained a very substantial review of optrode fiber-optic biosensors by Biran and Walt. In addition to these impressive reviews, there is a wide-ranging textbook edited by Lopez-Higuera [3]. In this paper, we present measurements using tensiography, in which we study the passage of light through the inside of drops. Tensiography can perhaps be defined as the measurement or monitoring of liquid properties from signals derived from a transducer system (not just optical) that graphically records shape and other variations in a growing drop that modulate the detected signal; optical tensiography was the first reported type and derives a signal from light injected inside a growing drop (usually pendant) supported on a drophead from a source fiber optic and collected by a second fiber optic suitably positioned in the drophead known as the collector fiber. It is clear that tensiography offers "a left-of-field technique" within the general classification of fiber optic sensors (FOS) techniques, because it has a range of operational modalities. Tensiography delivers physical measurands such as refractive index/dispersion [4], spectra (both fluorescence [5] and UV-visible/turbidity [6]) and importantly monitors specific processes and components in the drop such as adsorption of proteins onto solid substrates that is the subject of this paper. McMillan, O'Neill et al have recently shown the value of drop spectroscopy for monitoring for example the purity of biomolecular solutions [7]. The same authors pointed to the possibility of using just a single drop for monitoring dynamic surface chemistry of such things as proteins and enzymes and have adopted the term drop-under-test (DUT) to distinguish this drop methodology from those associated

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with the more widely used term liquid-under-test (LUT) [8]. It is feasible indeed to consider the adaptation of this tensiographic technique to incorporate biological components, for example, onto activated surfaces. Such adaptation is discussed at the end of this paper as it would establish tensiography within the strict definition of the term biosensing technique.

The important biophysical protein characterisation techniques based on photonic methods are UV-visible, fluorescence, circular dichroism (CD), static light scattering (SLS), dynamic light scattering (DLS), electrophoretic light scattering (ELS), small angle X-ray scattering (SAXS) and others approaches of lesser importance and popularity. These techniques in some way all impinge on the tensiographic technique discussed here, but collectively the light scattering techniques are the ones of the most relevance with wide application in chemistry, biology and physics [9]. Dynamic light scattering may be used to study the aggregation process and which can be used to determine the size distribution profile of small particles in colloidal, protein or indeed other complex solutions. The techique originates with the exhaustive experimental studies of the Carlow scientist Tyndall who founded the science of nephelometry. This science was impressively subsequently developed theoretically by Tyndall's fellow Royal Institution of G.B. professor Rayleigh [10]. The techique has now been developed continuously for more than a century to become a well-established technique in the arsenal of biochemistry [11]. The importance to some extent for this study is its ability to provide measurements on 'aggregates', a term that can encompass oligomers, assembly or agglomeration. Of course, molecular weight of molecules is traditionally measured using static light scattering. A wide array of application studies now exist for protein solutions which exhibit thermal denaturation, changes in quaternary structure, aggregation and virial coefficient. Measuring small poorly scattering molecules is a challenge to these scattering techniques, but larger molecules increase light scattering and are detectable at even small concentrations. From the late 1980s commercial DLS instruments appeared in biophysical laboratories and these instruments enabled size measurements of small volumes of proteins and other biomaterials in solution to be made, with small sample volumes and in relatively low concentration ranges. The present work should be seen perhaps as an extension of this low concentration and microvolume analysis that connects with the extensive and largely exploratory non-commercial FOS methods. A fiber optic probe for monitoring protein aggregation, nucleation and crystallisation has been developed by NASA [12].

Of relevance for example to the present work, are a number of important developments using interferometric methods to monitor protein layers; Zhang [13] is perhaps the one of the most relevance and furthermore provides an excellent review of FO biosensors. Protein adsorption to surfaces has been widely studied because of its importance to medical and biotechnological applications and this is one of the most important potential applications of this present work. The study by O'Neill et al. [14] into the efficacy of enzyme cleaning of polymer surfaces that are used for contact lens cleaning was indeed the departure point for the present work. It might also be worth noting here that Dual Polarisation Interferometry (DPI) is an important enabling tool for the study of polymer deposition processes and the molecular mechanisms behind any subsequent interactions of proteins and surfactants with the polymer layer in real-time [15]. Furthermore, the technique allows for in-vitro diagnostics for the detection, prognosis, prediction and monitoring of diseases, not least to deal with point of care testing.

Diagnostic assay design is routinely faced with the necessity of working with biomolecules at surfaces. The central relevance to the present study is that this technique can perhaps provide such real-time density and dimensional measurements, showing mass capture events and revealing conformational changes. Light scattering has been used to measure nanolayers in such work as Wooster and Augustin [16] on steric layers. Brahma, De and Bhattacharyya [17] have used Rayleigh scattering intensity of a protein solution in spectrofluorimeter measurement and this application study is particularly relevant here. The tensiograph could be developed to fit into a category of one of the optical techniques that are broadly classified into four main types - intensity sensors, diffraction-based sensor, interferometric sensors and polarization sensors. Such NDT methods are in turn classified according to the optical property that is altered by the measurand. Drop analyser methods often relate to refractive index/dispersion measurements and in this regard there have been some loosely related studies on drops. Massilo has made some studies on what he refers to as 'rainbow refractometry' that was applied to radially inhomogeneous spheres. He investigated the critical case of evaporating droplets [18]. This work is perhaps more important for the idea of rainbow refractometry as the drop analyser has been referred to extensively in the literature as the rainbow analyser.

Biomedical spectroscopic applications are probably the most important of all for the present work and these techniques have been reviewed by Utzinger and Richards-Kortum [19]. One of the main industrial applications for this work would be in studies on biomaterial implants [20,21]. When biomaterials are implanted, contact with the blood may lead to serum protein adsorption to the implant. This in turn can lead to platelet adsorption and blood coagulation [22]. Some previous studies have shown that albumin adsorbs more on to some hydrophobic surfaces, such as polystyrene and polyurethane, than on hydrophilic surfaces [23,24]. Other studies on hydrophobic polyethylene oxide and polyethylene glycol surfaces have shown the opposite trend [25,26]. These results would indicate that there are other factors influencing the adsorption mechanism. In recent years a number of techniques to study biological adsorption situations have been developed. These include flow chamber particle deposition with image analysis [27], aqueous phase atomic force microscopy [28], ellipsometry [29], and axisymmetric drop shape analysis by profile (ADSA-P) [30–33].

Central to the present study is the explanation presented in a previous paper Dunne et al. [34] by the authors giving an account of the initial adsorption and desorption features of Bovine Serum Albumin (BSA) on polymethyl methacrylate (PMMA), Polyether ether ketone (PEEK) and nylon substrates. Contact angle measurements were used to confirm the explanations presented in this earlier paper. On nylon and PEEK substrates, which are hydrophobic, the drop periods decreased to a plateau and stayed at approximately a constant value. This decrease in the measured drop period with a PMMA substrate was followed by a rapid increase. This process was only seen in such hydrophilic surfaces. This rise in what is clearly a surface process is attributed to structural rearrangement of the protein, with proteins changing from a globular state to an unravelled state at the solid substrate. The recent work of Landry et al. [35] is important as it provides an analysis of dependence of incident angle reflectivity of an ultra-thin layer of BSA protein on both transparent and opaque substrates. They noted the practical importance of the increased reflectivity near the Brewster angle on glass at 56°. Importantly, they found that the fractional reflectivity change to an ultrathin film (or modified surface layer) on a substrate is dramatically enhanced by a theoretically defined angular factor near the Brewster angle, or the pseudo-Brewster angle (this is small theoretically defined modification of the physics due to the BSA surface layer changing the optical interface) in a relationship that depends on the ratio of film thickness to wavelength (d/λ). The important point for the present studies is that the authors found enhanced reflectivity in their ellipsometer studies. A highly coloured double optical interface produced by a thinning draining soap film appear to be black when viewed in white light just before it bursts. This 'destruction of colour' arises