

# In-situ and real-time protein adsorption study by Spectroscopic Ellipsometry

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## Abstract

Protein adsorption is an important aspect for the improvement of many applications, such as medical implants, biosensor design, etc. The density, orientation and conformation of surface-bound proteins are believed to be key factors in controlling subsequent cellular adhesion. The aim of this work is the development of a methodology in order to study in-situ and real-time protein adsorption phenomenon, and describe fibrinogen adsorption on amorphous hydrogenated carbon (a-C:H) thin films developed by rf reactive magnetron sputtering under different deposition conditions. Spectroscopic Ellipsometry (SE) in Vis–UV energy region was implemented for this purpose. SE is a non-destructive, surface sensitive technique, with the capability of performing real-time measurements in air as well as in liquid environment, with great potential in biomedical studies. An appropriate ellipsometric model has been developed, in order to describe accurately the protein adsorption mechanisms in real-time. It was found that the thickness and density of fibrinogen are larger on the a-C:H thin film deposited under absence of bias voltage application. The differences in fibrinogen thickness and transition of fibrinogen from liquid to adsorbed state are presented and discussed in the terms of the surface and optical properties of a-C:H films.

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**Keywords:** Protein adsorption; Fibrinogen; Spectroscopic ellipsometry; Amorphous carbon

## 1. Introduction

Protein adsorption has been investigated extensively during the past decades because it is an important aspect for the improvement of many applications, such as medical implants, biosensor design, etc. Plasma protein adsorption is accepted as the first event that occurs when a foreign material comes into contact with blood [1]. The subsequent interactions of blood cells with adsorbed protein layer lead normally to coagulation, thrombus formation and embolization. The density, orientation, and conformation of surface-bound proteins are believed to be key factors in controlling subsequent cellular adhesion [2].

Several works have dealt with the biocompatibility of diamond-like carbon and tetrahedral amorphous carbon coatings, where good haemocompatibility properties have been reported [3–9]. This is very interesting in the field of biomaterials, which are an important aspect in the development of biomedical devices and implants. In a previous work of our research team, it was deduced that a-C:H thin films, developed with rf reactive magnetron sputtering, with  $sp^3$  content ~42%

and small amount of  $H_2$  in plasma during deposition, exhibit good haemocompatibility [10,11].

This deduction has led to the need of investigating further the phenomenon of protein adsorption on a-C:H thin films. The protein studied in this work is fibrinogen (Fib), which takes part in blood coagulation, facilitates adhesion and aggregation of platelets, and is important in the processes of both haemostasis and thrombosis [12]. In order to study the Fib adsorption mechanisms in real-time, Spectroscopic Ellipsometry (SE) was used. This is a non-destructive, surface sensitive technique, which can be applied both in air and in liquid environment. SE has the capability of using advanced modelling procedures, which can describe complicated systems, such as the biomaterial–protein system. Thus, SE is a favourable technique for the study of biological samples, with great potential in biomedical studies. First, SE was used for the fundamental characterization of the a-C:H thin films in terms of its optical properties and the Fib layers formed on them. Atomic Force Microscopy (AFM) was implemented to estimate the surface characteristics of the a-C:H thin films and the adsorbed Fib layers formed on them. An appropriate ellipsometric model was developed for the real-time investigation of Fib adsorption, providing valuable information about the

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thickness and density change of the Fib layers formed on the a-C:H thin films during adsorption and the results are discussed in terms of the different deposition conditions of the studied a-C:H thin films.

## 2. Experimental

The a-C:H thin films were deposited by rf magnetron sputtering on c-Si (100) substrates at room temperature in high vacuum ( $2 \cdot 10^{-7}$  mbar) in Ar/H<sub>2</sub> atmosphere, using a high purity carbon target [13]. The H<sub>2</sub> partial pressure in the deposition chamber during the deposition was 5% and the samples were grown without applying a negative bias voltage ( $V_b$ ) at the substrate (a-C:H sample UBMS — UnBiased Magnetron Sputtered) and with application of negative bias voltage  $V_b = -40$  V (a-C:H sample BMS — Biased Magnetron Sputtered).

The former one has exhibited good haemocompatibility properties [10,11]. Optical characterization was made using ex-situ phase modulated spectroscopic ellipsometer (by Horiba Jobin Yvon), capable of measuring in the Near IR to FUV energy region (0.7–6.5 eV), at variable angles of incidence (55–80°). Fibrinogen solution (Fib) with a concentration of 1 mg/ml, in Phosphate Buffer Saline (PBS) at pH 7.4 was prepared for the study of real-time protein adsorption on the a-C:H thin films. Especially for the real-time SE experiments of this work, measurements during the protein adsorption were performed extremely fast (measurement duration 500 ms, every 1 s) in a flow-cell (by Nanofilm), in the energy region 1.5–4.0 eV, at angle of incidence 60°.

Atomic Force Microscopy (AFM) measurements (by NT-MDT) in semi-contact mode were performed to estimate the surface characteristics of the a-C:H thin films and the Fib adsorbed on them.

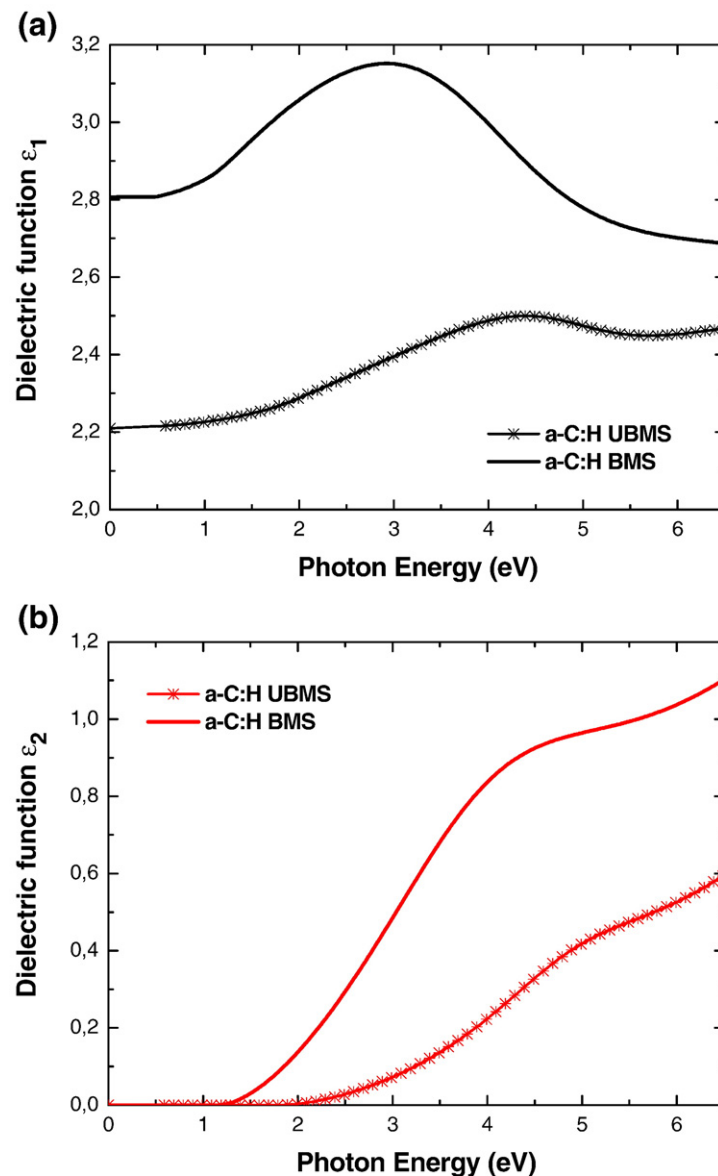


Fig. 1. Bulk dielectric function  $\epsilon(\omega)$ , real  $\epsilon_1(\omega)$  (a) and imaginary  $\epsilon_2(\omega)$  (b) part using the best-fit parameters for the UBMS and BMS a-C:H film.

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