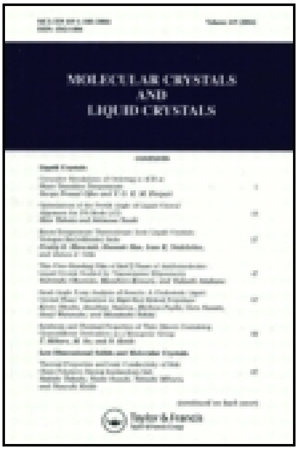

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The Behaviour of Pure Titanium in Albumin Solution

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Among the different biofluids in the human body, albumin is the most important plasma proteins circulating in the bloodstream and our paper are focused on the behaviorof pure titanium in albumin solution. The samples investigated were immersed for 10 days in a mixture of human body plasma and bovine serum albumin solution with concentrations of 2% and 10% in weight. The analysis by diffuse reflectance in the 220–2300 nm domain, and in the mid-infrared range (400–4000 cm^{-1}) was performed. The albumin adsorption at interface was put in evidence from spectral data and is found to be most important in the sample containing a higher concentration of albumin, regarding the relative band intensity. This is in agreement with the electrochemical results showing a better stability at a smaller albumin concentration according to a higher corrosion rate.

Keywords: albumine; pasive film; proteins; titanium

INTRODUCTION

The human body is considered a complex and aggressive environment, due to the content of different inorganic and organic compounds etc. It has been recognized that this situation can increase the corrosion processes of metals or alloys used as implants. Titanium is mainly used today in medical applications due to its lightweight, excellent mechanical properties and stability in various physiological media. An occurrence, its corrosion resistance is due to the formation of a passive oxide film. The disruption of this thin film constitutes an aspect of corrosion increase, which causes more metal ion release and more inflammation around metallic implant as a tissue response.

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Among the different biofluids in the human body, albumin is the most important plasma proteins circulating in the bloodstream and contributing in the osmotic blood pressure [1]. These proteins (polypeptides) are biopolymers of L-amino acids, each of them joined together by peptide bonds. Albumin plays a significant role in the maintenance of colloid osmotic pressure and the binding of long-chain fatty acids. It also acts as a carrier for nutritional factors and drugs. It is well-known that most proteins have a strong tendency to adsorb at a solid/liquid interface. This phenomenon plays one of the major roles in both biocompatibility of medical implant devices and biosensors development, due to problems related to surface fouling, bacterial growth, metal dissolution and interface charge distribution. However, only few studies have investigated the corrosive, toxicity and biocompatibility of the titanium implants in the albumin solution. Since BSA structure is similar to human serum albumin in 76% [1], the study of the in-vitro activity of metallic implants in BSA could be an attractive mean for a better understanding of the self-defence human body to stranger bodies used as implants. Our aim is to study the adsorption phenomena at the interface Ti/BSA, and to correlate this process with the stability [2] and biocompatibility behavior of Ti.

EXPERIMENTAL

Materials

The samples investigated are pure titanium which was immersed for 10 days in a mixture of phosphate buffer solution and bovine serum albumin solution (Merck) with concentrations of 2% and 10% in weight. To simplify, the samples are labeled respectively Ti-Alb2% and Ti-Alb10%. The chemical composition of titanium and albumin is given in Tables 1 and 2.

The samples were analyzed by diffuse reflectance in the 220–2300 nm domain using a UV-VIS-NIR JASCO 570 V spectrophotometer with integrative sphere, and in the mid-infrared range (400–4000 $\mathrm{cm^{-1}}$) by using a FTIR JASCO 620 spectrophotometer. For FTIR technique, the samples were mixed to KBr powder in an

TABLE 1 Chemical Composition of Titanium Electrodes, %

Composition	C	N	H	O	Al	Ti
Ti	0.1	0.036	0.015	0.2	0.05	Rest

TABLE 2 Chemical Composition of Albumin

Composition	Specification	Typical
Protein	$\geq 98\%$	99%
Purity (albumin)	$\geq 98\%$	100%
Solubility (4% H_2O)	Pass	Pass
Moisture	$\leq 5,0\%$	$< 3\%$
pH (10% H_2O)	6.5–7.5	6.8–7.2
Ash	$\leq 2,0\%$	$\leq 2,0\%$
Protease	$\leq$ 0.005 U/mg	Not detected
Endotoxin	$\leq$ 3 EU/mg	0.1 EU/mg
Na, Cl, K, P, Ca	Tested	Report on request
Heavy Metals (Pb)	$\leq$ 10 ppm	$<$ 1 ppm

adequate concentration and pressed in pellets. FTIR spectrum was performed with pure albumin powder in KBr pellets as blank experiment and in KBr pellet with titanium powder covered with adsorbed albumin as tested composites. The albumin concentrations were 2% and 10%.

The evaluation of albumin concentration in solution was effectuated also spectrophotometrically using the formation of complex albumin-brome cresol at wavelength 637 nm.

RESULTS AND DISCUSSION

IR-Spectroscopy

The transmission spectrum of bovine serum albumin obtained by FTIR analysis is displayed in the Figure 1. Characteristic bands of free and associated OH groups are observed at $\sim$3750 and 3500 cm^{-1}, respectively. Also CH stretching bands in the range 2800–300 cm^{-1} are observed. The most prominent amino-acids bands are situated at $\sim$1600–1700 cm^{-1} and 1500–1550 cm^{-1} and correspond respectively to Amide I (80% C=O stretching + 20% C−N stretching vibration) and Amide II (60% NH in-plane bending vibration + 40% C−N stretching vibration). Using the amide I, as a sensitive indicator of conformation and structure [3], the present study show two bands at $\sim$1685, 1645 and 1620 cm^{-1} attributed to β-turn, α-helix and β-sheet conformations. Infrared spectra are characterized by minor vibration bands as follows: 1740 cm^{-1} due to ester carbonyl group, amide III (40% C−N stretching vibration + 30% NH in-plane bending vibration) with free carboxylate ions and CH_3 and CH_2 bands between 1400–1450 cm^{-1}, OH aromatic with CC groups (amide III) at 1240 cm^{-1}, C=C groups at around 1100 cm^{-1}. Below 800 cm^{-1} correspond mainly

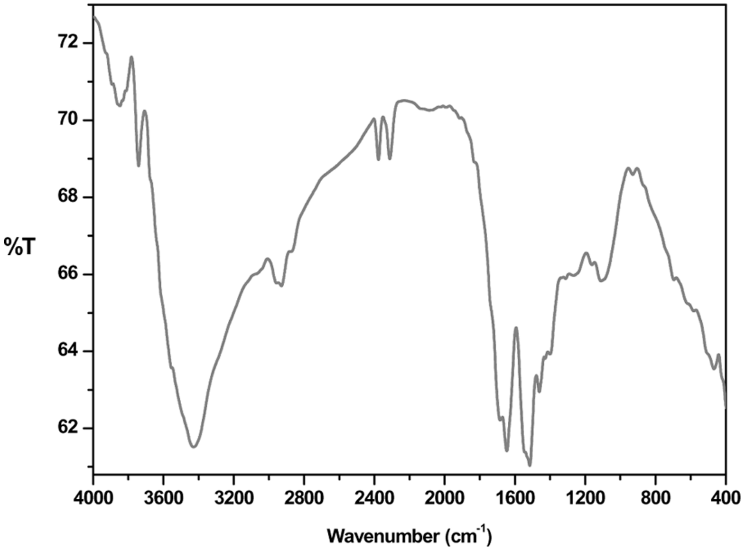


FIGURE 1 FTIR spectrum of bovine seric albumin.

to C–C bending, amide V, amide IV, and amide VI vibrations. This result is in accordance with those available in the literature [4,5]. The transmission spectra obtained on the titanium-albumin composite are presented in the Figure 2. The similar FTIR profile with the one obtained on pure albumin shows clearly adsorption of albumin groups was occurred in the titanium-based materials. However, spectra reveal more prominent Amide I, Amide II and Amide III groups and reflect structural modifications in comparison of pure albumin. The albumin ions release is found to be most important in the sample containing a higher concentration of albumin, regarding the relative band intensity. This is in agreement with the electrochemical results showing a better stability due to the formation of passive titanium oxide films [6–8]. The decrease of albumin concentration in the case of Ti2%Albumin is depending on temperature being maximum at 323 K (value is 0,97) For Ti 10%Albumin the decrease is maximum at the same temperature, the final value after electrochemical test being 7.98 it could be concluded these films exhibit a BSA-preferred property. This result supports a similar one reported with human seric albumin (HSA) [9]. The decrease of albumin concentration after a potentiodynamic electrochemical test [10] is due probably to anodic oxidation process with a passive stratum formation, and explains why at a smaller albumin concentration the corrosion rate is smaller [10].

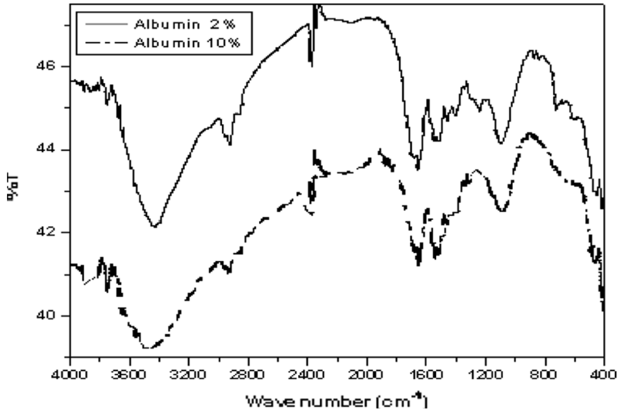


FIGURE 2 FTIR spectra of titanium-albumin composite for two concentrations of albumin, Ti-Alb2% and Ti-Alb10%.

The samples investigated present also clear additional bands at ~1200, 790, 723 and 450 cm^{-1} in comparison to pure albumin. These bands are most prominent with Ti-Alb2%. They are attributed to phosphate groups. The sample Ti-Alb2% presents some modifications in the absorbance intensity at 1480, 1344, and 1090 cm^{-1}, probably due to phosphate bands overlapping. How the albumin concentration could influence the phosphate adsorption is actually unknown. However, our result show that the adsorption behaviour of phosphate groups seems inhibited as the concentration of albumin increases. Complementary measurements regarding surface analysis were performed [10] and the composition of surface oxide surface was change, denoting an adsorption-desorption phenomenon, as a most important event at interface. Bonds between the titanium surface and the organic molecules are difficult to observe. No significant changes in the frequency are observed.

UV-VIS-NIR Spectroscopy

UV-VIS-NIR spectra reported below are recorded in reflectivity mode for which the light reflected by the sample is measured by an integrating sphere. The reflection spectra are displayed in the wavelength range 250–2300 nm. The result obtained on pure bovine seric albumin is shown in the Figure 3, and the assignment of bands presented in Table 3. We obtain a good agreement with the data reported in the literature [1].

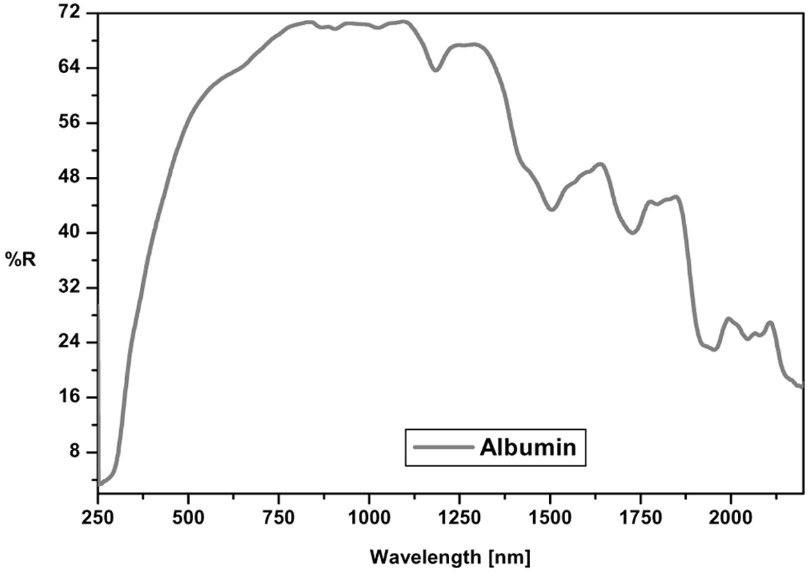


FIGURE 3 UV-VIS-NIR spectrum bovine seric albumin.

UV-VIS-NIR spectrum of titanium dioxide is shown in the Figure 4. The spectrum is characterized by a high reflectance emission in the VIS-NIR range, as currently observed [4]. Usually, the transmission

TABLE 3 Characteristic Bands in UV-VIS-NIR Diffuse Reflectance Spectra

Wave length (nm)	Attribution
250–350	NH ($\pi \rightarrow \pi^*$) + C = O ($n \rightarrow \pi^*$)
870	OH and CH groups
906	OH and CH groups
1025	2 × NH stretch + 2 × amide I
1185	CH second overtones (HC = CH + CH_3 groups)
1424	OH first overtone
1505	NH first overtone
1726	CH first overtone
1728	CH first overtone
1796	CH first overtone
1922	C = O second overtone (CONH) + OH
1955	C = O second overtone + OH
2044	NH symmetric stretch + amide II
2082	OH stretch + OH deformation
2173	2x amide I + amide III (CONHR)

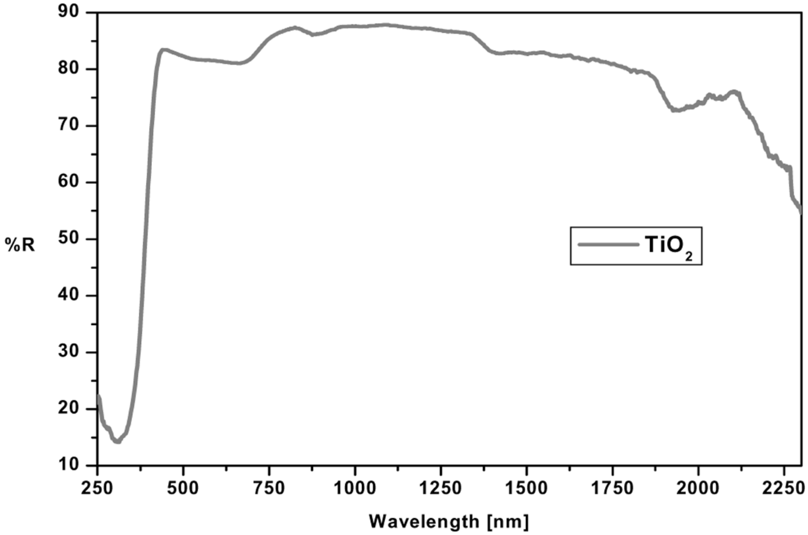
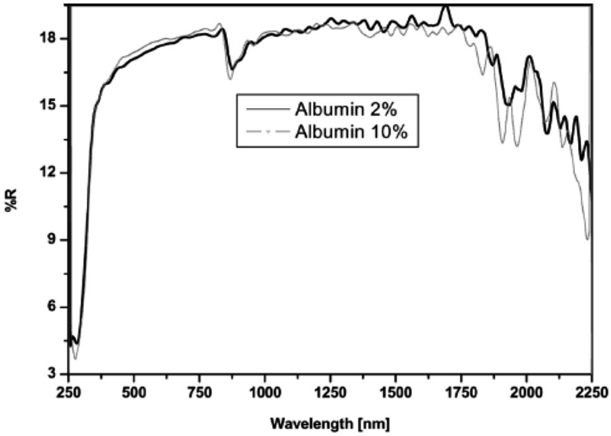


FIGURE 4 UV-VIS-NIR spectrum titanium dioxide.

edge in the visible range corresponds to the band gap of the semiconductor. In our case, we apply the Tauc law [4,14], a more accurate method, for determining the direct band gap of TiO_2. So, the plot of $(\alpha\ h\nu)^2$ versus $h\nu$ is presented in the Figure 6, where the absorption coefficient α is proportional to $\ln[(R_{max} - R_{min})/(R - R_{min})]$, $h\nu$ is the



FIGRUE 5 Determination of the band gap of titanium dioxide using UV-VIS-NIR data and tauc law.

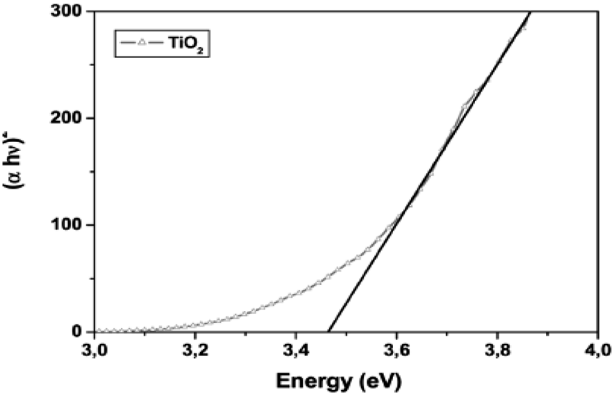


FIGURE 6 UV-VIS-NIR spectra of titanium-albumin composite concentration of albumin.

photon energy and R_{max}, R_{min} and R are the maximum, minimum and any intermediate reflectance, respectively. The band gap energy of 3.46 eV was obtained from the extrapolation of the straight line to the $(\alpha\ h\nu)^2 = 0$ axis (Fig. 5). This result is in good agreement with experimental data available of an anatase phase [4].

Figure 6 show UV-VIS-NIR spectra obtained on Ti-Alb2% and Ti-Alb10%. In the UV domain, bands at 268 and 280 nm are observed for the both samples with a prominence for Ti-Alb2%. Additional band at 275 nm is present in the UV domain of Ti-Alb10%. Most of the bands are from aromatic amino acids (phenylalanine, tyrosine, tryptophan). The main bands in the VIS-NIR domain are situated between 840–1050 nm and 1850–2020 nm. Bands at 1909 and 1966 nm from O−H first overtone and Amide II are more prominent for Ti-Alb10%. Results obtained confirm that the Ti-Alb10% effectively contains the most concentrated albumin solution.

We observed a slight hypsochrome shift of the bands in this sample in comparison of Ti-Alb2%. However spectral studies do not indicate great difference between Ti-Alb10% and Ti-Alb2% behavior.

CONCLUSIONS

1. Adsorption phenomena of phosphate and albumin groups were found in the two samples investigated (Ti-Alb 2% and Ti-Alb10%). The adsorption behaviour of phosphate groups seems inhibited as the concentration of albumin increases.

2. The evaluation of albumin concentration in solution effectuated also spectral using the formation of complex albumin-brome cresol confirms adsorption process at interface.

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