Brazilian Journal
of Chemical
Engineering

ISSN 0104-6632
Printed in Brazil
www.abeq.org.br/bjche

Vol. 26, No. 02, pp. 257 - 264, April - June, 2009

ADSORPTION OF HUMAN IMMUNOGLOBULIN G TO POLY (β-HYDROXYBUTYRATE) (PHB), POLY (L- LACTIC ACID) (PLLA) AND PHB/PLLA BLENDS

M. V. S. Lima, E. A. R Duek, and C. C. Santana*

Department of Biotechnogical Processes, School of Chemical Engineering,
State University of Campinas, UNICAMP, P.O. Box 6066, CEP 13083-970, Campinas - SP, Brazil
E-mail: santana@feq.unicamp.br

(Submitted: December 12, 2005 ; Revised: October 8, 2008 ; Accepted: October 16, 2008)

Abstract - Biodegradable polymeric materials can be used as temporary implants and may be able to carry out specific functions for a pre-determined period prior to their degradation *in vivo*. In order to be used they must present characteristics of biocompatibility. When a material comes into contact with blood, the instantaneous adsorption of protein occurs on its surface. Coverage of the surface by γ-globulins causes the adhesion of platelets and, consequently, provokes the process of blood coagulation. In this context**,** the objective of the present paper was to quantify the adsorption of human Immunoglobulin G (HIgG) to poly (β-hydroxybutyrate) (PHB), poly (L-lactic acid) (PLLA) and PHB/PLLA blends using the FTIR/ATR technique. The results showed the occurrence of HlgG adsorption for all three systems, PHB having the highest adsorption density, about Γ_{max} =2.57μg/cm^2, followed by PHB/PLLA blends (Γ_{max}=0.85μg/cm^2) and PLLA with the lowest values (Γ_{max}=0.25μg/cm^2).
Keywords: Adsorption; Biodegradable polymers; Human immunoglobulin G; Fourier transform infrared spectroscopy; Poly (L-lactic acid) (PLLA); Poly (β-hydroxybutyrate) (PHB).

INTRODUCTION

The introduction of polymeric biodegradable materials for medical applications is an important advance in biotechnology. These materials can be used as temporary implants for specific functions and their subsequent degradation *in vivo* means that there is no need for subsequent surgery to remove the implant, but the choice of the material depends on its application and biocompatibility.

The material used as implants receives the denomination of biomaterial and, according to Black (1982), "A biomaterial is any pharmacologically inert material, viable or unviable, natural product or manmade, that is a part of or is capable of interacting in a beneficial way with a living organism". Williams (1987) defined biomaterial as "Any nonliving materials used in medical devices intended to interact with biological systems".

Ideal blood compatible materials (materials used in contact with blood) do not activate the intrinsic blood coagulation system or attract or alter platelets or leukocytes. Blood compatibility of any material is dependent on surface charge, surface free energy, chemical group distribution, heterogeneity, surface texture, porosity, smoothness and flow conditions (Sharma, 2001).

Due to the relatively high diffusivity of proteins and protein surface affinity, a thin layer of protein is formed at the blood-material interface within a few seconds after blood contacts a foreign surface (Hoffman, 1982). Subsequent cellular events, such as adhesion and aggregation of platelets that initiate clot formation, are most likely mediated by this protein

*To whom correspondence should be addressed

layer instead of by the material surface itself. Hence, it is important to understand the composition and organization of this protein layer and the interrelationship with the material's surface properties before a material with better blood compatibility can be developed (Lin and Cooper, 1996).

Therefore, protein surface adsorption plays a critical role in the binding of protein to cell surface receptors, in the biocompatibility of clinical implants, in mammalian and bacterial cell adhesion to surfaces and in protein adsorption to bioprobes.

Therefore, the quantification of the interaction between synthetic polymer surfaces and plasma proteins in solution is important because foreign surface-induced coagulation is frequently observed (Brash and Lyman, 1969). For example, albumin-coated surfaces do not seem to attract platelets, whereas γ-globulin and fibrogen coatings cause not only platelet adhesion, but also aggregation and release of platelet constituents (Lyman et al., 1968; Kaeble and Moacanin, 1977; Vroman and Adams, 1971).

The degradable polyester poly(lactic acid) has received considerable attention because of its usefulness as absorbable sutures and in other biomedical applications (Hrkach et al.,1996). The main advantage of this material is that its degradation under physiological conditions yields products that can be metabolized by the organism.

Several studies have used ATR (attenuated total reflectance) - Fourier transform infrared (FTIR) spectroscopy as a technique to quantify protein adsorption to several surfaces, notably, Deng et al. (1986), Fink (1987), Straaten and Peppas (1991), Jeon et al. (1992), Fu et al. (1993), and Giacomelli et al.(1999). More recently, Yokoyama et al. (2003) used this technique to study the adsorption of hen egg white lysozyme onto a solid polytris(trimethylsiloxy) silylstyrene surface, while Xie et. al. (2002), studied the adsorption of bovine serum albumin onto calcium phosphate surfaces. This method has several advantages, including fast spectral acquisition, the capacity for data acquisition in aqueous physiological systems through background suppression, and its non-destructive nature. However, the use of this technique requires perfect contact between the sample and the internal reflection element (crystal) and this involves coating the crystal with the polymer layer. The extent of protein adsorption is monitored by analyzing the amide group (CONH) of the proteins, which exhibits characteristic bands at 1650 cm^{-1} (amide I), 1550 cm^{-1} (amide II) and 1300 cm^{-1} (amide III). The amide II band is the most widely used because it is relatively insensitive to conformational changes in the proteins and shows less interference from the water band (1640 cm^{-1}).

In this study, we describe the kinetics for the surface-adsorption of HIgG (human immunoglobulin G) onto poly(β-hydroxybutytrate) (PHB), poly(L-lactic acid) (PLLA) and PHB/PLLA 50/50 blend, using an ATR germanium (Ge) crystal as the internal reflection element, as well as equilibrium isotherm data at 37°C for PHB, PLLA and for the PHB/PLLA 50/50 blend. We used a continuous flow method to simulate physiological conditions (blood flow, temperature, pH and ionic strength) in order to follow the adsorption of protein onto these polymers. To simulate the physiological conditions, a continuous flow cell was built in the laboratory to couple to the FTIR - ATR equipment used for the tests, according to Chittur (1988).

MATERIAL AND METHODS

Material

Biodegradable Polymers:

The polymers used in the adsorption experiments were:

- Poly(lactic acid) (PLLA) obtained from Purac, with Mn = 100.000g/mol and 0.3mm of granulometry of analytical grade;
- Poly(β-hybroxybutyrate) (PHB) purchased from Aldrich, with Mn = 64.000g/mol, powder of analytical grade.

Figure 1 shows the molecular structures of the two optically active forms of poly (lactic acid) and also depicts the molecular structure of poly(β-hydroxybutyrate).

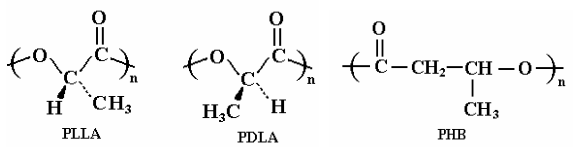

Figure 1: Molecular structures of the two optically active forms of poly (lactic acid) and molecular structure of poly(β-hydroxybutyrate).

Solvent

Analytical grade 1,1,1,3,3,3 - Hexafluoro-2-isopropanol (HFIP) from Aldrich was used to the prepare the films of PHB/PLLA blends.

Human IgG

Human immunoglobulin G (HIgG) was purchased from Sigma (98% purity).

Buffer Solution

The buffer solution used in the adsorption procedure was prepared with Milli-Q water (conductivity 18.2 MΩ.cm). Saline phosphate buffer (PBS) was used at pH 7.4. All of the reagents were of analytical grade and were purchased from Merck.

Methods

The adsorption of HIgG adsorption onto biomaterial surfaces (PHB, PLLA and PHB/PLLA blend) was assessed using FTIR/ATR in a continuous flow system that simulated physiological conditions. The experimental and analytical procedures were based on the guidelines of Chittur (1988), for polymeric films applied to germanium crystal surfaces by the spin-coating technique. According to this author, the protein adsorption density Γ can be quantified using Equation (1):

$$\Gamma = \frac{A/N - \varepsilon C_b d_e}{\varepsilon\left(2d_e/d_p\right)} \quad (1)$$

where: Γ is the protein adsorption density (mol/cm^2), A is the integrated absorbance due to internal reflections in the crystal element (mol/cm^2), N is the number of internal reflections, ε is the molar absorption index [dimensionless], d_p is the depth of penetration for the IR radiation (cm), d_e is the effective optical length of IR radiation (cm), C_b is the protein concentration in the sample (mol/cm^3) and b is the sample depth (cm).

The index ε can be calculated experimentally using the Beer-Lambert law, described by Equation (2):

$$A = \varepsilon b C_b \quad (2)$$

where b is the thickness of the sample (cm).

Figure 2 shows the continuous flow cell with the ATR crystal used in the experiments. The total height of the slit was 0.8 mm and allowed shear rates in the range of 500 - 700 s^{-1}, for flow rates of 75 mL/min. The angle of incidence of the germanium ATR crystal used was characteristically 45^o. The crystal was coated with the fused polymer using the spin coating technique (Young et al., 1988).

Based on Chittur (1988) and Young et al. (1988), the methods used in the tests of polymeric film confection and adsorption and in the quantification of the adsorbed proteins in these films followed the procedures presented below.

Procedure for Polymeric Film Preparation Using a Spin Coating Equipment

The blends PHB/PLLA 0/100, 50/50 e 100/0 (%m/m) were cast in the shape of films on a Ge crystal by using a Spin Coating Equipment, as represented in Figure 3. For this, to 0, 2% (m/v) solutions of the polymers were prepared in 1,1,1,3,3,3 – hexafluoro-2-isopropanol (HFIP) as organic solvent. After solubilization, 200 μL of the solution was dropped onto the substrate already coupled to the Spin Coating Equipment; the equipment was turned on in order to spread the solution and form an extremely fine film, using 4000 rpm of rotation during 30 seconds; this procedure was repeated 4 times to obtain film with four layers on the crystal, with a total thickness of 400 Å ± 50 Å (measured by using a Dektak^3 Profilemeter, Veeco Instruments Inc., USA) and an area equal to that of the Ge crystal used during the adsorption test (0.4mm in height x 7 cm in length). According to Chittur (1986), a film with just one layer is very fragile and can detach from the crystal or break during the adsorption test.

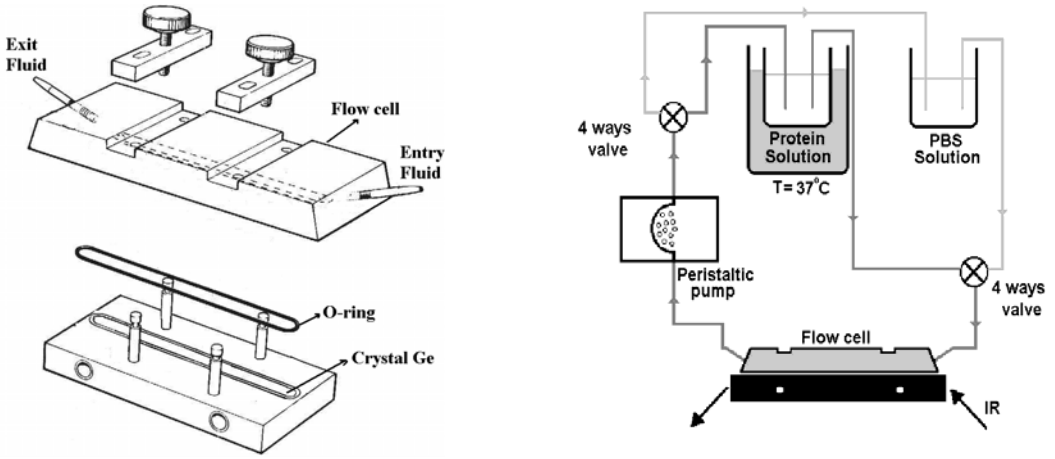

Figure 2: Schematic diagram of the continuous flow cell and experimental set-up for the FTIR/ATR experiments.

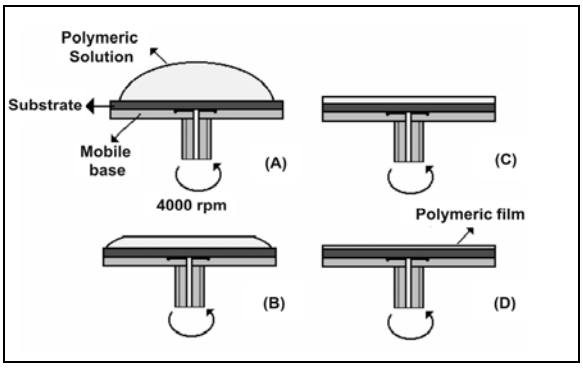

Figure 3: Schematic representation of film confection on the "Spin Coating" equipment in steps: A - drops of polymer solution, B - spreading of the polymer solution, C and D - evaporation of the solvent and formation of the thin film.

Procedure for the Testing of the Protein Adsorption HIgG on the Polymer Films

Protein adsorption tests on the polymer films were conducted in real time by spectrophotometry in the infrared (FTIR) employing a Nicollet Protegé 460 equipped with an ATR (attenuated total reflectance) cell. The calibration of the infrared light beam (IR) was performed using a standard of polystyrene (PS) according to the manufacturer's instructions. The continuous flow cell and continuous flow system were assembled in the laboratory and are shown in Figure 2. The tests and simultaneous analyses of the adsorption of the protein on the polymer film were done as follows: (i) a spectrum (crystal + film) (32 scans and resolution of 4 cm^{-1}) was collected: (ii) a flow of water was passed for 5 minutes, collecting 3 spectra (32 scans and resolution 4 cm^{-1}), in order to wash the film and analyze the band of water absorption at 1640 cm^{-1}; (iii) PBS solution was passed for 30 minutes, for ionic strength balance, and spectra (crystal + film + PBS) (32 scans and resolution of 4 cm^{-1}) collected; (iv) PBS solution was changed to protein solution (the concentrations ranged from 2.5 to 8.5 mg/mL) and spectra (crystal + film + saline solution + protein) collected during 2 hours (64 scans and resolution of 4 cm^{-1}); (v) protein solution was replaced by PBS solution, beginning the process of film washing for 2 hours in order to remove the proteins weakly adsorbed and eliminate the contribution from the protein in the proteic solution to the spectra, collecting spectra at pre-set intervals (64 scans and 4 cm^{-1}); (vi) at the end of the test, the cell was disassembled and the crystal with the film + protein was left in the laboratory environment for drying. When the crystal with the film + protein was dry, a spectrum (36 scans and 4 cm^{-1}) was collected in order to make sure that the polymeric film still remained on the crystal after the test.

The flow of solutions in the continuous flow system was about 33 mL/min, corresponding to a shear gradient ($\Delta\tau$) of 417 s^{-1}, comparable to that of blood, which is $\Delta\tau$ =420 s^{-1}. Such flow resulted in a Reynolds number equal to 70, confirming that the flow was laminar.

Spectral Treatment

The treatment of spectra was done according to Fink (1987). Before starting such treatment, all spectra obtained during the test were placed on the same scale with an automatic baseline correction and the calculations then performed as follows: (i) 1:1 subtraction of data from spectra (E) : E (protein + Saline solution + film + crystal) - E (saline solution + film + crystal) = E (protein); (ii) after the subtraction, the spectrum was multiplied by a normalization factor (N) aiming at standardization of all spectra for comparisons and calculations. A normalization factor (N) was calculated for each experiment, according to equation 3, because the intensity of the water absorption band at 1640 cm^{-1} varied from experiment to experiment, probably due to the continuous flow cell assembly and disassembly in each test.

$$N = \frac{I_{H_2O(1640)}}{(I_{T(1640)} - I_{S(1640)})} \quad (3)$$

where $I_{H2O\ (1640)}$ is the water absorption band intensity at 1640 cm^{-1}, $I_{T\ (1640)}$ is the absorption intensity at 1640 cm^{-1} of the total spectrum (crystal + polymer + buffer solution + adsorbed protein) and $I_{S\ (1640)}$ is the absorption intensity at 1640 cm^{-1} of the protein spectrum after the subtraction (crystal + polymer + buffer). (iii) After spectra had passed through the treatment described above (subtraction and

normalization), the region between 1000 and 2000 cm^{-1} was selected and a baseline was drawn between 1480 and 1700 cm^{-1}, as shown in Figure 4. Then the area of the region between 1480 and 1580 cm^{-1} was calculated by integration. This provided the area of the region of interest for each spectrum in each test. With the integrated areas, the adsorption density was estimated from Equation 1.

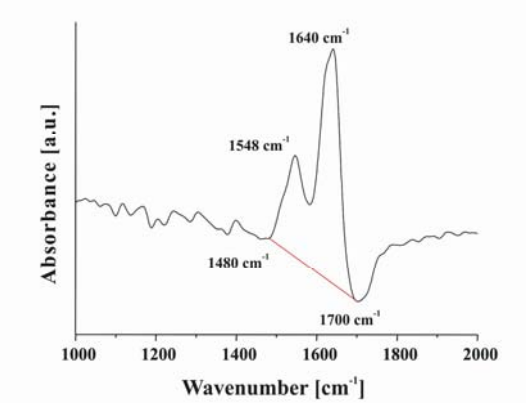

Figure 4: Typical absorbance subtraction spectrum for the FTIR/ATR experiments

For the quantification of adsorbed protein in the material, spectra obtained during the wash stage were used, because in this stage the spectra are related to the protein adsorbed on the material without the contribution of the protein in the proteic solution; the average values obtained during the wash stage were used for the quantification of adsorbed protein according to equation 1 (Chittur, 1988).

A curve of the adsorption kinetics versus the density of adsorbed protein was also constructed.

RESULTS

The amount of adsorbed protein was calculated as the difference between the peak areas after subtraction of FTIR/ATR spectra for the polymer film-coated on the germanium crystal containing the adsorbed protein and for the pure polymer with no adsorbed protein. A typical result for the subtraction spectra is shown in Figure 4. A linear correlation exists between the amount of protein adsorbed and the subtracted area of the amide II band (1640 cm^{-1}), and this allowed the protein adsorption density Γ to be obtained from the measurements.

The kinetics for the adsorption of different concentrations of HIgG to PHB, PLLA and PHB/PLLA blends are shown in Figures 5, 6 and 7, respectively.

The equilibrium isotherm data of PHB, PLLA and the blend PHB/PLLA 50/50 at 37°C are shown in Table 1 and the isotherm curves are depicted in Figure 8. The protein adsorption density Γ at equilibrium was obtained after washing to remove weakly and non-specifically bound proteins from the surface.

Table 1: Equilibrium isotherms data for HIgG adsorbed to the three polymers

HIgG/PHB C_b (mg/mL)	Γ (μg/cm²)	HIgG/PLLA C_b (mg/mL)	Γ (μg/cm²)	HIgG /blend 50/50 C_b (mg/mL)	Γ (μg/cm²)
2.5	1.23 ± 0.062	2.5	0.56 ± 0.028	3.5	1.09 ± 0.055
3.5	1.32 ± 0.066	3.5	1.14 ± 0.057	5.0	0.91 ± 0.046
5.0	1.31 ± 0.066	5.0	1.30 ± 0.065	7.5	0.98 ± 0.049
6.0	1.00 ± 0.050	6.0	1.20 ± 0.060	---------	---------
7.5	0.47 ± 0.024	7.5	1.24 ± 0.062	---------	---------
8.5	1.45 ± 0.072	---------	----------	---------	---------

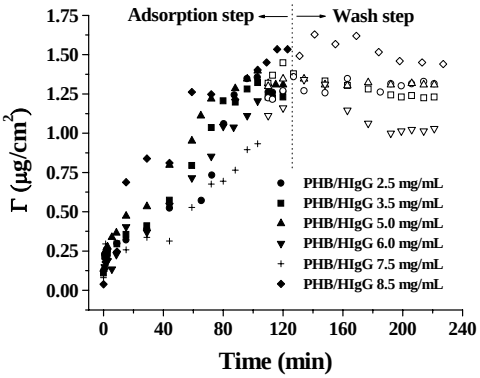

Figure 5: Adsorption kinetics for HIgG to PHB.

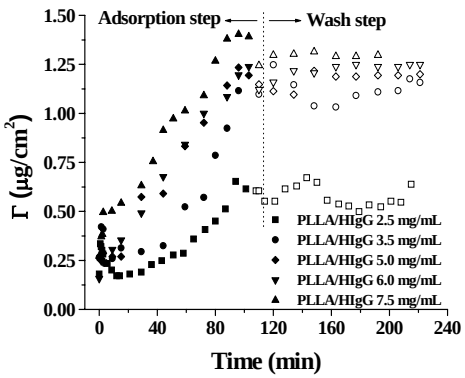

Figure 6: Adsorption kinetics for HIgG to PLLA.

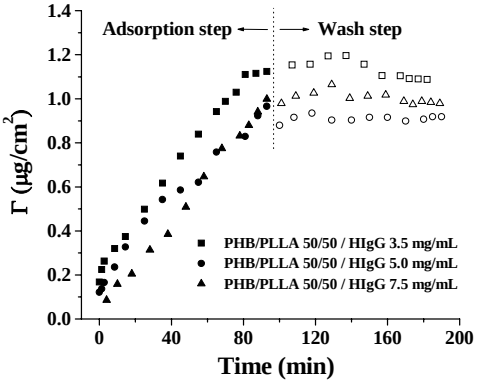

Figure 7: Adsorption kinetics for HIgG to 50/50 PHB/PLLA blend surfaces.

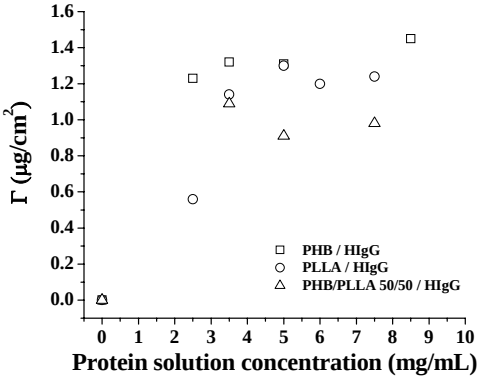

Figure 8: Adsorption isotherms for HIgG binding to PHB, PLLA and PHB/PLLA blends.

Analyzing the curves of adsorption kinetics of the systems PHB / HIgG, PLLA / HIgG and, PHB / PLLA / HIgG shown in Figures 5, 6 and 7, respectively, it is observed that the adsorption density presents a tendency to increase as a function of the increase in the proteic solution concentration, in the range of concentration studied. The adsorption density did not vary greatly with increasing proteic solution concentration in the systems studied, suggesting that the adsorption density of HIgG for a proteic solution concentration equivalent to that of blood (12mg/mL) would be very similar to the adsorption densities obtained in our experiments.

The kinetic data show a definite saturation of the surface after ~100 minutes (PLLA and blend PH/PLLA) and after ~120 minutes for PHB for all protein solution concentrations tested.

In Figure 8, a tendency to saturation of the adsorbed protein can be seen for the systems studied at the proteic solution concentration of approximately 6 mg/mL. It can be also verified that the adsorbed protein density is different for each system, probably due to differences in interaction between the material and protein.

Figure 8 also shows that the adsorption isotherm for the binding of HIgG has a distinct plateau that probably corresponds to formation of protein monolayers at the surface. This general behavior can be approximately correlated to the Langmuir isotherm model, which is mathematically described by Equation 4.

$$\Gamma = \frac{\Gamma_{máx} C^*}{(K_d + C^*)} \quad (4)$$

where C^* is the equilibrium concentration (mg/mL), Γ_{max} is the maximum density of adsorbed protein per unit area of the surface ($\mu g/cm^2$) and K_d is the dissociation constant. The values obtained for Γ_{max} and K_d from linear regression of the data for the tested polymers are shown in Table 2.

Table 2 shows that the values of maximum adsorption density for PHB indicate that this has the highest capacity for absorption of HIgG, followed by PHB / PLLA blend and finally by PLLA. The greater capacity of the blend adsorption in relation to PLLA may be due to the influence of PHB in the structure of PLLA when these are mixed.

Table 2: Values for Γ_{max} (maximum density of the protein adsorption per unit area) and K_d (dissociation constant)

System	Γ_{max} ($\mu g/cm^2$)	K_d
PHB/HIgG	2.57 ± 0.129	1.69
PLLA/HIgG	0.25 ± 0.013	0.04
50/50 PHB/PLLA / HIgG	0.85 ± 0.043	0.73

For comparison, Lassen and Malmsten (1997) reported a maximum adsorption value of 0.5 $\mu g/cm^2$ for HIgG at PP-AA surfaces at 30°C, utilizing fluorescence spectroscopic analysis (TIRF). Brash and Lymamn (1969), using the FT-IR/ATR technique, reported maximum adsorption values of HIgG to polystyrene, polyethylene and Silastic of 0.7 $\mu g/cm^2$, 1.0 $\mu g/cm^2$ and 1.8 $\mu g/cm^2$, respectively, at 30°C. In the present work PHB had the highest adsorption density, followed by blends (PHB/PLLA), while PLLA had the lowest values,

the values of HIgG adsorption density for these systems agreeing with the values observed in the literature.

CONCLUSION

At HIgG concentrations of 2.5 - 5 mg/mL, there was adsorption to PHB, PLLA and a 50/50 PHB/PLLA blend that reached a plateau at higher protein concentrations. The fit of the isotherm data for PHB, PLLA and the 50/50 PHB/PLLA blend followed the Langmuir model and showed saturation of the adsorbed protein for proteic solution concentrations of around 6 mg/mL. The use of fine films (thickness of 400 Å) produced by spin-coating increased the sensitivity of the FTIR/ATR technique. The use of a continuous method that simulated physiological conditions (blood flow, temperature, pH and ionic strength) made it possible to follow the protein adsorption in real time and in simulated conditions for the human body. The adsorption density values for HIgG onto biomaterial surfaces were very similar to those reported for other polymeric biomaterials presented in the literature (Straaten and Peppas, 1991 and Jeon et al., 1992). PHB had the highest adsorption density of about Γ_{max}=2.57μg/cm^2, followed by blends PHB/PLLA (Γ_{max}=0.85μg/cm^2); PLLA had the lowest values (Γ_{max}=0.25μg/cm^2).

ACKNOWLEDMENTS

Financial support from FAPESP in the form of scholarship and from PRONEX/CNPq (Biomaterials Group of Excellence) in the form of equipment and reagents are gratefully acknowledged.

NOMENCLATURE

Latin Letters

A	integrated absorbance due to internal reflections	mol/cm^2
b	sample depth	cm
C*	equilibrium concentration of protein in solution	mol/cm^3
C_b	bulk concentration of protein in solution	mol/cm^3
d_e	effective optical length	Cm
d_p	depth penetration for infrared radiation	cm
K_d	dissociation constant for the Langmuir isotherm	
N	number of internal reflections	

Greek Letters

Γ	adsorption density	mol/cm^2
Γ_{max}	maximum adsorption density	mol/cm^2
ε	molar adsorption index	dimensionless

Abreviations

ATR	attenuated total reflectance
FTIR	Fourier transform infrared spectroscopy
HFIP	1,1,1,3,3,3 - Hexafluoro-isopropanol
HIgG	Human immunoglobulin G
PHB	poly(β-hydroxybutyrate)
PLLA	poly(L-lactic acid)

REFERENCES

Black, J., The Education of the Biomaterialist: Report of a Survey, Journal of Biomedical Materials Research, 16, 159-167 (1982).

Brash, J. L. and Lyman, D. J., Adsorption of Plasma Proteins in Solution to Uncharged, Hydrophobic Polymer Surfaces, Journal of Biomedical Materials Research, 3, 175-189 (1969).

Chittur, K. K., FTIR-ATR for Protein Adsorption to Biomaterials, Biomaterials, 19, 357-369 (1998).

Deng, X. M., Castillo, E. J. and Anderson, J. M., Surface Modification of Soft Contact Lenses: Silanization, Wettability and Lysozime Adsorption Studies, Biomaterials, 7, 247-251 (1986).

Fink, D. J., Quantitative Surface Studies of Protein Adsorption by Infrared Spectroscopy, Analytical Biochemistry, 165, 147-154 (1987).

Fu, F. N., Fuller, M. P. and Singh, B. R., Use of Fourier Transform Infrared? Attenuated Total Reflectance Spectroscopy for the Study of Proteins Adsorption, Applied Spectroscopy, 47, 98-102 (1993).

Gendreau, R. M., Biomedical Fourier Transform Infrared Spectroscopy: Applications to Proteins, In: Spectroscopy in the Biomedical Sciences, Gendreau, R. M., Editor, CRC Press, Florida, 1-86 (1986).

Giacomelli, C. E., Bremer, M. G. E. G. and Norde, W., ATR-FTIR Study of IgG Adsorbed on

Different Silica Surfaces, Journal of Colloid and Interface Science, 220, 13-23 (1999).

Hoffman, A. S., In: Biomaterials: Interfacial Phenomena and Applications, Cooper, S. L. and Peppas, N. A. Editor, American Chemical Society, Washington, DC, 199, 3-8 (1982).

Hrkach, J. S., Ou, J., Lotan, N. and Langer, R., Poly (L-lactic acid-co-amino acid) Graft Copolymers: A class of functional Biodegradable Biomaterials, in: ACS Symposium Series 627, Chapter 8, 93-101 (1996).

Jeon, J. S., Sperline, R. P. and Raghavan, S., Quantitative Analysis of Adsorbed Serum Albumin on Segmented Polyurethane Using FTIR/ATR Spectroscopy, Applied Spectroscopy, 46, 1644-1648 (1992).

Kaelble, D.H. and Moacanin, J., A Surface Energy Analysis of Bioadhesion, Polymer, 18, 475-482 (1977).

Lassen B. and Malmsten, M., Competitive Protein Adsorption at Plasma Polymer Surfaces, Journal of Colloid and Interface Science, 186, 9-16 (1997).

Lin, J-C., Cooper, S. L., *In Vitro* Fibrinogen Adsorption from Various Dilutions of Human Blood Plasma on Glow Discharge Modified Polyethylene, Journal of Colloid and Interface Science, 182, 315-325 (1996).

Lyman, D. J., Brash, J. L., Chaikin, S. W., Klein, K. G., and Carini, M. The effect of chemical structure and surface properties of synthetic polymers on the coagulation of blood. II. Protein and platelet interaction with polymer surfaces. Transactions of the American Society for Artificial Internal Organs, 14, 250 (1968).

Pitt, W. G. and Cooper, S.L., Albumin Adsorption on Alkyl Chain Derivatized Polyurethanes: I. The Effect of C-18 Alkylation, Journal of Biomedical Materials Research, 22, 359-382 (1988).

Sharma, C. P., Blood-Compatible Materials: A Perspective, Journal of Biomaterials Applications, 15, 359-381 (2001).

Straaten, V. J. and Peppas, N.A., ATR-FTIR Analysis of Protein Adsorption on Polymeric Surfaces, Journal of Biomaterials Science - Polymer Edition, 2, 113-121 (1991).

Vroman, L. and Adams, A. L., Peculiar behavior at of blood and solid interfaces, Journal Polymer Science Part C, 34, 159-165 (1971).

Yokoyama, Y., Ishiguro, R., Maeda, H., Mukaiyama, M., Kameiyama, K. and Hiramatsu, K., Quantitative Analysis of Protein Adsorption on a Planar Surface by FTIR: lysozyme adsorbed on hydrophobic silicon-containing polymer, Journal of Colloid and Interface Science, 268, 23-32 (2003).

Williams, D. F. Definitions in Biomaterials, Elsevier Press, New York, v.4 (1987).

Young, B.R., Pitt, W. G. and Cooper, S. L., Protein Adsorption on Polymeric Biomaterials: I. Adsorption Isotherms, Journal of Colloid and Interface Science, 124, 28-43 (1988).

Xie, J., Riley, C., Kumar, M. and Chittur, K., FTIR/ATR Study of Protein Adsorption and Brushite Transformation to Hydroxyapatite, Biomaterials, 23, 3609-3616 (2002).