Vol. 18, No. 3 (2019) 1119-1132
Revista Mexicana de Ingeniería Química

# RHEOLOGICAL BEHAVIOR OF PLASMA POLYMERIZED IODINE-DOPED POLYPYRROLE PARTICLES SUSPENDED IN SOLUTIONS OF BOVINE SERUM ALBUMIN
# COMPORTAMIENTO REOLÓGICO DE PARTÍCULAS DE POLIPIRROL DOPADO CON YODO POLIMERIZADAS POR PLASMA SUSPENDIDAS EN SOLUCIONES DE ALBUMINA DE SUERO BOVINO

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Received: February 9, 2019; Accepted: March 14, 2019

## Abstract

Multiple studies have demonstrated the great potential of polymers derived from pyrrole (PPy) as biomaterials in the health area. Iodine doped polypyrrole synthesized by plasma (PPPy/I) has shown good *in vitro* results in cultures of various cell types as well as for treating traumatic spinal cord injury (TSCI) in laboratory animals. The objective of this study is to characterize the interactions between two agents that are potentially useful for repairing tissue, PPPy/I and the protein albumin of bovine serum (BSA); these materials and protein are known to behave separately, and they have not been used in combination as a treatment for TSCI. In this article, we present the rheological behavior of PPPy/I particulate suspensions in BSA solutions as well as the superficial physicochemical characterization of PPPy/I by X-ray Photoelectron Spectroscopy (XPS), Fourier-Transform Infrared Spectroscopy (FT-IR), Scanning Electron Microscopy (SEM) and Contact Angle analysis (CA). The results show the changes that occur in the surface chemistry of PPPy/I due to its interaction with BSA and how these interactions generate a complex system in suspension. The shear rate-dependent viscosity changes in the suspension provide the combination of PPPy/I-BSA features that are ideal for a combined treatment via injection.

*Keywords*: polypyrrole, albumin, rheometry, spinal cord injury.

## Resumen

Diversos estudios han demostrado el gran potencial de polímeros derivados de pirrol (PPy) como biomaterial en el campo de la salud. El polipirrol dopado con yodo sintetizado por plasma (PPPy/I) ha mostrado buenos resultados en *in vitro* para el cultivo de diversos tipos celulares, así como, en el tratamiento de la lesión traumática de medula espinal (LTME) en animales de laboratorio. El objetivo de este estudio es caracterizar las interacciones entre dos agentes potencialmente útiles para reparar el tejido, como el PPPy/I y la proteína albúmina de suero bovino (BSA), cuya acción es conocida por separado, pero que no han sido usados en combinación como tratamiento para la LTME. En este artículo, se presenta el comportamiento reológico de suspensiones de partículas de PPPy/I en soluciones de BSA, así como, la caracterización fisicoquímica superficial de PPPy/I por espectroscopia de fotoelectrones de rayos X (XPS), espectroscopia infrarroja por transformada de Fourier (FT-IR), microscopía electrónica de barrido (SEM) y análisis del ángulo de contacto (CA). Los resultados muestran el cambio en la química superficial de PPPy/I debido a sus interacciones con BSA y cómo estas interacciones generan en la suspensión un complejo sistema estructurado, cuyos cambios de viscosidad dependientes de la cizalla dan a la combinación PPPy/-BSA, características ideales para un tratamiento combinado a través de una suspensión inyectable.

*Palabras clave*: polipirrol, albumina, reometría, lesión de médula espinal.

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https://doi.org/10.24275/uam/izt/dcbi/revmexingquim/2019v18n3/Fabela
*issn-e*: 2395-8472

# 1 Introduction

Suspensions, called solid-liquid dispersions, are heterogeneous systems that are distinguishable by having two phases. The first phase is the continuous or external phase, which may be either a liquid (liquid suspension) or a semisolid (gels), and the second is the dispersed or internal phase, which is typically a particulate material that is practically insoluble in the continuous phase. In recent decades, these systems have been studied because of their potential biomedical applications as vehicles for injections, cell and drugs encapsulation and for their ability to take on the required shape of the site of interest (Romano *et al*., 2011; Yu *et al*., 2008). Furthermore, these semisolid suspensions have advantages such as their ability to maintain a high-water content in areas such as the extracellular matrix, controllable physicochemical properties and efficient mass transfer processes that carry great biological importance. Such semisolid systems may be obtained from natural polymers such as carboxymethyl cellulose (CMC), globular proteins and gums, or by synthetic polymers such as 2- hydroxyethyl methacrylate (HEMA), polyethylene glycol (PEG), N-isopropyl amine (NIPAAm) polyvinyl alcohol (PVA), and (Chitosan-g-Glycidyl Methacrylate)-xanthan hydrogel [(CTS-g-GMA)-X], among others (Chiu *et al*., 2009; Choi *et al*., 2009; Fabela-Sánchez *et al*., 2009; Ninan *et al*., 2013).

One way to characterize those systems is through the study of their rheological properties, which allows us to evaluate their ability to maintain and/or recover their original shape after they are subjected to deformation by stationary or dynamic shear. Both tests can analyze the changes in viscosity ($\eta$, $\eta^*$). The structural changes of dynamic shear can be studied through the behavior of the elastic or storage modulus (G') and the viscous or loss modulus (G"); with these parameters, it is possible to establish the existence of a state of gelation (Tadros, 2010). Using this information, it is possible to determine whether a gel can be used or not as a vehicle. It is possible to observe a thinning behavior by increasing the applied shear and a thickening behavior by decreasing shear. This pattern would be the rheological behavior during and following the injection process, assuming that the biological shear efforts are less than those that are applied to the system flow (Nielloud *et al*., 2000). Some of the other variables that should be considered to affect rheological performance are the size, shape, distribution and concentration of the suspended particles; system temperature; ionic strength; and surface and interparticle interactions (Agbenorhevi *et al*., 2011; Gunes *et al*., 2008; Mueller *et al*., 2010).

The protein serum albumin (SA) is a biomolecule of biotechnological interest due to its biological properties and its involvement in the transportation of nutrients and drugs, physiological regulation of pH and osmotic pressure, ability to act as a chelating agent (Di Bari *et al*., 2004; Peters Jr, 1995) and for its neuroprotective effect (Diaz-Ruiz *et al*., 2010; Gum *et al*., 2004), among other uses. Some of these properties have been exploited, mainly in the pharmaceutical and food sector, to obtain systems that are made of ordered multiphase structures, (Peters Jr, 1995) such as latex particles coated with polypyrrole (PPy) (Bousalem *et al*., 2004), which can be used as a drug delivery vehicle in a suspension of particles of PPy-alginate (Thanpitcha *et al*., 2011) or as a coating in metal stents and bars (Khan *et al*., 2007), as an example of some applications.

Various processes have been reported in the production of biomaterials that are derived from pyrrole. Those processes have been shown to produce some degree of biocompatibility of biomaterials in the short, medium and long term; for example, the capacity of pyrrole can be improved by changing its topography, chemistry, or surface energy (Harnett *et al*., 2007). In this regard, low-temperature plasma can be employed for polymer synthesis and has been an invaluable resource for coating surfaces which initially have a low or non-biocompatibility. Using this technique, it has been possible to develop new technologically important macromolecules that exhibit beneficial properties for biological applications (Huang *et al*., 2014; Russo *et al*., 2010). An example of this innovation is the surface modification of biomaterials for the immobilization and coupling of biomolecules (Bousalem *et al*., 2004; Khan *et al*., 2007; Kotwal *et al*., 2001; Stauffer *et al*., 2006). In the case of iodine-doped polypyrrole that is synthesized by plasma (PPPy/I), our group and others have tested its ability to interact with biological systems, both *in vitro* (Zuniga-Aguilar *et al*., 2014; Zuñiga-Aguilar *et al*., 2013) and in vivo (Cruz *et al*., 2012; Olayo *et al*., 2008), showing good biocompatibility results in both systems. Suspensions of PPPy/I particles were injected in vivo in rats with traumatic spinal cord injury and produced a good recovery of motor function in the rats after they were subjected to a spinal cord lesion and

polymer implant (Mondragon-Lozano *et al*., 2016). However, the implanted particles may migrate from the site of the lesion due to its shape and size of the lesion relative to the nonlesioned surrounding tissue. Therefore, it would be desirable to characterize both the morphological as the physical chemistry of the surface, to provide an environment that takes advantage of these polymer - protein interactions. These interactions may in turn trigger at the injured site the regrowth of tissue which would otherwise be lost due to the mobility of the particles. A second objective of the present study is to characterize the physicochemical interactions between polymer particles and a model protein (albumin) to better understand polymer-tissue interactions.

In the present work, we studied the interactions between the PPPy/I particles and BSA protein. The changes in surface chemistry through the contact angle on the thin PPPy/I films were determined after their exposure to solutions with different concentrations of BSA protein. Furthermore, the behavior of the PPPy/I particles that were suspended in solutions of BSA was analyzed by flow shear-rate tests under stationary and dynamic regimes. The results that were obtained from the physico-chemical analysis of the PPPy/I particles provided evidence that the polymer - protein interactions established a complex system that is capable of generating, in the womb of the suspensions that were tested, a structured arrangement that depends on the shear-rate to which they are being subjected. In this way, the changes in the behavior of the suspensions that were tested allowed us to determine which combination of PPPy/I-BSA presented the maximal flow conditions so that, in subsequent work, in vivo tests can be performed by microinjecting the selected suspension as part of a TSCI treatment, leveraging the neuroprotective properties of the PPPy/I particles and the BSA protein.

## 2 Materials and methods

### 2.1 Reagents

Pyrrole monomer, iodine and solvents of analytical reagent grade were purchased from Sigma Aldrich Co. (St Louis, Missouri, USA) and used for the subsequent experiments. Bovine serum albumin (BSA) was acquired from Equitech - Bio, USA (Cat. BAH68). The phosphate buffer solution (PBS) was acquired from Cellgro Corning, USA (Cat. 46 - 013 - CM).

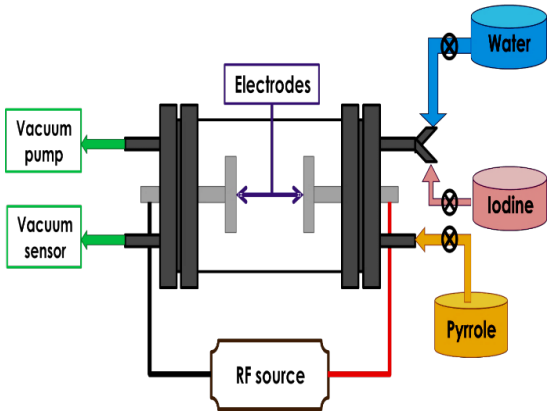


Fig. 1. Schematic representation of the plasma reactor used for the synthesis of PPPy/I.

### 2.2 Synthesis of PPPy/I

Synthesis of iodine-doped polypyrrole by plasma (PPPy/I) was performed in a cylindrical glass reactor (Fig. 1), as reported previously (Cruz *et al*., 1997; Morales *et al*., 2000). The system was supplied with 25 W of power, with a reaction time of 240 min, and pyrrole was supplied at intervals of 20 minutes, iodine at 3 minutes and water at 1 minute intervals. The material collected from the reactor was stored under a vacuum seal until final use.

### 2.3 PPPy/I characterization

Once synthesized, the PPPy/I film was analyzed by Fourier-Transform Infrared Spectroscopy (FT-IR), using a Perkin-Elmer 1600 FT spectrophotometer. Moreover, the rest of the polymer film was ground to a fine powder and was observed by Scanning Electron Microscopy (SEM) using the JEOL JSM-5900LV (20 kV). In addition, the corresponding elemental analysis was performed by XPS in a Thermo K-Alpha spectrometer with X-ray monochromator using an Al Ka source (1486.6 eV).

### 2.4 Albumin absorption test

Glass slides were coated with a thin film of PPPy/I and were immersed for 5 min in solutions of BSA in PBS at physiological pH, with increasing concentrations of protein from 0 to 30 mg/mL at room (25±1 °C) temperature. At the end of the exposure time, the excess protein was removed by rinsing with PBS. The glass slides were placed in a vacuum desiccator for 24 hours, and then an analysis of the surface modifications was performed by measuring the static

method contact angle; the data are presented as the average with standard deviation and were plotted in GraphPad Prism Version 7.

### *2.5 Rheometry*

Flow tests were performed for stationary and oscillatory shear in an AR-G2 Rheometer of controlled stress (TA Instrument, USA). Samples consisted of 10 mg of PPPy/I that were suspended in solutions at 0, 7, 14 or 21 mg/mL of BSA in PBS at a physiological pH. The geometries that were used in this study were concentric cylinders (gap of 500 microns), cone and plate (60 mm in diameter, cone with slope of 1 ° and 36 microns of truncation). Measurements were made at temperatures of 25 and 37 °C, and the temperature was controlled with a recirculating water system (RTE-110 Endocal, UK). The tests were performed in the strain-controlled mode. Frequency sweeps for the suspensions were conducted within the linear viscoelastic region at a constant strain of 95%, and the frequency range was 0.016 to 16 Hz. The steady shear tests were performed from 0.1 to 100 $s^{-1}$. The data were analyzed using Rheology Advantage Data Analysis TA Instruments Software Version 3.3.4 and plotted in GraphPad Prism Version 7.

## 3 Results and discussions

### *3.1 Morphology*

Once the PPPy/I film was ground, various morphological aspects, such as particles with different sizes and an irregular geometry with a tendency to form agglomerates, were observed, as shown in Fig. 2. From the different SEM images, it was determined that the length of the particles ranges between 0.7 and 9.1 $\mu$m and the average size and standard deviation was 2.3 ± 0.2 $\mu$m. These differences in size and shape are embroiled in the hydrodynamics of the PPPy/I particle suspensions; in addition, this diversity in size allows the particles to display a large surface area that is capable of interacting with the protein BSA, as will be demonstrated later.

### *3.2 Fourier transform infrared spectroscopy (FT-IR)*

A section of the PPPy/I film was analyzed by FT-IR before it was ground. The spectrum (Fig. 3) shows the presence of O-H and N-H groups in the bands at 3615, 3350 and 3217 $cm^{-1}$. The next two bands correspond to aliphatic C-H bonds. The following bands correspond to multiple C-O type bonds at 2361 $cm^{-1}$, and both C≡C and C≡N absorptions were observed at 2220 $cm^{-1}$, suggesting the loss of hydrogen atoms in the synthesis process. The bands at 1625 and 1550 $cm^{-1}$ are associated with C=C conjugated bonds, which together with the bands centered at 1450 and 1375 $cm^{-1}$ are characteristic of the pyrrole molecule. The same band of 1550 $cm^{-1}$ may overlap with the signal belonging to the N-H bonds. Moreover, the bands at 725 and 675 $cm^{-1}$ confirm the presence of unsaturated C=C type bonds. Finally, a shoulder located at 575 $cm^{-1}$ may be associated with iodine, which acts as a dopant in the synthesis of PPPy/I. The presence of groups with a major unsaturation grade, as well as peaks corresponding to groups with oxygen, are evidence of rupturing of the pyrrolic ring during the synthesis process, which allows for the great diversity of functional groups that make up the surface structure of the PPPy/I particles.

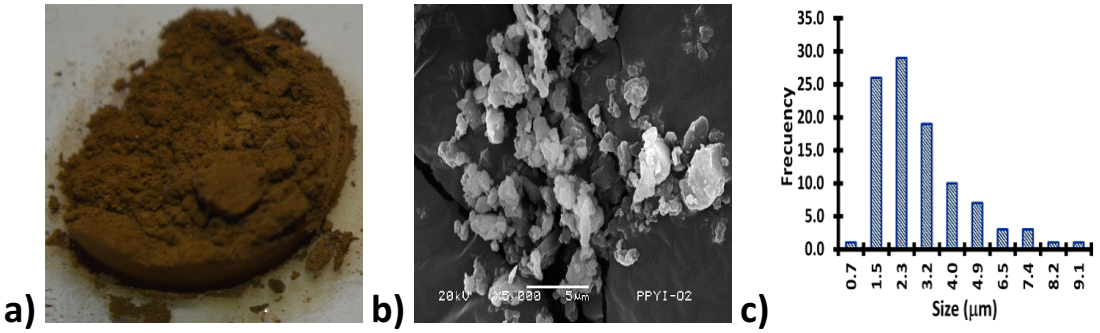


Fig. 2. Representative images of PPPy/I. (a) Photograph of the PPPy/I powder. (b) SEM Micrograph (bar 5 $\mu$m) and (c) Particle size distribution.

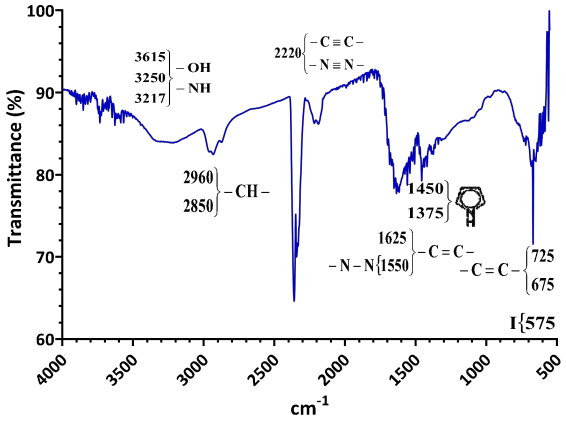


Fig. 3. FT-IR spectrum of the iodine-doped polypyrrole film.

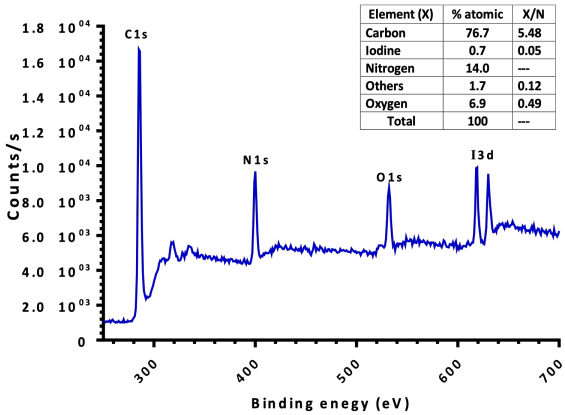


Fig. 4. XPS Survey spectrum and elemental analysis of PPPy/I.

## *3.3 XPS and elemental analysis*

A quantitative analysis such as XPS allowed us to estimate the elemental composition of the PPPy/I particles, as defined by the atomic percent of C, N, O and I in this system (Fig. 3). The C and N come from the pyrrole monomer. The C/N = 4 relationship of the pyrrole monomers was modified during the polymerization process, thereby obtaining a final C/N relationship of 5.48 in the PPPy/I particles. Changes in the C/N ratio suggest that, subsequent to the rupture of the pyrrolic rings in the plasma conditions, some of the fragments formed which contain N can escape from the reactor, resulting in a decrease of N atoms in the final product. The XPS analysis also detected the presence of iodine atoms; some of these may be covalently bound to the polymer but others were found trapped in the ionic form as dopants in the PPPy/I structure. Considering that the I/N ratio was estimated to give 20 nitrogen atoms for every iodine atom that was present in the polymer, its doping effect it is not very efficient.

An element that did not belong to the pyrrole monomeric molecule but was also detected was oxygen, which could have two main sources; the first could be due to the entry of the water stream in the synthesis process, while the second possibility is the interaction of the ambient oxygen once the reactor is opened, in which case the oxygen would have worked to neutralize of free radicals that were present on the surface of the synthesized polymer. In any case, the presence of O allows for the calculation of the O/N relationship that indicates the level of oxidation of the polymer. Since O/N=0.49, it is estimated there is the presence of an atom of O for every two atoms of N; i.e., for every two molecules of pyrrole, there enters at least one oxygen for the PPPy/I structure. In addition to this ratio, when performing the data analysis by deconvoluting the peak for the 1 s configuration of carbon that is obtained by XPS (Fig. 5), it is possible to establish the existence of C-C, C-N, C-O, C-OO and C-NN functional groups, which are present in the superficial layers of the PPPy/I particles; these groups match the set of signals that were observed in the FT-IR spectrum.

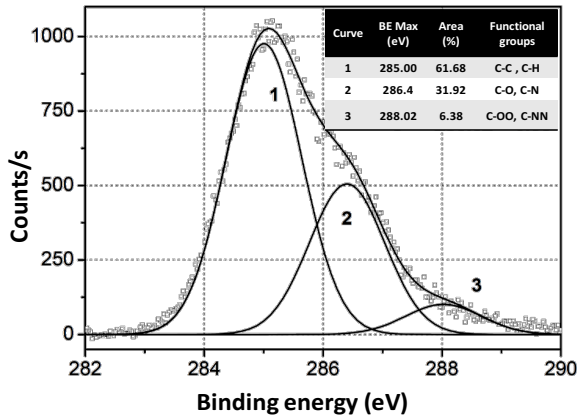


Fig. 5. Deconvolution of the main peak for the configuration C1s that were obtained by XPS analysis.

## *3.4 Contact angle*

Wettability and chemical affinity were assessed by contact angle on glass slides that were coated with a PPPy/I film, providing a characterization of the functional groups that were exposed at the polymer surface. This analysis shows the behavior of the polymer surface with and without its interaction with

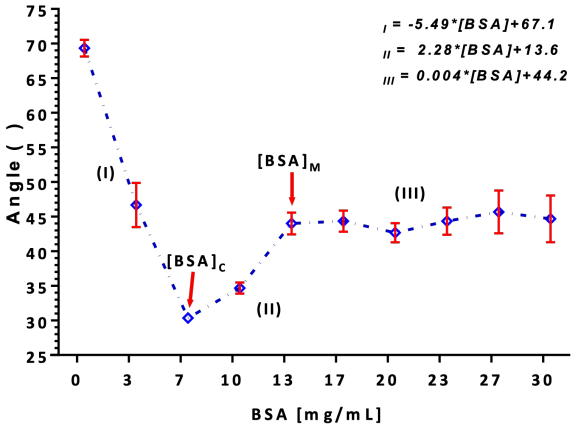


Fig. 6. Surface contact angle of the glass slide that was coated with a PPPy/I film in relation to the increasing concentrations of BSA at 25°C (average ± standard error).

the BSA protein solution at different concentrations. It is important to note that in this study, PBS was used as a dissolution medium for the protein and subsequently as the suspending medium when testing with the suspended flow of the particles, so the behavior of BSA differs somewhat from that reported in previous studies in the literature, which were carried out with distilled water, since the presence of salts modifies the behavior of proteins (Duan *et al*., 2013).

The determination of the contact angle is a tool that allows for the study of the performance of the surface chemistry, and such behavior is equivalent in thin films such as in particle suspensions of any material. The behavior of films and of particles in the PPPy/I suspensions depends on various factors, such as the concentration and temperature of the BSA solutions and the osmolarity and the concentration of Cl-, Na+ and K+ ions, which are present in solutions, resembling those found in the extracellular fluid of mammals. This measurement is important because these ions help to establish the surface electrochemical behavior of both PPPy/I and BSA; in the case of BSA, this medium favors the intramolecular interactions that stabilize the three-dimensional structure of the protein. The presence of an ionic medium should help in the processes of the adsorption/desorption of BSA on the surface of PPPy/I particles, as suggested by the literature (Zhang *et al*., 2011).

The surface chemistry features that were established through the XPS and FT-IR analyses are important since the surface is in direct contact with the surrounding medium. For biological systems, the ability of surfaces to adsorb/desorb from proteins is very important, particularly for serum albumin, which is the most abundant protein in the biological environment; for the specific case of PPPy/I, the adsorption process can be seen in Fig. 6. We considered that for a hydrophobic surface with an angle greater than 60 ° (Harnett *et al*., 2007; Keselowsky *et al*., 2003), it is possible to associate this surface with characteristics of PPPy/I since it initially shows a contact angle of 70.7±1.0 °, which is related to the presence of both unsaturated and aliphatic groups (Figs. 3 and 5).

Thus following the adsorption behavior that is shown in Fig. 6, it is possible to distinguish three zones depending on the concentration of BSA at which the films of PPPy/I were exposed; zones (I) and (II) converge at a minimum angle of 30.4±0.3°, suggesting the formation of a monolayer that is dependent on a critical concentration of protein ($[BSA]_C$) (Azioune *et al*., 2005; Bialopiotrowicz *et al*., 2001), which is approximately 7 mg/mL. From this point, the functional groups that are exposed in the new surface are capable of associating with other free molecules of BSA, which enables the formation of multiple layers of protein, a phenomenon that has been observed in the case of films that are exposed to a medium with a higher protein concentration, also known as a critical concentration of multilayer ([BSA]M), which is close to 13 mg/mL and corresponds to the beginning of the third zone that is depicted in the same figure.

Perhaps one way to represent the surface interaction of PPPy/I and BSA is shown in Fig. 7, where a possible mechanism of protein adsorption onto the surface of polymer has been established after observing the formation of the monolayer; the surface exposes polar groups of BSA containing NH, OH and COOH groups, and these groups are present in the amino acids that make up the protein structure and are highly dependent on physiological pH (Ahmed *et al*., 2015; Fanali *et al*., 2012), allowing the free BSA molecule to interact with a new surface chemical diversity. Nevertheless, when increasing the thickness of the protein layer, there are packing and hydration limits (Ahmed *et al*., 2015; Zhang *et al*., 2011) that establish a dynamic equilibrium where BSA molecules are released from last layers and replaced by new BSA molecules; this phenomenon occurs while the films are exposed to higher protein concentrations than that of the surrounding medium, which still contains proteins that are capable of interacting; this equilibrium indicates that at higher protein concentrations the observed angle does not

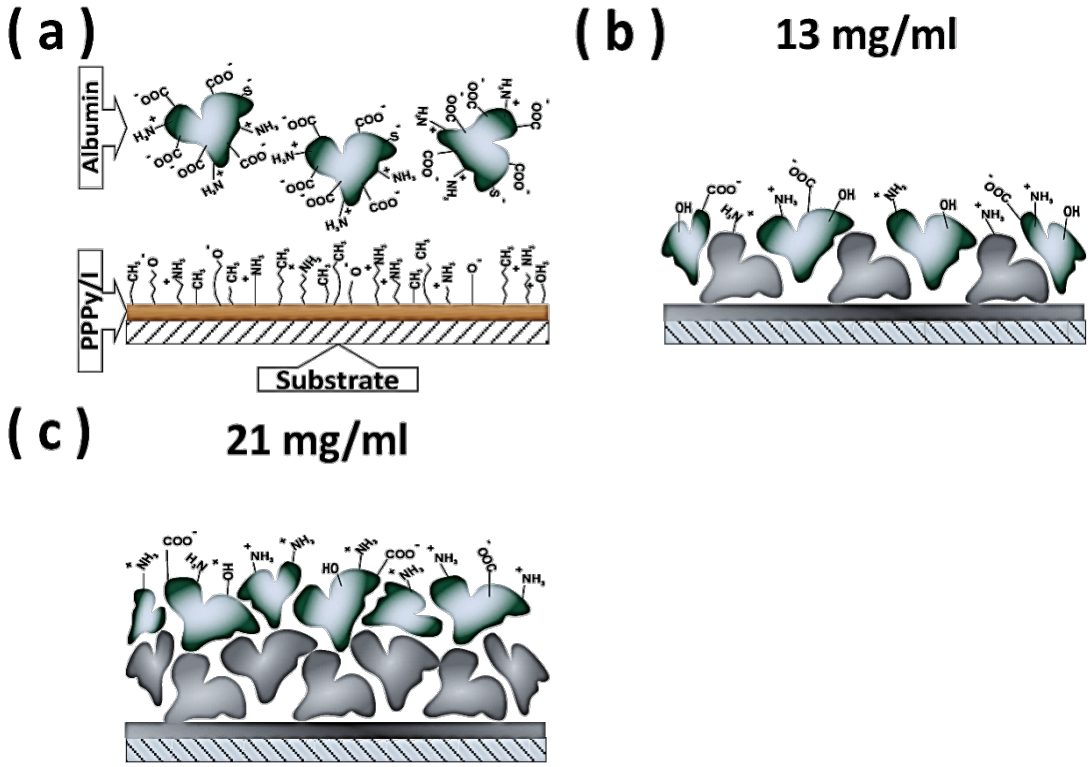


Fig. 7. Schematic representation of the process of adsorption of BSA onto PPPy/I films. (a) Initial phase of exposure of BSA to the surface of the PPPy/I, (b) the saturated monolayer on the surface of the polymer and (c) the arrangement of new molecules of BSA that are present at higher concentrations of exposure when the multilayer formation process reaches a concentration-independent limit.

substantially increase but instead maintains an average value of 42.6±2.0 ° (Vogler, 2012; Zhang *et al*., 2010).

A similar mechanism can be extrapolated to the suspensions of particles in protein solutions that were tested, with the difference that these suspensions would have a greater amount of BSA available to maintain the replacement system dynamic that was previously mentioned in the multilayer region, (Fig. 7) thereby obtaining a complex structured system that depends on multiple interactions between PPPy/I-BSA, PPPy/I-PPPy/ and BSA-BSA; this system of interactions is outlined in Fig. 8.

### *3.5 Rheological behavior*

Prior to conducting the polymer suspension flow tests, thermal stability tests of the albumin protein were performed with stationary shear tests. The stability of BSA in solutions at low and high protein concentrations was studied through a temperature scan under stationary shear conditions at a temperature between 25 and 39 °C. In this range no significant changes in the viscosity were observed, as seen in Fig. 9, which also shows the behavior of the solutions. Nevertheless, at 38 °C, the viscosity starts to drop due to protein denaturation; this shows that the temperatures (25 and 37 °C) at which the experiments were conducted did not result in significant conformational changes.

In rheological terms, the protein BSA presents a stable viscoelastic behavior under stationary shear conditions in the temperature range of 25 to 37 °C (Fig. 9) because the protein denaturation begins at approximately 38 °C according to the literature (Inoue *et al*., 1996). After determining the protein stability in the temperature range of interest for this study, a rheological test for oscillatory shear was performed at concentrations of 7, 14 and 21 mg/mL BSA in PBS (Fig. 10). At all concentrations, the elastic modulus (G') had a tendency to remain constant at low frequencies, and this effect was more remarkable when the solutions were at 37°C temperature (Fig. 10b). Furthermore, it has been reported that solutions of BSA have behavior that is stabilized by the strong

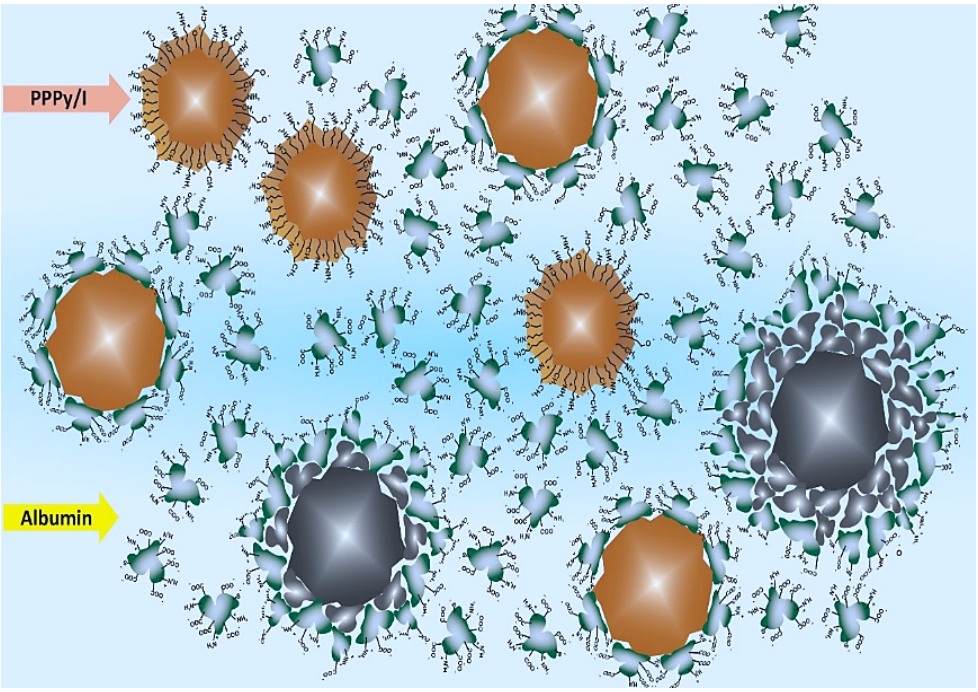


Fig. 8. Schematic representation of the multiple interactions that are present in the suspensions of PPPy/I particles in BSA solutions. All interactions are present within the continuous phase and affect the rheological behavior of the suspension, giving this an internal structure which more or less depends on the conditions to which it is subjected.

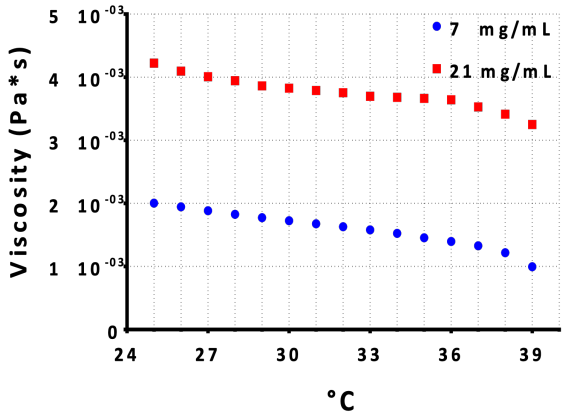


Fig. 9. Rheological study in stationary shear with a temperature scan of 25 to 39°C for solutions at 7 (●) and 21 (▲) mg/mL PBS.

molecular interactions between the proximal globular proteins, which involves not only a non-Newtonian behavior but also results in a yield stress that drifts to a structured system with rheological behavior due to the adsorption and aggregation that occurs in the majority of the solution (Castellanos *et al*., 2014; Chodankar *et al*., 2010). This behavior is characteristic for solutions showing a frequency independent of G', especially at a concentration of 14 mg/mL at 37 °C for our system. These properties that are advantageous for the generation of hauling and drug delivery systems or to promote a structured system by multiple interactions of the biomaterial PPPy/I and BSA at low shear stress, as in the case of this study.

The curves of viscosity for stationary shear of the suspensions of PPPy/I particles in solution at different concentrations of BSA at temperatures of both 25 and 37 °C are shown in Fig. 11. In all cases, shear thinning viscosity was obtained, which means that the suspensions are structured, and this structure is shear rate dependent. The measuring range of the rheometer did not allow for us to obtain the viscosity at a very low shear rate; however, it is possible to conclude that the system has a viscosity that decreases with shear rate. This property will allow it to be injected at a large shear rate, then at the end of the injection process the viscosity will increase by several orders of magnitude.

The interactions between PPPy/I-PPPy/I, PPPy/I-BSA and BSA-BSA that were mentioned earlier define the modification of rheological behavior of the suspensions (Ortiz-Zarama *et al*., 2016). For the rheological study of stationary shear, we can perform a data analysis using the model that has been proposed

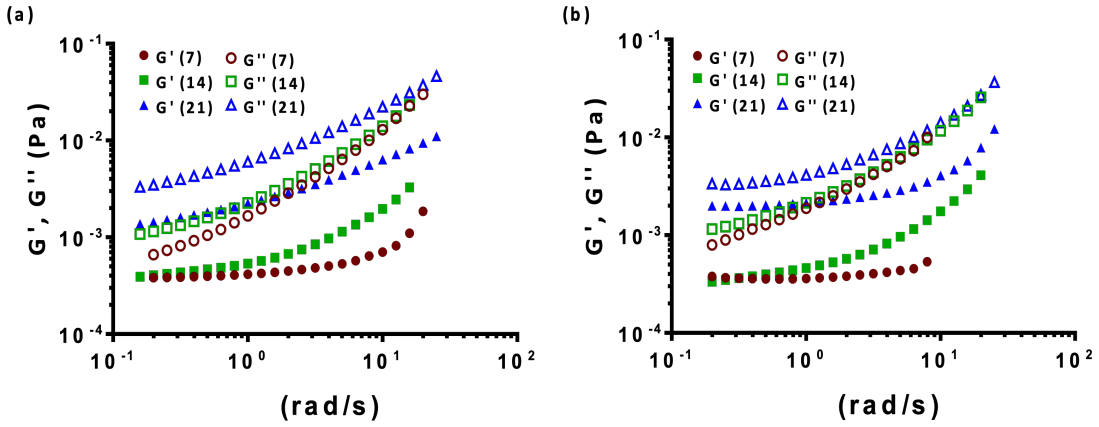


Fig. 10. Rheological study in oscillatory shear of suspensions of BSA solutions. The filled symbols correspond to elastic modulus (G', Pa) and empty symbols represent the loss modulus (G", Pa). The concentration of BSA in the solutions corresponds to 7 (●, o), 14 (■, □), or 21 (▲, △) mg protein per mL PBS. T=25°C (a) and T=37°C (b), respectively.

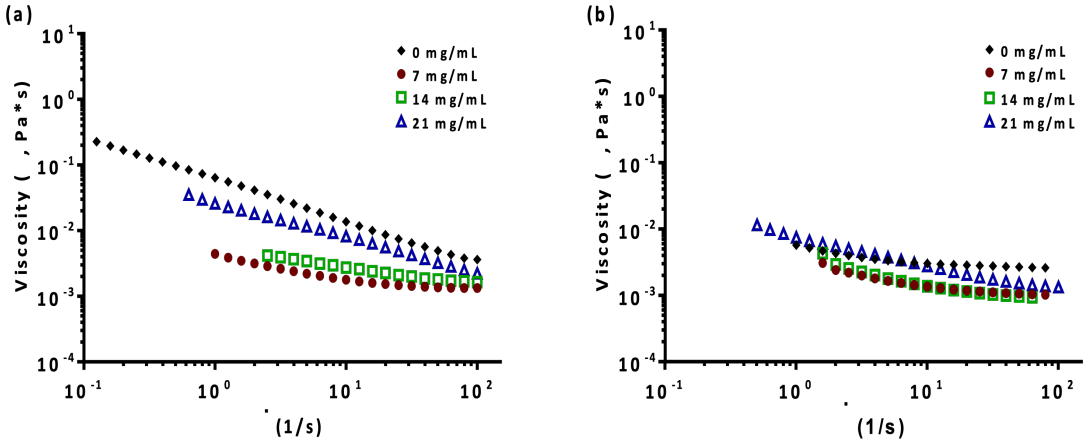


Fig. 11. Rheological study in stationary shear of the viscosity of the PPPy/I suspensions in BSA solutions. All suspensions containing 10 mg per mL PPPy/I and an additional 0 (♦), 7 (●), 14 (□), or 21 (△) mg BSA per mL PBS. T=25°C (a) and T=37°C (b), respectively.

by Cross (Cross, 1965). This is one of the more basic models (Eq. 1) that allows us to analyze the trend of the viscosity at a shear rate of zero. This model provides an estimate of the Newtonian region and generally proposes viscosity behavior over a wide range of shear rates, allowing us to estimate the shear thinning behavior of the suspension that is produced when the stress results in the arrangement of PPPy/I and BSA to become disorganized inside of the suspension (Guerra-DellaValle *et al*., 2009).

$$\eta = \eta_{\infty} + \frac{\eta_0 - \eta_{\infty}}{1 + (\lambda\dot{\gamma})^{\alpha}} \quad (1)$$

Where $\eta$= apparent viscosity, $\eta_0$ = zero shear viscosity, $\eta_{\infty}$ = infinite shear viscosity, $\lambda$ = the Cross time constant or the consistency, $\dot{\gamma}$ = shear rate and $\alpha$ = the Cross-rate constant.

The behavior of the suspensions that is estimated by the Cross model (Eq. 1) is shown in Fig. 12. It can be seen that at 37 °C the estimated viscosities are high enough that the low shear rate results in a behavior of thickening for the PPPy/I- BSA suspensions. The suspension of 10 mg/mL PPPy/I with 14 mg/mL BSA gives a higher viscosity than the suspension of 10 mg/mL PPPy/I without BSA; additionally, this same suspension has a very low viscosity at a high shear rate. However, per the model of Cross, the suspension with 7 mg/mL PPPy/I also presents a high viscosity in

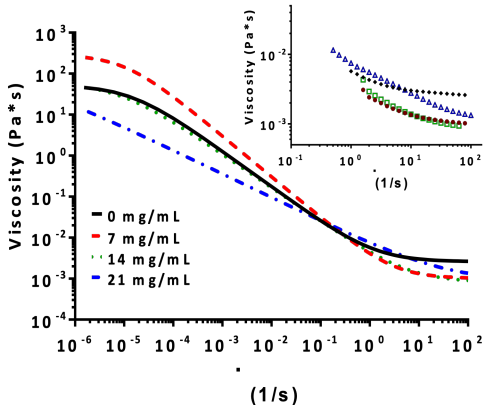


Fig. 12. Flow curves according to the Cross model. The upper pane displays the flow curves that were obtained experimentally at 37°C, while the main panel shows the setting of the data according to the Cross model for the same group of experimental data.

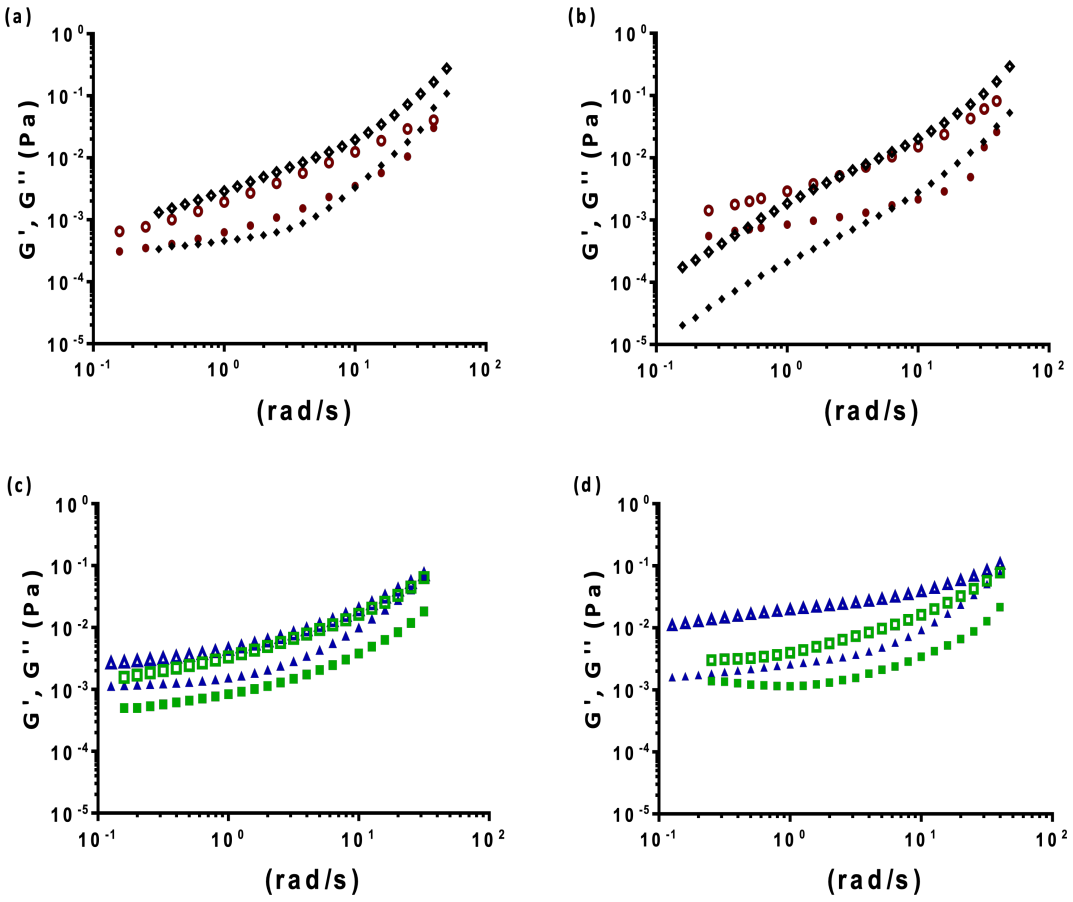


Fig. 13. Rheological study in oscillatory shear of PPPy/I suspensions with or without BSA in solution. The filled symbols correspond to the elastic modulus (G', Pa) and empty symbols represent the loss modulus (G", Pa); all suspensions contain 10 mg per mL PPPy/I. BSA concentrations correspond to 0 (♦, ◊), 7 (●,o), 14 (■, □), or 21 (▲, △) mg/mL, and the temperature was (a and c) T = 25°C and (b and d) T= 37°C.

stationary shear and low shear rates at 37 °C, but the dynamic behavior is less adequate for this study (Fig 13); apparently, the ability of these suspensions to interact as a structured system is present at lower frequencies than those that are required by the suspension of 14 mg/mL PPPy/I.

The data shown in Fig. 13 presents the dynamic rheology of the particle suspensions, and the graphs show the elastic modulus (G') and the loss modulus (G") as a function of frequency at 25 and 37 °C. At 25 °C, all of the suspensions trend to a zone where the G' is independent from the frequency, which establishes a behavior of a system that is internally structured like a solid at low frequencies. When the temperature increases to 37 °C, the polymer suspension without BSA does not tend to form a plateau at low frequencies, showing a similar behavior as an unstructured polymeric suspension or a suspension with a weak internal structure. However, all the other samples tend to form a plateau, in particular the suspension that includes 14 mg BSA, which shows a clear plateau. The suspension with a concentration of 21 mg BSA also shows a tendency towards frequency-independence except for frequencies much lower than those shown by 14 mg of BSA.

In this way, both the behavior under dynamic shear tests and the result by Cross modeling show a suspension with characteristics of interaction between polymer and protein, and both types of tests correspond to the combination of 10 mg/mL PPPy/I with 14 mg/mL BSA. Given the behavior of the suspensions that were observed under these temperature and shear conditions, it is expected that the in vivo conditions would shoe results that are equivalent to those reported previously (Cruz *et al*., 2012), where a pill solid was introduced and a compact PPPy/I polymer was administered at a site of injury near a complete section of spinal cord, and positive results in the functional motor recovery were observed.

## Conclusions

In this study, a superficial physico-chemical analysis of the PPPy/I polymer was performed, showing that due to the synthesis conditions a great diversity of functional groups is obtained on its surface, and most of these functional groups are of the hydrophobic type. The diverse chemical functionalities allowed us to study the behavior of this material under testing of its flow in both stationary and dynamic shear conditions in a medium as ionic as PBS. Additionally, it could be observed that due to the surface properties of the polymer and the possible charges that were established by its ionic medium, it has a great capacity to interact with the model protein BSA. This capacity for interaction allows the formation of a system of mono or multilayer proteins on the polymer and thus at the same time allows for us to obtain a modification of the superficial chemistry that is presented by PPPy/I. These superficial changes are of great importance to the extent that the flow properties of these polymer particle suspensions depend not only on the temperature or the conditions of ionic PBS but also to an even greater extent on the concentration of BSA that is present in the suspension.

The results reported here allow us to show the existence of a suspension of PPPy/I-BSA that can flow under high shear rate conditions (shear thinning) and is able to recover its viscosity once the shear rate considerably decreases (shear thickening). This feature of thinning/thickening of the viscosity is characteristic of a structured system for multiple interactions and is ideal for conducting *in vivo* tests by microinjection in animals with spinal cord injury, where the goal of such studies is to obtain synergistic outcomes of the potential neuroprotective effects of both PPP/I and BSA, a processes that involves the functional recovery of the ability to walk. In this manner, and in accordance with the results we obtained, we conclude that the combination of 10 mg/mL PPPy/I and 14 mg/mL BSA is the suspension best suitable for this purpose.

### *Acknowledgements*

The authors wish to thank to National Council for Science and Technology (CONACyT) by doctoral scholarship number 327854 and for partial support for the realization of this work through project CONACyT-15523.

### *Nomenclature*

| | |
|---|---|
| [BSA] | Concentration of Bovine Serum Albumin, mg/mL |
| BE | Binding Energy, eV |
| BSA | Bovine Serum Albumin |
| eV | Electronvolt |

| | |
|---|---|
| FT-IR | Fourier Transform Infrared Spectroscopy |
| G' | elastic or storage modulus, Pa |
| G" | viscous or loss modulus, Pa |
| Pa | Pascal, N* $s^{-2}$ |
| PBS | Phosphate Buffer Solution |
| PPPy/I | iodine-doped polypyrrole synthesized by plasma |
| SA | Serum Albumin |
| SEM | Scanning Electron Microscopy |
| T | Transmittance in percentage, % |
| XPS | X-ray Photoelectron Spectroscopy |
| *Greek symbols* | |
| $\theta$ | contact angle, ° |
| $\lambda$ | cross time constant or the consistency |
| $\alpha$ | cross-rate constant |
| $\omega$ | frequency of oscillation, rad*$s^{-1}$ |
| $\eta_\infty$ | infinite shear viscosity, Pa*s |
| *gaṁma* | shear rate |
| $\eta$ | apparent viscosity, Pa*s |
| $\eta_0$ | zero shear viscosity, Pa*s |

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