PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE
ESCUELA DE INGENIERIA

IMPACT OF WATER IN THE DEGRADABILITY AND HEMOCOMPATIBILITY OF POLYMERS FOR MEDICAL APPLICATIONS

MIN A BAG

Thesis submitted to the Office of Research and Graduate Studies in partial fulfillment of the requirements for the Degree of Master of Science in Engineering

Advisor:
LORETO M. VALENZUELA

Santiago de Chile, (July, 2017)

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"Only those who will risk going too far can possibly find out how far one can go."

– T.S. Elliot

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NOMENCLATURE

ΔH	Enthalpy change of melting of ice of *bulk* water
ΔH_m	Enthalpy change of melting of ice
ΔH_{cc}	Enthalpy change of the cold-crystallization of ice
$\%Free$	Weight percentage of *free* water
$\%Int$	Weight percentage of *intermediate* water
$\%NF$	Weight percentage of *non-freezable* water
Γ	Relation of permeability and diffusivity of water at interface
π	Non-dimensional permeability of the interface to water molecules
A	Amount of water that the interface is able to uptake at saturation
A_c	Calculated area of peak of NMR signal
A_m	Measured area of peak of NMR signal
A_w	Area of peak of NMR signal of pure water
ATR	Attenuated total reflection
C_e	Concentration of ester bonds
C_{e0}	Initial concentration of ester bonds
C_m	Concentration of monomers
C_w	Concentration of water
C_p	Specific heat capacity
D	Monomer diffusion coefficient
D_o	Monomer intrinsic diffusion coefficient
D_w	Water diffusion coefficient
DSC	Differential scanning calorimetry
EWC	Equilibrium water content
Fg	Fibrinogen
FT-IR	Fourier transform infrared
GA	Glycolic acid

IR	Infrared
k_1	Hydrolysis rate
k_2	Acid-catalyzed hydrolysis rate
L	Thickness of the plate
LA	Lactic acid
M_p	Molecular weight per polymer repeating unit
MTDSC	Modulated differential scanning calorimetry
M_v	Viscosity-average molecular weight
M_{water}	Molecular weight of water
M_w	Molecular weight
M_{w0}	Initial molecular weight
n	Acid terminal group dissociation
NMR	Nuclear magnetic resonance
NVP	N-vinyl-2-pyrrolidone
N_w	Number of water molecules per polymer repeating unit
N_{wf}	Number of *free* water molecules per polymer repeating unit
N_{wnf}	Number of *non-freezable* water molecules per polymer repeating unit
p	Intrinsic porosity
PAN	poly(acrylonitrile)
PANcNVP	poly(acrylonitrile)-co-N-2-vinyl-pyrrolidone
PBA	poly(n-butyl acrylate)
PCL	poly(ε-caprolactone)
PDO	poly(dioxanone)
PEA	poly(ethyl acrylate)
$Peak_H$	Height of peak in NMR signal
$Peak_W$	Width of the peak in NMR signal
PEG	poly(ethylene glycol)
PEHA	poly(2-ethylhexyl acrylate)

PGA	poly(glycolic acid)
PHEMA	poly(2-hydroxyethyl methacrylate)
PLA	poly(lactic acid)
PLGA	poly(lactic-co-glycolic acid)
PMEA	poly(2-methoxyethyl acrylate)
PMMA	poly(methyl methacrylate)
PPEA	poly(2-phenoxyethyl acrylate)
PRT	Plasma recalcification time
PTMC	poly(trimethylene carbonate)
PVA	poly(vinyl alcohol)
PVL	poly(δ-valerolactone)
Q_{cc}	Heat associated to cold-crystallization process
Q_m	Heat associated to melting process
SEM	Scanning electron microscope
T_g	Glass transition temperature
TGA	Thermogravimetric analysis
WU	Water content
w_{dry}	Weight of dry sample
W_f	Mass of *free* water
$w_{freezable}$	Weight percentage of *freezable* water
W_{int}	Mass of *intermediate* water
W_{nf}	Mass of *non-freezable* water
$w_{nonfreezable}$	Weight percentage of *non-freezable* water
$w_{polymer}$	Weight percentage of polymer
$WR_{freezable}$	Weight ratio of *freezable* water:polymer
$WR_{freezable}$	Weight ratio of *non-freezable* water:polymer
WU	Water uptake
w_{water}	Weight of sorbed water

w_{wet}	Weight of wet sample
XRD-DSC	X-ray diffraction with DSC

RESUMEN

El agua cumple un rol fundamental en el funcionamiento de varias actividades biológicas en el cuerpo. En implantes poliméricos degradables, el agua forma parte del proceso de degradación a través de la hidrólisis. En superficies, el agua se comporta de manera distinta al analizarlo a niveles moleculares. Existen tres estados del agua en superficie: agua *no-congelable*, la cual no se congela incluso a -100°C; agua *intermedia*, la cual se congela a temperaturas bajo 0°C; y agua *libre*, la cual se congela a 0°C. Cuando un material es implantado en el cuerpo, lo primero que ocurre es la absorción de agua, seguido por el contacto con la sangre. La hemocompatibilidad corresponde al efecto de un material en la sangre o sus componentes, y se presume que el agua es capaz de influenciar la hemocompatibilidad de implantes poliméricos. Sin embargo, se sabe muy poco de esta relación. En este trabajo se estudiaron dos efectos del agua en polímeros: (i) el efecto en la cinética de degradación de polímeros, para lo cual se diseñó un modelo matemático de degradación de poliésteres; y (ii) el efecto en la hemocompatibilidad de polímeros, para lo cual se seleccionaron y analizaron distintos estudios de agua y hemocompatibilidad en polímeros. Se realizó una estimación de parámetros del modelo matemático de degradación a partir de datos experimentales de la literatura. Los valores obtenidos se encontraron dentro del rango esperado de valores según estudios anteriores, mientras que el modelo presentó un buen ajuste a los datos experimentales obtenidos de la literatura. Por otro lado, para estudiar el efecto del agua en la hemocompatibilidad se seleccionaron estudios de la literatura de cinco polímeros degradables y dos polímeros no-degradables. Se concluyó que contenidos de agua *intermedia* mayores a 3% peso están relacionados con mayor hemocompatibilidad en los casos de cinco de los siete polímeros analizados; en el caso de los otros dos, los datos disponibles no permitieron afirmar ni negar esta conclusión. Estos resultados muestran que: (i) el agua influencia la cinética de degradación y su comportamiento puede describirse mediante un modelo matemático; y (ii) que los estados del agua influencian la hemocompatibilidad de materiales poliméricos. Estos resultados otorgan información relevante para la selección, diseño y desarrollo de materiales poliméricos en la industria médica.

ABSTRACT

Water has a key role in the functioning of many biological activities in the body. In degradable polymeric implants, water takes part of the degradation process through hydrolysis. Water in surfaces presents different behavior at a molecular level and three states of water are recognized: *non-freezable* water, which does not freeze even at -100°C; *intermediate* water, which freezes below 0°C; and *free* water, which freezes at 0°C like *bulk* water. When a device is implanted in the body, water interacts first by adsorbing at the surface, followed by the contact with blood. Hemocompatibility is the effect of a material on blood or its components and it is presumed that water is capable to mediate hemocompatibility in polymeric devices. However, very little is known about this relation. This work focuses on two effects of water in polymers: (i) the effect on the polymer degradation kinetics, for which a mathematical model of polyester degradation was built; and (ii) the effect on polymer hemocompatibility, for which water and hemocompatibility studies in polymers available in literature were selected and analyzed. The parameters of the mathematical model were estimated from experimental data available in literature. The obtained values were in agreement to prior studies found in literature. On the other hand, to study the effect of water on hemocompatibility, studies available in literature of five degradable polymers and two non-degradable polymers were selected. It was concluded that *intermediate* water content higher than 3% wt is related to higher hemocompatibility for five of the seven polymer surfaces. For the remaining two polymers, no enough data was available to affirm or deny this conclusion. These results show that: (i) water influences the degradation kinetics and their behavior can be described through a mathematical model; and (ii) that the states of water influence the hemocompatibility of polymeric devices. These findings provide relevant information for polymer selection, design and development of polymeric materials in the medical field.

Keywords: *Intermediate* water, *non-freezable* water, *free* water, water structure, platelet adhesion, fibrinogen adsorption.

1. INTRODUCTION

Water is considered the most important compound of life and it is the most abundant on earth. Over 70% of the surface of the planet is covered by water, in the form of solid, liquid and vapor (Robinson, 1996). It is also the main component of biological systems, being essential for many chemical reactions. Water is often regarded as the universal solvent because of its great versatility: it can dissolve proteins, ions, sugars, gases, organic liquids and lipids. What is fascinating about water is its uniqueness. Compared to other common liquids, water is characterized by having high boiling, melting and critical temperatures, large specific heat and high surface tension, among other properties (Ratner, 2012).

1.1. General aspects of water

Water is a small molecule composed by two hydrogen atoms covalently bonded to one oxygen atom. These bonds form a V-shaped structure with an angle of 104.6° (Figure 1-1) due to the two pairs of unused electrons of each oxygen atom, which tend to position as far from each other as they can to minimize the repulsion. The presence of these electrons causes a large dipole moment (i.e., uneven distribution of charges in the molecule), where the oxygen atom is slightly negatively charged, and the hydrogen atoms are slightly positively charged. This large dipole moment leads to a strong intermolecular interaction called hydrogen bonding, which is much weaker than the intramolecular covalent OH bond (23.3 kJ mol^{-1} vs. 492 kJ mol^{-1}, respectively) (Chaplin, 2010). The most predominant form is the 4-coordinated water molecule which forms hydrogen bonds with four different water molecules, i.e., each hydrogen atom pairs up with an oxygen atom of different water molecules, while the oxygen atoms pairs up with two hydrogen atoms of different water molecules (Bagchi, 2013).

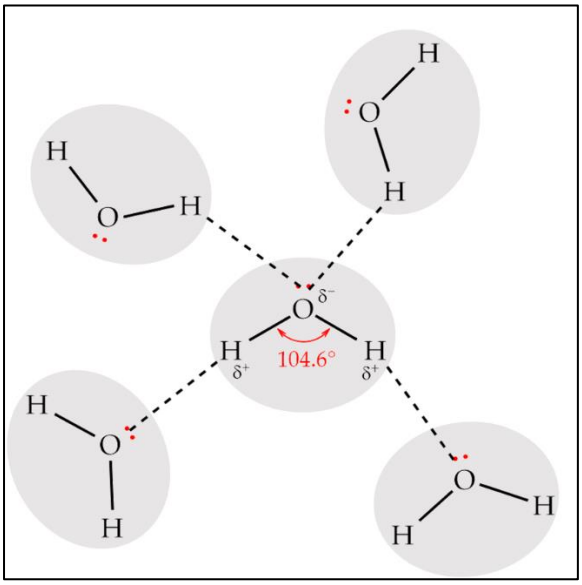

Figure 1-1. Structure of a 4-coordinated water molecule. Hydrogen atoms are positively charged (δ+) and oxygen atoms are negatively charged (δ-), which also presents a lone pair of electrons. Solid lines indicate covalent bonds and dashed lines, hydrogen bonds.

The behavior of pure water can be described by different models, which can be used to explain the peculiarities of water and its properties. In general, these models can be classified into two groups: (i) mixture or multicomponent models, where two or more water groups are present; and, (ii) continuum or uniformist models, in which each water molecule is influenced by the same intermolecular force (Jhon & Andrade, 1973). The flickering cluster model corresponds to a mixture model that suggests that clusters of hydrogen-bonded water are rapidly formed and swim in a medium of monomeric water molecules. However, this model fails to explain many of the water properties, as well as the nature of the clusters. On the other hand, the continuum model is a widely accepted model for water structure and explains water in terms of intact hydrogen bonds unlike the mixture model, where hydrogen bonds are broken between water molecules (Jhon & Andrade, 1973; Ratner, 2012).

1.2. Water in biological systems

Water is very important in all biological systems. It is the major component of the body, with around 57% of the human weight. Without water, biochemical reactions would not be possible, as well as many other biological activities because these use water as their solvent. When medical devices are implanted in the body, the material is in contact with water before any other biological component, such as proteins or cells (Baier, 1978). Some of these devices are expected to degrade to allow cell regrowth in certain areas, such as bones or muscle. In many cases, they are made of polymers which degrade with the aid of water. Thus, it is reasonable to think that water plays a major role in material-body interactions in medical devices, specially mediating the biodegradation and biocompatibility of these materials (Ratner, 2012).

1.2.1. Biochemical reactions and body function

Water is fundamental in many biochemical reactions. For example, it is part of the enzymatic activity when a substrate binds to the active site displacing water molecules. The thermodynamics of this displacement contributes to the overall thermodynamics of protein-ligand binding (Nguyen, Cruz, Gilson, & Kurtzman, 2014). The same principle is observed in the association of antibodies with antigens, and hormones with biological receptors in cells (Lindstrom, 2008). Water also stabilizes the native state of protein and the double-helix form of DNA(Bagchi, 2013). At a larger scale, water helps in the mechanical function of the body. In articular cartilage, water helps in the lubrication and transportation of nutrients through the cartilage to chondrocytes, and the great load that the articular cartilage is able to support is due to the frictional resistance and the pressure gradient in the collagen-water matrix of the cartilage (Fox, Bedi, & Rodeo, 2009).

1.2.2. Swelling of Medical devices

Hydrophilic non-metallic medical devices implanted in the body swell by absorbing water onto their surface before any protein or cell approaches them. The amount of water

uptaken by an implanted material is important for the material functioning as it impacts its degradation, interaction with blood and other biological components, its performance in tissue regeneration and the diffusion of molecules through the material. For example, the behavior of water affects the rate of dehydration of hydrogel based contact lenses and thus their performance (Ratner, 2012). In drug delivery systems, the slow release of drugs is due to the slow diffusion of water through the dry hydrogel matrix (J. Chen, Park, & Park, 1998).

1.2.3. Polymer degradation

In some cases, polymeric medical devices and implants are expected to serve a supporting temporary purpose and then degrade and disappear. These devices are attractive because they are not required to be surgically removed after their objective is accomplished. Polymer degradation refers to the cleavage of covalent bonds which causes the scission in the polymer backbone and lateral chains forming oligomers and finally monomers. There are different degradation mechanisms, such as photo-, thermal-, mechanical and chemical degradation. Hydrolysis is the most common chemical process by which polymer degradation occurs and describes the reaction involving water and the functional group possessing the labile bond, then the rate of hydrolysis is influenced by the concentration of these two reactants (Göpferich, 1996).

The design of degradable devices requires long *in vitro* and *in vivo* experiments to ensure its safety until they get fully resorbed. Therefore, computer modelling offers a virtual and helpful alternative for testing, accelerating the design and development processes. Some models have been developed for polymer hydrolytic degradation. The most common are phenomenological models, which are based on diffusion-reaction equations, where the diffusion of the degradation products and the hydrolysis reaction are described. This type of mathematical models have been develop to predict the degradation kinetics of porous scaffolds (Y. Chen, Zhou, & Li, 2011; Heljak, Swieszkowski, & Kurzydlowski, 2014),

fixation devices (Y. Wang, Pan, Han, Sinka, & Ding, 2008), and drug delivery devices (Y. Chen et al., 2011; Siepmann & Gopferich, 2001; Soares & Zunino, 2010).

1.3. Surface water

The water network can be disturbed by external elements such as ions, solutes and surfaces. When these elements get in contact with water, they reorganize its structure in order to minimize the energy of the system. In the case of surfaces, their interaction with water depends on properties such as structure, polarity, hydrophilicity or hydrophobicity, and composition of the surface. Water molecules reorder according to these factors, forming different conformations or layers on the vicinity of the surface. The influence of hydrophilic and hydrophobic surfaces on water structure has been widely studied as seen in the study by Li and collaborators, where the microscopic behavior of water on different surfaces of self-assembled monolayers was studied (Li, Du, & Yuan, 2013). They suggested that water molecules neighboring a hydrophilic surface are more rigid and have better ordering than pure water. In contrast, when faced with a hydrophobic surface, water molecules have almost the same mobility and distribution as of pure water.

The interaction of water molecules has an impact on different surfaces. Foods are often frozen to storage but their quality could be deteriorated after thawing because the change in texture due to the formation of ice. The higher the amount of tightly bound water, the less ice is formed in the food matrix, which contributes to better quality and stability of foods (Seetapan, Limparyoon, Fuongfuchat, Gamonpilas, & Methacanon, 2016). In metals, water is often believed to adsorb to the surface in a bilayer structure similar to ice structure ("*ice-like* water"). Noble metals, i.e. metals resistant to corrosion and oxidation, exhibit a weaker interaction with water molecules than less noble metals. This stable water bilayer can be regulated by changes in surface charge and influences the electrochemical performance of the metal (Schnur & Gross, 2009). For ceramics, those with higher surface energy interact with water molecules more tightly. The stabilization of this water

molecules contributes to the overall stability of the hydrated ceramic-water system (Levchenko, Li, Boerio-Goates, Woodfield, & Navrotsky, 2006).

In particular, on hydrated polymers, different water "types" or "states" have been observed. These can be classified depending on the mobility of water sorbed to the polymeric surface or its freezing temperature. Generally, there are three states of water, one with very low mobility and no freezing (even at -100°C); one with slightly higher mobility and freezing temperature below 0°C; and one with similar mobility and freezing temperature as bulk water.

1.3.1. States of water

The study of water in different surfaces has been driven by the interest in investigating the effects of water on the functionality of these surfaces. It includes hydrophobic/hydrophilic surfaces, metals, polymers, among others. Because of the large number of studies on this matter, a summary of the different denominations that water receives on surfaces is presented.

Generally, water is classified in three states according to mobility or freezing temperature criteria. Early studies using nuclear magnetic resonance (NMR) suggested the possibility of an ordered water structure in the vicinity of different surfaces, called *bound* or *ice-like* water due to its low molecular mobility. Later, other types of water were identified: one with higher mobility than the so-called *bound* water, and another that behaves similarly to *bulk* or pure water.

In most cases, the naming of the types of water has been based on its thermal behavior (i.e., crystallization), or on the mobility of their molecules (Table 1-1). Water can be classified according to low, intermediate and high mobility at atmospheric conditions, and based on this criterion Hechter and collaborators (Hechter, Wittstruck, McNiven, & Lester, 1960), Sterling and Masuzawa (Sterling & Masuzawa, 1968), and McBrierty and

collaborators (McBrierty, Martin, & Karasz, 1999) got to the similar classification of the states of water with similar names. These three classifications were based on NMR spectra of water, which is described in the next section.

The other criterion used is the freezing temperature of water and the most common used denominations are the one presented by Higuchi and Iijima (Higuchi & Iijima, 1985), Hatakeyama and Hatakeyama (Hatakeyama & Hatakeyama, 1998), and Tanaka and collaborators (Tanaka et al., 2000). *Non-freezing* water does not freeze (even at -100°C); *freezing bound* water freezes at temperatures below 0°C; and *free* water freezes at 0°C. Hirata and collaborators (Hirata, Miura, & Nakagawa, 1999) described hydration water as the one that freezes below 0°C or does not freeze at all. Therefore, hydration water is equivalent to *non-freezing* and *freezing bound* water, while *free* water is the same in both cases. Additionally, the mobility and the freezing temperature criteria can be considered equivalent, because more thermal energy in molecules translates in faster movement of them and thus molecular mobility increases with temperature. A third criterion was proposed by Aizawa and collaborators (Aizawa & Suzuki, 1971), and Jhon and Andrade (Jhon & Andrade, 1973) that was based on the thermal expansion of water molecules and the transition temperature of this property, i.e., the temperature at which thermal expansion changes.

Table 1-1. Denominations of the types of surface water based on different criteria.

Criteria	**Types of water**		**Reference**
	Name	Criteria	
Rigidity and mobility	*Ice-like* water[a]	Very low mobility	(Hechter et al., 1960)
	Intermediate between *ice-like* and *free* water [b]	Intermediate mobility	
	Free water[c]	High mobility	
	Solid water[a] (*glass-like* or *ice-like*)	Very low mobility	(Sterling & Masuzawa, 1968)

	Bound water[b]	Intermediate mobility	
	Free water[c] (very loosely bound or liquid)	High mobility	
	Tightly bound water[a]	Very low mobility at temperatures <230K	
	Loosely bound water[b]	Low mobility in the range 230-260K	(McBrierty et al., 1999)
	Free or *bulk-like* water[c]	Mobility similar to bulk water at around 273K	
Freezing temperature	*Hydration* water[d]	Freezes sub-zero or does not freeze	
	Free water[e]	Freezes at 0°C	(Hirata et al., 1999)
	Bulk-like water	Normal mobility as normal melting point is approached	
	Non-freezing water or *Non-freezing bound* water[f]	No crystallization (no freezing)	(Hatakeyama & Hatakeyama, 1998; Higuchi & Iijima, 1985; Tanaka et al., 2000)
	Freezing-bound water or *Intermediate* water[g]	Crystallization under 0°C	
	Free water[e]	Normal crystallization of water	
Thermal expansion	W3	Transition temperature: Non in -30 to 0°C	
	W2	Transition temperature: -20 to 0°C	(Aizawa & Suzuki, 1971; Jhon & Andrade, 1973)
	W1	Transition temperature: 0°C	

[a] *Ice-like* water, solid water and *tightly bound* water are equivalent.
[b] *Intermediate* between *ice-like* and *free* water, *bound* water and *loosely bound* water are equivalent.
[c] *Free* water in the three references are equivalent.
[d] Corresponds to f+g.
[e] Same *free* water by freezing temperature criterion.

Here, the three types of water are referred as: *non-freezable* water, *intermediate* water and *free* water, according to the freezing temperature criterion. *Non-freezable* and *intermediate* water are *bound* water, while *intermediate* and *free* water are *freezable* water

(Figure 1-2). In terms of structure and interaction with surfaces, the three water types are characterized by the following (Hatakeyama & Hatakeyama, 1998; Tsuruta, 2010):

- *Non-freezable* water is tightly bound to the surface and the water-surface interactions are very strong, while water-water interactions are very weak.
- *Intermediate* water interacts moderately with the surface (stronger than *free* but weaker than *non-freezable* water), involving both water-surface and water-water interaction.
- *Free* water hardly interacts with the surface and there is mainly water-water interaction.

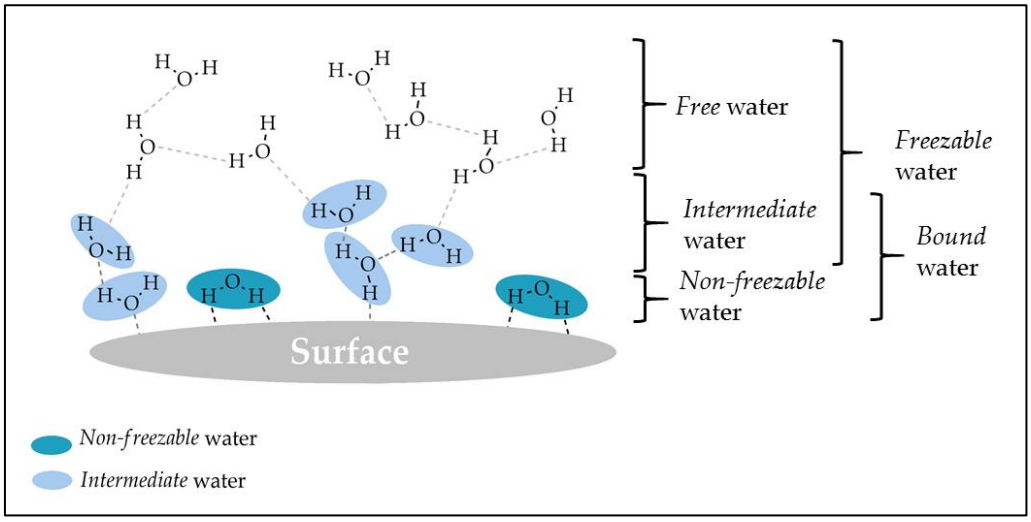

Figure 1-2. Types of water on surfaces with the denominations, their structure and interactions on surfaces.

1.3.2. Water measurement techniques

Water content and water uptake are two denominations for total water sorbed in a material. These can be can be measured by thermogravimetrical analysis (TGA), and their difference relies on whether they consider the dry or wet sample. Then, water uptake (WU) and water content (WC) can be calculated as follows:

$$WU = \frac{w_{water}}{w_{dry}} = \frac{w_{wet} - w_{dry}}{w_{dry}} \quad (1.1)$$

$$WC = \frac{w_{water}}{w_{wet}} = \frac{w_{wet} - w_{dry}}{w_{wet}} \quad (1.2)$$

where w_{water}, w_{wet} and w_{dry}, correspond to the weight of sorbed water, wet sample and dry sample, respectively.

There are several analytical methods to identify and/or quantify the different types of water on a surface. The three main techniques used are differential scanning calorimetry (DSC), NMR and infrared (IR) spectroscopy. These techniques are further described below.

1.3.2.1. Differential Scanning Calorimetry

The identification of the states of water on a surface and their quantities can be analyzed using DSC. It is widely used because the identification of *intermediate* water is easy due to the clear peaks associated with its phase transition. It has been used by Hatekayama and Hatekayama (Hatakeyama & Hatakeyama, 1998) to study the state of water in several insoluble and soluble polymers, such as cellulose, polyhydroxysterene, hyaluronic acid and poly(vinyl alcohol). Besides polymers, DSC can also be used to identify the states of water on metals (Johari, Hallbrucker, & Mayer, 1996), ceramics (Peng, Qisui, Xi, & Chaocan, 2009), and food (Tylewicz et al., 2016; Xu, Li, & Yu, 2014).

DSC allows the observation of the phase transitions in a thermogram from which various information can be extracted: (i) changes in heat capacity, (ii) magnitude of the heat (exothermic or endothermic), (iii) shape of the exotherms or endotherms, and (iv) the temperature at which these phenomena occur (McBrierty et al., 1999). Figure 1-3 shows a DSC thermogram of a hydrated surface containing the three types of water: *non-freezable*, *intermediate* and *free* water. The exothermic peak below 0°C corresponds to cold-crystallization of water and indicates the presence of *intermediate* water. The

endothermic peak at around 0°C corresponds to the melting of *free* water and *intermediate* water when it corresponds.

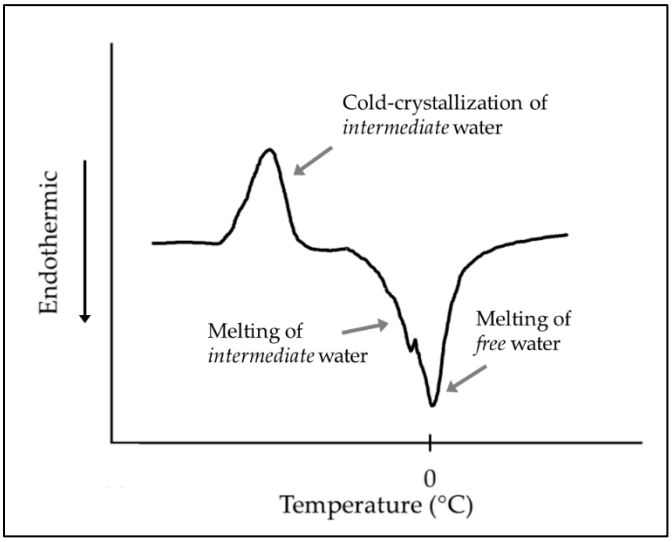

Figure 1-3. Scheme of a DSC thermogram of a hydrated surface with three states of water. Non-freezable water not visible in thermograms. Modified from (Tanaka, Hayashi, & Morita, 2013).

The thermogram hands out useful information to calculate the amount of the types of water. The mass of *intermediate* (W_{int}) and *free* water (W_f) can be calculated according to the following equations:

$$W_{int} = {}^{Q_{cc}}/_{\Delta H_{cc}} \tag{1.3}$$

$$W_f = {}^{Q_m}/_{\Delta H_m} - W_{int} \tag{1.4}$$

where ΔH_{cc} and ΔH_m are enthalpy changes in the cold-crystallization and the melting of ice, respectively; and, Q_{cc} and Q_m are the heat absorbed during the cold-crystallization process and the melting process, which are obtained from the area of the respective peaks in the thermogram. The enthalpy changes (ΔH_{cc} and ΔH_m) are assumed to be the same as that of bulk water (334 J g^{-1}) (Ping, Nguyen, Chen, Zhou, & Ding, 2001).

The mass of *non-freezable* water (W_{nf}) is calculated as follows:

$$W_{nf} = EWC(wt\%) - W_{int} - W_f \quad (1.5)$$

where EWC is the equilibrium water content of the sample.

The number of *non-freezable* (N_{wnf}) and *freezable* (N_{wf}) water molecules per polymer repeating unit can be calculated using the DSC information, as previously described (Ping et al., 2001; Zhao et al., 2013). The weight ratio ($WR_{nonfreezable}$) of *non-freezable* water/polymer, and the weight ratio ($WR_{freezable}$) of *freezable* water/polymer are to be calculated using the following equations:

$$WR_{nonfreezable} = \frac{w_{nonfreezable}}{w_{polymer}} = \frac{(EWC - w_{freezable})}{w_{polymer}} \quad (1.6)$$

$$WR_{freezable} = \frac{w_{freezable}}{w_{polymer}} \quad (1.7)$$

where $w_{nonfreezable}$, $w_{freezable}$ and $w_{polymer}$, are the weight percentages of *non-freezable* water, *freezable* water and polymer, respectively. $w_{freezable}$ is obtained from the DSC thermograms and calculated as follows:

$$w_{freezable} = \frac{Q_m}{\Delta H_m} \cdot 100\% \quad (1.8)$$

Finally, N_{wnf} and N_{wf} are obtained using the equations:

$$N_{wnf} = \frac{M_p}{M_{water}} \cdot WR_{nonfreezable} \quad (1.9)$$

$$N_{wf} = \frac{M_p}{M_{water}} \cdot WR_{freezable} \quad (1.10)$$

where M_p is the molecular weight per polymer repeating unit, and M_{water} is the molecular weight of water.

1.3.2.2. Nuclear magnetic resonance

NMR is used to measure the dynamic behavior of water, in contrast to the static state of water that DSC measures. Most of the early investigations on water states used NMR for their identification, as in the case of Hechter and collaborators (Hechter et al., 1960) with agar gels. It can also be used for other materials such as muscle tissue (Fung & McGaughy, 1974) and food, including vegetables (Xu et al., 2014), fruits (Tylewicz et al., 2016), and dough (MacRitchie, 1976).

NMR is based on the line broadening observed when molecular mobility is interfered. For *free* water, the resonance lines result narrow, but when water molecules interact with a solid, the mobility of the molecules may be impeded or nonexistent on the NMR time scale, resulting in broad resonance lines (Schmitt, Flanagan, & Linhardt, 1994) (Figure 1-4). In general, a decrease in the signal intensity corresponds to an increased amount of *bound* water on the surface (Miwa, Ishida, Tanaka, & Mochizuki, 2010).

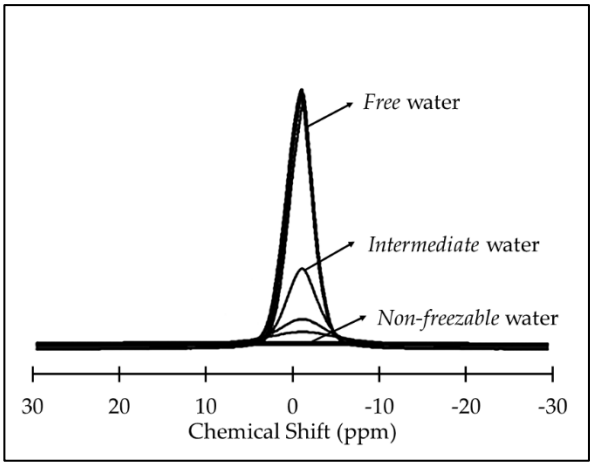

Figure 1-4. ^{2}H-NMR spectra of water on a surface at different temperatures. The narrower peaks correspond to temperatures above 0°C, while broad lines correspond to temperatures below 0°C. Modified from (Miwa et al., 2010).

The NMR spectra of the different water states are as follows (Miwa et al., 2010):

- For *non-freezable* water the spectra is broad, showing low mobility due to strong interaction with the surface.
- For *free* water the spectra is narrow, very similar to *bulk* water, which means it has high mobility.
- For *intermediate* water the spectra is somewhere in between the spectra of the other two types of water, meaning that the mobility is intermediate.

The weight percentages of the different states of water can be calculated based on NMR data, though not many studies base their calculations on this technique. Sterling & Masuzawa (1968) presented the methodology to determine them. From the NMR signal, height ($Peak_H$) of the peak and width ($Peak_W$) at half the height of the peak are measured. Then, the area of the peak is measured:

$$A_m = Peak_W \cdot Peak_H \tag{1.11}$$

The width (W_w) and the area (A_w) of the peak of pure water are constant and considered 0.22 and 11.34, respectively. The calculated area (A_c) of the peak of the sample is based on content of water in the sample:

$$A_c = WC \cdot A_w \tag{1.12}$$

Then, the percentages of the water types can be calculated as follows:

$$\%NF = 100 \cdot \left(1 - \frac{A_m}{A_c}\right) \tag{1.13}$$

$$\%Int = 100 - (\%NF + \%Free) \tag{1.14}$$

$$\%Free = 100 \cdot \left(\frac{Peak_H \cdot W_w}{A_m}\right) \tag{1.15}$$

where $\%NF$, $\%Int$ and $\%Free$ correspond to the percentages of *non-freezable*, *intermediate* and *free* water, respectively.

1.3.2.3. Fourier transform infrared spectroscopy

Fourier transform infrared (FT-IR) spectroscopy is used to analyze the interaction of water molecules with functional groups of the surface. However, the information obtained cannot be used to calculate the amount of the different states of water. In general, IR analyzes the interaction of an infrared light with a molecule by detecting the energy absorbance or transmittance of the molecule due to its vibration. It is widely used to identify different molecules or molecular structure because functional groups vibrate at different energy levels (Stuart, 2004). The O-H stretching band region at around 3800-3000 cm^{-1} is of much interest because the interaction with water occurs through hydrogen bonding. Shifts of the wavenumber are associated to a change of the hydrogen bonding strength: the stronger the hydrogen bond, the lower the wavenumber shift (Morita, Tanaka, & Ozaki, 2007). FT-IR can be used in conjunction to attenuated total reflection (ATR) to analyze thin samples (Tanaka et al., 2013). With ATR-IR the infrared beam passes through a crystal, reflecting with the surface in contact with the sample positioned on top of it. The reflection produces an evanescent wave that goes through the sample. When the sample absorbs energy from the evanescent wave, this is attenuated, and the attenuated beam is analyzed to obtain the IR spectra.

1.3.2.4. Other techniques

Other techniques have been used to study the types of water on surfaces. Raman spectroscopy can be used to analyze the structure and hydrogen bonding of sorbed water and it is similar to IR. Raman spectroscopy detects changes in the polarizability of the molecular bonds, whereas IR detects changes in the dipole moment. The analysis extracted from these two are complementary and therefore provide a better insight of the water structure (Hiromi Kitano et al., 2005). Recent research has been developed to clarify the origin of cold-crystallization of water combining wide-angle X-ray diffraction with DSC (XRD-DSC) (Kishi, Tanaka, & Mochizuki, 2009). The XRD determines the atomic and

molecular structure of a crystal by the diffraction of incident X-rays, which with the combination of DSC allows identification of the origin of phase transition.

1.4. Biocompatibility

Biocompatibility is defined as "the ability of a material to perform with an appropriate host response in a specific application" (Williams, 1987). This definition includes the characteristics that is expected for a material to have: (i) the material in the body must have a specified function and therefore it has to perform it; (ii) the host response that the material provokes has to be acceptable; and (iii) the response to a specific material will vary in different situations because the performance of the material depends on a specific application, in a specific tissue (Williams, 1999, 2012). Given that biocompatibility changes according to the situation, it should not be considered as a property of a biomaterial, but a characteristic of a material-tissue interaction (Williams, 2012).

There are several factors that affect biocompatibility. Some of them are composition of the material, production processes, structure (e.g., woven, knitted, polished, film, tube, sponge, sphere), surface characteristics (e.g., smoothness, porosity, rheology), surface treatments/coatings, location of the device, and expected host response on the interface (e.g., macrophage activation, biofilm formation, encapsulation, platelet adhesion) (Williams, 2012). Considering these factors a more detailed definition of biocompatibility was proposed by Williams (Williams, 2012):

"Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy."

Biocompatibility evaluation can be performed analyzing different biological effects that the material has in the body. The assessment of biocompatibility according to the ISO

10993 (U.S. Department of Health and Human Services, Food and Drug Administration, 2016) is based on the material characteristics and its potential risk in the body under the conditions it is used. The biocompatibility test categories presented by the ISO 10993 are: hemocompatibility, cytotoxicity, degradation, implantation, sensitization, irritation or intracutaneous reactivity, acute system toxicity, material-mediated pyrogenicity, subacute or subchronic toxicity, genotoxicity, chronic toxicity, carcinogenicity, and reproductive or developmental toxicity.

Hemocompatibility is the effect of a material on blood or blood components such as breakdown of blood cells, immunologic response and thrombus formation. In this work, hemocompatibility is evaluated by the adsorption of plasma proteins and platelet adhesion. It is known that when an implant takes contact with blood, plasma proteins adsorb to the surface. Fibrinogen (Fg), albumin and immunoglobulins are the most abundant, and along with fibronectin, vitronectin and the von Willebrand factor, these proteins are mediators of the platelet adhesion and thrombosis around the biomaterial (Badugu, Lakowicz, & Geddes, 2004). In particular, fibrinogen, the main protein in blood plasma, is fundamental in blood coagulation and thrombosis induced by biomaterials. Activation of fibrinogen leads to platelet immobilization, activation and aggregation, although the availability of platelet-binding sites is more important than the total amount of adsorbed fibrinogen in mediating platelet adhesion (Badugu et al., 2004). Either way, high fibrinogen adsorption and high platelet adhesion imply that the biomaterial is not inert in blood, hence it has low hemocompatibility.

Before an implant takes contact with blood, water is firstly sorbed onto the surface, thus it is presumed that water plays a fundamental role in material-body interactions. However, only few studies consider water on the surface as a relevant factor on biocompatibility, and even fewer relate its states on the surface with its hemocompatibility. Studying the relation between water states and hemocompatibility of materials will allow to predict the body response against the implanted material.

1.5. Hypothesis and Objectives

Water on hydrated polymers influences polymer degradation and hemocompatibility, and the presence of the states of water (i.e. *non-freezable, intermediate* and *free* water) plays a key role in blood-material interactions.

The two general objectives of this study and their specific aims are:

1. To design a mathematical model that describes polymer degradation including the effect of water that can relate to biological response.
    1.1. To construct a mathematical model that includes the effect of water on polymer degradation.
    1.2. To estimate the parameters of the models and verify the model with experimental data available in literature.

2. To study the relation between water and its states on polymer surfaces with their hemocompatibility, specifically fibrinogen adsorption and platelet adhesion.
    2.1. To select relevant studies of the states of water and hemocompatibility on polymers from literature.
    2.2. To assess the cause of the formation of water states on polymers.
    2.3. To evaluate the relation between water states and hemocompatibility on selected polymers.

This study is expected to describe the effect of water on polymer degradation through mathematical modelling. On the other hand, it is expected to provide a deeper insight on the hydration states of water and their relation with the biocompatibility of polymeric devices by analyzing the effects of the presence or absence of the states of water on fibrinogen adsorption or platelet adhesion in different polymers. These findings will present relevant information that will aid in the process of polymer selection for medical devices and polymeric materials design.

1.6. Organization of the Document

The present document is organized as follows. Section 2 describes the construction of the degradation model, its results and discussion. Section 3 presents the effect of water on hemocompatibility found in each polymer which are divided in two groups: degradable and non-degradable polymers. Then, the obtained results are discussed in three separated topics: (i) states of water in polymers, (ii) platelet adhesion on polymers, and (iii) effect of water states in polymers on biological response. Finally, Section 4 highlights the main conclusions of this study and the future challenges on this field. The annex includes the article submitted to the *International Journal of Molecular Sciences*. It is important to clarify that all experimental data presented in this document was extracted from literature and not obtained directly by the author.

2. MATHEMATICAL MODEL OF POLYMER DEGRADATION AND WATER UPTAKE

The degradation of polymeric devices is of special interest in tissue regeneration and drug delivery applications. As the polymer degrades, biological components take over the device and allows regeneration. Once polymeric devices are implanted in the body, their degradation rate are influenced by water uptake, protein adsorption and cellular adhesion (Woo, Chen, & Ma, 2003). Among the most commonly degradable polymers in industry are polyesters, which correspond to a group of polymer containing ester groups in their main chains. Current research applications of synthetic degradable polyesters comprise drug delivery, sutures, orthopedic and dental implants and fixation devices (Ratner, 2012). Poly(glycolic acid) (PGA), poly(lactic acid) (PLA) and their copolymers correspond to the most investigated and used aliphatic polyesters in the biomaterial field. The hydrophobicity of lactic acid reduces the water uptake decreasing the degradation rate as compared to PGA. These polymers degrade by hydrolysis of the ester bond, which can also be catalyzed by acidic or basic groups or enzymes (Dunne, Corrigan, & Ramtoola, 2000). The hydrolysis of PGA, PLA and their copolymers can be catalyzed by their own degradation products or monomers (autocatalysis) because these correspond to acidic groups. In the bulk of the polymeric matrix, the reduction of pH accelerates the degradation process by accumulation of acidic degradation products.

Computer modelling of polymer degradation helps the design process of degradable materials and shortens the times of device development. Phenomenological models based on reaction-diffusion equations are the most common among mathematical models. They describe the hydrolysis reaction and the diffusion of degradation products through the polymer matrix. For a drug delivery application, Soares & Zunino (2010) presented a mixture model that describes water-dependent degradation and erosion of polymers, as well as drug release from the polymer. They analyzed the polymer as a polydisperse system, i.e., a system constituted by a collection of molecules of different sizes instead of solely by molecules of a single chain size. By making this consideration they were able to account for different diffusion rates of chains with different sizes through the polymer

matrix. Y. Wang et al. (2008) presented a phenomenological model for the degradation of PGA, PLA and their copolymers, used in orthopedic fixation with various geometries. They based their model on the existing understanding of biodegradation mechanisms: hydrolysis, autocatalysis and diffusion. In other studies, they included the interaction between crystallization and degradation and the analysis of the mechanical properties of the polymers (Pan, 2014).

In this work, a mathematical model based on the two models mentioned (Soares & Zunino, 2010; Y. Wang et al., 2008) was developed, which describes the effect of water content on the degradation mechanisms of aliphatic polyesters, specifically PGA, PLA and their copolymers used for tissue regeneration. The aim of adding water to this model was to mathematically relate polymer degradation to biological response because, as mentioned above, the degradation rate is influenced by protein adsorption and cellular adhesion, where water acts as a mediator of this interaction.

2.3. Model Formulation

The mathematical model developed is based on the one proposed by Y. Wang et al. (2008), which involves the reaction and diffusion phenomena of the species in the polymer degradation process. In their study, the species considered were monomers, as product of the degradation, and ester bonds. In this work, water was also considered as it is relevant for hydrolysis and can also be related to biological response. Two main assumptions were made: (i) size distribution of polymer chains and hydrolysis products are ignored, i.e. monomers, and (ii) the degree of crystallinity of the polymers is assumed to be constant.

The model is formed by a system of partial differential equations describing the concentration of monomers, ester bonds and water in time. The governing equation for monomer concentration is as follows:

$$\frac{dC_m}{dt} = k_1 C_e C_w + k_2 C_e C_m^n + \nabla(D\nabla C_m) \tag{2.1}$$

where C_m is the monomer concentration, t is time, k_1 is the hydrolysis rate, C_e is the ester bond concentration, C_w is the water concentration, k_2 is the acid-catalyzed hydrolysis rate, n is the acid terminal group dissociation (equal to 0.5) and D is the monomer diffusion. Monomer concentration depends on the production of monomers by un-catalyzed hydrolysis (first term) and by autocatalyzed hydrolysis (second term). The term in the right corresponds the diffusion of monomers through the polymer matrix, assuming Fick's laws for diffusion. The diffusion coefficient D can be related to the porosity of the matrix by the following equation:

$$D = D_o(1 + \alpha p) \tag{2.2}$$

where D_o is the intrinsic diffusion coefficient and p is the intrinsic porosity. Here, α is an empirical parameter and its value is fixed at 4.5 by Y. Wang et al. (2008). The porosity is generated by the loss of short chains (Pan, 2014) and can be estimated as:

$$p = 1 - \frac{C_m + C_e}{C_{e0}} \tag{2.3}$$

where C_{e0} corresponds to the initial concentration of ester bonds.
Then, equation 2.1 can be rewritten as:

$$\frac{dC_m}{dt} = k_1 C_e C_w + k_2 C_e C_m^n + \nabla \left\{ D_o \left[1 + \alpha \left(1 - \frac{C_m + C_e}{C_{e0}} \right) \right] \nabla C_m \right\} \tag{2.4}$$

The reduction of ester bond concentration is given by the un-catalyzed hydrolysis and the acid-catalyzed hydrolysis of polymer chains. Then the governing equation for ester bond concentration is as follows:

$$\frac{dC_e}{dt} = -(k_1 C_e C_w + k_2 C_e C_m^n) \tag{2.5}$$

The ester bond concentration relates to the molecular weight of the polymer matrix in the following manner:

$$\frac{C_e}{C_{e0}} = \frac{M_w}{M_{w0}} \tag{2.6}$$

Water concentration depends on the un-catalyzed hydrolysis and the diffusion of water through the polymer matrix, as shown in the next equation:

$$\frac{dC_w}{dt} = -k_1 C_e C_w + \nabla(D_w \nabla C_w) \quad (2.7)$$

where D_w corresponds to water diffusion.

2.3.1. Initial conditions

Before any degradation has taken place in the polymeric matrix, no monomer has been produced and its concentration is zero, i.e. $C_m(x,t)|_{t=0} = 0$, while ester bond concentration was considered as the initial molecular weight of the polymer matrix, i.e. $C_e(x,t)|_{t=0} = M_{w0}$. As the matrix starts out dry, $C_w(x,t)|_{t=0} = 0$.

2.3.2. Boundary conditions

At the center of the plate ($x = 0$) no diffusion of any specie (monomers, ester bonds and water) occurs through this boundary. Then:

$$\left.\frac{\partial C_m}{\partial x}\right|_{x=0} = 0, \quad \left.\frac{\partial C_e}{\partial x}\right|_{x=0} = 0, \quad \left.\frac{\partial C_w}{\partial x}\right|_{x=0} = 0 \qquad \text{for } t > 0. \quad (2.8)$$

At the border of the plate ($x = L$), where L corresponds to the thickness of the plate, monomers diffuse from the center of the matrix to the border due to concentration gradient, and their concentration is close to zero $C_m(x,t)|_{x=L} = 0$. For ester bonds, i.e. oligomers, the border of the plate is impermeable, then $\left.\frac{\partial C_e}{\partial x}\right|_{x=L} = 0$.

In the case of water, it permeates through the interface according to the following boundary condition (Soares & Zunino, 2010):

$$\left[D_w \frac{\partial C_e}{\partial x} + \Gamma \pi \left(C_w(x,t)|_{x=L} - A\right)\right] = 0 \quad (2.9)$$

where π corresponds to the non-dimensional permeability of the interface to water molecules and *A* to the amount of water that the interface is able to uptake at saturation, i.e. the equilibrium *WC* (*EWC*). Γ corresponds to a non-dimensional number that relates the permeability and diffusivity of water at the boundary:

$$\Gamma = \frac{L\,\pi}{D_w} \tag{2.10}$$

2.4. Simulation

The simulation of the model was performed using the software Matlab® and the pdepe function, which solves parabolic-elliptic partial differential equations. A one-dimensional case was considered utilizing experimental data presented by (Grizzi, Garreau, Li, & Vert, 1995) of a 2 mm thick PLA plate.

2.4.2. Parameter estimation

From the experimental data obtained from (Grizzi et al., 1995), five parameters of the model (*Water model*) and the three parameters of the original model (*Wang's model*) were estimated (Table 2-1). The input data corresponded to the initial molecular weight, the *EWC* of the polymer and the thickness of the sample, extracted from the experimental data.

Table 2-1. Estimated parameters and R^2 of degradation and water uptake data of the *Water model* and *Wang's model*.

Parameter	Unit	*Water Model*	*Wang's model*
D_0	$\frac{m^2}{week}$	8.70 x 10^{-7}	3.90 x 10^{-8}
k_1	$\frac{\left({}^{m^3}/_{mol}\right)}{week}$	1.30 x 10^{-3}	2.53 x 10^{-5}

k_2	$\frac{\sqrt{m^3/_{mol}}}{week}$	2.50 x 10^{-3}	2.43 x 10^{-3}
D_w	$\frac{m^2}{week}$	4.83 x 10^{-11}	--
π	$\frac{m^2}{week}$	2.38 x 10^{-5}	--
R^2 degradation		0.98	0.99
R^2 water		0.81	--

Comparing the estimated parameters for both models, shown in Table 2.1., the diffusion rate of monomers (D_0) of the *Water model* is one order of magnitude greater than *Wang's model*, which means that monomers exit the polymer matrix faster probably because the water in the polymer accelerates diffusion. The auto-catalyzed hydrolysis rate (k_2) in both cases are similar, while the non-catalyzed hydrolysis rate (k_1) of the *Water model* is two orders of magnitude greater than the other. This could be due to the more relevant role that was given to water in the first model causing a greater contribution to the degradation rate than in the second one. Also, as monomer diffuse at slower rate in *Wang's model* this could result in a higher concentration of monomers in the polymer matrix causing more auto-catalyzed hydrolysis than non-catalyzed, as opposed in the *Water model*, where monomers diffuse faster to the outside of the matrix.

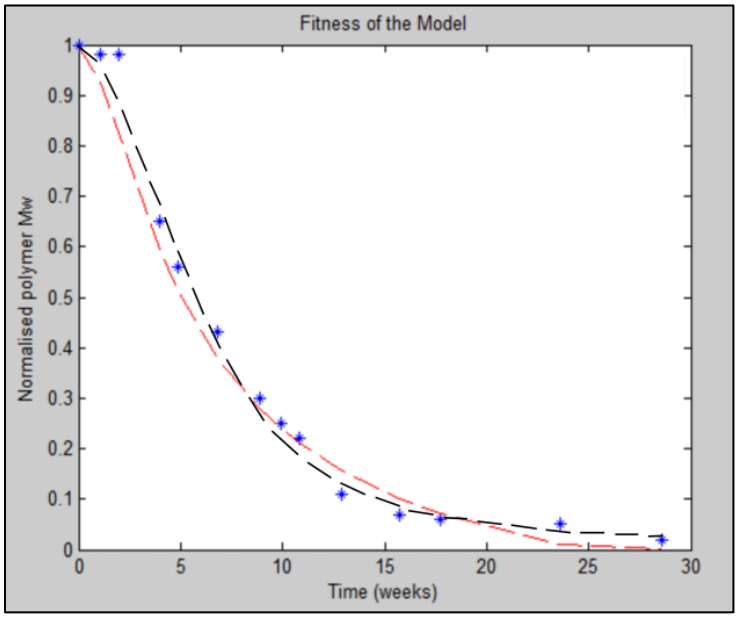

Figure 2-1. Comparison between the experimental (blue asterisks) and predicted (dashed lines) degradation as functions of time. Red lines: *Water model*, $R^2 = 0.98$; blue lines: *Wang's model*, $R^2=0.99$. Experimental data obtained from (Grizzi et al., 1995).

Figure 2-1. shows the fitness of both models according to the estimated parameters. Both models have a good fit with the experimental data (the value of R^2 of the *Water model* and *Wang's model* are 0.98 and 0.99, respectively). In figure 2-2. the fitting of the *Water model* with water concentration data is shown. Here, the value of R^2 is 0.81, the fitting accuracy is lower than degradation prediction. Other border conditions describing water behavior at the interface are to be tested to obtain a better fitting of the model.

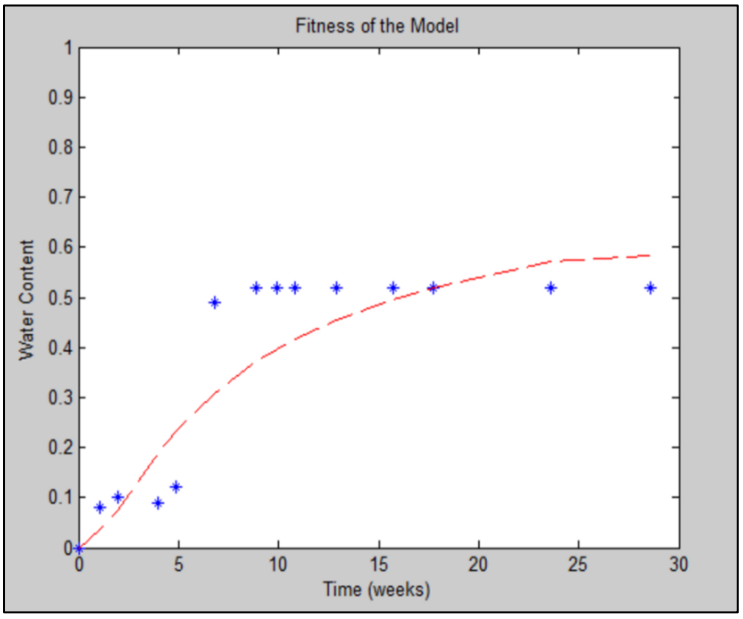

Figure 2-2. Comparison between the experimental (blue asterisks) and predicted by the *Water Model* (red dashed lines) water content as functions of time. R^2=0.81. Experimental data obtained from (Grizzi et al., 1995).

2.4.3. Species distribution evolution in polymer matrix

A three-dimensional surface plot for each three variables (C_m, C_e and C_w) was obtained (Figures 2-3 to 2-5) using the *Water Model*. In these figures the behavior of monomers, ester bonds and water concentration with respect to time and relative distance from the center of the plate are observed. Analyzing the surface plots, one can observe that the three species behave according to what is expected to physically occur within the polymer matrix. Monomer concentration (Figure 2-3) is zero at the beginning of the degradation and increase until a maximum value coinciding with the depletion of ester bonds. Then, the concentration starts to decrease in time and within the matrix as monomers diffuse to the outside of it. Ester bond concentration (Figure 2-4) decreases rapidly throughout the matrix because the *EWC* is reached in a short period of time. At the border, ester bonds accumulate because of the impermeable border condition but the plot also shows that ester bonds concentration is undefined, thus the mathematical problem, i.e. border condition, related should be fixed.

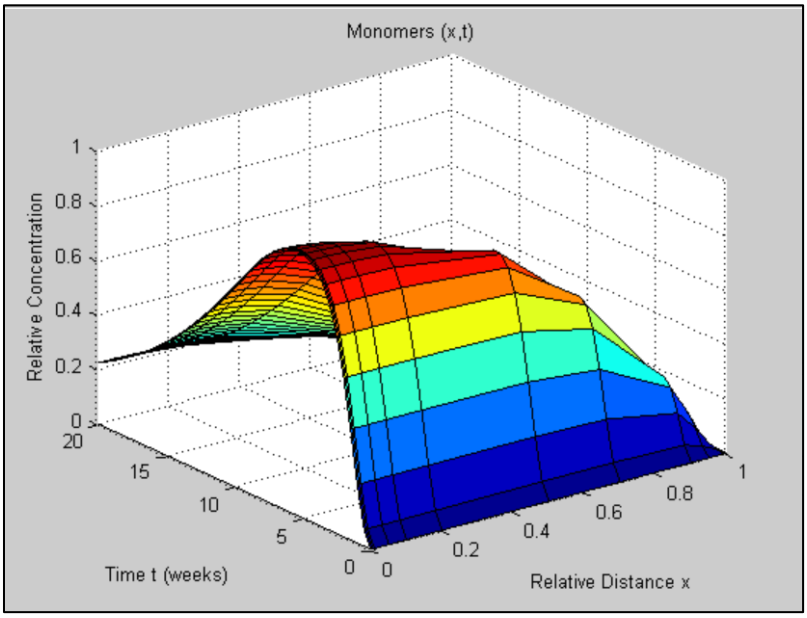

Figure 2-3. Three-dimensional surface plot of monomer relative concentration with respect to time and relative distance.

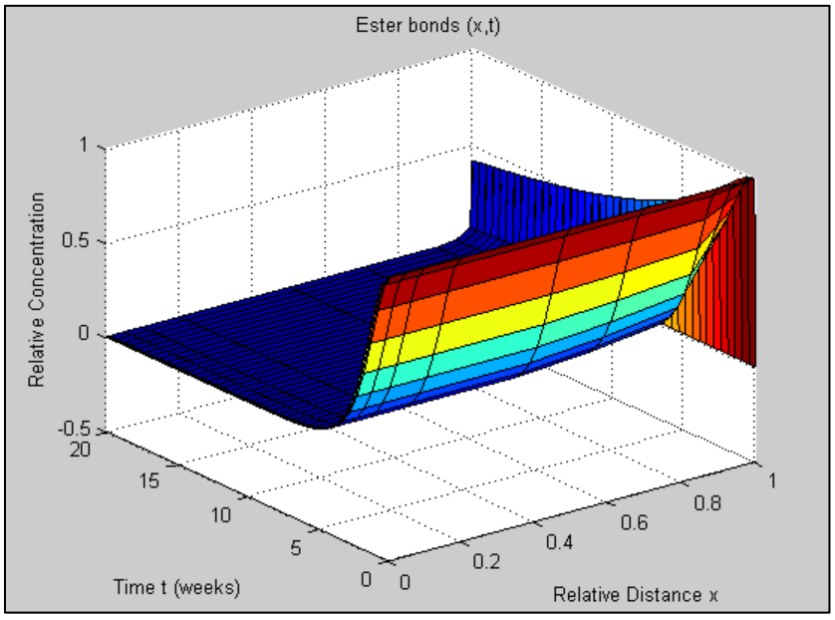

Figure 2-4. Three-dimensional surface plot of ester bond relative concentration with respect to time and relative distance.

Water (Figure 2-5) reaches its maximum concentration very fast at the border according to the experimental data and then, it slowly diffuses through the matrix to the center. According to the estimated parameters, the permeability rate (π) is higher than the water diffusion rate (D_w) (2.48 x 10^{-5} and 4.83 x 10^{-11}, respectively), therefore the rapid water uptake and slow water diffusion to the center are expected. However, because water concentration is low at $x = 0$ and $t = 0$, the degradation should be slower than at the outer sections of the matrix, as oppose what is shown in Figure 2-4. There are two possible causes for this misbehavior: (i) water actually diffuses faster through the polymeric matrix being available for hydrolytic degradation in the entire matrix, or (ii) degradation, i.e., ester bond concentration decrease, is not considering local concentration of water but total amount of water in the matrix.

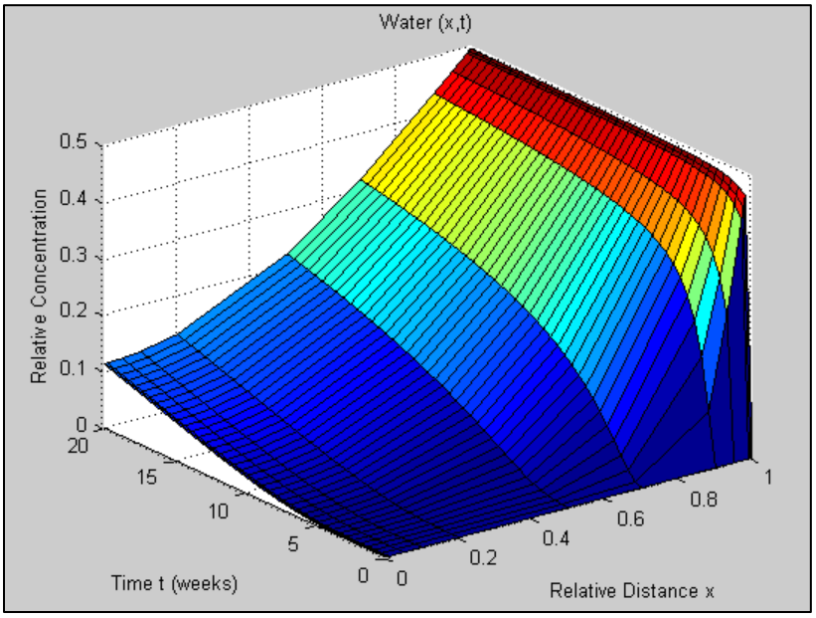

Figure 2-5. Three-dimensional surface plot of water relative concentration with respect to time and relative distance. Note that the maximum value of relative concentration axis is 0.5.

2.5. Conclusions

In this section, a mathematical model for polymer degradation was presented. This model included the contribution of water in the degradation process in order to relate it to the biological response. The design of this model was based on a previous degradation model, which was modified adding water in the degradation process. After the construction of the model, experimental data of PLA degradation previously reported was used for parameter estimation of the two models. Both models fit well to the experimental data and the parameter values agreed with the degradation and diffusion kinetics of the polymer matrix. Three-dimensional surface plots of the *Water model* were analyzed and, in general, monomers, ester bonds and water concentration behaved according to what is expected.

This model was able to describe PLA degradation, however further simulation is needed to verify its accuracy in other polyesters such as PGA and copolymers, which present different degradation and water uptake kinetics. Also, degradation experiments can be carried out to obtain empirical values of the parameters and validate the model.

Polymer degradation is also influenced by biological response, such as protein adsorption and cell adhesion. Since water is the responsible for the interaction of polymeric devices and biological components, the mathematical model presented here can be further modified and improved to describe the effect that these components have on the degradation. From current available data, it is not possible to obtain a mathematical relation between these. In the next section, a thorough analysis of the effect of water on the hemocompatibility of polymers is presented, which will shed the first light on the relation between degradation, water and biological response, for future development of this model.

3. EFFECT OF WATER ON HEMOCOMPATIBILITY OF POLYMERS

3.1. Study Selection and Data Analysis

Different water and hemocompatibility studies on polymers were selected from the literature. In the case of water, the selection was restricted to studies with available data on the content of water states or DSC thermograms within a temperature range from about -100°C to above 0°C. For hemocompatibility studies, the selection was based on the polymers which water studies were selected before. The type of sample structure of the polymer (film or hydrogel) was also considered in the selection.

The contents of *non-freezable*, *intermediate* and *free* water were directly extracted from the selected studies, as well as hemocompatibility data. Other relevant information on the sample characteristics, such as molecular weight, sample weight, sample fabrication, were recorded. When water states data was not available, it was obtained by the analysis of DSC curves of the polymer. The content of *freezable* (*intermediate* and *free*) and *non-freezable* water were calculated with equations 1.1, 1.2 and 1.6 to 1.10 as described by Ping et al. (2001) and Zhao et al. (2013).

3.2. Results on the effect of the states of water on hemocompatibility

From the selected studies, data of water states and hemocompatibility of five degradable polymers (L-tyrosine derived polyarylates, poly(ethylene glycol) (PEG), a group of aliphatic carbonyls, and poly(lactic-co-glycolic acid) (PLGA)) and two non-degradable polymers (poly(meth)acrylates and poly(vinyl alcohol)s (PVAs)) were extracted and calculated. In this section, the analysis and discussion of the impact of water states on the hemocompatibility (i.e. protein adsorption or platelet adhesion) of each polymer is presented. Also, a brief description of the characteristics and current applications of each polymer is included.

3.2.1. Degradable polymers

3.2.1.1. L-tyrosine derived polyarylates

L-tyrosine-derived polyarylates are a family of 112 A-B-type copolymers with an alternating sequence of tyrosine-derived diphenol and an aliphatic diacid. These were firstly synthesized by Kohn and collaborators (Brocchini, James, Tangpasuthadol, & Kohn, 1997; Fiordeliso, Bron, & Kohn, 1994) (Figure 3-1). For this library, the number of oxygen or carbon atoms in the polymer backbone and pendent chain affects properties such as the glass transition temperature (T_g) and the water contact angle. These polymers have been used to fabricate bone pins showing no significant inflammatory response in *in vivo* resorption studies (Hooper, Macon, & Kohn, 1998). Also, drug eluting implants near the eye area have been fabricated ("Lux Biosciences Gains Exclusive Worldwide License for Polyarylate Patent Estate From Rutgers University for Ophthalmic Use," 2006), as well as FDA approved devices for hernia repair and infection control in 2006 ("FDA approves first medical device using Rutgers biomaterial," 2006). Recently, potential topical psoriasis therapy using drug loaded nanospheres have been developed which are based on amphiphilic block copolymers of PEG and L-tyrosine-derived polyarylate oligomers (Kilfoyle et al., 2012).

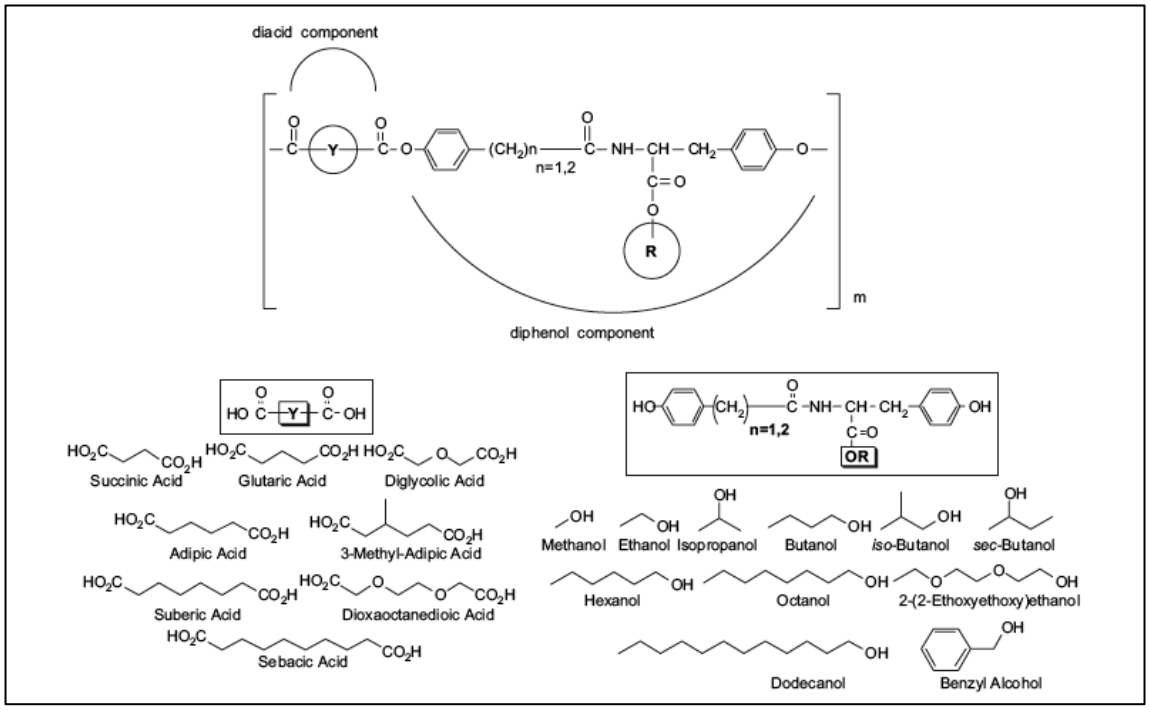

Figure 3-1. Structure of L-tyrosine derived polyarylates. Symbol Y represents diacids (left) and symbol R represents tyrosine-derived diphenols (right). The number of methyl groups in the diphenol is variable (n = 1 for HTR, n = 2 for DTR) (L M Valenzuela, Michniak, & Kohn, 2011).

Previously, the influence of different properties of polymeric films on their WU behavior was investigated, due to the high variability of the latter reported in various studies (L M Valenzuela et al., 2011). From this study, the DSC curves for 21 of the used polymers were obtained, along with the WU data. From the former, the sample weight and the enthalpy change of the melting process of *freezable* water (Q_m) were extracted. Then, the WC and the number of *freezable* (N_{wf}) and *non-freezable* (N_{wnf}) water molecules per polymer repeating unit were calculated, as described in section 2.2.1. Depending on the N_{wf}, polymers were classified into two groups: (i) low WC, from 0 to 0.31 and N_{wf} less than 10, and (ii) high WC, from 0.45 to 0.6 and N_{wf} more than 10.

The analysis of the behavior of N_{wnf} and N_{wf} related to the WC of the polymer shows that the majority of them have similar behavior (Figure 3-2), where N_{wnf} rises until a threshold from which it tends to remain constant, while N_{wf} keeps rising as the polymer absorbs more water. However, when WC is very low (around 0.05) N_{wnf} is higher than N_{wf}. This probably occurs because at lower WC the total amount of water is close to the amount of *non-freezable* water allowed in the polymer, and *freezable* water is in smaller or similar amounts. Also, in Figure 3-2, N_{wf} does not reach any saturation point like N_{wnf} because the calculation were performed considering data until reaching the maximum WC of the polymer.

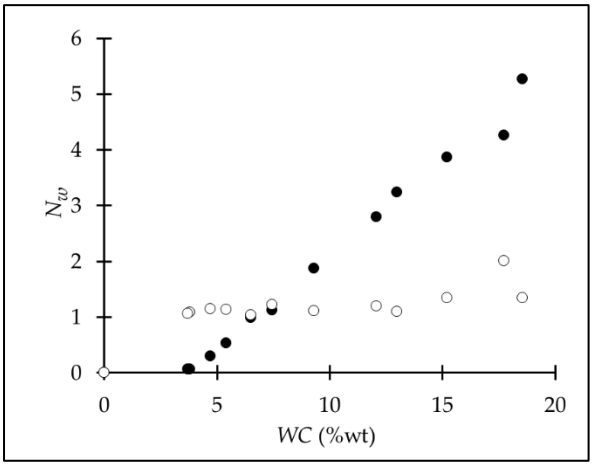

Figure 3-2. Example behavior of non-freezable water (N_{wnf}, o), and freezable water (N_{wf}, •) in relation to WC of poly(DTH adipate).

Fibrinogen adsorption data was obtained from Weber and collaborators (Weber, Bolikal, Bourke, & Kohn, 2004) (Table 3-1). Figure 3-3 shows the relation between N_w and fibrinogen adsorption of *non-freezable* and *freezable* water. In the case of *non-freezable* water, there is no apparent relationship between the fibrinogen adsorbed to the surface, but for the low WC group there is a tendency to increase as the amount of water is increased. On the other hand, when only *freezable* water is analyzed, the low WC group has the same tendency as the previous case, although the correlation is lower in the latter.

The high WC group shows a direct relation with the fibrinogen adsorption with a R^2 of 0.89, however due to the low number of data for this group (only 4) it is not enough to make a conclusive statement about this relationship.

Table 3-1. Data of each polyarylate measured and calculated from (Loreto M. Valenzuela et al., 2012; Loreto M Valenzuela, Knight, & Kohn, 2016). Fg adsorption data extracted from (Weber et al., 2004).

Polymer	N_{wf}	N_{wnf}	WC **Max (wt%)**	Fg **adsorption (% of control)**	**Group**
poly(DTO succinate)	0.14	0.77	4	121.97	
poly(DTB succinate)	0.28	0.76	4	129.36	
poly(HTE adipate)	0.82	1.31	8	125.19	
poly(DTO adipate)	1.08	0.8	6	78.30	
poly(DTM adipate)	1.92	1.77	13	142.69	
poly(DTM sebacate)	1.98	1.03	12	99.14	
poly(DTH suberate)	2.25	1.03	10	91.68	
poly(DTB adipate)	3.45	1.76	19	127.12	
poly(DTB glutarate)	3.95	1.59	19	123.38	Low
poly(HTH adipate)	4.13	1.55	16	76.20	
poly(DTE glutarate)	4.13	1.91	22	151.44	
poly(DTH adipate)	4.26	2.01	18	82.27	
poly(DTiP adipate)	5.39	1.87	22	121.76	
poly(DTE adipate)	5.45	4.26	27	131.21	
poly(DTBn adipate)	6.98	2.40	30	142.16	
poly(HTE succinate)	9.14	3.27	30	182.15	
poly(DTBn methyl adipate	9.77	2.30	31	138.98	
poly(DTBn suberate)	15.71	2.26	47	92.10	High

poly(DTM (R)(+) methyl adipate)	23.67	3.51	56	125.70	
poly(DTsB glutarate)	23.70	1.80	49	132.32	
poly(DTsB (R)(+) methyladipate)	36.98	1.59	58	153.27	

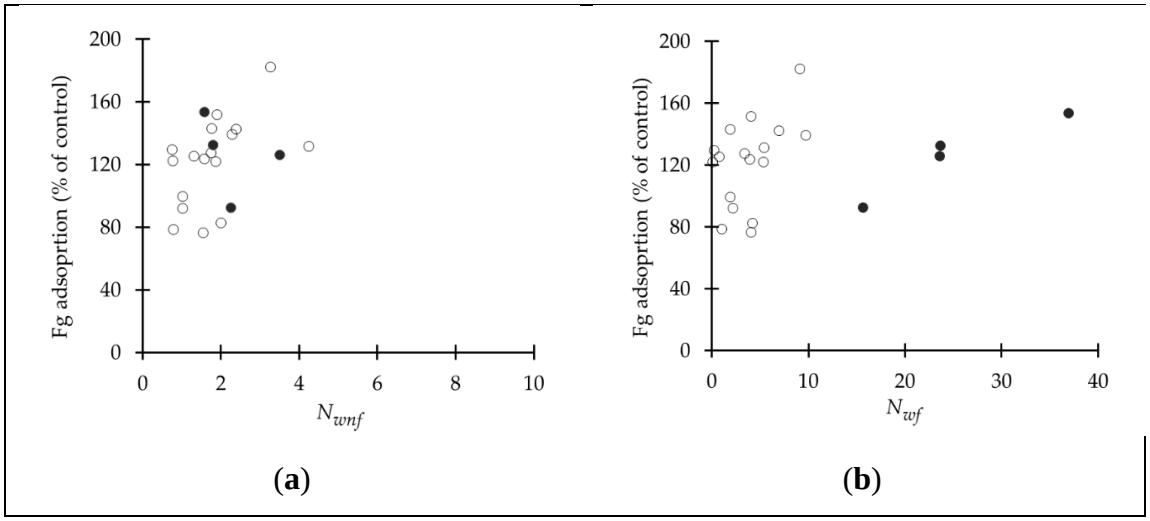

Figure 3-3. Fg adsorption vs N_w in polyarylates (a): non-freezable water. (b): freezable water. (•) high WC, (o) low WC. X-axes are not in same scale.

Polyarylates with longer ester and diacid groups adsorb less fibrinogen than those with shorter ester and diacid groups (Kohn, Darr, & Schut, 2011). It is hypothesized that this phenomenon occurs because longer chains allow the polymer to fold upon itself forming more hydrogen bonding between functional groups of the chain and water, as it occurs with PEG chains (Antonsen & Hoffman, 1992). This interaction causes the formation of *intermediate* water that decreases the adsorption of proteins.

From the data analyzed (L M Valenzuela et al., 2011, Weber et al., 2004) it can be concluded that at higher WC values, higher *freezable* water content is responsible for the higher fibrinogen adsorption and low hemocompatibility of polyarylates. There is not enough available data to conclude anything regarding *intermediate* water and its relation

to hemocompatibility because we were only able to calculate the amount of *freezable* water, namely *intermediate* and *free* water together. However, it is known that longer chains promote the formation of *intermediate* water, which decreases fibrinogen adsorption, and further study on the structure of water in polyarylates through NMR and IR is necessary.

3.2.1.2. Poly(ethylene glycol)

PEG is formed by the polymerization of ethylene oxide and corresponds to a neutral polyether (Figure 3-4). This hydrophilic polymer is especially relevant in the biomedical field because of its nontoxicity in the human body. In aqueous solution, PEG presents high mobility with a large exclusion volume; it also has a great capacity of water uptake, which depends on its molecular weight (M_w). This polymer has been extensively used, as blend or as a copolymer, to improve the biocompatibility and water solubility of other polymers with little chemical modification (Harris, 1992). In tissue engineering, PEG has been used to form hydrogels, mostly in diblock, triblock and multiblock copolymers with poly(lactic-co-glycolic acid) (PLGA) to improve the degradability of PEG based hydrogels (Z. Chen et al., 2014). In drug delivery systems, PEG is used as a stabilizer by coupling it to proteins, polypeptides, DNA, RNA, among others (Knop, Hoogenboom, Fischer, & Schubert, 2010). Some relevant PEGylated products include those for severe combined immunodeficiency (Adagen®), acute lymphoblastic leukemia (Oncospar®) (Duncan, 2014). Recent PEGylated products include therapy for age-related macular degeneration (Macugen®) and for Crohn's disease and rheumatoid arthritis (Cimzia®) (Knop et al., 2010).

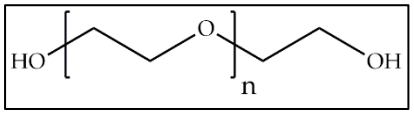

Figure 3-4. Molecular structure of PEG.

The thermal behavior of aqueous PEG solutions with different M_w was obtained by DSC measurements by Antonsen and Hoffman (Antonsen & Hoffman, 1992). They explored and analyzed the effect of the M_w on the amount of *bound* water and the low-temperature behavior of these solutions. They hypothesized that low M_w PEG chains are only associated with *non-freezable* water and as the M_w increases, the polymer chains fold upon themselves allowing *intermediate* water molecules to form. These results agree with the observations made by Yamauchi and Tamai (Yamauchi & Tamai, 2003) where at M_w lower than 500, *intermediate* water was not detected, and then slowly increased with the M_w until a constant amount of about 4% of total water was reached at around M_w 2200 (Figure 3-5).

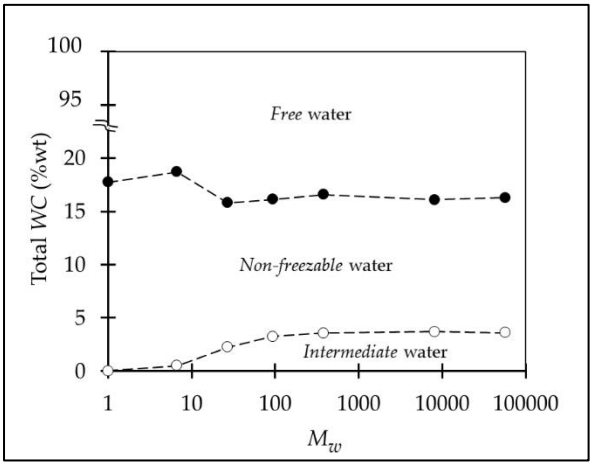

Figure 3-5. Water states distribution in PEG at different M_w. (•) Non-freezable water; (o) intermediate water. Modified from (Yamauchi & Tamai, 2003).

Kitano and collaborators (H Kitano, Ichikawa, Ide, Fukuda, & Mizuno, 2001) conducted a FT-IR study to analyze the O-H stretching band peaks for sorbed water to PEG films of various M_w and elucidate the effect of OH end groups of the polymer on the interaction with water molecules. In the IR spectra obtained (Figure 3 in (H Kitano et al., 2001)), different peaks of the O-H stretching band were observed, from which they proposed five types of sorbed water molecules: *binding* water, monomeric water binding to the ether oxygen atom of the polymer (peak 1); dimeric water, water molecule which is hydrogen-

bonded to another water molecule binding to PEG (peak 2) or water molecule hydrogen-bonded to both a water molecule and the ether oxygen of PEG (peak 3); *bridging* water, the association with two ether oxygen atoms (peak 4); and, hydrogen-bonded water to the end OH group (peak X). The latter was only observed in PEG films of low viscosity-average molecular weight ($M_v \leq 28K$) due to the increase of OH ends of the polymer. Observing the proposed hydration structure of each water types (Figure 3-6 and Figure 3-7) and comparing to the three types of water, the following analogy can be drawn: peak 4 or *bridging* water corresponds to *non-freezable* water, while peaks 2 and 3 (dimeric water) correspond to *intermediate* water. Peak 1, although very similar to *bulk* water in the IR spectra, it also corresponds to *intermediate* water given the higher mobility than bridging water. In the case of peak X, its IR spectra is very similar to peak 4, meaning water molecules are *tightly bound* to the polymer chain.

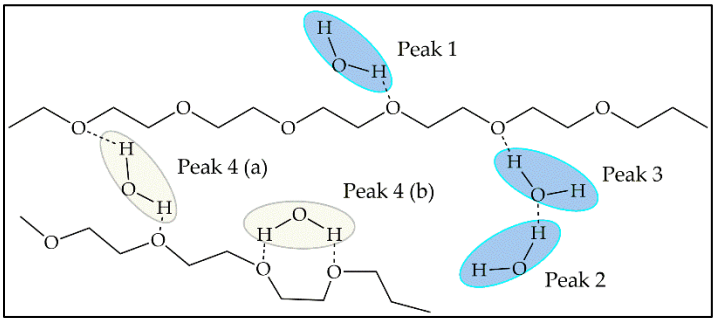

Figure 3-6. Proposed structure of water on hydrated PEG as shown in (H Kitano et al., 2001). Intermediate water showed in blue; non-freezable water showed in yellow.

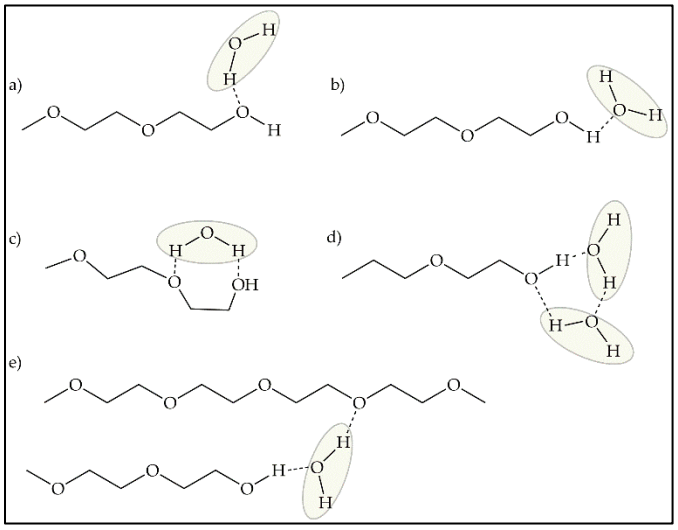

Figure 3-7. Proposed structure of water at the end of an hydrated PEG chain (peak X) as shown in (H Kitano et al., 2001).

PEG presents good hemocompatibility. Both protein adsorption and platelet adhesion are a function of the M_w below 2000 (Antonsen & Hoffman, 1992). At low M_w protein adsorption and platelet adhesion are high (Nagaoka et al., 1985) because the amount of *intermediate* water is negligible. As described above, the increase of M_w causes the formation of *intermediate* water in the polymer matrix until it reaches a maximum and stays constant at higher M_w. At high M_w, the folding of the chain and the subsequent formation of *intermediate* water molecules prevent the adhesion to the polymer and to *non-freezable* water of high-molecular-weight molecules like plasma proteins (Antonsen & Hoffman, 1992), avoiding the activation of these and hence, providing good hemocompatibility to the polymer. As shown in figure 3-8, the longer the chain length of PEG immobilized to poly(methyl methacrylate) (PMMA), the lower the amount of adsorbed proteins and adhered platelets (Bergstrom et al., 1992).

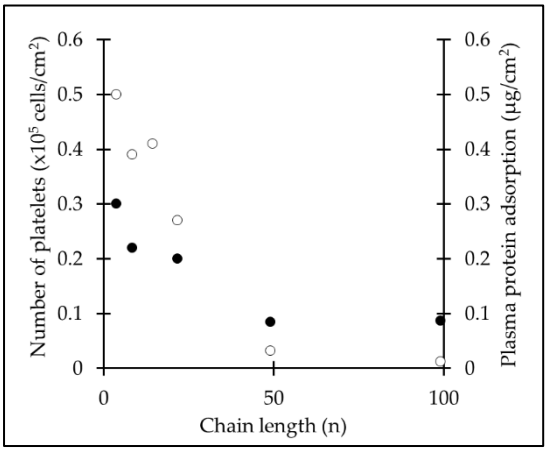

Figure 3-8. Effect of PEG chain length, immobilized to PMMA, on platelet adhesion (•) and plasma protein adsorption (o). Modified from (Bergstrom et al., 1992).

3.2.1.3. Aliphatic Carbonyls

Aliphatic carbonyl polymers have ester or carbonate linkages facilitating the breakdown of their monomers and thus their degradation. This type of polymers is broadly studied and applied in the biomedical field for the development of drug delivery systems and tissue engineering. Poly(dioxanone) (PDO) is mostly used for the manufacture of absorbable sutures (Hong et al., 2006) and is currently studied in the development of degradable intravascular stents (C. E. Wang & Zhang, 2016) and nanofibrous scaffolds (Goonoo et al., 2015). Poly(ε-caprolactone) (PCL) and poly(trimethylene carbonate) (PTMC) are used in tissue engineering scaffolds and sutures (Hong et al., 2006). These polymers have high biocompatibility, however there is little research available on the relation of the states of water on these polymers and their compatibility.

Tanaka and collaborators (Fukushima et al., 2015) studied PDO, PCL and PTMC in addition to poly(δ-valerolactone) (PVL) (Figure 3-9) in order to elucidate the differences in their backbone structure on hydration and hemocompatibility. They found that a higher amount of *intermediate* water in PDO is related to its good hemocompatibility and the

presence of the ether bonds in the main chain of PDO are involved in the hydration and formation of *intermediate* water.

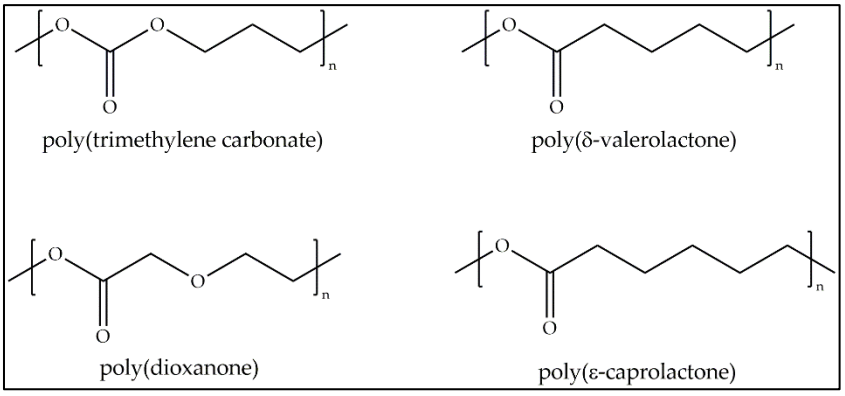

Figure 3-9. Molecular structure of aliphatic carbonyl polymers.

The available data of EWC and amount of types of water in PCL (M_w 70-100 k), PDO (M_w information not provided), PTMC (M_w 22 k) and PVL (M_w 28.5 k) were related to the amount of adhered platelets. In general terms, no relation between the total WU, *non-freezable* water and *free* water with platelet adhesion was found. However, there is a tendency of lower platelet adhesion when intermediate water is higher in quantity (Figure 3-10).

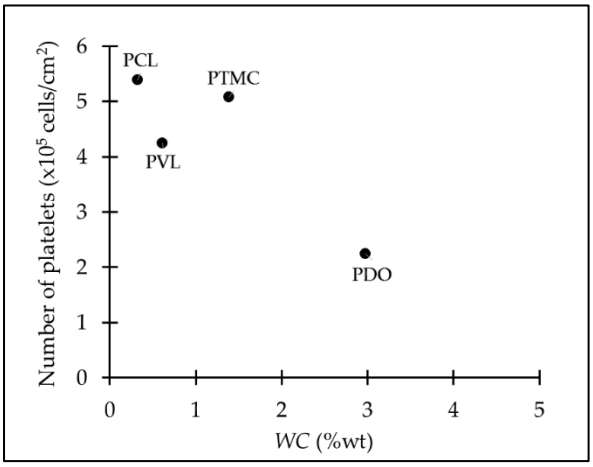

Figure 3-10. Relation of intermediate water content and platelet adhesion in aliphatic carbonyl polymers. Data extracted from (Fukushima et al., 2015).

The number of adhered platelets decreases considerably when *intermediate* water content is about 3% wt, which occurs for PDO. The difference between PDO and the rest of the polymers is the ether bond in its backbone and it is believed that this bond contributes to the formation of hydrogen bonds and the increased amount of *intermediate* water, improving its hemocompatibility (Figure 3-11). On a first glance at Figure 3-10, one could conclude that there is an inverse relationship between the number of platelet and the amount of *intermediate* water. However, the actual amount of *intermediate* water on PTMC might be overestimated because the author hypothesized that this water is not in the hydration layer formed by the polymeric chains and water, but it spreads over this layer (Fukushima et al., 2015). Therefore, the data of PTMC in Figure 3-10 should be closer to the Y-axis next to PCL and PVL, and the inverse relation is not conclusive anymore.

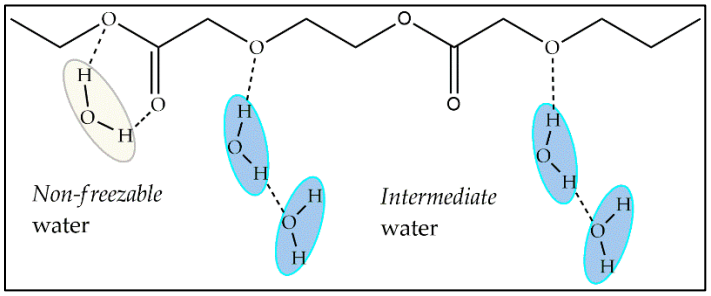

Figure 3-11. Proposed structure of non-freezable (yellow) and intermediate (blue) water sorbed on PDO. Based on (Fukushima et al., 2015).

3.2.1.4. Poly(lactic-co-glycolic) acid

PLGA is the result of the copolymerization of lactic acid (LA) and glycolic acid (GA) and corresponds to a saturated poly(α-hydroxy ester) (Figure 3-12). This polymer presents good biocompatibility and biodegradability, which can be tailored by controlling the M_w of the polymer and the ratio between lactic and glycolic acid (Bartzoka, Crestini, & Lange, 2015). Commonly used LA:GA ratios are 25:75, 50:50, 75:25 and 85:15, all of them with different T_g values, degree of crystallinity, and degradation rates. Due to the wide range of properties this copolymer can have, it is one of the most popular in the pharmaceutical and medical industry. Many PLGA-based products have been approved by the FDA, such as sinus implants for the treatment of chronic rhinosinusitis (Parikh et al., 2014), and injectable suspension containing PLGA microspheres for the treatment of patients with acromegaly (McKeage, 2015).

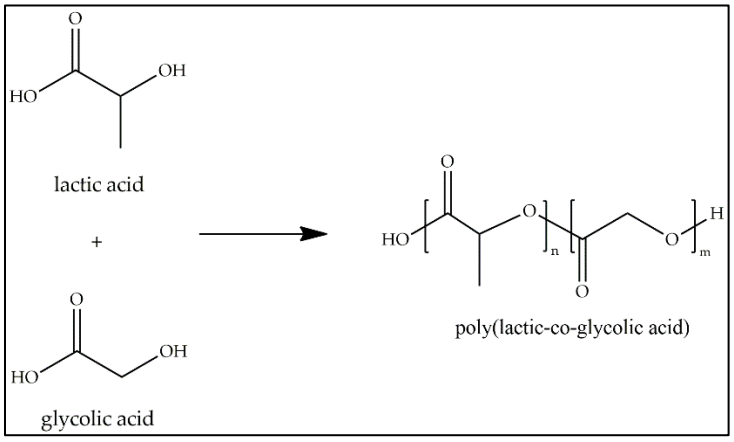

Figure 3-12. Molecular structure of PLGA and its monomers.

Blasi and collaborators (Blasi, D'Souza, Selmin, & DeLuca, 2005) studied the effect of water on the T_g of PLGA 50:50 in the early stage of hydration and the physical state of water within the hydrated polymer. They characterized the thermal behavior of the hydrated polymer using modulated DSC (MTDSC), a technique that allows the application of an oscillatory heating (or cooling) profile with improved resolution and enhanced sensitivity in comparison to a conventional DSC (Reading, Luget, & Wilson, 1994). From the cooling ramp of the MTDSC only one exothermic peak appears at around -20°C, indicating the crystallization of water. The absence of a peak at 0°C indicates that the peak at -20°C does not correspond to cold-crystallization of *intermediate* water, but to supercooling of *free* water, thus no *intermediate* water is present in PLGA 50:50.

In terms of water structure, Blasi and collaborators (Blasi et al., 2005) suggested that water molecules could directly interact with the polymer chain through hydrogen bonds with its hydrophilic groups (C=O and COO-). However, *intermediate* water was not identified, probably because of a lack of binding sites. *Intermediate* water, as studied in other polymers, can form either by binding weakly with a hydrophilic group or with another water molecule directly bound to the polymer. In this case, we suggest that *non-freezable* water is simultaneously binding with two hydrophilic groups, as shown in Figure 3-13. Therefore, the remaining water can only bind with each other, forming *free* water.

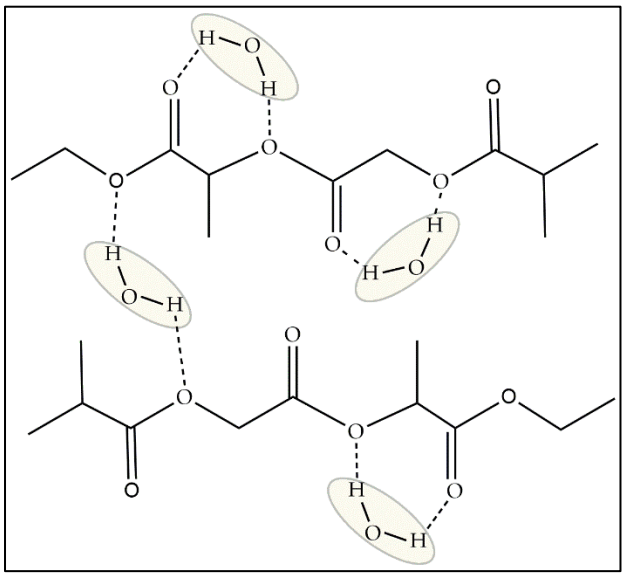

Figure 3-13. Proposed structure of non-freezable water sorbed on PLGA.

The hemocompatibility of PLGA 50:50 is very low with an increased fibrinogen adsorption with respect to noncoated polypropylene (about 150%) (Weber et al., 2004). Moreover, Liu and collaborators compared PLGA 85:15 and PLGA 85:15 immobilized with silk fibroin and demonstrated that the platelet adhesion, activation and the thrombogenic ability of the polymer by itself was higher than that of the treated one (Liu et al., 2011). For example, untreated PLGA adhered over $550x10^5$ platelets/cm^2 after 2h incubation, while treated PLGA adhered only about $50x10^5$ platelets/cm^2. Other biocompatibility aspects studied by them include vascular endothelial cell attachment and morphology, cell viability, transcription level of genes, and expression of proteins. When silk fibroin is immobilized to the polymer (Liu et al., 2011) an additional hydrophilic group is available for water molecules to bind. Then, i*ntermediate* water would form by the presence of the carbonyl group of the fibroin (Figure 3-14), which would explain the improved hemocompatiblity and other biocompatibility aspects of treated PLGA. More studies on the water states in modified PLGA polymers are necessary to clarify if this improvement is due to the presence of *intermediate* water.

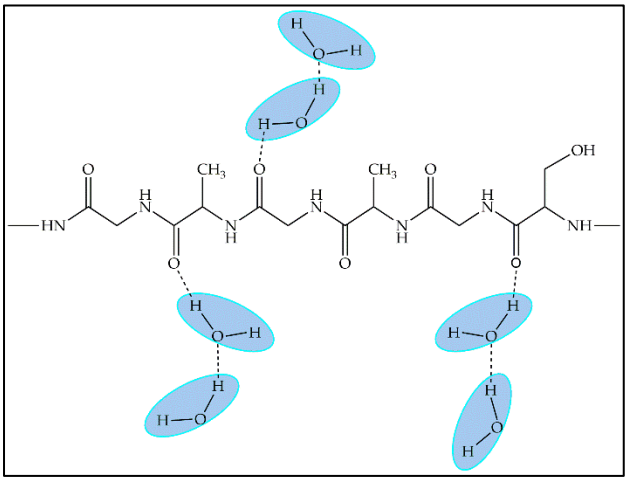

Figure 3-14. Proposed structure of intermediate water sorbed on silk fibroin.

3.2.1.5. Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is one of the few vinyl polymers that are water soluble and biodegradable (Figure 3-15). This polymer used in many industries such as plastic, textile, paper, food, biomedical and pharmaceutical. In the biomedical field, studies on the use of PVA for contact lenses, skin and artificial meniscus have been developed (Muppalaneni, 2013). While in the pharmaceutical industry, it is mainly used for drug delivery systems and many PVA-based products are currently available. Most of these products are in the form of tablets, but there are also transdermal/topical forms, and ophthalmic and implantable devices (Muppalaneni, 2013).

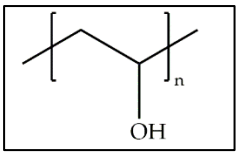

Figure 3-15. Molecular structure of PVA.

Hodge and collaborators (Hodge, Edward, & Simon, 1996) studied the states of water by DSC in PVA films. They observed that all the water sorbed into the polymer inhabits the

amorphous region but it also destroys crystalline ones. Therefore, as the polymer sorbs more water the crystalline region decreases. They also calculated the amount of each water state at different water content. When total water content is below 22%, all of this corresponds to *non-freezable* water. Above this value, *intermediate* water appears and reaches a maximum of 2.5% while *free* water also appears increasing linearly until its saturation level of about 60% of total water (Figure 3-16).

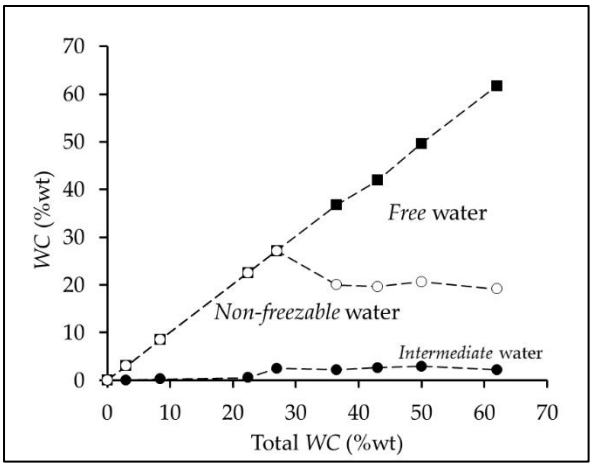

Figure 3-16. Water states distribution in PVA at different WC. (•) Intermediate water; (o) non-freezable water; (▪) free water. Modified from (Hodge et al., 1996).

Hemocompatibility of PVA is considered good and the adhered platelets inactive on the polymer surface (Ino et al., 2013). This polymer is attractive for tissue engineering applications due to its mechanical properties; however, many studies modify it by the addition of other polymers such as gelatin or dextran (Alexandre et al., 2014; Muppalaneni, 2013), in order to improve its hemocompatibility. When comparing PVA platelet adhesion (about $40x10^5$ platelets/cm^2) (Ino et al., 2013) with other polymers presented in this study (e.g., PCL: about $5x10^5$ platelets/cm^2), we can consider that platelet adhesion on PVA is high (i.e., low hemocompatibility). Taking into account the low *intermediate* water content in PVA films reported by Hodge and collaborators (Hodge et al., 1996), where this value reached a maximum of 2.5%, we can conclude that low

intermediate water content is related with high platelet adhesion, although it is not activated by the contact with the polymeric surface.

3.2.2. Non-degradable polymers

3.2.2.1 Poly(meth)acrylates

Polymethacrylates correspond to copolymers of methacrylic acid and acrylates. The copolymerization in different ratios provides a wide range of products with different tensile strength and elongations. In general, methacrylates have a higher tensile strength and lower elongation than their corresponding acrylate (Ellis & Smith, 2008). In the medical field, polymethacrylates are used for the manufacture of contact lenses, artificial joints, dental implants, among others. The most studied polymer among these group is PMMA and its applications include bone cement, intraocular lenses and artificial kidney (Oda et al., 2014). Eudragit® from Evonik is one of the commercial products of polymethacrylates and is used for functional solid oral dosage with specific drug delivery (S. Chen et al., 2017; Huanbutta, Nernplod, Akkaramongkolporn, & Sriamornsak, 2016).

Tanaka and collaborators have carried out extensive research on the biocompatibility of poly(meth)acrylates, especially poly(2-methoxyethyl acrylate) (PMEA). They studied the structure of water in hydrated PMEA and compared it to water of poly(2-hydroxyethyl methacrylate) (PHEMA) and polyacrylates analogs (Tanaka, 2004). They analyzed the phase transitions of water by DSC and performed a platelet adhesion test on the polymers. Their results show that PMEA shows a high hemocompatibility in comparison to the other polymers and this phenomenon appears to be dictated by the presence of *intermediate* water on the surface of the polymer and not by the total amount of water.

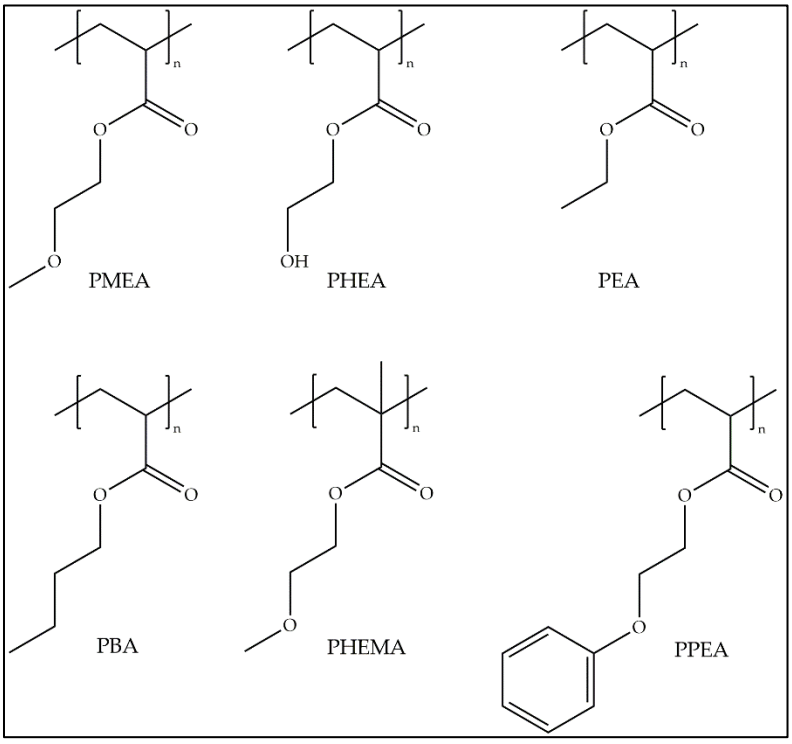

Figure 3-17. Molecular structure of poly(meth)acrylates.

From this study, the available data of the different polymers were analyzed, namely: PMEA, PHEMA, poly(ethyl acrylate) (PEA), poly(2-phenoxyethyl acrylate) (PPEA), poly(2-ethylhexyl acrylate) (PEHA), and poly(n-butyl acrylate) (PBA) (Figure 3-17). EWC and amount of types of water were analyzed separately with the number of adhered platelets. In general terms, there is no relation between the total EWC, *non-freezable* water and *free* water with platelet adhesion. However, as concluded by Tanaka and collaborators, the presence of *intermediate* water decreases the number of adhered platelets to the surface (Figure 3-18), hence the hemocompatibility is related with the presence of *intermediate* water.

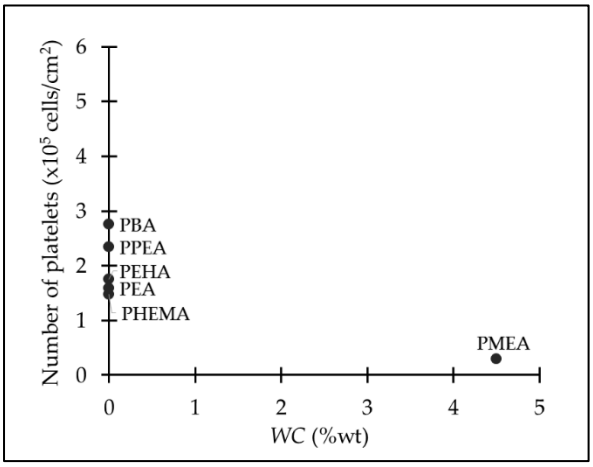

Figure 3-18. Relation of intermediate water content and platelet adhesion in poly(meth)acrylates. Data extracted from (Tanaka, 2004).

The analysis of ^{2}H-NMR and ^{13}C-NMR of hydrated PMEA (Miwa et al., 2010) shows that *non-freezable* water has a low mobility because it interacts strongly with the polymer chain, which allows this water to not freeze even below -100°C. *Free* water has high mobility and it barely interacts with the polymer, resembling *bulk* water. From ATR-IR studies it was elucidated that *non-freezable* water interacts with the C=O groups in PMEA side chain, while *free* water only interacts through hydrogen bonds with other water molecules and has no interaction with the polymer (Morita et al., 2007). In the case of *intermediate* water, it interacts with the methoxy moiety (O-CH_3) in the side chain terminal, both by hydrogen-bonding interaction. Though, this interaction is weaker than the one with *non-freezable* water; therefore, the ^{2}H-NMR and ^{13}C-NMR analysis show that *intermediate* water has intermediate mobility in comparison to the other types of water. It is hypothesized that because of these characteristics, the layer of *intermediate* water formed on the surface is more stable than *free* water and when it is sufficiently thick, it does not allow direct contact of cells or protein with polymer surface or *non-freezable* water (Tanaka et al., 2013).

In general, *non-freezable* and *free* water are present in all surfaces of polymethacrylates. When fibrinogen approaches the surface through contact with *non-freezable* water it

activates, it causes the adhesion of platelets to the polymer (Tanaka et al., 2013). Due to the high mobility and its resemblance to bulk water, *free* water is not able to prevent this from happening. However, when *intermediate* water is present, it avoids the activation of plasma proteins, due to the moderate mobility of its molecules; and therefore, it plays a key role in their hemocompatibility.

3.2.2.2. Poly(acrylonitrile)-co-N-2-vinyl-pyrrolidone

Polyacrylonitrile (PAN) is obtained from the polymerization of a vinyl group linked to a nitrile (acrylonitrile), and is mostly used as a copolymer in the production of plastics, as a precursor of carbon fiber and as separation membrane material in hemodialysis (Serkov & Radishevskii, 2008; L. S. Wan, Xu, Huang, Wang, & Ye, 2005). Some studies show the improvement of biocompatibility of PAN by immobilizing other polymers such as PEG and N-vinyl-2-pyrrolidone (NVP) (Nie, Xu, Huang, Ye, & Wu, 2003). NVP is composed by a five-membered lactam (γ-Lactam) bound to a vinyl group; it has been used as a coating on cardiovascular devices showing improved biocompatibility (Francois, 1996). Copolymers of acrylonitrile and NVP have been synthesized and applied in liver support systems (Krasteva et al., 2002) and as nanocarriers in drug delivery systems (Staufenbiel et al., 2015).

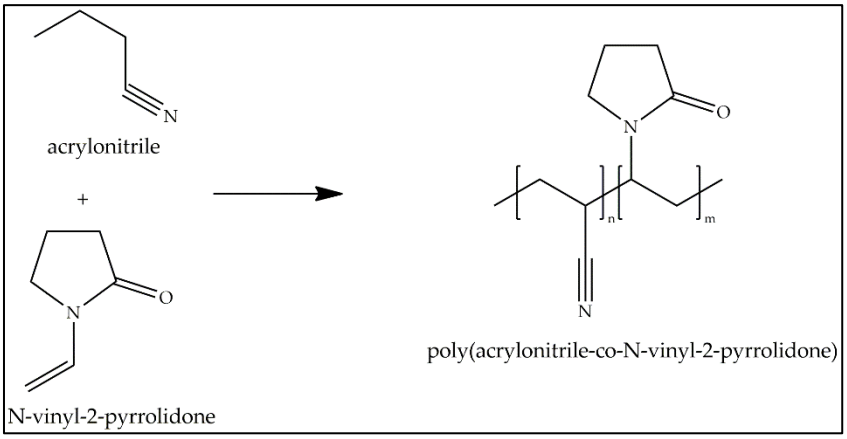

Figure 3-19. Molecular structure of PANcNVP and its monomers.

Wan and collaborators (L.-S. Wan, Huang, & Xu, 2007; L. S. Wan et al., 2005) compared the swelling behavior of PANcNVP films (Figure 3-19) with different NVP content: 7%, 15%, 22% and 31%, with their hemocompatibility by platelet adhesion and plasma recalcification time (PRT) test. They observed that higher contents of NVP meant increased water uptake in the polymer, with increased amount of *non-freezable* and *freezable* water both determined by DSC and TGA (Table 3-2). They also conducted a FT-IR in transmission mode and in ATR to examine the diffusion and structure of water in the copolymers. From their calculations they identified three types of water in the surface of fully hydrated polymers (high NVP content): type III water (*free* water), with relatively weak hydrogen bonding with the nitrile group of PAN; type II water (*intermediate* water), monomeric or dimeric water molecules interacting with a carbonyl group in NVP; and, type I water (*non-freezable* water), bound to a carbonyl group and two or more water molecules. When analyzing the different hydrated copolymers, higher contents of NVP relate with higher amounts of *non-freezable* water, shown by the peak at wavenumbers below 3300 cm^{-1} in the FT-IR spectra. The presence of the carbonyl group of the NVP allows the binding of more stable water molecules, namely, *non-freezable* water.

Table 3-2. Water content of total, non-freezable and freezable water in PANcNVP. Extracted from (L. S. Wan et al., 2005).

Content of NVP (wt%)	**Total water (wt%)**	***Non-freezable* water (wt%)**	***Freezable* water (wt%)**
0	29.7	4.6	25.1
7	30.6	5.2	25.4
15	43.3	9.6	33.7
22	55.5	16.0	39.5
31	58.3	19.3	39.0

Hemocompatibility tests showed that with higher amounts of NVP the number of platelets adhered to the surface was less than with samples with lower amounts of NVP, and these platelets conserved much of their original shape, indicating an inactivated state. The high content of NVP also increased the PRT, which indicates that coagulation around the polymer occurs slower than in copolymers with low NVP (L. S. Wan et al., 2005) (Figure 3-20). Relating this information with the water states data, the study suggests that the improved hemocompatibility is due to the higher amount of *non-freezable* water present in the polymer-water system. But in the calculation of *non-freezable* and *freezable* water the authors did not differentiate the latter between *intermediate* and *free* water. They did however identify the three different states of water in the polymer-water system through the IR analysis. Both *non-freezable* and *intermediate* water can be responsible for the improved hemocompatibility of the polymer and determining the amount of *intermediate* water with DSC or NMR will provide more information on its role on hemocompatibility.

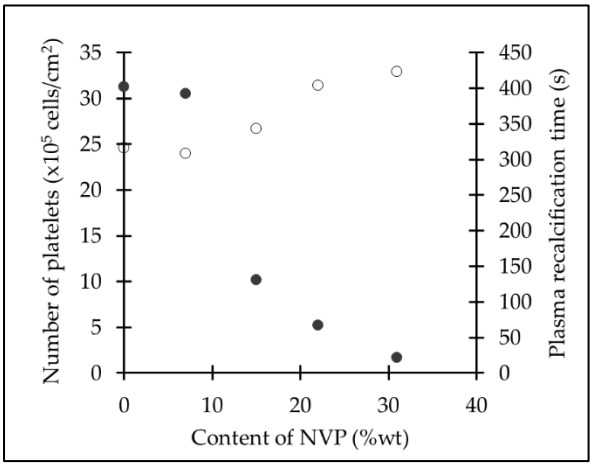

Figure 3-20. Relation of NVP content with platelet adhesion (•) and PRT time (o) on PANcNVP. Modified from (L. S. Wan et al., 2005).

3.3. Discussion

3.3.1. Water states in polymers

Among the studies presented above, some analyzed the states of water as *non-freezable* and *freezable* water, understanding the latter as the sum of *intermediate* and *free* water. In the case of some polymethacrylates and PLGA *intermediate* water was not identified. However, *non-freezable* water was always present in the polymer-water matrix analyzed. Also, based on the analysis in polyarylates, PEG and PVA, as the polymer hydrates, *non-freezable* water increases until it reaches a limit from which it remains constant at higher hydration levels. This occurs because as the polymer gets more hydrated, there are not enough hydrogen bonding sites to form *non-freezable* water.

The structure of the polymer determines the formation of the different types of water. Functional groups in the polymeric chain interact with different strength levels with water through hydrogen bonds. As seen in aliphatic carbonyls, the carbonyl group bonds to water forming *non-freezable* water but no *intermediate* water. The same happens in PANcNVP and the carbonyl group of the NVP. The ether group of PDO and PMEA binds to water and due to its moderate strength, *intermediate* water is formed. However, *intermediate* water can also form when a water molecule binds to another which is directly bound to the polymer. In this case, the indirectly bound water molecule has higher mobility than the directly bound one.

The length of the polymeric chains of the M_w also affects the formation of the different states of water. When M_w is higher, larger polymeric chains tend to fold upon themselves and functional groups of the chain are closer to each other. This induces the formation of *intermediate* water as bridging these groups and supplying more stability to the folded polymer. Additionally, if the end group of a polymer has the capacity to form hydrogen bonds, then in low M_w chains, where more end groups are available, more water molecules can bind to them and more *non-freezable* water is formed.

3.3.2. Platelet adhesion on polymers

Most of the biological response data presented here was about platelet adhesion. A polymer with high amount of adhered platelets is considered to have low hemocompatibility as in the case of PLGA, which has about 550 x 10^5 platelets/cm^2 (Liu et al., 2011). However, when comparing polymers with lower platelet adhesion, there is a lack of consensus on what is low, moderate or high hemocompatibility. Figure 4-1 shows the number of adhered platelets on different polymers. In the study of Tanaka and collaborators (Tanaka, 2004) they state that PMEA has excellent hemocompatibility while the other poly(meth)acrylates (PHEMA, PEA, PEHA, PPEA and PBA) have low hemocompatibility. However, when compared to other polymers, like PAN or PVA, which are considered to have moderate hemocompatibility, poly(meth)acrylates have high hemocompatibility.

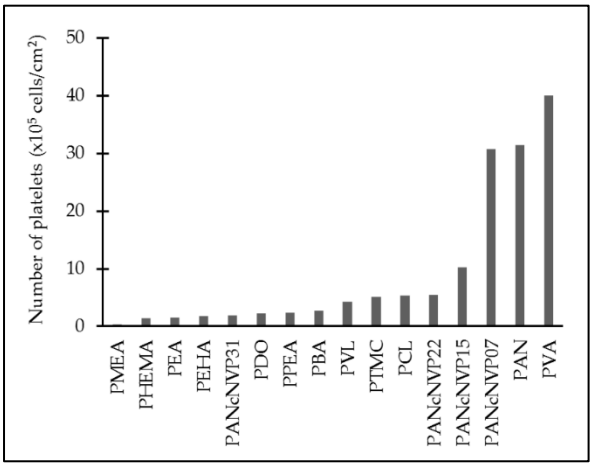

Figure 3-21. Platelet adhesion on different polymers.

3.3.3. Effect of water states on hemocompatibility

In most polymers studied, when *intermediate* water is present, low platelet adhesion is observed, which means high hemocompatibility. This relation is clear in PEG, poly(meth)acrylates, aliphatic carbonyls and PLGA, although there is no mathematical relation between the content of *intermediate* water and the amount of adhered platelets.

Intermediate water in the polymer is more stable and has lower mobility than *free* water, thus when it is sufficiently thick it prevents the activation of the protein by avoiding direct contact with *non-freezable* water. The main conclusions of each polymer are summarized in Table 4-1.

Tanaka and collaborators (Tanaka, 2004) did not detect any *intermediate* water in poly(meth)acrylates except for PMEA. However, these polymers have high hemocompatibility in relation to other polymers such as PAN and therefore it would be expected that poly(meth)acrylates (PBA, PPEA, PEHA, PHEMA and PEA) to have some *intermediate* water of about 3% wt. The apparent absence of *intermediate* water in these polymers could be determined by the absence of an exothermic peak of cold-crystallization in the heating DSC thermogram. However, the cooling thermogram can show the presence of *intermediate* water by a second peak below 0°C, which the authors might not have considered. Additionally, in the heating thermograms a single peak with a shoulder on the lower temperature region is often considered as the effect of the melting of *intermediate* water, which is present in some heating thermograms of poly(meth)acrylates, therefore it cannot be concluded that there is no presence of *intermediate* water at all.

Considering PEG, aliphatic carbonyls and poly(meth)acrylates, a limit of about 3% wt for *intermediate* water can be established, from which platelets adhere less to the surface and the polymer has higher hemocompatibility, i.e., at contents of *intermediate* water above 3% wt, platelet adhesion is low, and below 3% wt, platelet adhesion is high. In order to find the mathematic relationship between these two properties, *intermediate* water content should be calculated not only for the afore mentioned poly(meth)acrylates (where no *intermediate* water was detected), but also with polyarylates and PANcNVP.

For polymers such as PANcNVP and polyarylates, the available data is not sufficient to make similar conclusions, where the presence of *intermediate* water means low platelet adhesion. However, in polyarylates, at high water content, high *freezable* water relates

directly with platelet adhesion. This *freezable* water corresponds to *intermediate* and *free* water, therefore considering the previous observation for poly(methyl methacrylates), it can be suggested that in polyarylates at high water content, higher *freezable* water contains less *intermediate* water, which would explain high platelet adhesion at high *freezable* water content. For PANcNVP, Wan and collaborators concluded that high content of *non-freezable* water means low platelet adhesion on the polymers. This conclusion was made after calculating the content of *non-freezable* and *freezable* water in the polymer and relating it to the content of NVP, and then with platelet adhesion. As in polyarylates, they calculated *intermediate* water and *free* water as *freezable* water; they also detected the presence of all three water types in the hydrated polymer. However, they did not calculate the content of these two states by separate, in which case it could happen that at higher NVP content *intermediate* water is high relating it with low platelet adhesion.

In the case of PVA, the presence of *intermediate* water implies moderate platelet adhesion. This polymer has a maximum content of *intermediate* water of about 2.5% wt, lower than the limit of 3% wt, therefore hemocompatibility can be considered as low. In fact, data on platelet adhesion shows that PVA adhere about 40 x 10^5 platelets/cm^2, a higher value than the one reported for poly(meth)acrylates, PEG and aliphatic carbonyls, but lower than PLGA. Then, in this case, the relation is that low *intermediate* water content relates to high platelet adhesion. It is hypothesized that this relation is due to low stability of *intermediate* water in this polymer, resembling *free* water, which is not enough to prevent contact of proteins with *non-freezable* water. Another alternative is that *intermediate* water is clustered in the polymer matrix, leaving free regions of this type of water and allowing direct contact of the proteins.

Finally, the improvement on biocompatibility of PLGA 85:15 by silk fibroin may be due to the formation of *intermediate* water. Although, the states of water in the polymer have not been identified nor calculated, it is hypothesized that the presence of the carbonyl group in silk fibroin allows *intermediate* water to form, improving the hemocompatibility. Hence, the presence of that water could be also improving the other biocompatibility

aspects of the scaffold, i.e., cell attachment and morphology, cell viability, transcription level of genes, and expression of proteins. This would imply that the presence of *intermediate* water not only could improve hemocompatibility of polymers, but also promote other biocompatibility aspects. This preliminary conclusion should be demonstrated with further research on the states of water and their impact on biocompatibility.

Table 3-3. Summary of principal observations and conclusions in each polymer.

Polymer	Types of water measured	Biological response measured	Observations	References	Conclusions
PEG	*Free*, *intermediate* and *non-freezable*		*Intermediate* water is negligible at low M_w and increases with M_w until a constant value	(Antonsen & Hoffman, 1992; Yamauchi & Tamai, 2003)	Presence of *intermediate* water means low protein adsorption and platelet adhesion
		Platelet adhesion and plasma protein adsorption	Low protein adsorption and platelet adhesion	(Antonsen & Hoffman, 1992; Nagaoka et al., 1985)	
Aliphatic carbonyls	*Free*, *intermediate* and *non-freezable*	Platelet adhesion	There is lower platelet adhesion when *intermediate* water is present.	(Fukushima et al., 2015)	Presence of *intermediate* water means low platelet adhesion (Fukushima et al., 2015)
Poly(meth)acrylates	*Free*, *intermediate* and *non-freezable*	Platelet adhesion	*Intermediate* water present only in PMEA is responsible for its excellent hemocompatibility.	(Tanaka et al., 2000)	Presence of *intermediate* water means low platelet adhesion (Tanaka et al., 2000)

PLGA	*Free*, *intermediate* and *non-freezable*		No presence of *intermediate* water	(Blasi et al., 2005)	Absence of *intermediate* water means high platelet adhesion.
		Fibrinogen adsorption and platelet adhesion	High fibrinogen adsorption and platelet adhesion	(Liu et al., 2011; Weber et al., 2004)	
		Cell attachment, morphology, viability; transcription level of genes and expression of proteins	PLGA with silk-fibroin has better biocompatibility	(Liu et al., 2011)	Presence of carbonyl group in fibroin allows *intermediate* water formation and better biocompatibility
PVA	*Free*, *intermediate* and *non-freezable*		PVA films have low *intermediate* water content	(Hodge et al., 1996)	Low *intermediate* water content means high platelet adhesion (inactive state)
		Platelet adhesion	High platelet adhesion but in inactive state.	(Ino et al., 2013)	
PANcNVP	*Free*, *intermediate* and *non-freezable*		Three types of water present	(L. S. Wan et al., 2005)	High content of *non-freezable* water means less platelet adhesion (L.-S. Wan et al., 2007)
			Higher NVP means higher *non-freezable* water content	(L.-S. Wan et al., 2007)	
		Platelet adhesion and PRT	Higher amounts of NVP led to less platelet adhesion and increase of PRT	(L. S. Wan et al., 2005)	*Intermediate* water could influence hemocompatibility (not enough

					data for conclusions)
Polyarylates	*Freezable* and *non-freezable*		In polymers with WC over 10%, *non-freezable* water reaches a threshold lower than *freezable* water.	(L M Valenzuela et al., 2011)	At high WC, high *freezable* water means high fibrinogen adsorption
		Fibrinogen adsorption	Polymers with longer ester and diacid chains adsorb less fibrinogen	(Weber et al., 2004)	*Intermediate* water (implicit in *freezable* water) could influence hemocompatibility (not enough data for conclusions)

4. GENERAL CONCLUSIONS

First, a mathematical model for polymer degradation, which included the contribution of water in the degradation process was presented. Parameter estimation was carried out with experimental data of PLA degradation previously reported. The values of the estimated parameters were in accordance to the degradation of the polymer and diffusion of the species, and the model had a good fit with respect to the degradation data ($R^2 = 0.98$), in contrast to water uptake data ($R^2 = 0.81$). Monomers, ester bonds and water concentration in the polymer matrix with respect to time and the relative distance to the center was presented in surface plots. Their general behavior described well the degradation and diffusion processes inside the matrix in time, although improvements in the description of water behavior is needed. Also, further simulation is necessary to verify the accuracy of the model in other polyesters.

Regarding water states in polymers, at low total water content *non-freezable* water is higher than that of *freezable* water and it increases until a constant value which remains below the content of *freezable* water as total content of water increases. When water approaches to the polymeric surface the first molecules to sorb correspond to *non-freezable* water and then it is followed by the sorption of *freezable* water in the form of *intermediate* and *free* water. However, *intermediate* water can be absent or in very small content as seen in some of the polymers included in this study. The content of the different states of water are subject to functional groups of the polymer chain, where different groups bond to water with different strength; and the length of the chain, where longer chains tend to fold upon themselves and aid in the formation of *intermediate* water.

In PEG, poly(meth)acrylates, aliphatic carbonyls, PLGA and PVA, *intermediate* water content of at least 3% wt relates to low platelet adhesion and higher hemocompatibility than those with less or none. This is due to the better stability that *intermediate* water has than *free* water, which prevents platelets to contact *non-freezable* water and to activate. In polyarylates and PANcNVP the available information was not enough to make the same

conclusion, however with further research it is expected that platelet adhesion in these polymers will also be negatively affected by the presence of *intermediate* water.

This study shows that surface water influences polymer hemocompatibility and that water states are the principal components in the water-hemocompatibility relation. More specifically, *bound* water acts as the intermediary between fibrinogen and the polymer surface; then, fibrinogen activates as it binds to this water, allowing platelets to adhere to the surface. *Free* water at this level would act like *bulk* water and would not impede fibrinogen to approach *bound* water. However, when *intermediate* water is present or when its content is enough, i.e. 3% wt, its higher stability than *free* water prevents fibrinogen and platelets to reach *bound* water and thus activate. Accordingly, *intermediate* water plays a key role in platelet adhesion where higher contents of this type of water means lower adhesion. Therefore, the hydration states of polymers must be considered when selecting polymers for a desired application.

4.1. Future Challenges

It is evident that many challenges remain to clarify the relation between water states and hemocompatibility. An immediate challenge, considering only polymers presented in this work, is the identification and measurement of *intermediate* water and *free* water separately utilizing techniques like DSC and IR, with special focus on polyarylates, PANcNVP and poly(meth)acrylates in order to support the conclusion that polymers with a content of *intermediate* water above 3% wt have better hemocompatibility than those below this value.

The hydration states can be related with more aspects of biocompatibility than only hemocompatibility. Some examples of biological responses include protein adsorption of non-plasmatic proteins; adhesion, growth, migration and differentiation of cells widely studied in tissue regeneration; and gene expression. Thereby, more information and details are needed on the role of water in biocompatibility and body-material interaction. Finally,

the information of the effect of water in biocompatibility can be complemented with the degradability of polymers, in which case, a mathematical relation could be obtained and added to the degradation model presented here. The ultimate goal of future studies would be to develop and provide an integrated model of water uptake, polymer degradation and biological response, that will accelerate polymeric device design and development in the medical industry.

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ANNEX: ARTICLE MANUSCRIPT

Review

Impact of the Hydration States of Polymers on Their Hemocompatibility for Medical Applications: A Review

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Abstract: Water has a key role in the functioning of all biological systems, it mediates many biochemical reactions, as well as other biological activities such as material biocompatibility. Water is often considered as an inert solvent, however at the molecular level, it shows different behavior when sorbed onto surfaces like polymeric implants. Three states of water have been recognized: *non-freezable* water, which does not freeze even at −100 °C; *intermediate* water, which freezes below 0 °C; and, *free* water, which freezes at 0 °C like *bulk* water. This review describes the different states of water and the techniques for their identification and quantification, and analyzes their relationship with hemocompatibility in polymer surfaces. *Intermediate* water content higher than 3 wt % is related to better hemocompatibility for poly(ethylene glycol), poly(meth)acrylates, aliphatic carbonyls, and poly(lactic-co-glycolic acid) surfaces. Therefore, characterizing water states in addition to water content is key for polymer selection and material design for medical applications.

Keywords: *intermediate* water; *non-freezable* water; *free* water; water structure; platelet adhesion; fibrinogen adsorption.

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1. Introduction

Water is considered to be the most important compound of life and is the most abundant compound on earth. Over 70% of the surface of the planet is covered by water, in the form of solid, liquid, and vapor [1]. It is also the main component of biological systems, being essential for many chemical reactions. Water is often regarded as the universal solvent because of its great versatility: it can dissolve proteins, ions, sugars, gases, organic liquids, and lipids. What is fascinating about water is its uniqueness. Compared to other common liquids, water is characterized by having high boiling, melting, and critical temperatures, large specific heat, and high surface tension,

among other properties [2]. In the first section of the review, general aspects of water and its behavior in biological systems and surfaces are described.

1.1. General Aspects of Water

Water is a small molecule composed of two hydrogen atoms covalently bonded to one oxygen atom. These bonds form a V-shaped structure with an angle of 104.6° (Figure 1) due to the two pairs of unused electrons of each oxygen atom, which tend to position as far from each other as they can to minimize the repulsion. The presence of these electrons causes a large dipole moment
(i.e., uneven distribution of charges in the molecule), where the oxygen atom is slightly negatively charged, and the hydrogen atoms are slightly positively charged. This large dipole moment leads to a strong intermolecular interaction called hydrogen bonding, which is much weaker than the intramolecular covalent OH bond (23.3 kJ mol^{-1} vs. 492 kJ mol^{-1}) [3]. The most predominant form is a 4-coordinated water molecule which forms hydrogen bonds with four different water molecules, i.e., each hydrogen atom pairs up with an oxygen atom of different water molecules, while the oxygen atoms pair up with two hydrogen atoms of different water molecules [4].

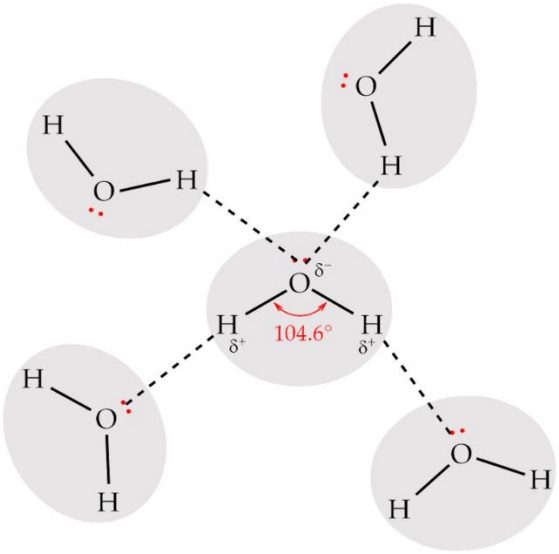


Figure 1. Structure of a 4-coordinated water molecule. Hydrogen atoms are positively charged (δ+) and oxygen atoms are negatively charged (δ-), which also present a lone pair of electrons. Solid lines indicate covalent bonds and dashed lines, hydrogen bonds.

The behavior of pure water can be described by different models, which can be used to explain the peculiarities of water and its properties. In general, these models can be classified into two groups: (i) mixture or multicomponent models, where two or more water groups are present; and, (ii) continuum or uniformist models, in which each water molecule is influenced by the same intermolecular force. The flickering cluster model corresponds to a mixture model that suggests that clusters of hydrogen-bonded water are rapidly formed and swim in a medium of monomeric water molecules. However, this model fails to explain many of the water properties, as well as the nature of the clusters. On the other hand, the continuum model is a widely accepted model for

water structure and explains water in terms of intact hydrogen bonds unlike the mixture model, where the hydrogen bonds are broken between the water molecules [2,5].

1.2. Water in Biological Systems

Water is very important in all biological systems. It is the major component of the body, at around 57% of the human weight. Without water, biochemical reactions would not be possible, as well as many other biological activities. For example, water is part of the functioning of enzymes when a substrate binds to the active site displacing water molecules [2]. When medical devices are implanted in the body, the material is in contact with water before any other biological component, such as proteins or cells [6]. Thus, it is reasonable to think that water plays a major role in material–body interactions in medical devices, specially mediating the biocompatibility of these materials [2].

1.3. Surface Water

The water network can be disturbed by external elements such as ions, solutes, and surfaces. When these elements become in contact with water, they reorganize its structure in order to minimize the energy of the system. In the case of surfaces, their interaction with water depends on properties such as structure, polarity, hydrophilicity or hydrophobicity, and composition of the surface. Water molecules reorder according to these factors, forming different conformations or layers at the vicinity of the surface. The influence of hydrophilic and hydrophobic surfaces on water structure has been widely studied as seen in the study by Li and collaborators, where the microscopic behavior of water on different surfaces of self-assembled monolayers was studied [7]. They suggested that water molecules neighboring a hydrophilic surface are more rigid and have better ordering than pure water. In contrast, when faced with a hydrophobic surface, water molecules have almost the same mobility and distribution as of pure water.

In particular, on hydrated polymers, different water "types" or "states" have been observed. These can be classified depending on the mobility of the water sorbed to the polymeric surface or its freezing temperature. Generally, there are three states of water, one with very low mobility and no freezing (even at −100 °C); one with slightly higher mobility and freezing temperature below 0 °C; and one with similar mobility and freezing temperature as bulk water. Further details about their characteristics and multiple denominations are given in the next section.

1.4. Biocompatibility

The concept of biocompatibility has been analyzed and defined thoroughly in order to comprise most of the factors that are implied in the material–body interaction. The most common definition is: "the ability of a material to perform with an appropriate host response in a specific application" [8]. This definition, although very generic, includes some essential characteristics that are expected for a material to have: (i) the material in the body must have a specified function and therefore it has to perform it; (ii) the host response that the material provokes has to be acceptable; and (iii) the response to a specific material will vary in different situations because the performance of the material depends on a specific application, in a specific tissue [9,10]. Given that biocompatibility changes according to the situation, it should not be considered as a property of a biomaterial, but a characteristic of a material-tissue interaction [10].

There are several factors that affect biocompatibility. Some of them are: composition of the material, production processes, structure (e.g., woven, knitted, polished, film, tube, sponge, sphere), surface characteristics (e.g., smoothness, porosity, rheology), surface treatments/coatings, location of the device, and expected host response on the interface (e.g., macrophage activation, biofilm formation, encapsulation, platelet adhesion) [10].

Taking these factors into account a more detailed definition of biocompatibility was proposed by Williams [10]:

"Biocompatibility refers to the ability of a biomaterial to perform its desired function with respect to a medical therapy, without eliciting any undesirable local or systemic effects in the recipient or beneficiary of that therapy, but generating the most beneficial cellular or tissue response in that specific situation, and optimizing the clinically relevant performance of that therapy."

Biocompatibility evaluation can be performed by analyzing different biological effects that the material has in the body. The assessment of biocompatibility according to the ISO 10993 [11] is based on the material characteristics and its potential risk in the body under the conditions it is used. The biocompatibility test categories presented by the ISO 10993 are: cytotoxicity, hemocompatibility, degradation, implantation, sensitization, irritation or intracutaneous reactivity, acute system toxicity, material-mediated pyrogenicity, subacute or subchronic toxicity, genotoxicity, chronic toxicity, carcinogenicity, and reproductive or developmental toxicity. A brief description of the most relevant aspects of biocompatibility for this review is presented below:

- Citotoxicity: cell damage caused by direct contact with the material or by leached compounds.
- Hemocompatibility: effect on blood or blood components such as breakdown of blood cells, immunologic response, and thrombus formation.
- Degradation: breakdown of the device, which products might cause toxicity.
- Implantation: local effects of the implant on tissue.

In this review, hemocompatibility is evaluated by the adsorption of plasma proteins and platelet adhesion. It is known that when an implant makes contact with blood, plasma proteins adsorb to the surface. Fibrinogen (*Fg*), albumin, and immunoglobulins are the most abundant, and along with fibronectin, vitronectin and the von Willebrand factor, these proteins are mediators of the platelet adhesion and thrombosis around the biomaterial [12]. In particular, fibrinogen, the main protein in blood plasma, is fundamental in blood coagulation and thrombosis induced by biomaterials. Activation of fibrinogen leads to platelet immobilization, activation, and aggregation, although the availability of platelet-binding sites is more important than the total amount of adsorbed fibrinogen in mediating platelet adhesion [12]. Either way, high fibrinogen adsorption and high platelet adhesion imply that the biomaterial is not inert in blood, hence it has low hemocompatibility.

There are few studies that consider water as a relevant factor on biocompatibility, and even fewer relate its states on the surface with its biocompatibility. This review highlights the role water and its states have on biocompatibility, specifically on the hemocompatibility of polymer surfaces.

2. Types of Water

2.1. Denominations

The study of water in different surfaces has been driven by the interest in investigating the effects of water on the functionality of these surfaces. As mentioned above, it includes

hydrophobic/hydrophilic surfaces, metals, polymers, among others. Because of the large number of studies on this matter, a summary of the different denominations that water receives on surfaces is presented.

Generally, water is classified in three states according to mobility or freezing temperature criteria. Early studies using nuclear magnetic resonance (NMR) suggested the possibility of an ordered water structure in the vicinity of different surfaces, called *bound* or *ice-like* water due to its low molecular mobility. Later, other types of water were identified: one with higher mobility than the so-called *bound* water, and another that behaves similarly to *bulk* or pure water.

In most cases, the naming of the types of water has been based on its thermal behavior (i.e., crystallization), or on the mobility of their molecules (Table 1). Water can be classified according to low, intermediate, and high mobility at atmospheric conditions, and based on this criterion Hechter and collaborators [13], Sterling and Masuzawa [14], and McBrierty and collaborators [15] arrived at similar classification of the states of water with similar names. These three classifications were based on the NMR spectra of water, which are described in the next section.

The other criterion used is the freezing temperature of water and the most common used denominations are the one presented by Higuchi and Iijima [16], Hatakeyama and Hatakeyama [17], and Tanaka and collaborators [18]. *Non-freezing* water does not freeze (even at −100 °C); *freezing bound* water freezes at temperatures below 0 °C; and *free* water freezes at 0 °C. Hirata and collaborators [19] described hydration water as the one that freezes below 0 °C or does not freeze at all. Therefore, hydration water is equivalent to *non-freezing* and *freezing bound* water, while *free* water is the same in both cases. Additionally, the mobility and the freezing temperature criteria can be considered equivalent, because more thermal energy in molecules translates in faster movement of them and thus molecular mobility increases with temperature. A third criterion was proposed by Aizawa and collaborators [20], and Jhon and Andrade [5] which was based on the thermal expansion of water molecules and the transition temperature of this property, i.e., the temperature at which thermal expansion changes.

Table 1. Denominations of the types of surface water based on different criteria.

Criteria	Types of Water		Reference
	Name	**Criteria**	
Rigidity and mobility	*Ice-like* water [a]	Very low mobility	[13]
	Intermediate between ice-like and *free* water [b]	Intermediate mobility	
	Free water [c]	High mobility	
	Solid water [a] (*glass-like* or *ice-like*)	Very low mobility	[14]
	Bound water [b]	Intermediate mobility	
	Free water [c] (very loosely bound or liquid)	High mobility	
	Tightly bound water [a]	Very low mobility at temperatures <230K	[15]
	Loosely bound water [b]	Low mobility in the range 230–260K	
	Free or *bulk-like* water [c]	Mobility similar to bulk water at around 273K	
Freezing temperature	*Hydration* water [d]	Freezes sub-zero or does not freeze	[19]
	Free water [e]	Freezes at 0 °C	
	Bulk-like water	Normal mobility as normal melting point is approached	

	Non-freezing water or *Non-freezing bound* water [f]	No crystallization (no freezing)	[16–18]
	Freezing-bound water or *Intermediate* water [g]	Crystallization under 0 °C	
	Free water [e]	Normal crystallization of water	
Thermal expansion	*Hydrated* water	Transition temperature: Non at −30 to 0 °C	[5,20]
	Interfacial water	Transition temperature: −20 to 0 °C	
	"Normal" or *bulk* water	Transition temperature: 0 °C	

[a] *Ice-like* water, *solid* water and *tightly bound* water are equivalent. [b] *Intermediate* between *ice-like* and *free* water, *bound* water and *loosely bound* water are equivalent. [c] *Free* water in the three references are equivalent. [d] Corresponds to f + g. [e] Same *free* water by freezing temperature criterion.

In this review, we refer to the different types of water as: *non-freezable* water, *intermediate* water and *free* water, according to the freezing temperature criterion. *Non-freezable* and *intermediate* water are *bound* water, while *intermediate* and *free* water are *freezable* water (Figure 2). In terms of structure and interaction with surfaces, the three water types are characterized by the following [17,21]:

- *Non-freezable* water is tightly bound to the surface and the water-surface interactions are very strong, while water–water interactions are very weak.
- *Intermediate* water interacts moderately with the surface (stronger than *free* but weaker than *non-freezable* water), involving both water-surface and water–water interactions.
- *Free* water hardly interacts with the surface and there is mainly water–water interaction.

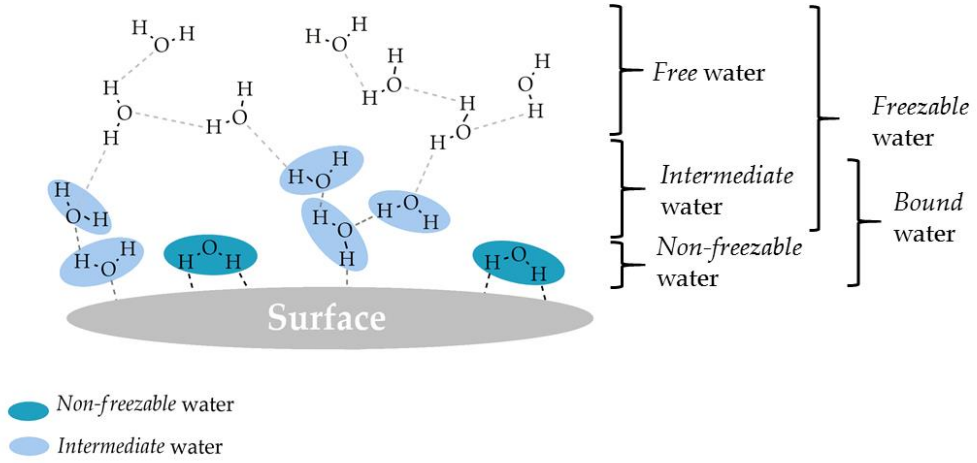


Figure 2. Types of water on surfaces with the denominations, their structure and interactions on surfaces.

2.2. Water Measurement Techniques

Water content and water uptake are two denominations for total water sorbed in a sample. These can be can be measured by thermogravimetrical analysis (TGA), and their difference depends on whether the dry or wet sample is considered. Then, water uptake (WU) and water content (WC) can be calculated as follows:

$$WU = \frac{w_{water}}{w_{dry}} = \frac{w_{wet} - w_{dry}}{w_{dry}} \quad (1)$$

$$WC = \frac{w_{water}}{w_{wet}} = \frac{w_{wet} - w_{dry}}{w_{wet}} \quad (2)$$

where w_{water}, w_{wet} and w_{dry}, correspond to the weight of sorbed water, wet sample and dry sample, respectively.

There are several analytical methods to identify and/or quantify the different types of water on a surface. The three main techniques used are differential scanning calorimetry (DSC), NMR, and infrared (IR) spectroscopy. These techniques are further described below.

2.2.1 Differential Scanning Calorimetry

The identification of the states of water on a surface and their quantities can be analyzed using DSC. It is widely used because the identification of *intermediate* water is easy due to the clear peaks associated with the phase transition. It has been used by Hatekayama and Hatekayama [17] to study the state of water in several insoluble and soluble polymers, such as cellulose, polyhydroxysterene, hyaluronic acid, and poly(vinyl alcohol). Besides polymers, DSC can also be used to identify the states of water on metals [22], ceramics [23], and food [24,25].

DSC allows the observation of the phase transitions in a thermogram from which various information can be extracted: (i) changes in heat capacity, (ii) magnitude of the heat (exothermic or endothermic), (iii) shape of the exotherms or endotherms, and (iv) the temperature at which these phenomena occur [15]. Figure 3 shows a DSC thermogram of a hydrated surface containing the three types of water: *non-freezable*, *intermediate,* and *free* water. The exothermic peak below 0 °C corresponds to the cold-crystallization of water and indicates the presence of *intermediate* water. The endothermic peak at around 0 °C corresponds to the melting of *free* water and *intermediate* water where it corresponds.

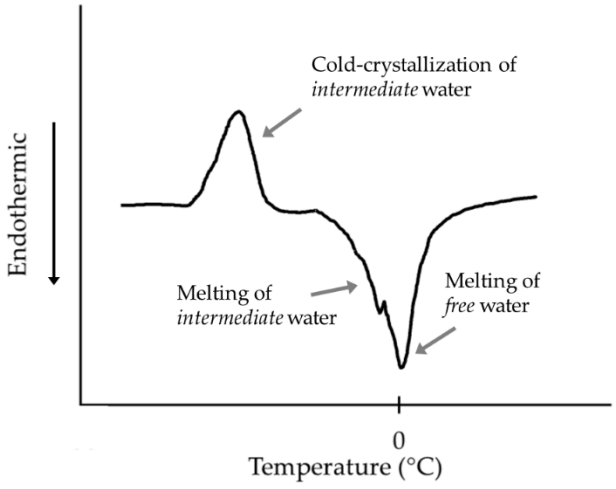


Figure 3. Scheme of a differential scanning calorimetry (DSC) thermogram of a hydrated surface with three states of water. *Non-freezable* water not visible in the thermograms. Modified from [26].

The thermogram gives useful information to calculate the amount of the types of water. The mass of *intermediate* (W_{int}) and *free* water (W_f) can be calculated according to the following equations:

$$W_{int} = {}^{Q_{cc}}/_{\Delta H_{cc}} \quad (3)$$

$$W_f = {}^{Q_m}/_{\Delta H_m} - W_{int} \quad (4)$$

where ΔH_{cc} and ΔH_m are enthalpy changes in the cold-crystallization and the melting of ice, respectively; and, Q_{cc} and Q_m are the heat absorbed during the cold-crystallization process and the melting process, which are obtained from the area of the respective peaks in the thermogram. The enthalpy changes (ΔH_{cc} and ΔH_m) are assumed to be the same as that of bulk water (334 J g^{-1}) [27].

The mass of *non-freezable* water (W_{nf}) is calculated as follows:

$$W_{nf} = EWC(wt\%) - W_{int} - W_f \quad (5)$$

where EWC is the equilibrium water content of the sample.

The number of *non-freezable* (N_{wnf}) and *freezable* (N_{wf}) water molecules per polymer repeating unit can be calculated using the DSC information, as previously described [27,28]. The weight ratio ($WR_{nonfreezable}$) of *non-freezable* water/polymer, and the weight ratio ($WR_{freezable}$) of *freezable* water/polymer c be calculated using the following equations:

$$WR_{nonfreezable} = \frac{w_{nonfreezable}}{w_{polymer}} = \frac{(EWC - w_{freezable})}{w_{polymer}} \quad (6)$$

$$WR_{freezable} = \frac{w_{freezable}}{w_{polymer}} \quad (7)$$

where $w_{nonfreezable}$, $w_{freezable}$,and $w_{polymer}$, are the weight percentages of *non-freezable* water, *freezable* water, and polymer, respectively. $w_{freezable}$ is obtained from the DSC thermograms and calculated as follows:

$$w_{freezable} = \frac{Q_m}{\Delta H_m} \cdot 100\% \quad (8)$$

Finally, N_{wnf} and N_{wf} are obtained using the equations:

$$N_{wnf} = \frac{M_p}{M_{water}} \cdot WR_{nonfreezable} \quad (9)$$

$$N_{wf} = \frac{M_p}{M_{water}} \cdot WR_{freezable} \quad (10)$$

where M_{p} is the molecular weight per polymer repeating unit, and M_{water} is the molecular weight of water.

2.2.2. Nuclear Magnetic Resonance

NMR is used to measure the dynamic behavior of water, in contrast to the static state of water tmeasured by DSC. Most of the early investigations on water states used NMR for their identification, as in the case of Hechter and collaborators [13] with agar gels. It can also be used

for other materials such as muscle tissue [29] and food, including vegetables [24], fruits [25], and dough [30].

NMR is based on the line broadening observed when molecular mobility is interfered with. For *free* water, the resonance lines are narrow, but when water molecules interact with a solid, the mobility of the molecules may be impeded or nonexistent on the NMR time scale, resulting in broad resonance lines [31] (Figure 4). In general, a decrease in the signal intensity corresponds to an increased amount of *bound* water on the surface [32].

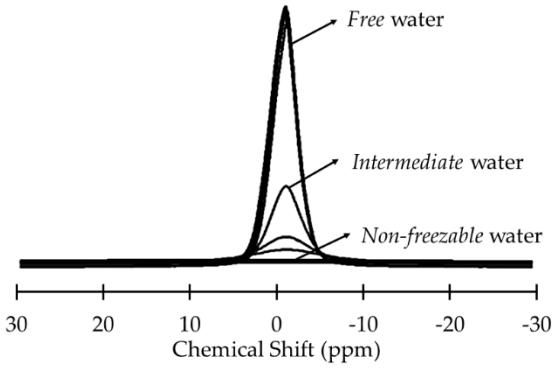


Figure 4. ^{2}H-NMR spectra of water on a surface at different temperatures. The narrower peaks correspond to temperatures above 0 °C, while broad lines correspond to temperatures below 0 °C. Modified from [32].

The NMR spectra of the different water states are as follows [32]:

- For *non-freezable* water the spectra is broad, showing low mobility due to strong interaction with the surface.
- For *free* water the spectra is narrow, very similar to *bulk* water, which means it has high mobility.
- For *intermediate* water the spectra is somewhere in between the spectra of the other two types of water, meaning that the mobility is intermediate.

The weight percentages of the different states of water can be calculated based on NMR data, though not many studies base their calculations on this technique. Sterling and Masuzawa [16] presented the methodology to determine them. From the NMR signal, height ($Peak_H$) of the peak and width ($Peak_{\mathrm{W}}$) at half the height of the peak are measured. Then, the area of the peak is measured:

$$A_{\mathrm{m}} = Peak_{\mathrm{W}} \cdot Peak_{\mathrm{H}} \tag{11}$$

The width (W_{w}) and the area (A_{w}) of the peak of pure water are constant and considered to be 0.22 and 11.34, respectively. The calculated area (A_c) of the peak of the sample is based on the content of water in the sample:

$$A_c = WC \cdot A_{\mathrm{w}} \tag{12}$$

Then, the percentages of the water types can be calculated as follows:

$$\%NF = 100 \cdot \left(1 - \frac{A_{\mathrm{m}}}{A_{\mathrm{c}}}\right) \quad (13)$$

$$\%Int = 100 - (\%NF + \%Free) \quad (14)$$

$$\%Free = 100 \cdot \left(\frac{H \cdot W_{\mathrm{w}}}{A_{\mathrm{m}}}\right) \quad (15)$$

where $\%NF$, $\%Int$ and $\%Free$ correspond to the percentages of *non-freezable*, *intermediate,* and *free* water, respectively.

2.2.3. Fourier Transform Infrared Spectroscopy

Fourier transform infrared (FT-IR) spectroscopy is used to analyze the interaction of water molecules with functional groups of the surface. However, the information obtained cannot be used to calculate the amount of the different states of water. In general, IR analyzes the interaction of infrared light with a molecule by detecting the energy absorbance or transmittance of the molecule due to its vibration, which is known as the transmission sampling method. It is widely used to identify different molecules or molecular structure because functional groups vibrate at different energy levels [33]. The O-H stretching band region at around 3800–3000 cm^{-1} is of much interest because the interaction with water occurs through hydrogen bonding. Shifts of the wavenumber are associated with a change of the hydrogen bonding strength: the stronger the hydrogen bond, the lower the wavenumber shift [34]. The attenuated total reflection (ATR) is another sampling method of FT-IR, which is used to analyze thin and soft samples [26], as opposed to the transmission method which is used to analyze thin films and solids. With ATR-IR the infrared beam passes through a crystal, reflecting with the surface in contact with the sample positioned on top of it. The reflection produces an evanescent wave that goes through the sample. When the sample absorbs energy from the evanescent wave, this is attenuated, and the attenuated beam is analyzed to obtain the IR spectra.

2.2.4. Other Techniques

Other techniques have been used to study the types of water on surfaces. Raman spectroscopy can be used to analyze the structure and hydrogen bonding of sorbed water and is similar to IR. Raman spectroscopy detects changes in the polarizability of the molecular bonds, whereas IR detects changes in the dipole moment. The analysis extracted from these two are complementary and therefore provide a better insight of the water structure [35]. Recent research has been developed to clarify the origin of cold-crystallization of water combining wide-angle X-ray diffraction with DSC (XRD-DSC) [36]. The XRD determines the atomic and molecular structure of a crystal by the diffraction of incident X-rays, which with the combination of DSC allows identification of the origin of the phase transition.

3. Studies on the States of Water on Polymers and Their Effect on Biocompatibility

In this section, we analyze and discuss the impact of water states on the hemocompatibility, namely protein adsorption and/or platelet adhesion, of different polymers used for medical applications. In the first subsection, we include degradable polymers such as L-tyrosine derived polyarylates, poly(ethylene glycol) (PEG), a group of aliphatic carbonyls, and poly(lactic-co-

glycolic acid) (PLGA). In the second subsection, some non-degradable polymers are analyzed, such as poly(meth)acrylates and poly(vinyl alcohol)s.

3.1. Degradable Polymers

3.1.1. L-Tyrosine Derived Polyarylates

L-Tyrosine-derived polyarylates are a family of 112 A-B-type copolymers with an alternating sequence of tyrosine-derived diphenol and an aliphatic diacid. They were first synthesized by Kohn and collaborators [37,38] (Figure 5). For this library, the number of oxygen or carbon atoms in the polymer backbone and pendent chain affects properties such as the glass transition temperature (*T*g) and the water contact angle. These polymers have been used to fabricate bone pins showing no significant inflammatory response in in vivo resorption studies [39]. Also, drug eluting implants near the eye area have been fabricated [40], as well as FDA approved devices for hernia repair and infection control in 2006 [41]. Recently, potential topical psoriasis therapy using drug loaded nanospheres have been developed which are based on amphiphilic block copolymers of PEG and L-tyrosine-derived polyarylate oligomers [42].

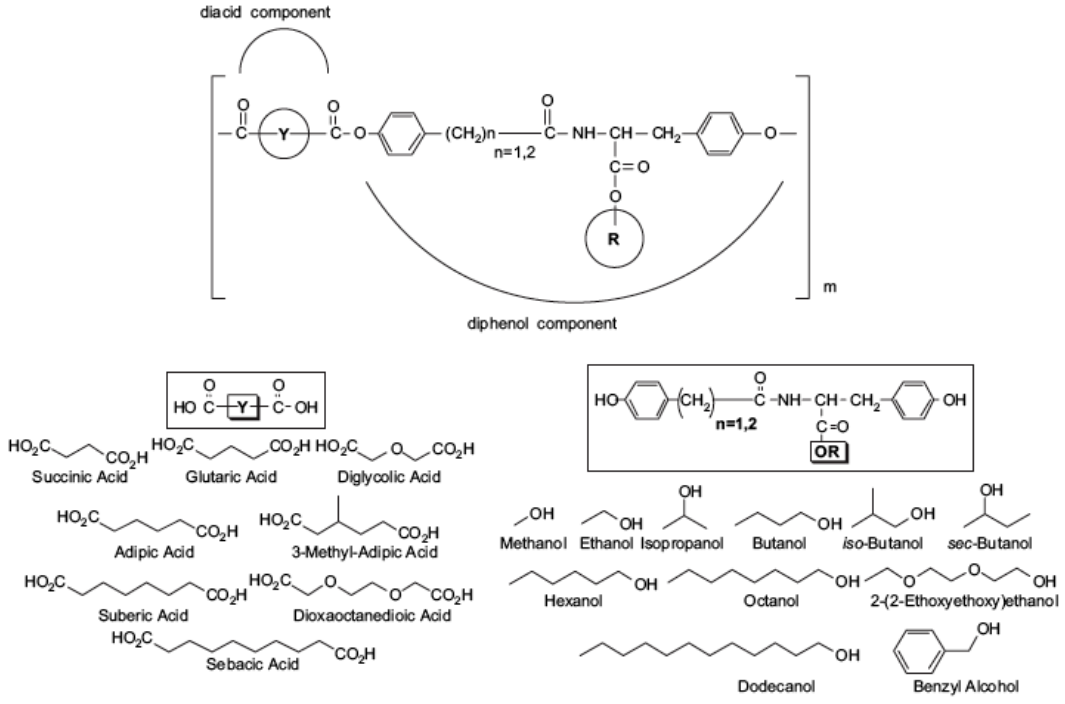


Figure 5. Structure of L-tyrosine derived polyarylates. Symbol Y represents diacids (lower left) and symbol R represents tyrosine-derived diphenols (lower right). The different compositions of R are: M = methyl, E = ethyl, B = butyl, H = hexyl, O = octyl, iP = isopropyl, sB = sec-butyl, Bn = benzyl. The number of methyl groups in the diphenol backbone is variable (n = 1 for HT, n = 2 for DT), where HT and DT stand for 4-hydroxyphenylacetic acid-tyrosine and desaminotyrosyl-tyrosine, respectively [43].

Previously, we investigated the influence of different properties of polymeric films on their WU behavior, due to the high variability of the latter reported in various studies [43]. From this study, the DSC curves for 21 of the used polymers were obtained, along with the WU data. From the former, the sample weight and the enthalpy change of the melting process of *freezable* water (Q_m) were extracted. Then, the WC and the number of *freezable* (N_{wf}) and *non-freezable* (N_{wnf}) water molecules per polymer repeating unit were calculated, as described in section 2.2.1. Depending on the N_{wf}, polymers were classified into two groups: (i) low WC, from 0 to 0.31 and N_{wf} less than 10, and (ii) high WC, from 0.45 to 0.6 and N_{wf} more than 10.

The analysis of the behavior of N_{wnf} and N_{wf} related to the WC of the polymer shows that the majority of them have similar behavior (Figure 6), where N_{wnf} rises until a threshold from which it tends to remain constant, while N_{wf} keeps rising as the polymer absorbs more water. However, when WC is very low (around 0.05) N_{wnf} is higher than N_{wf}. This probably occurs because at lower WC the total amount of water is close to the amount of *non-freezable* water allowed in the polymer, and *freezable* water is in smaller or similar amounts.

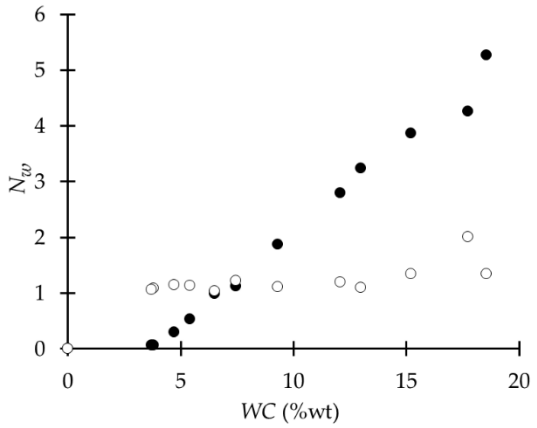


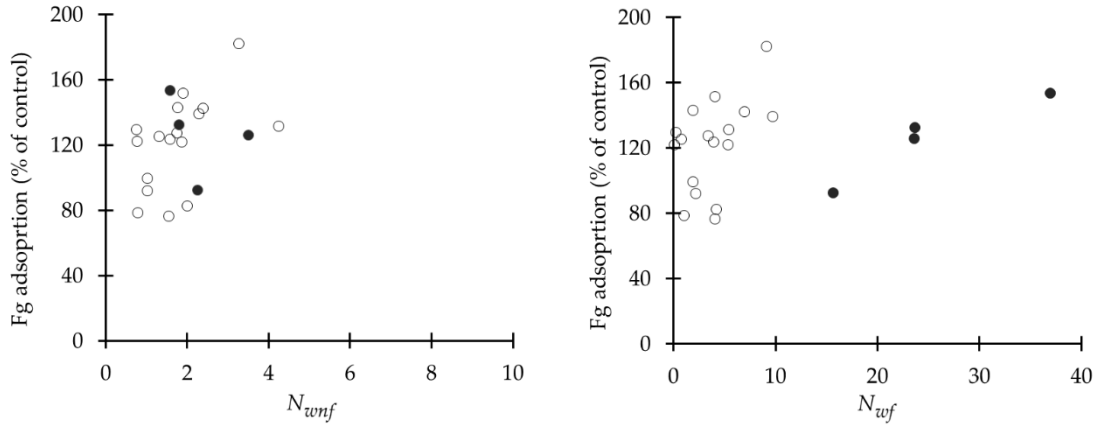
Figure 6. Example behavior of *non-freezable* water (N_{wnf}, o), and *freezable* water (N_{wf}, •) in relation to water content (WC) of poly(DTH adipate).

Fibrinogen adsorption data was obtained from Weber and collaborators [44] (Table 2). Figure 7 shows the relation between N_w and fibrinogen adsorption of *non-freezable* and *freezable* water. In the case of *non-freezable* water, there is no apparent relationship between the fibrinogen adsorbed to the surface, but for the low WC group there is a tendency to increase as the amount of water is increased. On the other hand, when only *freezable* water is analyzed, the low WC group has the same tendency as the previous case, although the correlation is lower in the latter. Although only four data points are available, the high *WC* group shows a direct relation between *WC* and fibrinogen adsorption. More experiments are required to make a conclusive statement about this relationship.

Table 2. Data of each polyarylate measured and calculated from [45,46]. Fibrinogen (*Fg*) adsorption data extracted from [44].

Polymer	N_{wf}	N_{wnf}	WC **Max (wt %)**	*Fg* **Adsorption (% of Control)**	**Group**
poly(DTO succinate)	0.14	0.77	4	121.97	Low

poly(DTB succinate)	0.28	0.76	4	129.36	
poly(HTE adipate)	0.82	1.31	8	125.19	
poly(DTO adipate)	1.08	0.80	6	78.30	
poly(DTM adipate)	1.92	1.77	13	142.69	
poly(DTM sebacate)	1.98	1.03	12	99.14	
poly(DTH suberate)	2.25	1.03	10	91.68	
poly(DTB adipate)	3.45	1.76	19	127.12	
poly(DTB glutarate)	3.95	1.59	19	123.38	
poly(HTH adipate)	4.13	1.55	16	76.20	
poly(DTE glutarate)	4.13	1.91	22	151.44	
poly(DTH adipate)	4.26	2.01	18	82.27	
poly(DTiP adipate)	5.39	1.87	22	121.76	
poly(DTE adipate)	5.45	4.26	27	131.21	
poly(DTBn adipate)	6.98	2.40	30	142.16	
poly(HTE succinate)	9.14	3.27	30	182.15	
poly(DTBn methyl adipate	9.77	2.30	31	138.98	
poly(DTBn suberate)	15.71	2.26	47	92.10	
poly(DTM (R)(+) methyl adipate)	23.67	3.51	56	125.70	High
poly(DTsB glutarate)	23.70	1.80	49	132.32	
poly(DTsB (R)(+) methyladipate)	36.98	1.59	58	153.27	



(**a**) (**b**)

Figure 7. *F*g adsorption vs. N_w in polyarylates (**a**): *non-freezable* water; (**b**): *freezable* water. (•) high WC, (o) low WC. X-axes are not in same scale.

Polyarylates with longer ester and diacid groups adsorb less fibrinogen than those with shorter ester and diacid groups [47]. We hypothesize that this phenomenon occurs because longer chains allow the polymer to fold upon itself forming more hydrogen bonding between functional groups of the chain and water, as occurs with PEG chains [48]. This interaction could cause the formation of *intermediate* water that decreases the adsorption of proteins.

From the data analyzed it can be concluded that at higher WC values, higher *freezable* water content is responsible for the higher fibrinogen adsorption and low hemocompatibility of polyarylates. There is not enough available data to conclude anything regarding *intermediate* water and its relation to hemocompatibility because we were only able to calculate the amount of

freezable water, namely *intermediate* and *free* water together. We hypothesized that longer chains promote the formation of *intermediate* water, which decreases fibrinogen adsorption. In order to confirm this, further studies on water structure in polyarylates through NMR and IR are necessary.

3.1.2. Poly(ethylene glycol)

Poly(ethylene glycol) (PEG) is formed by the polymerization of ethylene oxide and corresponds to a neutral polyether (Figure 8). This hydrophilic polymer is especially relevant in the biomedical field because of its nontoxicity in the human body. In aqueous solution, PEG presents high mobility with a large exclusion volume; it also has a great capacity for water uptake, which depends on its molecular weight (M_w). This polymer has been extensively used, as a blend or as a copolymer, to improve the biocompatibility and water solubility of other polymers with little chemical modification [49]. In tissue engineering, PEG has been used to form hydrogels, mostly in diblock, triblock, and multiblock copolymers with poly(lactic-co-glycolic acid) (PLGA) to improve the degradability of the PEG hydrogels [50]. In drug delivery systems, PEG is used as a stabilizer by coupling it to proteins, polypeptides, DNA, RNA, among others [51]. PEG has also been utilized as a coating for biosensors, especially those applied for blood glucose sampling and monitoring for their anti-fouling properties [52,53]. Some relevant PEGylated products include those for severe combined immunodeficiency and (Adagen®, Gaithersburg, MD, USA) acute lymphoblastic leukemia (Oncospar®, St. Helier, Jersey) [54]. Recent PEGylated products include therapy for age-related macular degeneration (Macugen®, New York City, NY, USA) and for Crohn's disease and rheumatoid arthritis (Cimzia®, Smyrna, GA, USA) [51].

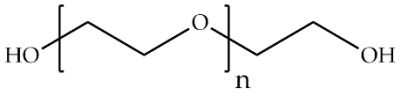


Figure 8. Molecular structure of poly(ethylene glycol) (PEG).

The thermal behavior of aqueous PEG solutions with different M_w was obtained with DSC measurements by Antonsen and Hoffman [48]. They explored and analyzed the effect of the M_w on the amount of *bound* water and the low-temperature behavior of these solutions. They hypothesized that low M_w PEG chains are only associated with *non-freezable* water and as the M_w increases, the polymer chains fold upon themselves allowing *intermediate* water molecules to form. These results agree with the observations made by Yamauchi and Tamai [55] where at M_w lower than 500, *intermediate* water was not detected, and then slowly increased with the M_w until a constant amount of about 4% of total water was reached at around M_w 2200 (Figure 9).

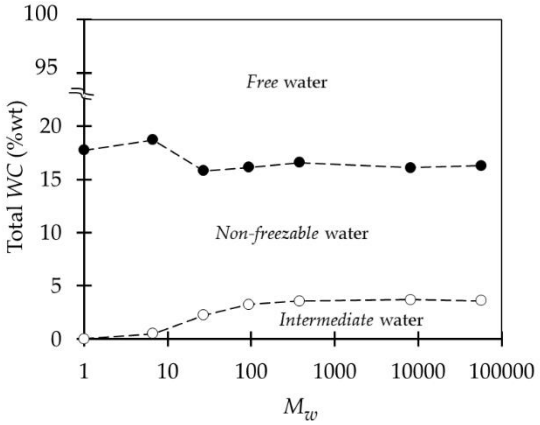


Figure 9. Water states distribution in PEG at different M_w. (•) *Non-freezable* water; (o) *intermediate* water. Modified from [55].

Kitano and collaborators [56] conducted a FT-IR study to analyze the O-H stretching band peaks for sorbed water to PEG films of various M_w and elucidate the effect of OH end groups of the polymer on the interaction with water molecules. In the IR spectra obtained (Figure 3 in [56]), different peaks of the O-H stretching band were observed, from which they proposed five types of sorbed water molecules: *binding* water, monomeric water binding to the ether oxygen atom of the polymer (peak 1); dimeric water, a water molecule which is hydrogen-bonded to another water molecule binding to PEG (peak 2) or a water molecule hydrogen-bonded to both a water molecule and the ether oxygen of PEG (peak 3); *bridging* water, association with two ether oxygen atoms (peak 4); and, hydrogen-bonded water to the end OH group (peak X). The latter was only observed in PEG films of low viscosity average molecular weight ($M_v \leq 28K$) due to the increase of OH ends of the polymer. Observing the proposed hydration structure of each water types (Figures 10 and 11) and comparing to the three types of water, the following analogy can be drawn: peak 4 or *bridging* water corresponds to *non-freezable* water, while peaks 2 and 3 (dimeric water) correspond to *intermediate* water. Peak 1, although very similar to *bulk* water in the IR spectra, also corresponds to *intermediate* water given the higher mobility than bridging water. In the case of peak X, its IR spectra is very similar to peak 4, meaning water molecules are *tightly bound* to the polymer chain.

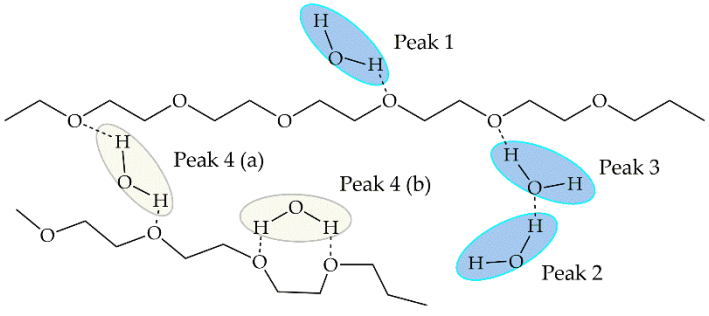


Figure 10. Proposed structure of water on hydrated PEG as shown in [56]. *Intermediate* water showed in blue; *non-freezable* water showed in yellow.

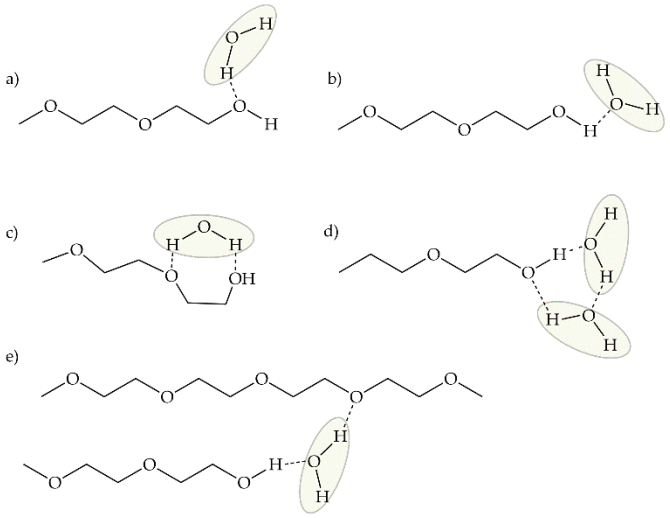


Figure 11. Proposed structure of water at the end of an hydrated PEG chain (peak X) as shown in [56].

PEG presents good hemocompatibility. Both protein adsorption and platelet adhesion are a function of the M_w below 2000 [48]. At low Mw protein adsorption and platelet adhesion are high [57] because the amount of *intermediate* water is negligible. As described above, the increase of M_w causes the formation of *intermediate* water in the polymer matrix until it reaches a maximum and stays constant at higher M_w. At high M_w, the folding of the chain and the subsequent formation of *intermediate* water molecules prevents adhesion to the polymer and to *non-freezable* water of high molecular-weight molecules like plasma proteins [48], avoiding the activation of these and hence, providing good hemocompatibility to the polymer. As shown in figure 12, the longer the chain length of PEG immobilized to poly(methyl methacrylate) (PMMA), the lower the amount of adsorbed proteins and adhered platelets [58].

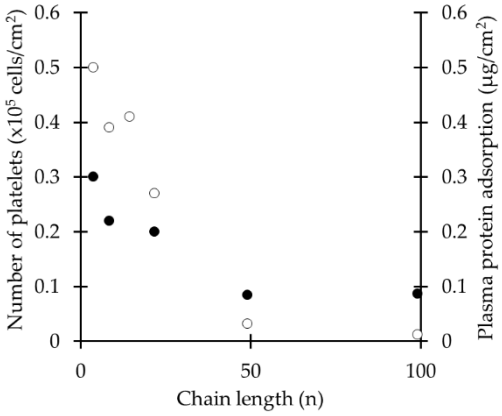


Figure 12. Effect of PEG chain length, immobilized to poly(methyl methacrylate) (PMMA) on platelet adhesion (•) and plasma protein adsorption (o). Modified from [58].

3.1.3. Aliphatic Carbonyls

Aliphatic carbonyl polymers have ester or carbonate linkages facilitating the breakdown of their monomers and thus their degradation. This type of polymer has been broadly studied and applied in the biomedical field for the development of drug delivery systems and tissue engineering. Poly(dioxanone) (PDO) is mostly used for the manufacture of absorbable sutures [59] and has been recently studied in the development of degradable intravascular stents [60] and nanofibrous scaffolds [61]. Poly(ε-caprolactone) (PCL) and poly(trimethylene carbonate) (PTMC) are used in tissue engineering scaffolds and sutures [59]. These polymers have high biocompatibility, however there is little research available on the relation of the states of water on these polymers and their compatibility.

Tanaka and collaborators [62] studied PDO, PCL and PTMC (see Nomenclature Section) in addition to poly(δ-valerolactone) (PVL) (Figure 13) in order to elucidate the differences in their backbone structure on hydration and hemocompatibility. They found that a higher amount of *intermediate* water in PDO is related to its good hemocompatibility and the presence of ether bonds in the main chain of PDO are involved in the hydration and formation of *intermediate* water.

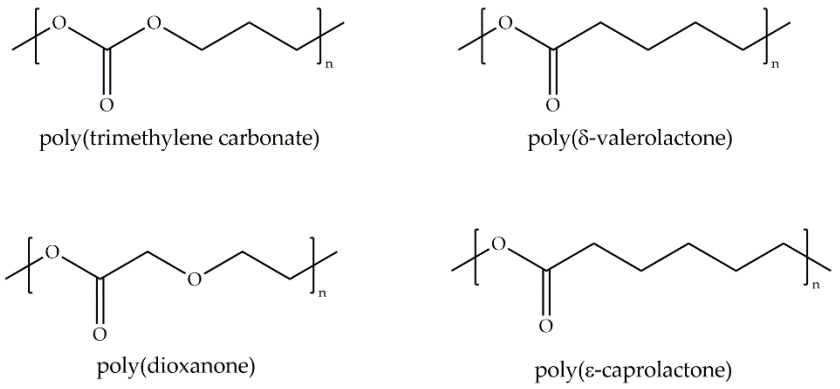


Figure 13. Molecular structure of aliphatic carbonyl polymers.

The available data of equilibrium water content (EWC) and amount of types of water of PDO, PCL, PTMC and PVL were related to the amount of adhered platelets. In general terms, no relation between the total WU, *non-freezable* water and *free* water with platelet adhesion was found. However, there is a tendency of lower platelet adhesion when intermediate water is higher in quantity (Figure 14).

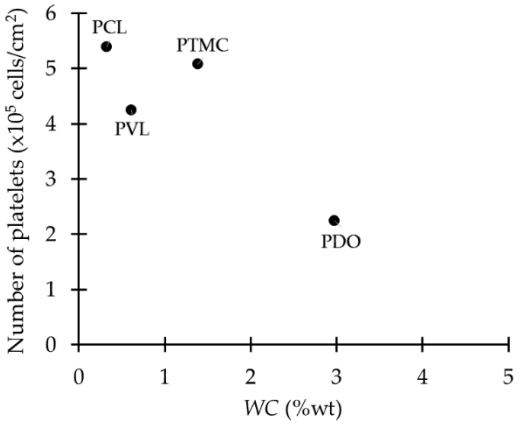


Figure 14. Relation of *intermediate* water content and platelet adhesion in aliphatic carbonyl polymers. Data extracted from [62].

The number of adhered platelets decreases considerably when *intermediate* water content is about 3 wt%, which occurs for PDO. The difference between PDO and the rest of the polymers is the ether bond in its backbone and it is believed that this bond contributes to the formation of hydrogen bonds and the increased amount of *intermediate* water, improving its hemocompatibility (Figure 15). At first glance at Figure 14, one could conclude that there is an inverse relationship between the number of platelets and the amount of *intermediate* water. However, the actual amount of *intermediate* water on PTMC might be overestimated because the author hypothesized that this water is not in the hydration layer formed by the polymeric chains and water, but spreads over this layer [62]. Therefore, the data of PTMC in Figure 14 should be closer to the Y-axis next to PCL and PVL, and the inverse relation is not conclusive anymore.

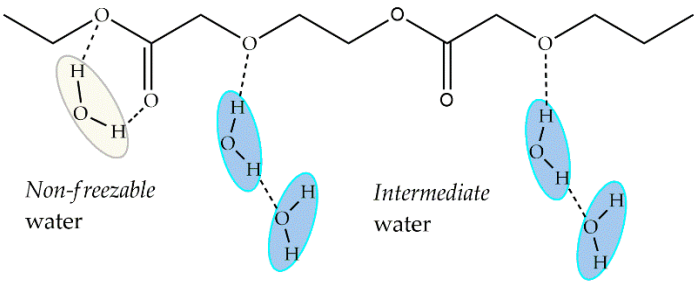


Figure 15. Proposed structure of *non-freezable* (yellow) and *intermediate* (blue) water sorbed on poly(dioxanone) (PDO). Based on [62].

3.1.4. Poly(lactic-co-glycolic) Acid

PLGA is the result of the copolymerization of lactic acid (LA) and glycolic acid (GA) and corresponds to a saturated poly(α-hydroxy ester) (Figure 16). This polymer presents good biocompatibility and biodegradability, which can be tailored by controlling the *M*w of the polymer and the ratio between lactic and glycolic acid [63]. Commonly used LA:GA ratios are 25:75, 50:50, 75:25, and 85:15, all of them with different *T*g values, degree of crystallinity, and degradation rates. Due to the wide range of properties this copolymer can have, it is one of the

most popular in the pharmaceutical and medical industry. Many PLGA-based products have been approved by the FDA, such as sinus implants for the treatment of chronic rhinosinusitis [64], and injectable suspensions containing PLGA microspheres for the treatment of patients with acromegaly [65].

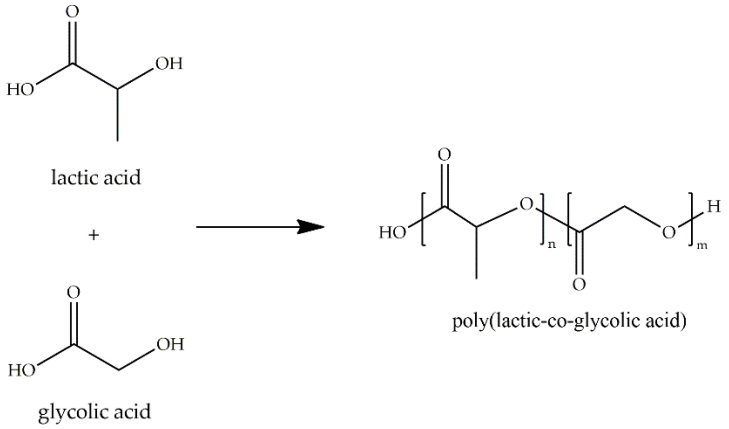


Figure 16. Molecular structure of poly(lactic-co-glycolic acid) (PLGA) and its monomers.

Blasi and collaborators [66] studied the effect of water on the *Tg* of PLGA 50:50 in the early stage of hydration and the physical state of water within the hydrated polymer. They characterized the thermal behavior of the hydrated polymer using modulated DSC (MTDSC), a technique that allows the application of an oscillatory heating (or cooling) profile with improved resolution and enhanced sensitivity in comparison to conventional DSC [67]. From the cooling ramp of the MTDSC only one exothermic peak appears at around −20 °C, indicating the crystallization of water. The absence of a peak at 0 °C indicates that the peak at −20 °C does not correspond to cold-crystallization of *intermediate* water, but to supercooling of *free* water, thus no *intermediate* water is present in PLGA 50:50.

In terms of water structure, Blasi and collaborators [66] suggested that water molecules could directly interact with the polymer chain through hydrogen bonds with its hydrophilic groups (C=O and COO–). However, *intermediate* water was not identified, probably because of a lack of binding sites. *Intermediate* water, as studied in other polymers, can form either by binding weakly with a hydrophilic group or with another water molecule directly bound to the polymer. In this case, we suggest that *non-freezable* water simultaneously binds with two hydrophilic groups, as shown in Figure 17. Therefore, the remaining water molecules can only bind with one other, forming *free* water.

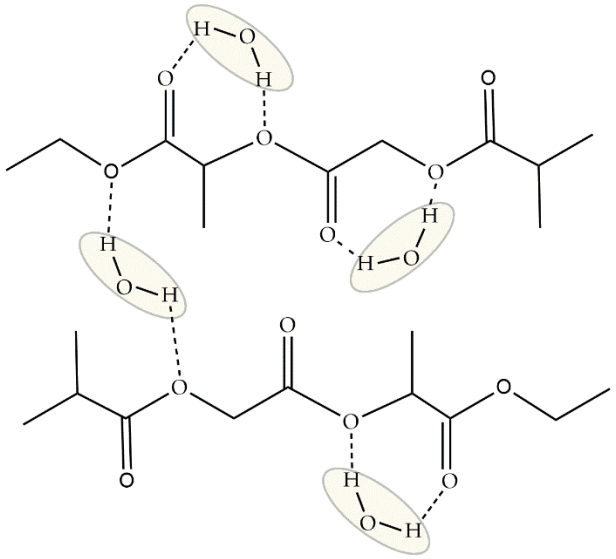


Figure 17. Proposed structure of *non-freezable* water sorbed on PLGA.

The hemocompatibility of PLGA 50:50 is very low with an increased fibrinogen adsorption with respect to noncoated polypropylene (about 150%) [44]. Moreover, Liu and collaborators compared PLGA 85:15 and PLGA 85:15 immobilized with silk fibroin and demonstrated that the platelet adhesion, activation and the thrombogenic ability of the polymer by itself was higher than that of the treated one [68]. For example, untreated PLGA adhered over 550 × 10^5 platelets/cm^2 after 2 h incubation, while treated PLGA adhered only about 50 × 10^5 platelets/cm^2. Other biocompatibility aspects studied by them include vascular endothelial cell attachment and morphology, cell viability, transcription level of genes, and expression of proteins. When silk fibroin is immobilized to the polymer [68] an additional hydrophilic group is available for water molecules to bind. Then, i*ntermediate* water would form due to the presence of the carbonyl group of the fibroin (Figure 18), which would explain the improved hemocompatiblity and other biocompatibility aspects of treated PLGA. More studies on the water states in modified PLGA polymers are necessary to clarify if this improvement is due to the presence of *intermediate* water.

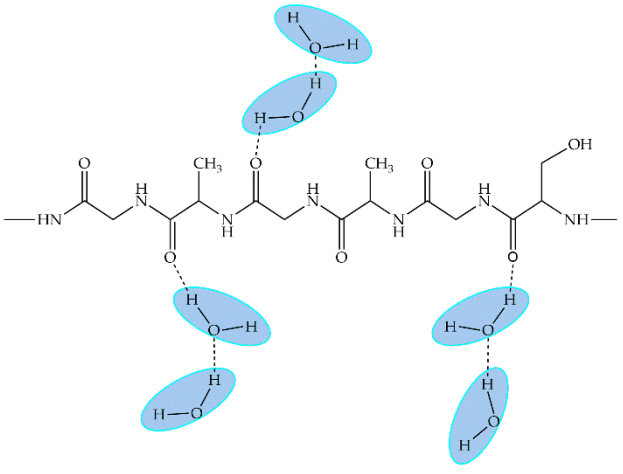


Figure 18. Proposed structure of *intermediate* water sorbed on silk fibroin.

3.1.5. Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is one of the few vinyl polymers that are water soluble and biodegradable (Figure 19). This polymer is used in many industries such as plastic, textile, paper, food, biomedical, and pharmaceutical. In the biomedical field, studies on the use of PVA for contact lenses, skin and artificial meniscus have been developed [69], while in the pharmaceutical industry, it is mainly used for drug delivery systems and many PVA-based products are currently available. Most of these products are in the form of tablets, but there are also transdermal/topical forms, and ophthalmic and implantable devices [69].

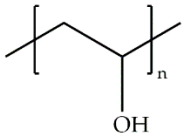


Figure 19. Molecular structure of poly(vinyl alcohol) (PVA).

Hodge and collaborators [70] studied the states of water by DSC in PVA films. They observed that all the water sorbed into the polymer inhabits the amorphous region but also destroys crystalline regions. Therefore, as the polymer sorbs more water the crystalline region decreases. They also calculated the amount of each water state at different water content. When the total water content is below 22%, all of this corresponds to *non-freezable* water. Above this value, *intermediate* water appears and reaches a maximum of 2.5% while *free* water also appears increasing linearly until its saturation level of about 60% of total water (Figure 20).

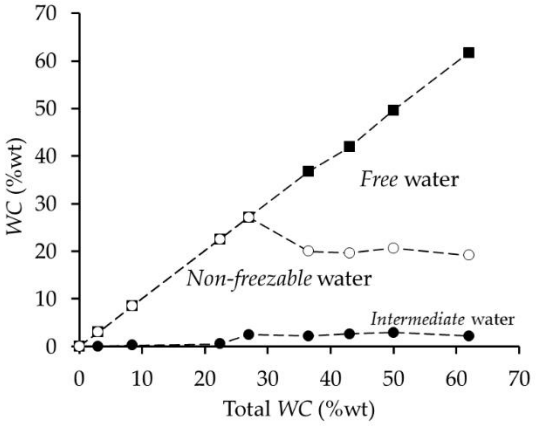


Figure 20. Water states distribution in PVA at different WC. (•) *Intermediate* water; (o) *non-freezable* water; (▪) *free* water. Modified from [70].

The hemocompatibility of PVA is considered good and the adhered platelets inactive on the polymer surface [71]. This polymer PVA is an attractive polymer for tissue engineering applications due to its mechanical properties.; however, many studies have modified PVA by the addition of other polymers such as gelatin or dextran [69,71], in order to improve its hemocompatibility. When comparing PVA platelet adhesion (about 40×10^5 platelets/cm^2) [72] with other polymers presented in this review (e.g., PCL: about 5×10^5 platelets/cm^2), we can

consider that platelet adhesion on PVA is high (i.e., low hemocompatibility). Taking into account the low *intermediate* water content in PVA films reported by Hodge and collaborators [70], where this value reached a maximum of 2.5%, we can conclude that low *intermediate* water content is related to high platelet adhesion, although it is not activated by contact with the polymeric surface.

3.2. Non-Degradable Polymers

3.2.1. Poly(meth)acrylates

Polymethacrylates correspond to copolymers of methacrylic acid and acrylates. Copolymerization in different ratios provides a wide range of products with different tensile strengths and elongations. In general, methacrylates have a higher tensile strength and lower elongation than their corresponding acrylate [73]. In the medical field, polymethacrylates are used for the manufacture of contact lenses, artificial joints, dental implants, among others. The most studied polymer in these groups is PMMA and its applications include bone cement, intraocular lenses, and artificial kidney [74]. Eudragit® (Essen, Germany) from Evonik is one of the commercial products of polymethacrylates and is used for functional solid oral dosage with specific drug delivery [75,76].

Tanaka and collaborators have carried out extensive research on the biocompatibility of poly(meth)acrylates, especially poly(2-methoxyethyl acrylate) (PMEA). They studied the structure of water in hydrated PMEA and compared it to water of poly(2-hydroxyethyl methacrylate) (PHEMA) and polyacrylates analogs [77]. They analyzed the phase transitions of water by DSC and performed a platelet adhesion test on the polymers. Their results show that PMEA shows a high hemocompatibility in comparison to the other polymers and this phenomenon appears to be dictated by the presence of *intermediate* water on the surface of the polymer and not by the total amount of water.

From this study, the available data of the different polymers were analyzed, namely: PMEA, PHEMA, poly(ethyl acrylate) (PEA), poly(2-phenoxyethyl acrylate) (PPEA), poly(2-ethylhexyl acrylate) (PEHA), and poly(n-butyl acrylate) (PBA) (Figure 21). EWC and amount of types of water were analyzed separately with the number of adhered platelets. In general terms, there is no relation between the total EWC, *non-freezable* water, and *free* water with platelet adhesion. However, as concluded by Tanaka and collaborators, the presence of *intermediate* water decreases the number of adhered platelets to the surface (Figure 22), hence the hemocompatibility is related to the presence of *intermediate* water.

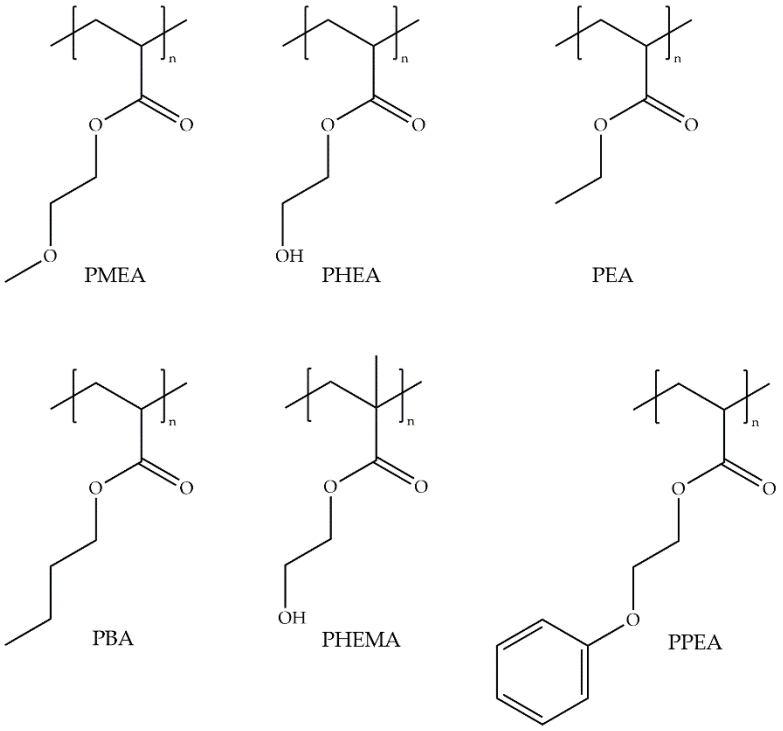


Figure 21. Molecular structure of poly(meth)acrylates.

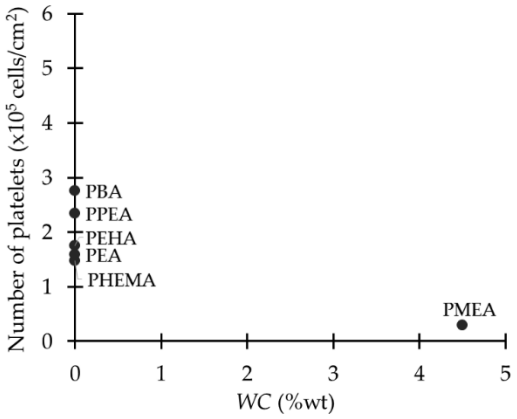


Figure 22. Relation of *intermediate* water content and platelet adhesion in poly(meth)acrylates. Data extracted from [77].

The analysis of ^{2}H-NMR and ^{13}C-NMR of hydrated PMEA [32] shows that *non-freezable* water has a low mobility because it interacts strongly with the polymer chain, which stops this water freezing even below −100 °C. *Free* water has high mobility and it barely interacts with the polymer, resembling *bulk* water. From ATR-IR studies it was elucidated that *non-freezable* water interacts with the C=O groups in the PMEA side chain, while *free* water only interacts through hydrogen bonds with other water molecules and has no interaction with the polymer [34]. In the case of *intermediate* water, it interacts with the methoxy moiety (O-CH_3) in the side chain terminal, both

by hydrogen-bonding interaction. Although, this interaction is weaker than the one with *non-freezable* water, the ^{2}H-NMR and ^{13}C-NMR analysis show that *intermediate* water has intermediate mobility in comparison to the other types of water. It is hypothesized that because of these characteristics, the layer of *intermediate* water formed on the surface is more stable than *free* water and when it is sufficiently thick, it does not allow direct contact of cells or protein with the polymer surface or *non-freezable* water [26].

In general, *non-freezable* and *free* water are present in all surfaces of polymethacrylates. When fibrinogen approaches the surface through contact with *non-freezable* water it activates, it causes the adhesion of platelets to the polymer [26]. Due to the high mobility and its resemblance to bulk water, *free* water is not able to prevent this from happening. However, when *intermediate* water is present, it avoids the activation of plasma proteins, due to the moderate mobility of its molecules; and therefore, it plays a key role in their hemocompatibility.

3.2.2. Poly(acrylonitrile)-co-*N*-2-vinyl-pyrrolidone

Polyacrylonitrile (PAN) is obtained from the polymerization of a vinyl group linked to a nitrile (acrylonitrile), and is mostly used as a copolymer in the production of plastics, as a precursor of carbon fiber and as separation membrane material in hemodialysis [78,79]. Some studies show the improvement of the biocompatibility of PAN by immobilizing other polymers such as PEG and N-vinyl-2-pyrrolidone (NVP) [80]. NVP is composed of a five-membered lactam (γ-Lactam) bound to a vinyl group; it has been used as a coating on cardiovascular devices showing improved biocompatibility [81]. Copolymers of acrylonitrile and NVP have been synthesized and applied in liver support systems [82] and as nanocarriers in drug delivery systems [83].

Wan and collaborators [79,84] compared the swelling behavior of PANcNVP films (Figure 23) with different NVP content: 7%, 15%, 22%, and 31%, with their hemocompatibility by platelet adhesion and plasma recalcification time (PRT) test. They observed that higher contents of NVP meant increased water uptake in the polymer, with increased amount of *non-freezable* and *freezable* water both determined by DSC and TGA (Table 3). They also conducted an FT-IR in transmission mode and in ATR to examine the diffusion and structure of water in the copolymers. From their calculations they identified three types of water in the surface of fully hydrated polymers (high NVP content): type III water (*free* water), with relatively weak hydrogen bonding with the nitrile group of PAN; type II water (*intermediate* water), monomeric or dimeric water molecules interacting with a carbonyl group in NVP; and, type I water (*non-freezable* water), bound to a carbonyl group and two or more water molecules. When analyzing the different hydrated copolymers, higher contents of NVP relate to higher amounts of *non-freezable* water, shown by the peak at wavenumbers below 3300 cm^{-1} in the FT-IR spectra. The presence of the carbonyl group of NVP allows the binding of more stable water molecules, namely, *non-freezable* water.

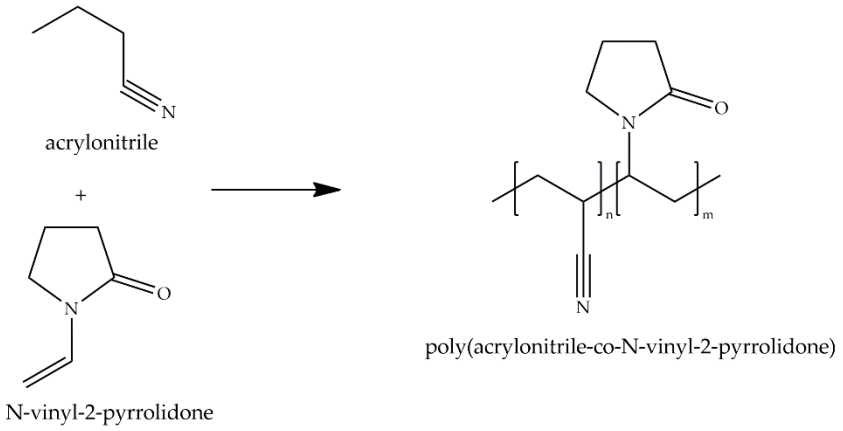


Figure 23. Molecular structure of poly(acrylonitrile)-co-N-2-vinyl-pyrrolidone (PANcNVP) and its monomers.

Table 3. Water content of total, *non-freezable* and *freezable* water in PANcNVP. Extracted from [79].

Content of NVP (wt%)	Total Water (wt%)	*Non-Freezable* Water (wt%)	*Freezable* Water (wt%)
0	29.7	4.6	25.1
7	30.6	5.2	25.4
15	43.3	9.6	33.7
22	55.5	16.0	39.5
31	58.3	19.3	39.0

Hemocompatibility tests showed that with higher amounts of NVP the number of platelets adhered to the surface was less than with samples with lower amounts of NVP, and these platelets conserved much of their original shape, indicating an inactivated state. The high content of NVP also increased the PRT, which indicates that coagulation around the polymer occurs more slowly than in copolymers with low NVP [79] (Figure 24). Relating this information with the water states data, the study suggests that the improved hemocompatibility is due to the higher amount of *non-freezable* water present in the polymer-water system. However, in the calculation of *non-freezable* and *freezable* water the authors did not differentiate the latter between *intermediate* and *free* water. They did however identify the three different states of water in the polymer-water system through IR analysis. Both *non-freezable* and *intermediate* water can be responsible for the improved hemocompatibility of the polymer and determining the amount of *intermediate* water with DSC or NMR will provide more information on its role on hemocompatibility.

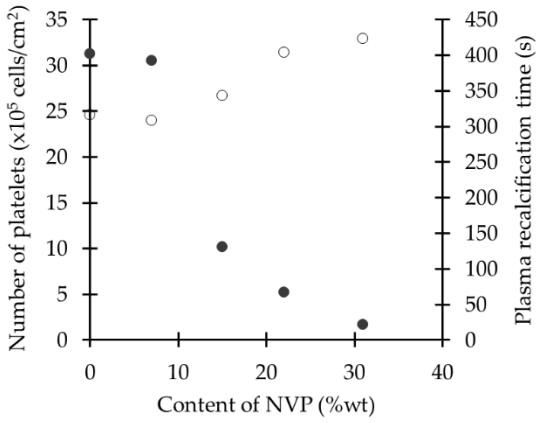


Figure 24. Relationship of *N*-vinyl-2-pyrrolidone (NVP) content with platelet adhesion (•) and plasma recalcification (PRT) time (o) on PANcNVP. Modified from [79].

4. Discussion

4.1. Water States in Polymers

Among the studies presented above, some of them analyzed the states of water as *non-freezable* and *freezable* water, understanding the latter as the sum of *intermediate* and *free* water. In the case of some polymethacrylates and PLGA, *intermediate* water was not identified. However, *non-freezable* water was always present in the polymer-water matrix analyzed. Also, based on the analysis in polyarylates, PEG and PVA, as the polymer hydrates, *non-freezable* water increases until it reaches a limit from which it remains constant at higher hydration levels. This occurs because as the polymer becomes more hydrated, there are not enough hydrogen bonding sites to form *non-freezable* water.

The structure of the polymer determines the formation of the different types of water. Functional groups in the polymeric chain interact with different strength levels with water through hydrogen bonds. As seen in aliphatic carbonyls, the carbonyl group bonds to water forming
non-freezable water but no *intermediate* water. The same happens in PANcNVP and the carbonyl group of the NVP. The ether group of PDO and PMEA binds to water and due to its moderate strength, *intermediate* water is formed. However, *intermediate* water can also form when a water molecule binds to another which is directly bound to the polymer. In this case, the indirectly bound water molecule has higher mobility than the directly bound one.

The length of the polymeric chains of the *M*w also affects the formation of the different states of water. When M_w is higher, larger polymeric chains tend to fold upon themselves and functional groups of the chain are closer to each other. This induces the formation of *intermediate* water bridging these groups and supplying more stability to the folded polymer. Additionally, if the end group of a polymer has the capacity to form hydrogen bonds, then in low *M*w chains, where more end groups are available, more water molecules can bind to them and more *non-freezable* water is formed.

4.2. Biological Response in Polymers

Most of the biological response data presented here was about platelet adhesion. A polymer with a high amount of adhered platelets is considered to have low hemocompatibility as in the case of PLGA, which has about 550 × 10^5 platelets/cm^2 [68]. However, when comparing polymers with lower platelet adhesion, there is a lack of consensus on what is low, moderate or high hemocompatibility. Figure 25 shows the number of adhered platelets on different polymers. In the study of Tanaka and collaborators [77] they state that PMEA has excellent hemocompatibility while the other poly(meth)acrylates (PHEMA, PEA, PEHA, PPEA, and PBA) have low hemocompatibility. However, when compared to other polymers, like PAN or PVA, which are considered to have moderate hemocompatibility, poly(meth)acrylates have high hemocompatibility.

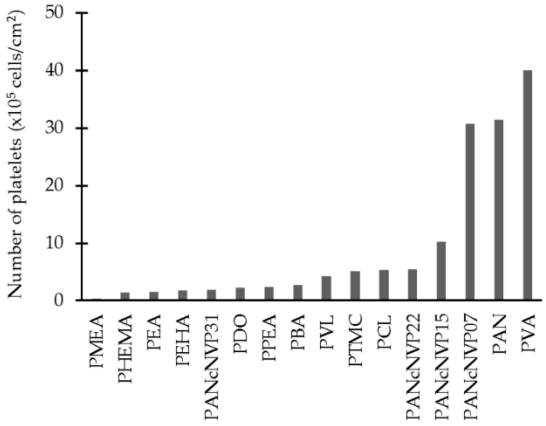


Figure 25. Platelet adhesion on different polymers.

4.3. Effect of Water States on Biological Response

In most polymers studied, when *intermediate* water is present, low platelet adhesion is observed, which means high hemocompatibility. This relation is clear in PEG, poly(meth)acrylates, aliphatic carbonyls, and PLGA, although there is no mathematical relation between the content of *intermediate* water and the amount of adhered platelets. *Intermediate* water in the polymer is more stable and has higher mobility than *free* water, thus when it is sufficiently thick it prevents the activation of the protein by avoiding direct contact with *non-freezable* water. The main conclusions of each polymer are summarized in Table 4.

Tanaka and collaborators [77] did not detect any *intermediate* water in poly(meth)acrylates except for PMEA. However, these polymers have high hemocompatibility in relation to other polymers such as PAN and therefore it would be expected that poly(meth)acrylates (PBA, PPEA, PEHA, PHEMA, and PEA) have some *intermediate* water of about 3 wt %. The apparent absence of *intermediate* water in these polymers could be confirmed by the absence of an exothermic peak of cold-crystallization in the heating DSC thermogram. However, the cooling thermogram shows the presence of *intermediate* water with a second peak below 0 °C, which the authors might not have considered. Additionally, in the heating thermograms a single peak with a shoulder on the lower temperature region is often considered as the effect of the melting of *intermediate* water, which is present in some heating thermograms of poly(meth)acrylates, therefore it cannot be definitely concluded that there is no presence of *intermediate* water.

Considering PEG, aliphatic carbonyls and poly(meth)acrylates, a limit of about 3 wt % for *intermediate* water can be established, from which platelets adhere less to the surface and the polymer has higher hemocompatibility, i.e., at contents of *intermediate* water above 3 wt %, platelet adhesion is low, and below 3 wt %, platelet adhesion is high. In order to find the mathematic relationship between these two properties, *intermediate* water content should be calculated not only for the afore mentioned poly(meth)acrylates (where no *intermediate* water was detected), but also for polyarylates and PANcNVP.

For polymers such as PANcNVP and polyarylates, the available data is not sufficient to make similar conclusions, where the presence of *intermediate* water means low platelet adhesion. However, in polyarylates, at high water content, high *freezable* water relates directly to platelet adhesion. This *freezable* water corresponds to *intermediate* and *free* water, therefore considering the previous observation for poly(methyl methacrylates), it can be suggested that in polyarylates at high water content, higher *freezable* water contains less *intermediate* water, which would explain high platelet adhesion at high *freezable* water content. For PANcNVP, Wan and collaborators concluded that high content of *non-freezable* water means low platelet adhesion on the polymers. This conclusion was made after calculating the content of *non-freezable* and *freezable* water in the polymer and relating it to the content of NVP, and then to platelet adhesion. As in polyarylates, they calculated *intermediate* water and *free* water as *freezable* water; they also detected the presence of all three water types in the hydrated polymer. However, they did not calculate the content of these two states separately, in which case it could happen that at higher NVP content *intermediate* water is high, relating it to low platelet adhesion.

In the case of PVA, the presence of *intermediate* water implies moderate platelet adhesion. This polymer has a maximum content of *intermediate* water of about 2.5 wt %, which is lower than the limit of 3 wt %, therefore hemocompatibility can be considered as low. In fact, data on platelet adhesion shows that PVA adheres about 40×10^5 platelets/cm^2, a higher value than the one reported for poly(meth)acrylates, PEG and aliphatic carbonyls, but lower than PLGA. Then, in this case, the relation is that low *intermediate* water content relates to high platelet adhesion. It is hypothesized that this relation is due to the low stability of *intermediate* water in this polymer, resembling *free* water, which is not enough to prevent the contact of proteins with *non-freezable* water. Another alternative is that *intermediate* water is clustered in the polymer matrix, leaving free regions of this type of water and allowing direct contact of the proteins.

Finally, the improvement on biocompatibility of PLGA 85:15 by silk fibroin may be due to the formation of *intermediate* water. Although, the states of water in the polymer have not been identified nor calculated, it is hypothesized that the presence of the carbonyl group in silk fibroin allows *intermediate* water to form, improving the hemocompatibility. Hence, the presence of that water could also improve the other biocompatibility aspects of the scaffold, i.e., cell attachment and morphology, cell viability, transcription level of genes, and expression of proteins. This would imply that the presence of *intermediate* water not only could improves hemocompatibility of polymers, but also promotes other biocompatibility aspects. This preliminary conclusion should be demonstrated with further research on the states of water and their impact on biocompatibility.

Table 4. Summary of principal observations and conclusions in each polymer.

Polymer	Types of Water Measured	Biological Response Measured	Observations	References	Conclusions

PEG	*Free*, *intermediate* and non-*freezable*		*Intermediate* water is negligible at low *M*w and increases with *M*w until a constant value	[48,55]	Presence of *intermediate* water means low protein adsorption and platelet adhesion
		Platelet adhesion & plasma protein adsorption	Low protein adsorption and platelet adhesion	[48,57]	
Aliphatic carbonyls	*Free*, *intermediate* and non-*freezable*	Platelet adhesion	There is lower platelet adhesion when *intermediate* water is present.	[62]	Presence of *intermediate* water means low platelet adhesion [62]
Poly(meth)acrylates	*Free*, *intermediate* and non-*freezable*	Platelet adhesion	*Intermediate* water present only in PMEA is responsible for its excellent hemocompatibility.	[18]	Presence of *intermediate* water means low platelet adhesion [18]
PLGA	*Free*, *intermediate* and non-*freezable*		No presence of *intermediate* water	[66]	Absence of *intermediate* water means high platelet adhesion.
		Fibrinogen adsorption & platelet adhesion	High fibrinogen adsorption and platelet adhesion	[44,68]	
		Cell attachment, morphology, viability; transcription level of genes and expression of proteins	PLGA with silk-fibroin has better biocompatibility	[68]	Presence of carbonyl group of fibroin allows *intermediate* water formation and better biocompatibility
PVA	*Free*, *intermediate* and non-*freezable*		PVA films have low *intermediate* water content	[70]	Low *intermediate* water content means high platelet adhesion (inactive state)
		Platelet adhesion	High platelet adhesion but in inactive state.	[72]	
PANcNVP	*Free*, *intermediate* and non-*freezable*		Three types of water present Higher NVP means higher *non-freezable* water content	[79] [84]	High content of *non-freezable* water means less platelet adhesion [84] *Intermediate* water could influence hemocompatibility (not enough data for conclusions)
		Platelet adhesion & PRT	Higher amounts of NVP led to less platelet adhesion and increase of PRT	[79]	
Polyarylates	*Freezable* and *non-freezable*		In polymers with WC over 10%, *non-freezable* water reaches a threshold lower than *freezable* water.	[43]	At high WC, high *freezable* water means high fibrinogen adsorption *Intermediate* water (implicit in *freezable*
		Fibrinogen adsorption	Polymers with longer ester and diacid chains adsorb less fibrinogen	[44]	

	water) could influence hemocompatibility (not enough data for conclusions)

5. Conclusions

Water and its states influence polymer hemocompatibility. In polymers such as PEG, aliphatic carbonyls, poly(meth)acrylates, PLGA, and PVA, *intermediate* water content of at least 3 wt% relates to low platelet adhesion and higher hemocompatibility than those with less or none, which is due to the better stability that this state of water has in comparison to *free* water, preventing platelets contacting *non-freezable* water and activating it. However, it is necessary to conduct further studies for the identification and measurement of *intermediate* water utilizing techniques like DSC and IR to support this conclusion. Furthermore, future challenges include the analysis of the relation of water states with other aspects of biocompatibility than hemocompatibility, and of the mechanism by which *non-freezable*, *intermediate*, and *free* water interact with biological components to provide more information on the role of water in biological response.

This review presents relevant information that will aid in the process of polymer selection and polymeric materials design given a certain application. Hydration states of water are not only relevant for polymers in the medical field but also for other materials and areas such as in the food industry, sewage treatment, among others. Water must not be considered as an inert solvent and, on the contrary, it must be noted that water does interact with solutes, forming different states, which interact with the material and are able to modulate their properties. Therefore, it is very important to consider water and its states in any process that has contact with water.

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Nomenclature

ΔH	Enthalpy change of melting of ice of *bulk* water
ΔH_m	Enthalpy change of melting of ice
ΔH_{cc}	Enthalpy change of the cold-crystallization of ice
$\%Free$	Weight percentage of *free* water
$\%Int$	Weight percentage of *intermediate* water
$\%NF$	Weight percentage of *non-freezable* water
A_{c}	Calculated area of peak of NRM signal
A_{m}	Measured area of peak of NRM signal
A_{w}	Area of peak of NRM signal of pure water
ATR	Attenuated total reflection
C_p	Specific heat capacity
DSC	Differential scanning calorimetry
DTB	Desaminotyrosyl-tyrosine butyl ester
DTBn	Desaminotyrosyl-tyrosine benzyl ester
DTE	Desaminotyrosyl-tyrosine ethyl ester
DTH	Desaminotyrosyl-tyrosine hexyl ester
DTiP	Desaminotyrosyl-tyrosine isopropyl ester
DTM	Desaminotyrosyl-tyrosine methyl ester

DTO	Desaminotyrosyl-tyrosine octyl ester
DTsb	Desaminotyrosyl-tyrosine sec-butyl ester
EWC	Equilibrium water content
Fg	Fibrinogen
FT-IR	Fourier transform infrared
GA	Glycolic acid
HTE	4-hydroxyphenylacetic acid-tyrosine ethyl ester
HTH	4-hydroxyphenylacetic acid-tyrosine hexyl ester
IR	Infrared
LA	Lactic acid
M_p	Molecular weight per polymer repeating unit
MTDSC	Modulated differential scanning calorimetry
M_v	Viscosity-average molecular weight
M_{water}	Molecular weight of water
M_w	Molecular weight
NMR	Nuclear magnetic resonance
NVP	N-vinyl-2-pyrrolidone
N_w	Number of water molecules per polymer repeating unit
N_{wf}	Number of *free* water molecules per polymer repeating unit
N_{wnf}	Number of *non-freezable* water molecules per polymer repeating unit
PAN	Poly(acrylonitrile)
PANcNVP	Poly(acrylonitrile)-co-N-2-vinyl-pyrrolidone
PBA	Poly(n-butyl acrylate)
PCL	Poly(ε-caprolactone)
PDO	Poly(dioxanone)
PEA	Poly(ethyl acrylate)
$Peak_H$	Height of peak in NMR signal
$Peak_W$	Width of the peak in NMR signal
PEG	Poly(ethylene glycol)
PEHA	Poly(2-ethylhexyl acrylate)
PHEMA	Poly(2-hydroxyethyl methacrylate)
PLGA	Poly(lactic-co-glycolic acid)
PMEA	Poly(2-methoxyethyl acrylate)
PMMA	Poly(methyl methacrylate)
PPEA	Poly(2-phenoxyethyl acrylate)
PRT	Plasma recalcification time
PTMC	Poly(trimethylene carbonate)
PVA	Poly(vinyl alcohol)
PVL	Poly(δ-valerolactone)
Q_{cc}	Heat associated to cold-crystallization process
Q_m	Heat associated to melting process
SEM	Scanning electron microscope
T_g	Glass transition temperature
TGA	Thermogravimetric analysis
WU	Water content
w_{dry}	Weight of dry sample
W_f	Mass of *free* water
$w_{freezable}$	Weight percentage of *freezable* water

W_{int}	Mass of *intermediate* water
W_{nf}	Mass of *non-freezable* water
$w_{nonfreezable}$	Weight percentage of *non-freezable* water
$w_{polymer}$	Weight percentage of polymer
$WR_{freezable}$	Weight ratio of *freezable* water:polymer
$WR_{freezable}$	Weight ratio of *non-freezable* water:polymer
WU	Water uptake
w_{water}	Weight of sorbed water
w_{wet}	Weight of wet sample
XRD-DSC	X-ray diffraction with DSC

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