Digest Journal of Nanomaterials and Biostructures Vol. 5, No 4, October-December 2010, p. 1089-1100

POLYETHERURETHANE MEMBRANES MODIFIED WITH RENEWABLE RESOURCE AS A POTENTIAL CANDIDATE FOR BIOMEDICAL APPLICATIONS

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A series of polyetherurethanes (PEU) based on polytetramethylene ether glycol (PTMEG), methylene diphenyl diisocyanate (MDI) and butanediol (BD) modified with diverse proportion of hydroxypropyl cellulose (HPC) was synthesized by solution prepolymer method. The molar ratio of polyether: diisocyanate: chain extender was of 1:4:3, which produces a molar concentration in urethane groups about of $3 \cdot 10^{-3}$ mole/g. To improve the wetting properties, the PEU matrix was filled with different amount of HPC and then examined by contact angles measurements and dynamic vapour sorption (DVS). The structure of these materials was confirmed by Fourier Transform Infrared (FTIR) spectroscopy and the morphological aspects of these membranes were studied by Scanning Electron Microscope (SEM) analysis. Fibrinogen adsorption and clot weight on these polyurethanes with different HPC content (0–10 wt %) were also investigated. The clot weight, after the triggering coagulation, was determined through recalcification with $CaCl_2$. These preliminary results suggest that this PEU with HPC might be better devices than traditional biodegradable polyurethanes for tissue engineering due to its better blood compatibility.

(Received October 22, 2010; accepted November 27, 2010)

Keywords: Cellulose derivative; Nanoporous membrane; Polyetherurethane; Renewable resource

1. Introduction

Composite materials are heterogeneous materials having two or more distinct components with different properties, separated of well defined structural barriers, which complement each other, and result a material with better characteristics [1,2]. The composite consists of a matrix, which is continuous and surrounds the filler, and provides the reinforcement such that the resulting composite property is a function of the properties of both the matrix and filler. The composite, with polymers as matrices, have found applications in many areas such as automotive, construction, aerospace and medicine [3-5].

In recent years, the polyurethanes have a large interest as composite materials, especially to modify mechanical, thermal and electrical properties or to improve the hydrophilicity surface [6-13]. Cellulose and its derivatives are of a great interest to obtain of new composite materials. Therefore, the composites based on cellulose derivatives are frequently used in design and construction of medical devices. Knowledge and understanding of most properties of these composites will make it possible to design new materials that provide better quality, such as: specific biodegradability or biostability, high stiffness and tear resistance [14,15]. In many

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situations it is necessary that these materials to be biodegradable or biostable. Therefore, introducing of renewable resources in a synthetic polymer matrix, have as result obtaining of biomaterials. So, because of their biocompatibility and degradability, the polyurethane composites are recommended as membranes for dialysis, in cardiovascular surgery as vascular prostheses, artificial heart components, orthopaedic prostheses etc [16]. Furthermore, polyurethane blends with natural polymers were shown to be easily degraded and to be metabolized by microorganisms, leading to the production of CO_2, H_2O, glucose, aromatic ether, glucopyranose derivatives, and nitrate [17].

Therefore, in this paper, a series of PEU composites were synthesized and then are discussed about the influence of HPC content with respect to the main characteristics of these composite materials, especially to enhance of biocompatibility. The study was focused on determining the hydrophilic surface, the average pores size and water vapour sorption capacity of films. Fibrinogen adsorption and clot weight on these polyurethanes completes this work.

2. Experimental

2.1. *Materials*

Polytetramethylene ether glycol (PTMEG), trade name as Terathane - Mw 1400, generous gift from INVISTA BV, Nederland; methylene diphenyl diisocyanate (MDI) – Fluka; 1,4-butanediol (BD) - Fluka; hydroxypropyl cellulose (HPC) Mw ~ 80000 - Aldrich; N,N-dimethylformamide (DMF) - Fluka. Commercial DMF was dried over anhydrous K_2C0_3, and then was distilled from calcium hydride (CaH_2) and kept over 4 Å molecular sieves. Polyol and chain extender were checked for moisture and if it was necessary, it was lowered at 0.3%.

2.2. *Characterizations*

FTIR was used to examine changes in the molecular structures of the samples after mixing. The spectra were measured on a Bruker Vertex 70 FT-IR instrument, equipped with a Golden Gate single reflection ATR accessory, spectrum range 600-4000 cm^{-1}, at ambient temperature.

Contact angles were measured by the static drop technique at room temperature, using a KSV CAM 101 goniometer, equipped with a special optical system and a CCD camera connected to a computer to capture and analyze the contact angle (five measurements for each surface). A drop of liquid (~1 μl) was placed, with a Hamilton syringe, on a specially prepared plate of substratum and the image was immediately sent via the CCD camera to the computer for analysis. The angle formed between the liquid/solid interface and the liquid/vapour interface is the contact angle. Temperature and moisture was constant during the experiment (25°C and 65 % respectively).

Dynamic vapours sorption (DVS) capacity of the samples has been measured in dynamic regime by using an IGAsorp apparatus (a fully automated gravimetric analyzer, supplied by Hiden Analytical, Warrington - UK). This apparatus is used to study water sorption at atmospheric pressure by passing a humidified stream of gas over the sample, and can be applied to a wide range of studies from fundamental research to routine quality assurance/control. The IGAsorp is a standard sorption equipment, which has a sensitive microbalance (resolution 1μg and capacity 200 mg), which continuously registers the weight of the sample together with the temperature and relative humidity around the sample. Isothermal studies can be performed as a function of humidity (0-95%) in the temperature range 5°C to 85°C, with an accuracy of ± 1% for 0 - 90% RH and ± 2% for 90 - 95%RH. The relative humidity (RH) is controlled by wet and dry nitrogen flows around the sample. The RH is held constant until equilibrium or until a given time is exceeded, before changing the RH to the next level.

The surface and fracture morphology were performed studying the membrane samples by scanning electron microscopy (SEM). The measurements were taken using a Quanta 200 instrument equipped with an energy dispersive X-ray device, EDX. The porosity and average pore

diameter of the polyetherurethane samples were determined with a Micrometrics Instrument, Mercury Intrusion Porosimeter.

2.3. *Synthesis of PEU filled with HPC*

PTMEG of molecular weight of 1400 g/mole, which was dried for two hours under vacuum at 80 ^{o}C temperatures, was used as polyol. The diisocyanate component used in this study was HDI and as chain extender was utilized BD. The molar ratio of this polymer was 1:4:3 (PTMEG: MDI: BD). The prepolymer solution was made using DMF as solvent. At 0.01 mole of PTMEG was added 100 g DMF anhydrous. When the temperature of this solution is 60 degrees, was added slowly, under vigorous stirred 0.04 mole of HDI. After one hour the prepolymer was extended with 0.03 mole BD. The PEU solution was kept under stirring (60 rpm) and 60 ^{o}C temperatures, approximately three hours. When the solutions become to be viscous was added a stopper solution, formed by 0.01 mole ethanol abs. and 5 mL DMF. Finally, the solution was filtered through a glass fritte G_4 and then kept in desiccators under vacuum at room temperature. After 24 hours the polyurethane solution was divided in six parts. One part was kept as blank sample and in the other ones it was added HPC in range of 0.1 to 0.5 g (solution in DMF - 2g solution contain 0.1g HPC). The solutions of PEU-HPC were maintained under stirring for one hour and then were again introducing in desiccators under vacuum for another hour at room temperature. From these prepared solutions were obtained membranes by precipitation of the films deposited on glass support, in warm water (45 ^{o}C). These membranes were dried at 50 ^{o}C temperature for 24 hrs at vacuum (1-2 mmHg), before characterizations. PEU samples were synthesized by solution polymerization method (Scheme 1).

$HO\text{-}[CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}O]_n\text{-}H$ + OCN-R-NCO

Terathane (PTMEG), n=18.8 ↓ Diisocyanate

↓ DMF

$OCN\text{-}R\text{-}NH\text{-}CO\text{-}O\text{-}[CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}O]_n\text{-}OC\text{-}NH\text{-}R\text{-}NCO$

Urethane prepolymer solution

↓ 1,4-butanediol

$...\text{-}O\text{-}CO\text{-}NH\text{-}R\text{-}NH\text{-}CO\text{-}O\text{-}[CH_2\text{-}CH_2\text{-}CH_2\text{-}CH_2\text{-}O]_n\text{-}OC\text{-}NH\text{-}R\text{-}NH\text{-}CO\text{-}O\text{-}(CH_2)\text{-}...$

Polyurethane solution were R: $-C_6H_4\text{-}CH_2\text{-}C_6H_4-$

PEU + Hydroxypropyl cellulose (HPC) (n~50) ⟶ PEU filled whit HPC

Scheme 1. Synthetic route for preparation of polyetherurethane filled with HPC

3. Results and discussion

3.1. FTIR analysis

Influence of the HPC amount on FTIR spectra corresponding to samples analyzed is not significant (Figure 1), all the samples having similar spectra. Main signals of HPC are overlapping on polyurethane signals (HPC content was less than 5%). A shoulder at 3500-3450 cm^{-1} show the OH signal. The spectra confirm, in principal, the formation of polyetherurethane structures. This is shown by the disappearance of the signal corresponding NCO (isocyanate) group at 2260 cm^{-1}. The spectra of polyetherurethane samples are characterized by amide I bands (C=O stretch) at 1729 cm^{-1} and 1701 cm^{-1}, an amide II band (the joint vibration resulting from combination of NCO and NH) at 1530 cm^{-1}, C-O stretches at 1222 cm^{-1}, 1105 cm^{-1}, and 1078 cm^{-1}, a C=C stretch (MDI aromatic ring) at 1598 cm^{-1}, and NH stretch at 3326 cm^{-1}. The peaks for CH_2 stretch appears at 2940 cm^{-1}, 2855 cm^{-1} and 2797 cm^{-1}, respectively at 1479 cm^{-1}, 1414 cm^{-1} and 1368 cm^{-1}. At 1310

cm^{-1} a signal for C-N vibration aromatic secondary amine appears. The peak of benzene ring with two adjacent hydrogen atoms is present at 816 cm^{-1} and CH_2 rocking vibration at 771 cm^{-1}.

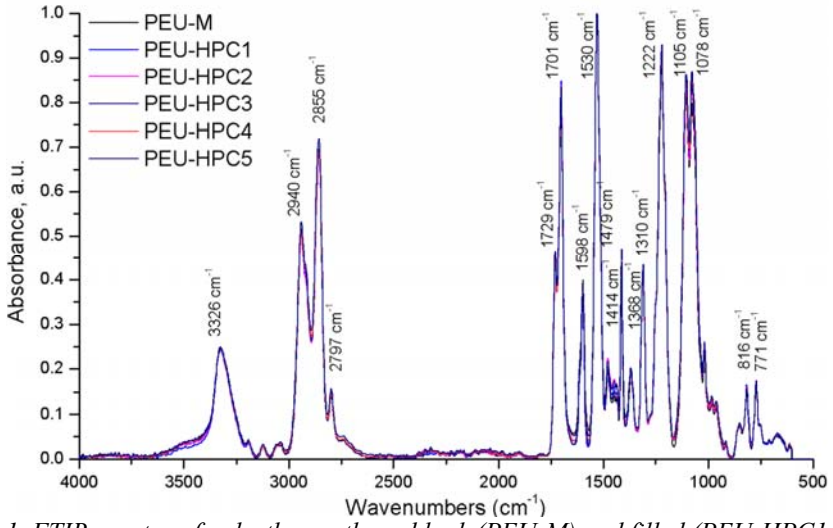

Fig. 1. FTIR spectra of polyetherurethane blank (PEU-M) and filled (PEU-HPC1… 5)

3.2. Wetting properties

3.2.1. Contact angle, work of adhesion and surface free energy

The knowledge of the surface free energy of polymers plays a prominent role in many high technology applications like: biomedical, microelectronics, thin film coating, etc. In the contact angle measurement process, which enables the determination of the surface free energy, the selection of appropriate test liquids is sometimes sophisticated. In many medical applications, polymers, in general, play an important role for the production of high-quality devices. In this connection, the PEU-HPC materials have of particular importance. In these processes, sometimes, problems occur because the polymer surfaces have relatively poor wetting properties. This is due to the relatively low surface free energy of these materials and the absence of polar surface groups. To improve the wetting properties, the polyurethane matrix is mixed with HPC. In this way, polar groups are introduced on the surface, and the surface free energy increases. The knowledge of the surface free energy of a polyurethane composite with its polar and dispersive portions is therefore of crucial importance when producing biomedical devices. To determine the surface free energy of a polymer with its polar and dispersive portions, the contact angle is measured with a number of test liquids and evaluated according to the Wu's method [18, 19]. The method requires the use of at least two test liquids with known surface tension and its polar and dispersive contributions. Each additional liquid will increase the accuracy of the estimation. A series of PEU films with 0-10% HPC was examined. In Table 1 the measured contact angles are showed.

Table 1. Contact angle of samples with different liquids in degrees.

Samples	Water, deg	Ethylene Glycol, deg	Diiodomethane, deg
PEU-M	94.12±0.20	80.33±0.17	68.16±0.09
PEU-HPC 1	90.09±0.12	72.89±0.38	64.07±1.41
PEU-HPC 2	79.74±1.47	74.95±1.20	64.47±1.84
PEU-HPC 3	72.13±0.98	78.62±0.55	61.53±0.15
PEU-HPC 4	74.84±1.90	75.35±2.82	73.01±1.47
PEU-HPC 5	77.42±1.32	72.38±1.35	65.63±1.21

The results show clearly that there are some differences in the contact angles, done by the influence of the HPC amount. The contact angles of water are less than 90 degree so, inserting of HPC in polyurethane structure increases the wetting properties. For calculation of the surface tension parameters and work of adhesion the following equations were used:

$$\gamma_{SV} = \gamma_{SL} + \gamma_{LV} \cdot \cos\theta \quad \text{Young equation} \quad (1)$$

$$W_a = \gamma_{SV} + \gamma_{LV} - \gamma_{SL} \quad \text{Dupré equation} \quad (2)$$

$$W_a = \gamma_{LV}(1 + \cos\theta) \quad \text{Young-Dupré equation} \quad (3)$$

$$\gamma_{SV} = \gamma_{SV}^{d} + \gamma_{SV}^{p} \quad \text{Fowkes equation} \quad (4)$$

$$\frac{1+\cos\theta}{2} \cdot \frac{\gamma_{LV}}{\sqrt{\gamma_{LV}^{d}}} = \sqrt{\gamma_{SV}^{p}} \cdot \sqrt{\frac{\gamma_{LV}^{p}}{\gamma_{LV}^{d}}} + \sqrt{\gamma_{SV}^{d}} \quad \text{Owens - Wendt geometric mean equation} \quad (5)$$

where θ is the contact angle determined for water and ethylene glycol, subscripts "*SL*", "*LV*" and "*SV*" denote the interfacial surface-liquid, liquid-vapour and surface-vapour tensions, while superscripts "*p*" and "*d*" denote the polar and dispersive components of total surface tension, γ_{sv}. By plotting of the left side of equation (5) as a function of $(\gamma_{LV}^{p} / \gamma_{LV}^{d})^{1/2}$ we get a straight line with the slope equal to $(\gamma_{SV}^{p})^{1/2}$ and intercept on the *y* axis equal to $(\gamma_{SV}^{d})^{1/2}$. The total free surface energy is merely the sum of its two component forces. Table 2 present the values obtained for the surface free energies of the polyurethane composite films.

Table 2.Work of adhesion, surface free energies and their dispersive and polar contributions

Samples	Surface Free Energy, mN/m	Dispersive Portion, mN/m	Polar Portion, mN/m	Work of adhesion, mN/m
PEU-M	16.49	8.54	7.95	67.57
PEU-HPC 1	20.09	12.26	7.83	72.69
PEU-HPC 2	27.14	2.57	24.57	85.76
PEU-HPC 3	45.40	0.01	45.39	95.13
PEU-HPC 4	35.50	0.65	34.85	91.83
PEU-HPC 5	28.99	2.75	26.24	88.66

From the table it is evident that the amount of HPC for moderate quantities (samples PEU-HPC 1-3) provokes an increase of the surface free energy, while for large quantities the surface free energy becomes decreased.

3.2.2. Humidity absorption study

The water vapour sorption behaviour and physical stability of polyetherurethane samples filled with cellulose derivative were determined by the IGAsorp system. The interaction of materials with water vapour is of interest to a broad spectrum of science and industry. Almost all materials have some interaction with moisture that is present in their surroundings. Materials are broadly classed as hydrophobic or hydrophilic, but detailed measurements of the interaction are required to investigate a given specimen and this generally requires determination of the water vapors sorption isotherm. The effects of water can be both harmful and beneficial depending on the material and how it is used. Water sorption is a general term that includes the range of possible interactions for water molecules with surfaces and bulk matter. The measurement that is most commonly made is the percentage weight content of moisture retained in the sample at a given relative humidity (% RH) and temperature and it is the measurement of these isotherms over a given range of temperatures that can be used to characterize the sample for defined environmental conditions. The sample is placed within a weighing basket and positioned on the microbalance. The chamber is then closed and the sample sealed in position. Drying of the sample before

sorption measurements is carried out at 25 ºC in flowing nitrogen (250 ml/min) until the weight of the sample is in equilibrium at RH<1%. This gives m_o at 25 ºC. After drying, the sorption measurements can begin with the absorption curve. The vapour pressure was increased and decreased after an established program. For absorption-desorption phase we used a program with 10% humidity steps between 0 and 90%RH, each having a pre-established equilibrium time between 40 and 50 min (minimum time and time out, respectively). The maximum time for each sequence (absorption-desorption) was set to 14 hrs in these experiments. The cycle was ended by decreasing the vapour pressure in steps to obtain also the desorption isotherms.

Water vapours sorption behaviour of this sample was analyzed using moisture sorption isotherms. The role played by water molecules in the sample was interpreted on the basis of two models, Brunauer-Emmet-Teller (BET) [20] and Guggenheim-Anderson-de Boer (GAB) [21-23] which allows a good fit of the water sorption data. The sorption-desorption isotherms of polyetherurethane samples are presented in Figure 2.

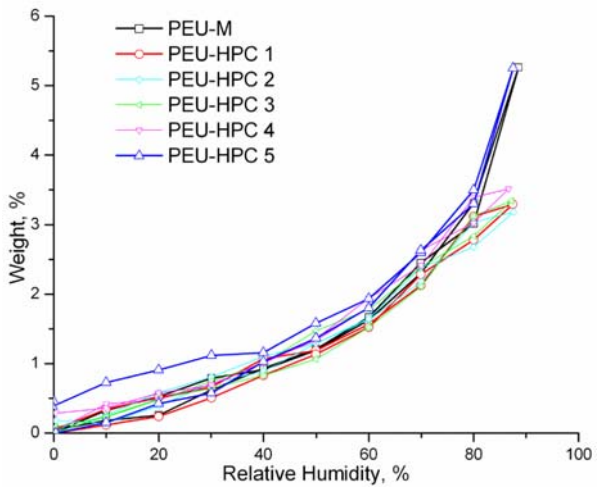

Fig. 2. Sorption-desorption isotherms of (PEU-M) and filled (PEU-HPC1… 5)

The BET model is the most widely used technique for predicting moisture sorption by solids and is used especially for evaluate the surface area of solid materials. Generally, this method describes the isotherms until a relative humidity of 50%, depending on the type of sorption isotherm and on the type of material. This model is limited because it cannot describe very well the water sorption in multilayer.

The GAB model presume that there is an intermediate layer, which has different adsorption, and liquefaction heats and also that there is a finite number of adsorption layers. GAB model is used for finding out the monolayer sorption values and for determinations of solid surface area. The GAB equation covers a larger range of humidity conditions.

For this, a program with 10 % humidity steps between 0 and 90 % RH was used. The cycle was ended by decreasing the vapour pressure in steps to obtain also the desorption isotherms. For desorption, 10 % humidity steps were used for entire humidity range.

The BET (6) and GAB (7) equations are very often used for modelling of the sorption isotherms:

$$W = \frac{W_m \cdot C \cdot p/p_0}{(1 - p/p_0) \cdot (1 - p/p_0 + C \cdot p/p_0)} \tag{6}$$

$$W = \frac{W_m \cdot C \cdot K \cdot p/p_0}{(1 - K \cdot p/p_0) \cdot (1 - K \cdot p/p_0 + C \cdot K \cdot p/p_0)} \tag{7}$$

where: W- the weight of adsorbed water,
W_m- the weight of water forming a monolayer,
C – the sorption constant,
p/p_o- the relative humidity,
K – the additional constant for the GAB equation.

Both of these methods have been used in many fields where the theory of mono and multilayer adsorption is very applied to the water sorption in order to find useful information about the compounds of interest. Contrarily to the GAB model, which represents the sorption isotherm on a wide range of water activity values (a_w), the BET model enables the representation of the sorption isotherms only in a range of activities from: 0 to 0.35 according to Brunauer and all [20]. For water activities lower than 0.35, the BET model is better fitting the experimental results than the GAB model. The data obtained from sorption/desorption isotherms are summarized in Table 3.

Table 3. The main surface parameters evaluated by sorption isotherms

Sample	Total water vapours sorption capacity, W (% db)	BET data		GAB data		Average pore size, (μm)	
		Area (m^2/g)	Monolayer (g/g)	Area (m^2/g)	Monolayer (g/g)	BET	GAB
PEU-M	5.26	27.869	0.007938	24.530	0.006987	3.77	4.28
PEU-HPC 1	3.29	19.646	0.005660	25.260	0.007195	3.35	2.60
PEU-HPC 2	3.18	41.514	0.011820	42.571	0.012120	1.53	1.49
PEU-HPC 3	3.34	27.276	0.007769	19.781	0.005634	2.45	3.38
PEU-HPC 4	3.51	31.907	0.009088	24.801	0.007064	2.20	2.83
PEU-HPC 5	5.25	22.267	0.006342	20.838	0.005935	4.71	5.04

The average pore size was estimated based on desorption branch assuming cylindrical pore geometry by using the following equation:

$$r_{pm} = \frac{2 \cdot n}{100 \cdot \rho_a \cdot A} \tag{8}$$

where r_{pm} is the average pore size, A is the BET or GAB surface area, n is the percentage uptake, and ρ_a is the adsorbed phase density.

Table 4. Water uptake capacity of polyetherurethane samples determined by immersion

Sample	Weight before drying, (mg)	Weight after drying, (mg)	Water uptake capacity, (wt %)
PEU-M	6.6135	1.8349	260.43
PEU-HPC 1	5.4145	1.2051	349.30
PEU-HPC 2	6.7738	1.4575	364.76
PEU-HPC 3	5.8258	1.1726	396.83
PEU-HPC 4	7.9938	1.5228	424.94
PEU-HPC 5	6.3626	1.1935	433.09

By analyzing of the experimental results of water vapours sorption/desorption the order of the dynamic water sorption capacity was PEU-M<PEU-HPC1<PEU-HPC2<PEU-HPC3<PEU-HPC4<PEU-HPC5, while the order of BET and GAB area values are different: PEU-HPC1<PEU-HPC5<PEU-HPC3<PEU-M<PEU-HPC4<PEU-HPC2, and respectively PEU-HPC3<PEU-HPC5<PEU-M<PEU-HPC4<PEU-HPC1<PEU-HPC2. The difference between the order by water sorption capacity and area can be caused by the nature of the polar groups. The average pore size also influence the sorption capacity, the order by this criterion being for BET: PEU-HPC2<PEU-HPC4<PEU-HPC3<PEU-HPC1<PEU-M<PEU-HPC5, respectively for GAB: PEU-HPC2<PEU-HPC1<PEU-HPC4<PEU-HPC3<PEU-M<PEU-HPC5. From the presented results, it is evident

that because of many parameters like amount of HPC from PEU, surface area and pore size, etc these influence in complex mode the sorption capacity of the samples.

In this study the samples are relatively thin as 1 mm. Therefore diffusion into the edges of the sample may be neglected and the diffusion can be modelled based on a one-dimensional Fickian model, which it is describe by following equation:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{dx^2} \tag{9}$$

where C is the concentration of the diffusing substance, t is time, D is the diffusion coefficient and x is the length parallel to the concentration gradient.

Thin film samples can be considered to be flat plates of thickness l. Both surfaces (top and bottom) are assumed to be at a constant concentration C_1 with an initial concentration of C_o within the film. For example, if the medium is initially in equilibrium and the concentration at all surfaces it is rapidly changed (i.e. by changing the pressure or humidity in a sorption experiment). The limit conditions stated mathematically are:

$C=C_1$ for: x= -l/2, x=l/2 and t≥0; $C= C_0$ for: -l/2<x>l/2 and t=0; $\frac{\partial C}{\partial x} = 0$ for: x=0 and t≥0.

Using the above boundary conditions Fick's Law can be solved to give the time dependence of concentration as shown below:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\sum\frac{1}{(2n+1)^2}\cdot e^{-\frac{D(2n+1)^2\pi^2 t}{l^2}} \tag{10}$$

where: t is the time measured from when the concentration is changed, l is the plate thickness, M_o is the initial equilibrium mass and ΔM is the change in the mass from M_o to the new equilibrium mass M_t. The equation (10) describes the change in mass due to the movement of the diffusing species responding to the sudden change in pressure/humidity around the sample. The half thickness (l/2) is used as the diffusion length above since in a typical sorption experiment the entire plate is measured and the substance is assumed to diffuse uniformly to the central plane. For M_t/M_∞ <0.5, equation (10) becomes [24]:

$$\frac{M_t}{M_\infty} = \frac{4}{l}\cdot\sqrt{\frac{D\cdot t}{\pi}} \tag{11}$$

and for M_t/M_∞ >0.5, same equation becomes:

$$\frac{M_t}{M_\infty} = 1 - \frac{8}{\pi^2}\cdot e^{-\frac{D\pi^2 t}{l^2}} \tag{12}$$

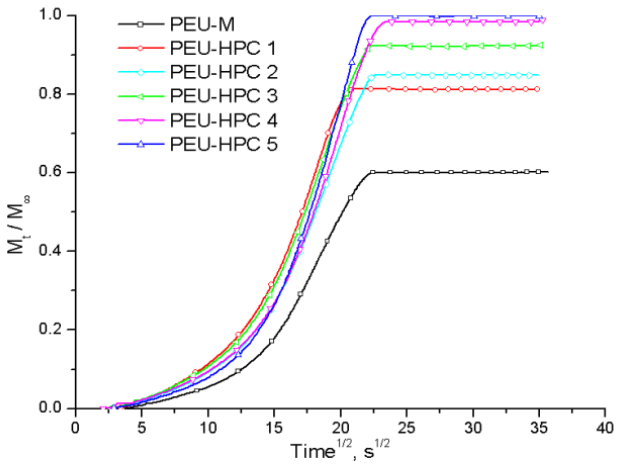

Fig. 3. Graphical representation of PEU samples from normalized mass changing vs. time $^{1/2}$

Of data taken from the Figure 3, were determined diffusion coefficients. Table 5 show the values of diffusion coefficients which were calculated after Eq. (11) for M_t/M_∞= 0.4 and respectively for M_t/M_∞ = 0.6 after Eq. (12).

Table 5. Diffusion coefficient values calculated form normalized mass changing vs. time $^{1/2}$

Sample	M_t/M_∞, <0.5/>0.5	$t^{1/2}$, $s^{1/2}$	$l^2 \cdot 10^8$ m^2	D, [M_t/M_∞<0.5, Eq. (11)/M_t/M_∞>0.5, Eq. (12)] m^2/s
PEU-M	0.4/0.6	18.595/22.350	5.76	$5.23335 \cdot 10^{-12}$ / $8.25185 \cdot 10^{-12}$
PEU-HPC1	0.4/0.6	15.961/18.086	6.25	$7.70743 \cdot 10^{-12}$ / $1.36735 \cdot 10^{-11}$
PEU-HPC2	0.4/0.6	16.937/19.251	10.24	$1.12144 \cdot 10^{-11}$ / $1.97732 \cdot 10^{-11}$
PEU-HPC3	0.4/0.6	16.194/18.340	13.69	$1.64000 \cdot 10^{-11}$ / $2.91265 \cdot 10^{-11}$
PEU-HPC4	0.4/0.6	16.870/18.994	4.00	$4.41549 \cdot 10^{-12}$ / $7.93435 \cdot 10^{-12}$
PEU-HPC5	0.4/0.6	16.700/18.588	3.24	$3.64974 \cdot 10^{-12}$ / $6.71064 \cdot 10^{-12}$

3.3. SEM study

Figure 4 shows some SEM images of samples (surface and fracture) for PEU-M (without HPC, *a* and *b*), PEU-HPC1 (with low amount of HPC, *c* and *d*) and respectively, PEU-HPC5 (with high amount of HPC, *e* and *f*). All samples were cryogenically fractured in liquid nitrogen.

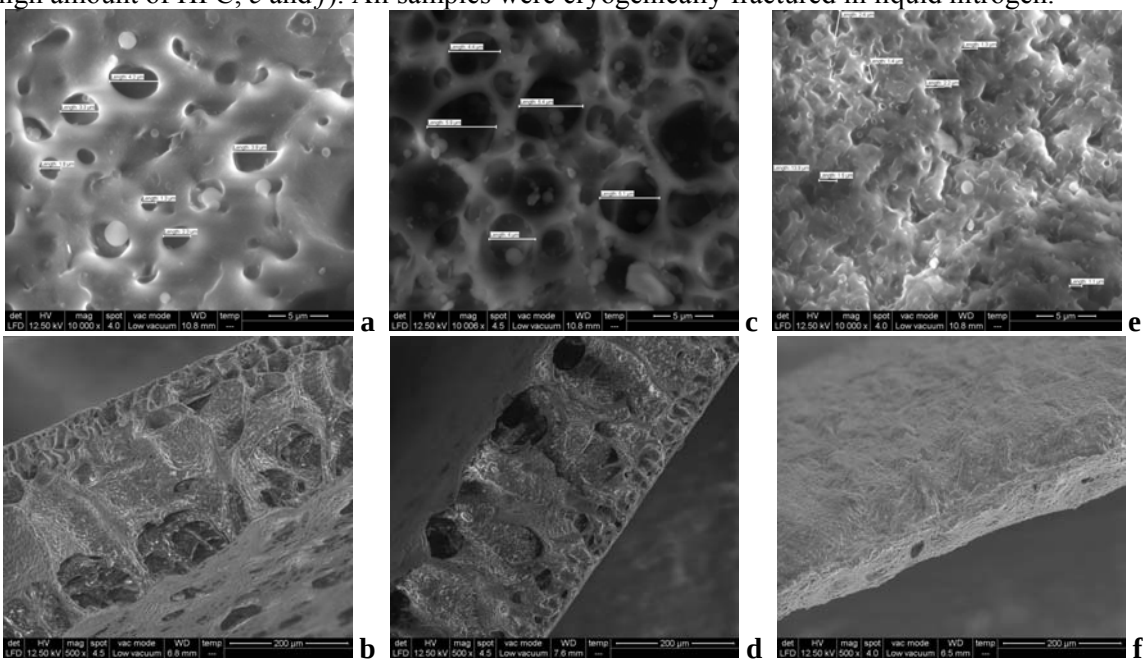

Fig.4. SEM micrographs of PEU-M (a- surface; b- fracture), PEU-HPC1(c- surface; d- fracture) and PEU-HPC5 (e- surface; f- fracture)

The samples PEU-M and respectively, PEU-HPC1 shows surfaces with large pores, while the PEU-HPC5 presents a surface with more small and uniform pores. The porosity of the polyurethane samples, increases with increase of the HPC content. In this way can be obtain materials, with interconnected pores, important in allowing cell colonization.

3.4. Biological study

3.4.1. Proteins adsorption

Understanding of the adsorption mechanism of proteins on the surface of a material is important to achieve a biocompatible material. Proteins adsorption causes platelets adhesion thereby surface-induced thrombosis. This phenomenon is favoured by electrostatic and hydrophilic

interactions between proteins and synthetic material surface. Other important factors are the surface electric charge, roughness and functional groups [25]. Proteins are macromolecules possessing functional groups within their hydrophobic or hydrophilic. In aqueous solution, hydrophobic portion is protected from water, while the hydrophilic portion is exposed at the surface of adsorbent [26].

Fibrinogen (*FB*) is a protein that plays a key role in blood clotting. For adsorption experiment was used 3 mg/ml bovine serum fibrinogen (*BSF*-from Sigma Co, 95% clotable) in 0.9% sodium chloride solution (similar with the physiological concentration in human blood) and human blood plasma (obtaining from human blood on 3.8% sodium citrate 9:1 v/v). A fresh solution of *FB* was always prepared for every adsorption experiment. Prior to adsorption experiment, the samples were brought to equilibrium with 0.9% sodium chloride solution (up to reaching of a maximum hydration, minimum 72 h). In order to perform adsorption experiments, polyurethane films with known surface area were introduced into tubes containing 0.5 ml *FB* solution or sanguine human plasma and kept them at 37 ^{o}C for 1 h. After incubation, the films of polyurethane were removed and the amount of remaining protein in solution was determined by using Na_2SO_4 reaction and assayed spectrophotometrically with Piccos UV–VIS [27]. The adsorbed amount of *FB* was calculated with the following equation:

$$AP = (C_o - C_e)V/S \quad (13)$$

where: *AP* is adsorbed protein (mg/cm^2), C_o and C_e are the initial and equilibrium concentrations of *FB* solution (mg/ml), *V* is the volume of protein solution (ml) and *S* is the surface of the polyurethane sample (0.5 cm x 0.5 cm).

In the literature many research studies are dealing with polyurethanes regarding contact angle measurements, water swelling properties and protein adsorption. Water plays an important role in determining biocompatibility characteristics of the synthetic material. It is very well known that high water levels on the surface of the biomaterial providing a low interfacial tension with blood consequently reduce fibrinogen adsorption and cell adhesion on the surface similarly to biological tissues.

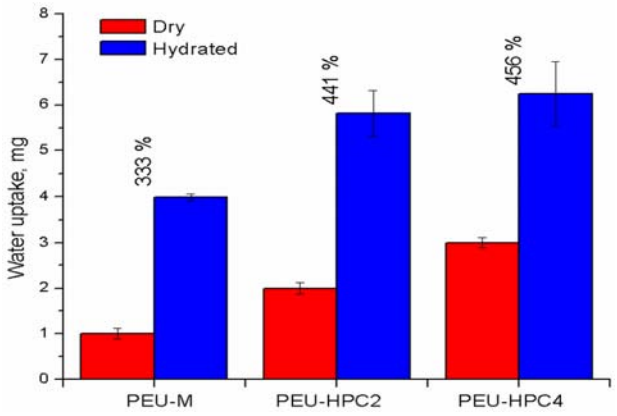

Fig. 5. Water swelling of representative PEU samples (after 24 hrs immersion)

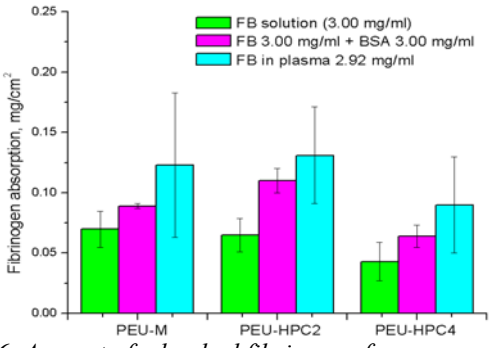

Fig. 6. Amount of adsorbed fibrinogen from simple solution, mixture and sanguine plasma

In Figure 5 were presented the water sorption values of PEU samples after 24 hrs immersion. The membranes were prepared through precipitation in water of simply PEU and mixed with HPC in different proportions. Increasing of HPC content in polyurethane matrix produces increasing of swelling properties.

The experimental data related to the amount of adsorbed fibrinogen of polymer samples with a simple physiological solution (*FB*: 3.00 mg/ml), mixed solution (*FB*: 3.00 mg/ml; *BSA*: 3.00 mg/ml) and blood plasma (*FB*: 2.92 mg/ml) are presented in Figure 6.

Determination of the adhered fibrinogen from blood plasma was coupled with the determination of the clot weight, followed by transformation of the fibrinogen in fibrin and clot formation. It is observed that the amount of fibrinogen adsorbed from blood plasma is slightly higher than in simple solution or mixture *FB-BSA*. Anyway, it is remarked that clot weight stand in physiological normal limits. The differences between adsorptions of the fibrinogen solution and

blood plasma, suggest that the fibrinogen adsorption properties of the polyurethane samples, under physiological condition are affected by the concurrent affinities for other plasma proteins, which do not disturb the haemostatic mechanisms.

3.4.2. Clot weight in contact with polymer

We followed the phenomenon of global coagulation, as the principle method for determining the time Howell-Gram method (modified in the laboratory), using whole blood. We determined the clot weight after triggering coagulation by recalcification with $CaCl_2$ (Table 6).

Table 6. Clot weight of blank, collagen and PEU samples

Sample	Clot weight, mg	Relative clot weight, %
Blank clot (240 s without polymer)	24.86±4.70	100.00
Film collagen bovine (positive sample)	42.50±3.50	58.49
PEU-M	38.40±2.92	64.74
PEU-HPC2	36.20±3.50	68.67
PEU-HPC4	31.60±6.61	78.67

Whole blood combined with an anticoagulant (sodium citrate 3,8%) in ratio of 1/9 (v/v) was collected by venous puncture from a donor, volunteer, healthy and non-smoker. Polymer films with size of 5x5 mm were incubated in 0.9% sodium chloride physiological solution for 30 min at room temperature for hydration. After incubation period, each sample of polymer was weighed and put a plate on the bottom of tissue culture plastics. Over polymer was added 0.1 ml of blood. Incubate in wet conditions at temperatures of 37 ^oC for 30 min. After incubation time, the reaction of coagulation is triggered by adding 0.05 ml of 0.025 M $CaCl_2$ solution. After 240 s (optimal time determined by exploratory coagulation earlier) clot was stopped by adding 1 ml distilled water. Fresh clot formed with polymer extracted from the Petri dish with a spatula, to remove excess liquid with filter paper and then weighed. By difference of final and initial weight of the sample was determined the clot weight. For each experiment was used a blank test obtained in the same conditions but in the absence of polymer. Determine their optimal time coagulation for a clot sized, easy to handle, but not to peak. This time was set to 240 s (4 min). Table 6 present the relative clot weight on the film reported to the blank test, taken as 100, which was used as the degree of antithrombogenicity of the film - Imai method [28].

The clot weight after incubation time can be arranged in the following antithrombogenic order: Blank clot > PEU-HPC4 >PEU-HPC2 > PEU-M > Collagen film, so studied polyurethane samples did not affect the clot formation mechanisms.

4. Conclusions

The biomedical materials, in general, are required to possess blood compatibility and biocompatibility. In this study, some polyetherurethanes based on natural renewable polymer resources have been prepared. Introducing of natural polymer in polyurethane matrix improves the biomaterial properties. In this context, the influence of HPC on the PEU samples enhanced the wetting properties proved by contact angle determinations and dynamic vapour sorption isotherms. The biocompatibility of these polymers was evaluated by fibrinogen adsorption and weight blood clot tests. PEU filled with HPC manifest relatively thromboresistant qualities and adequate swelling and dynamic vapour sorption properties. These positive results together with improving of hydrophilicity recommend these materials as candidate for biomedical applications.

Acknowledgements

This work was supported by CNCSIS –UEFISCSU, project number PNII – IDEI code 988/2008 contract nr. 751/2009.

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