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Development of a biodegradable bioadhesive containing urethane groups

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Abstract Surgical adhesives consist on an attractive alternative to suturing or stapling since they can accomplish other tasks, such as haemostasis and the ability to seal air leakages. The application of adhesives would also reduce the surgeries procedure time since they represent an easier and faster method to establish tissue adhesion. The aim of this work was the development of a biodegradable urethane pre-polymer that presents the capacity of reacting with the amino groups present in the biological molecules. Urethanes based on polycaprolactone diol (PCL) were synthesized by reaction of the molecule either with isophorone diisocyanate (IPD-isocyanate) or hexamethylene diisocyanate (HDI-isocyanate). The characterization of the materials was accomplished by: ATR–FTIR (Attenuated Total Reflectance–Fourier Transform Infrared), determination of swelling capacity, stability of NCO groups in the presence of humidity conditions, reaction with aminated substrates (as a simulation of the living tissues) and determination of surface energy by contact angle measurement. The haemocompatibility of the PU was also evaluated by thrombosis and haemolysis tests.

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Introduction

Traumatic or surgical induced continuity solutions in tissues are usually restored by the apposition of a biologic or synthetic material. The use of an adhesive or a glue would be a suitable way to deal with the most frequent challenges in surgical practice-bind together two hollow viscus (anastomosis), stop the bleeding flow from the crude surface of a solid organ (liver, spleen) and close effectively and quickly wall incisions [1].

Despite the recent advances in these fields, namely automatic suture devices (suture threads or staples), surgical strips and some glues, a place exists for an adhesive that can fit the real non-solved points in the surgical daily practice, which are

(a) hollow viscus anastomosis—easy apposition of the two components to be anastomosed, immediate seal of the interface, tension strength compatible with fast functional recovery, none or scarce induction of adhesion between adjacent viscera and oriented scar formation with reduced risk of stenosis;
(b) solid organ section or surface fractures—effective bleeding control and seal off without fluid leakage;
(c) wall incision closure—easy to apply and effective in the maintenance of a tension strength until a complete scar formation.

As a result of these limitations, surgeons have thought about using medical tissue adhesives. These surgical adhesives must obey some clinical requirements. They must hold the two sides of the tissue together, until it is no longer necessary, and then they should be degraded to biocompatible products [2]. The most used surgical glues nowadays are the fibrin [3–5] based adhesives and the cyanoacrylates [6–8]. The fibrin sealants are advantageous

since they are both biocompatible and biodegradable, and are not associated with inflammation, foreign body reactions, tissue necrosis, or extensive fibrosis. However, fibrin based adhesives present several problems, e.g., immunogenicity and risk of blood transmission diseases such as hepatitis, HIV and BSE. On the other hand, cyanoacrylates have been reported as been strong adhesives but they are not bioabsorbable or biocompatible. Another drawback of this type of adhesives is the fact that they degrade in aqueous media to produce formaldehyde, which causes inflammation and has got carcinogenicity potential. Other options are now coming into light such as gelatine [9, 10] and albumin based glues [11], and among the synthetic materials, urethane-based adhesives have been considered to be the most promising [1]. The main advantages of the urethanes are their biocompatibility and the ability of the pre-polymers to react with the amino groups of the proteins existent in the living tissues. This reaction results in the formation of urea linkages and also on the promotion of adhesion. An adhesive based on polyurethanes was synthesised and studied by Lipatova et al. [1]. These authors showed that their degradation products did not present any toxic effect and that the glue was auto sterile and assured intensive haemostasis. Its potential as surgical adhesive was tested in several fields, such as renal surgery [12], endocrinology [13] and pancreatic occlusion [14].

In this paper we wish to report the synthesis of urethanes based on polycaprolactone diol (PCL). A pre-polymer with terminal isocyanate groups (responsible for the ability of the adhesive to react with living tissue proteins) was obtained. PCL consists on biodegradable aliphatic polyester whose products of degradation are either metabolised by been included in the tricarboxylic acid cycle or eliminated by renal secretion [15]. This polymer has been used in several medical applications already approved by the US Food and Drug Administration [16, 17]. Nowadays, PCL is being applied not only in the development of drug delivery systems [18], and resorbable sutures [19], but also as a material for tissue regeneration [20].

In this work, two different polymers with urethane groups were prepared by reacting PCL and two different isocyanates. The characterization of the synthesised materials was accomplished by different techniques: ATR–FTIR (Attenuated Total Reflectance–Fourier Transform Infrared), swelling capacity determination, study of the stability of NCO groups under humidity conditions, reaction with aminated substrates and determination of surface energy by contact angle measurement. The haemocompatibility of the PU was also evaluated by thrombosis and haemolysis tests.

Experimental procedure

Materials

All the reagents were purchased from Sigma/Aldrich Chemical Company (Spain) and used with no further treatment. Rabbit venous blood used in haemocompatibility studies was colleted in polypropylene tubes with a 9:1 blood ACD (Acid Citrate Dextrose) solution [21] ratio and was used immediately after collection.

Synthesis

Urethanes based on polycaprolactone diol with molecular weight of 530 (PCL, Fig. 1) were synthesized by modification of their hydroxyl groups either with isophorone diisocyanate (IPD-isocyanate) or hexamethylene diisocyanate (HDI-isocyanate) (Fig. 2). The ratio of NCO:OH groups used was 2:1 and the chosen solvent was chloroform because of its high volatility.

The reactions were performed by stirring the two components at 60 °C, in the absence of air (under a nitrogen atmosphere), The ATR–FTIR technique showed that after 24 h of reaction all the PCL hydroxyl groups had reacted with the NCO groups of the isocyanate.

The ATR–FTIR technique was also employed to confirm the formation of the urethane groups as well as the presence of free isocyanate groups in the pre-polymers. All these analysis were performed on a Magma-IR™ Spectrometer 750 from Nicolet Instrument Corp., equipped with a Golden Gate Single Reflection Diamond ATR. Spectra were recorded on an average of 128 scans at a resolution of 4 cm^{-1}.

Water sorption capacity

In order to determine the swelling, the polymers were primarily dried until constant weight at 60 °C under vacuum conditions. The weight of the dried sample was obtained. These samples were then placed in a container with a saturated solution of pentahydrated copper sulphate and were weighted at different times until a maximum weight was achieved. The swelling ratio was evaluated by using Eq. 1.

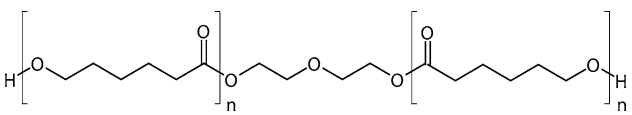


Fig. 1 Chemical structure of the polycaprolactone diol

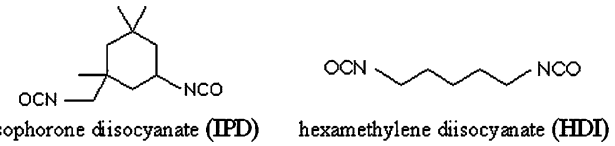


Fig. 2 Structure of the two diisocyanates used to produce the urethanes

$$\text{Swelling ratio}\,(\%) = \left(\frac{W_s - W_d}{W_d}\right) \times 100 \qquad (1)$$

where W_s and W_d correspond to the weight of the swollen sample and dried sample, respectively.

Stability of NCO groups under humidity conditions

The stability of the NCO groups present in the urethane pre-polymers in moist medium, was evaluated by keeping three samples of samples of each polymer under a 100% water saturated atmosphere.

The length of the peak at 2,260 cm^{-1}, which corresponds to the NCO free groups, was measured by the ATR–FTIR technique within different intervals, until the isocyanate groups were no longer detected.

Reaction with aminated substrates

One of the advantages of the urethanes is the ability of the pre-polymers to react with the amino groups of the proteins existent in the living tissues. This reaction results in the formation of urea linkages and also on the promotion of adhesion (Fig. 3).

In order to evaluate the binding capacity of the adhesives, they were placed between gelatine sheets that present a great amount of amino groups and therefore simulate the living tissues (Fig. 4).

The gelatine sheets were then subjected to the binding strength test, using a Chatillon TCD 1000. The pulling velocity was 20 mm/min, and the assays were carried out at room temperature. The software program coupled to the apparatus registered the force and length variation. The tests terminated with the fracture of the gelatine sheets or their separation in case adhesion failed to occur.

Determination of surface energy by contact angle measurement

Measurement of contact angles and surface energies provides a better understanding of the interactions between solids and liquids. These interactions play a key role in understanding adhesion, material wettability and even biocompatibility [22].

The surface energies of solids can be accessed by measuring the contact angles between the solid surfaces and liquids with well determined surface tensions (Fig. 5).

The equilibrium of forces at the edge of a resting drop can be described by the interfacial energies of the corresponding surfaces as realized by Young [23]. From the

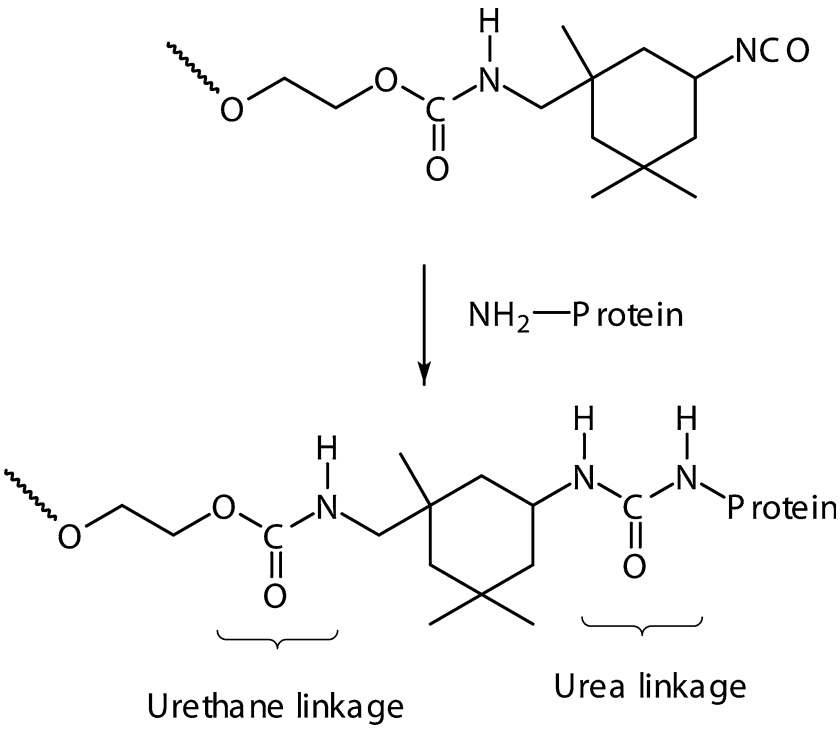


Fig. 3 Reaction between a pre-polymer and the amino groups of a protein resulting in a urea linkage

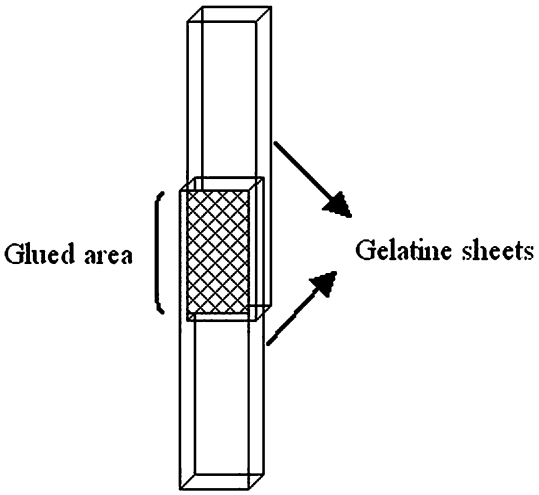


Fig. 4 Schematic representation of the glued gelatine sheets

equilibrium of these forces both the surface and the interfacial tensions can be obtained:

$$\sigma_S = \sigma_{SL} + \sigma_L \cos\theta \tag{2}$$

Here σ_S, σ_{SL} and σ_L represent the surface tensions between the solid and the saturated vapour of the liquid, the interfacial tension between the drop and the solid and the surface tension of the drop versus the saturated vapour [24].

Owens, Wendt, Rabel and Kaelble observed [25] that the interfacial tension has got two components, taking in account the interactions between the molecules. Based in their studies they concluded that there are two main types of interactions: polar and dispersive. Polar interactions include Coulomb interactions between permanent dipoles and also the ones established between permanent and induced dipoles. Dispersive interactions correspond to the time fluctuations of charge distribution within molecules. The sum of both contributions establishes the surface energy and surface tension of, respectively, solids and liquids:

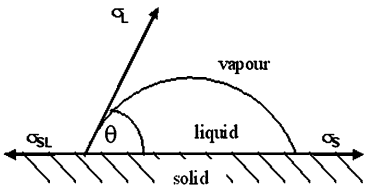
$$\sigma_S = \sigma_S^D + \sigma_S^P \quad \sigma_L = \sigma_L^D + \sigma_L^P \tag{3}$$

Here σ_L^D and σ_L^P represent the dispersive and polar parts of the liquid, while σ_S^D and σ_S^P represent the respective contributions of the solid [24].

According to Owens, Wendt, Rabel and Kaelble, the interfacial energy (σ_{SL} in 2) can be calculated from the contributions of the liquid and the solid by forming a geometric mean. It is then obtained:

$$\sigma_{SL} = \sigma_S + \sigma_L - 2\left(\sqrt{\sigma_S^D\sigma_L^D} + \sqrt{\sigma_S^P\sigma_L^P}\right) \tag{4}$$

Substituting this term for σ_{SL} in Eq. 1 results in the following equation:



Where: σ_{SL} = interfacial tension between the drop and the solid

σ_L = surface tension of the drop versus the saturated vapour

σ_S = surface tensions between the solid and the saturated vapour of the liquid

Fig. 5 Scheme of a drop on the surface of a solid

$$\sigma_S = \sigma_S + \sigma_L - 2\left(\sqrt{\sigma_S^D\sigma_L^D} + \sqrt{\sigma_S^P\sigma_L^P}\right) + \sigma_L \cos\theta \tag{5}$$

Finally it is possible to obtain an equation of a straight line of the form

$$y = ax + b \tag{6}$$

$$\text{Where}: y = \frac{(1+\cos\theta)}{2}\frac{\sigma_L}{\sqrt{\sigma_L^D}}; a = \sqrt{\sigma_S^P}; \quad x = \sqrt{\frac{\sigma_L^P}{\sigma_L^D}} \text{ and } b = \sqrt{\sigma_S^D}. \tag{7}$$

Therefore, plotting y vs. x it becomes possible to calculate σ_S^P from the slope of the fitted line and σ_S^D from the intersection with the vertical axis. In order to achieve this, the contact angle of at least two liquids on the unknown solid must be determined.

In this work, surface energies of the urethanes and gelatine sheets were evaluated, by static contact angle (θ) measurements in an OCA 20 from Dataphysics in order to compare them with the ones obtained from literature for skin and blood. The tests were performed on the air-facing surfaces of the samples with four liquids: water, formamide, ethylene glycol and propylene glycol using the sessile drop method. Nine measurements on different points were performed to calculate the mean static contact angle θ and its standard deviation.

According to the Owens–Wendt–Rabel and Kaelble relationship, the dispersive σ_S^D and polar σ_S^P components of the urethanes as well as the ones of the gelatine sheets were determined.

Haemocompatibility

The haemocompatibility was evaluated in vitro according to the International Standard Organization (ISO) 10993-4 [26], and the following categories of blood interactions were studied: thrombogenicity and haemolytic potential.

Thrombogenicity

The evaluation of thrombus formation on polymeric surfaces was carried out using the gravimetric method of Imai and Nose [27]. Anticoagulated rabbit blood was used for this purpose. This sample was prepared by adding 1 mL of ACD solution to 9 mL of fresh rabbit blood. Before performing the tests, the urethanes were kept in contact with PBS (Phosphate Buffered Saline pH 7.4) at a constant temperature of 37 °C. After 24 h of incubation, the PBS was removed and the ACD blood was added to the polymers surface and also to an empty Petri dish that acted as a positive control. Blood clotting tests were initiated by adding 0.02 mL of a 10 M calcium chloride solution and were stopped by the addition of 5 mL of water after 45 min. The resultant clots were fixed with 5 mL of a formaldehyde solution 36%, dried with tissue paper and finally weighted.

Haemolysis

The haemolysis tests were performed as described in American Society for Testing and Materials (ASTM) (ASTM F 756-00, 2000). Three samples of each urethane (21 cm^2) were placed in polypropylene test tubes and 7 mL of PBS were added. After 72 h of incubation at 37 °C, the PBS was removed and 1 mL of diluted ACD rabbit venous blood (9.02 mg/mL) was added to each sample. ACD blood was also added both to the PBS extraction solution and to three samples of pre-polymers with no previous treatment with PBS and they were all maintained at 37 °C for 3 h. Positive and negative controls were prepared by adding the same amount of ACD blood to 7 mL of water and PBS, respectively. Each tube was gently inverted twice each 30 min to maintain contact of the blood with the material. After incubation, each fluid was transferred to a suitable tube and centrifuged at 2,000 rpm for 15 min. The haemoglobin released by haemolysis was measured by the optical densities (OD) of the supernatants at 540 nm using a spectrophotometer UV–Vis (Jasco V-550). The percentage of haemolysis was calculated as described in Eq. 8.

$$\%\,\text{Haemolysis} = 100\left(\frac{\text{OD}_{\text{test}} - OD_{\text{negative control}}}{\text{OD}_{\text{positive control}} - \text{OD}_{\text{negative control}}}\right) \times 100 \quad (8)$$

Results and discussion

Synthesis

Two different urethanes were synthesised by reacting PCL either with IPD-isocyanate or with HDI-isocyanate. The polymers were prepared in a 2/1 ratio of NCO/OH groups to make sure that the urethanes would end up with terminal isocyanate groups (Figs. 6, 7).

The success of the reaction was monitored by ATR–FTIR by detection of the urethane peak at 1,525 cm^{-1}. It was also determined that all the PCL hydroxyl groups had reacted with the NCO groups of the isocyanate since the band that corresponded to the OH groups (3,490 cm^{-1}) was no longer detected in the urethanes spectra. The presence of NCO free groups on the pre-polymers was also proved by the same technique, once one could detect a peak around 2,250 cm^{-1}, which corresponds to the existence of these groups. The ATR–FTIR spectrums are presented in the Fig. 8.

However, it was observed, that the intensity of the peak correspondent to the free isocyanate groups was less intense for the urethane synthesised with HDI-isocyanate. In fact, the diisocyanate structure is important for the NCO group reactivity [28]. Isophorone diisocyanate (IPD-isocyanate) presents an important characteristic: unequal reactivity of the primary and secondary isocyanate groups [29]. For this reason, the polyol reacts with the primary

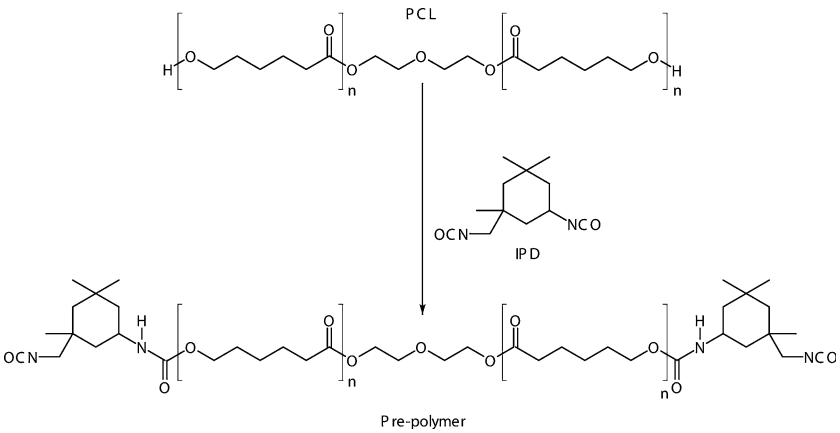


Fig. 6 Polyurethane with terminal NCO groups based in PCL and IPD-isocyanate

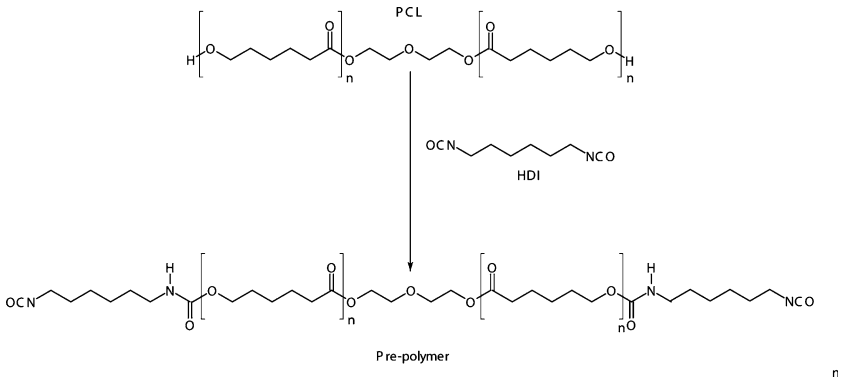


Fig. 7 Polyurethane with terminal NCO groups based in PCL and HDI-isocyanate

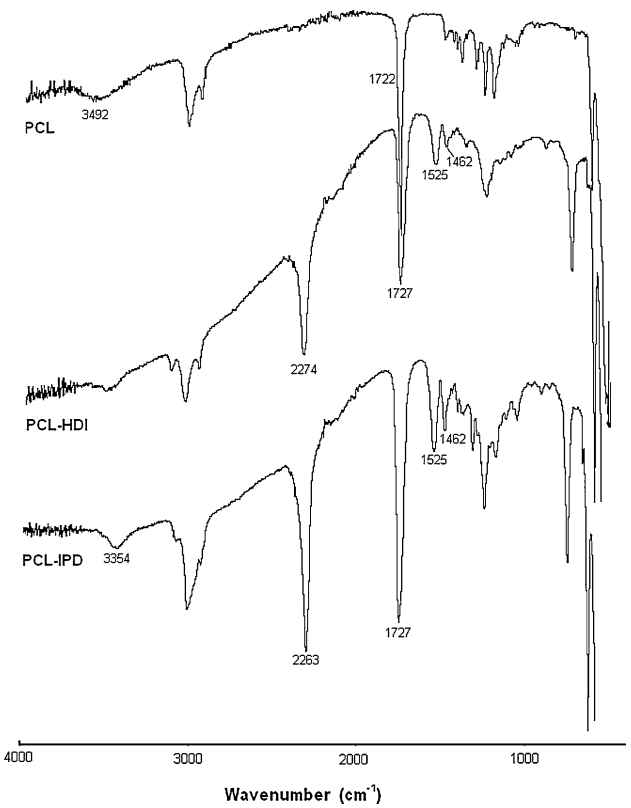


Fig. 8 ATR–FTIR spectrum obtained for the PCL and the urethanes synthesised

isocyanate group of IPD-isocyanate, leaving the secondary isocyanate group intact for subsequent reaction with the biological tissues. This unequal reactivity of the primary and secondary isocyanate groups helps control crosslinking and therefore a more intense NCO peak at 2,250 cm^{-1} was detected. On the other hand, HDI-isocyanate although aliphatic, both isocyanate groups have equal reactivity [30]. Since the diisocyanate was used in excess, the NCO groups reacted not only with the hydroxyl groups of the PCL but they also reacted with the urethane groups, resulting in branched polymer chains or, the crosslinking of the polymer. This is probably the reason why the peak around 2,250 cm^{-1}, which corresponded to NCO free groups, resulted in a smaller length peak in this urethane.

Water sorption capacity

The swelling ratio (%) was also calculated for the two polyurethanes synthesized: PCL + IPD, PCL + HDI. The values obtained are represented in Chart 1.

The swelling capacity of a polymer is a very important parameter when dealing with biological applications. If a polymer swells two much when in contact with living tissues, this may mean that it will damage the surrounding tissues due to the increase in its volume. This phenomenon can lead to a deficient healing process and possible secondary effects such as infections and inefficient cicatrisation.

Considering the results obtained when this parameter is evaluated, one could observe that the percentage of swelling was very low to both polymers. For this reason we can suggest that the synthesised urethanes will not increase their volume in a matter that can prevent their use for the pretended purpose. However, they still present some hydrophilicity, which can contribute to their biocompatibility.

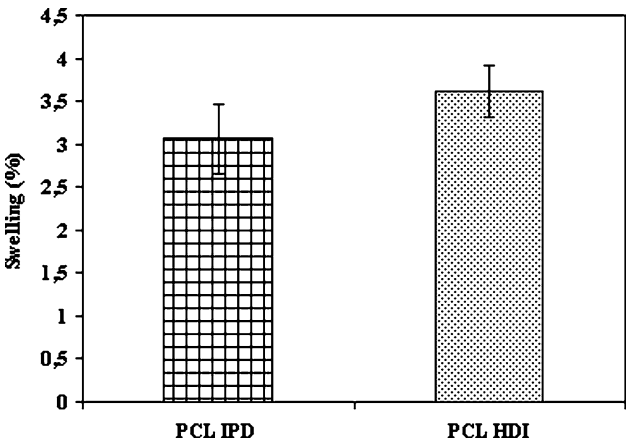


Chart 1 Swelling ratio of the urethanes

Stability of NCO groups under humidity conditions

Considering that one of the main advantages of the urethanes with isocyanate end groups is their ability to favour the adhesion of the living tissues due to the reaction with the amino groups of their proteins, the importance of analysing the stability of NCO free groups becomes evident, even in dramatic conditions, like the ones represented by an water saturated atmosphere.

The isocyanate end groups present in this type of polymers are able to react with water resulting in the formation of an unstable carbamic acid that decomposes to carbon dioxide and an aminic ending polymer (Fig. 9).

However, further reactions may occur resulting, in a first step, in the formation of urea groups (Fig. 10).

Finally, the isocyanate groups may react with these urea units, and a consequence of this is that the adhesive which was first linear now becomes crosslinked (Fig. 11) [31].

Although this moisture curing of the adhesive will necessarily occur in the living tissues, it is also important to avoid this occurrence while the adhesive is still not being used, like while being manipulated or even in storage.

The stability of the NCO groups was evaluated by placing the samples in a water saturated atmosphere and following the evolution of the length of the peak at 2,250 cm^{-1} by ATR–FTIR technique, which corresponds to the NCO free groups. The results obtained are presented in Chart 2.

When analysing the chart, we can verify that only after 6 days all the NCO groups were completely hydrolysed in the urethanes. We could also verify that even after 1 day under water saturated atmosphere the isocyanate peak length was nearly the same. Considering that the tests were

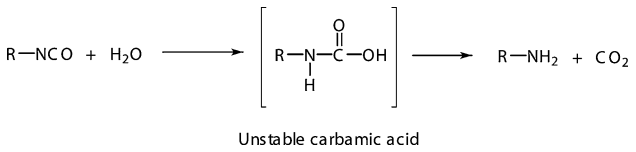


Fig. 9 Reaction between isocyanate groups and water

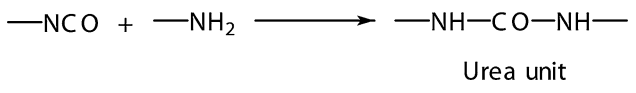


Fig. 10 Reaction between isocyanate and aminic groups with formation of a urea unit

Fig. 11 Reaction between isocyanate and urea units resulting in crosslinking of the urethane

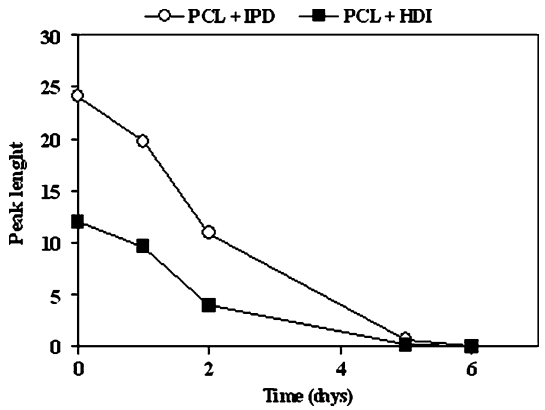


Chart 2 Evolution of the peak length corresponding to the NCO free groups

performed in water saturated conditions, the NCO groups present themselves as being stable enough to be kept in storage conditions as long as humidity is avoided until its application is pretended and also during the surgeon manipulation.

When considering the application conditions, it is important to keep in mind that water will be present in the living tissues. However it is known that the isocyanate reactivity with water and primary hydroxyl group is comparable, but still much smaller than with amines [28], (Table 1).

Based on this statement, it is expected that the reaction between NCO and amino groups in the living tissues occurs much faster than the one between NCO and water although it may be unavoidable that some does happen.

Reaction with aminated substrates

The binding capacity of the adhesives was determined by placing them between gelatine sheets. These were then subjected to the binding strength test which terminated either with the fracture of the gelatine sheets or their separation if adhesion failed to occur. A sample of gelatine

Table 1 Relative reactivity of isocyanate groups with hydrogenated compounds

Hydrogenated compound	General structure	Relative reaction rate (at 25 °C and without catalyst)
Primary aliphatic amine	R–NH_2	100,000
Secondary aliphatic amine	RR′NH	20,000–50,000
Primary hydroxyl	RCH_2–OH	100
Water	HOH	100
Secondary hydroxyl	RR′CH–OH	30
Tertiary hydroxyl	RR′R″C–OH	0.5
Amide	RCO–NH_2	0.1

alone was also tested and acted as the control. The values of the maximum force were registered and are presented in Chart 3.

The results obtained with this test proved to be different for the two urethanes. The PCL + IPD polymer was able to bind the two gelatine sheets together since the break occurred in one of the gelatine pieces without compromising the glued section. In fact, the break occurred with almost no elongation and the maximum peak force obtained corresponds to the force necessary to fracture the gelatine sheet itself (control).

The urethane PCL + HDI, however, failed in establishing the adhesion necessary to bind the aminated substrates together and when the binding strength test was performed they were easily separated by the section containing the polymer.

As already reported on literature [28], the reaction of isocyanate groups with amines, forming urea linkages, is very fast and does not require catalysis. However, as stated before, the isocyanate structure is important for the NCO group reactivity.

The ATR–FTIR technique had previously proved that the NCO content was not the same in both urethanes. In fact, the pre-polymer PCL + HDI presented a smaller peak associated to these functional groups. For this reason it is possible to suggest that the concentration of NCO free groups in this polymer was not sufficient to establish adhesion between the two gelatine sheets.

Determination of surface energy by contact angle measurement

For any adhesive to adhere to a substrate, one fundamental thermodynamic requirement has to be satisfied first: the measured surface energy of the adhesive must be equal to or less than that of the adherent. Unless this condition is satisfied, a material cannot adhere to the substrate [32].

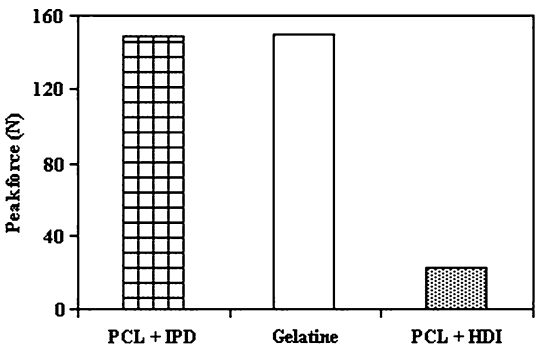


Chart 3 Values of peak force for the gelatine glued with the urethanes and for the gelatine alone

The main purpose of this measurement was to evaluate how well the adhesive would spread when placed over the aminated substrate, over skin itself and over a bleeding surface. For skin, the critical surface energy varies between 38 and 56 mN/m depending on the temperature and relative humidity of the skin [32]. The surface tension of blood assessed in a group of 150 healthy people (72 men and 78 women aged from 20 to 65 years) by the drop-weight method at a temperature of 22 °C was 63,8 mN/m, [33].

In order to evaluate this parameter, surface tensions of the urethanes and of the gelatine sheets were evaluated, by static contact angle (θ) measurements with four liquids: water, formamide, ethylene glycol and propylene glycol. According to the Owens–Wendt–Rabel and Kaelble relationship, the dispersive σ_S^D and polar σ_S^P components of the urethanes as well as the ones of the gelatine sheets were determined. The obtained results as well as the ones for skin and blood are presented in Table 2.

The obtained results showed that the surface energies of gelatine, skin and blood are much higher than the ones of the urethanes. This means that the forces between the molecules that make up the urethanes are weak and consequently adhesion between them and any of these surfaces is therefore likely to happen. The adhesive forces between any of these surfaces and the urethanes will overcome the cohesive forces of these prepolymers and intermolecular proximity between the adhesives and the surface will, by this reason, occur.

Haemocompatibility

Thrombogenicity

The capacity of thrombus formation of the urethanes was evaluated by a gravimetric method. The weights of the blood clots formed after 45 min are presented in Chart 4.

It was observed that clot formation was rather high for both urethanes, even higher than the control. Considering that glass, which constituted the positive control is considered a highly thrombogenic material, it can be concluded that all our polyurethanes are also classified as

Table 2 Surface energy values and correspondent dispersive and polar components

Substrate	Surface energies and surface tensions (mN/m)		
	σ_S	σ_S^D	σ_S^P
Gelatine	44.24	5.00	39.24
Skin [31]	38–56	–	–
Blood [32]	63.80	–	–
PCL + IPD	28.54	16.06	12.48
PCL + HDI	26.85	14.73	12.11

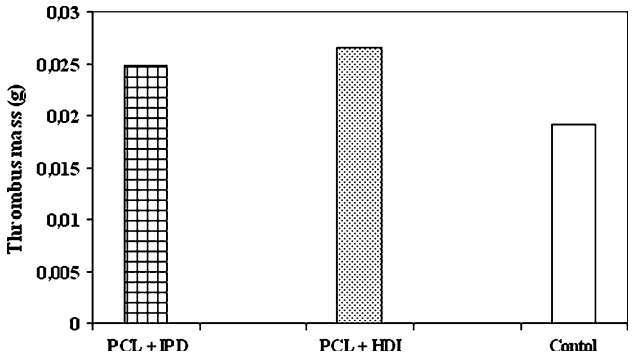


Chart 4 Weight of thrombus formed after 45 min

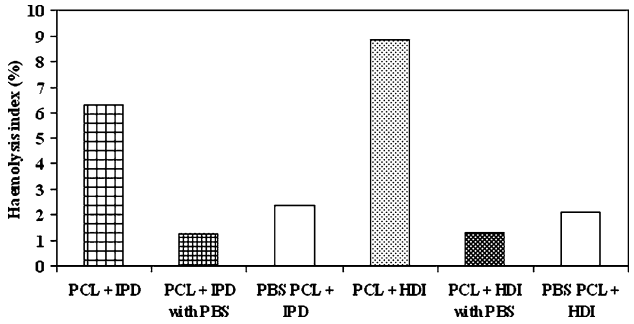


Chart 5 Values of haemolysis of the urethanes not subjected to extraction, the urethanes incubated in PBS and of the PBS in contact with them

thrombogenic. This characteristic is directly related to the low surface energy presented by the urethanes. Surface energy can be understood as a measure of hydrophobicity/hydrophilicity. High surface energy means that the surface is hydrophilic, and low surface energy characterizes the surfaces as hydrophobic. At low surface energy materials, proteins adsorb strongly and irreversibly and at high surface energy materials, proteins adsorb weakly and reversibly [34]. Once the protein adhesion constitutes the first step to initiate the coagulation cascade that ends in thrombus formation [35], it was predictable that the urethanes would present a high thrombogenic character. For the majority of the biomaterials, especially the ones designed to act as implants, this would be an undesirable feature, but considering that the adhesives would be applied in blooding conditions, its haemostatic character is of a great importance. These materials would largely contribute to stop the bleeding, initiating coagulation, and therefore help the cicatrisation of the wound.

Haemolysis

During haemolysis assay, test and control materials were placed in contact with ACD rabbit blood under identical conditions and the increase in released haemoglobin was measured.

The haemolytic index was evaluated for the urethanes contacting directly with blood, urethanes which were kept with PBS (extraction solution) and finally for PBS extraction solution (indirect contact) (Chart 5).

According to ASTM F 756-00 [36] materials can be classified in the categories described in Table 3.

The synthesised urethanes can be classified as haemolytic, when they are not subjected to extraction. When incubated in PBS solution the values of haemolysis became very low and the membranes were no longer haemolytic. These values indicate that the haemolysis was caused by products that were washed away effectively by the PBS and not by the urethanes themselves. This was probably

Table 3 Classification of haemolytic grade of samples according to their % haemolysis

Haemolysis (%) above negative control	Haemolytic grade
0–2	Non-haemolytic
2–5	Slightly haemolytic
> 5	Haemolytic

caused by monomers that did not react during the synthesis and also by the solvent that was used to prepare the adhesives. However it is important to mention that even being haemolytic when not subjected to extraction, the values of haemolysis are not very high and the values presented by the urethanes were lower than 9%.

Haemolysis is regarded as an especially significant screening test, once it provides quantification of small levels of plasma haemoglobin that may not be measurable under in vivo conditions. However, it is not possible to define a universal level of acceptable or unacceptable amounts of haemolysis [26]. Although by definition a blood-compatible material should be non-haemolytic, the truth is that in practice several medical devices cause haemolysis. This means that when such haemolytic effect takes place, it is important to make sure that clinical benefits overcome the risks and that the values of haemolysis are within acceptable limits.

Conclusions

Two different urethanes were synthesised by reacting polycaprolactone diol (PCL) with isophorone diisocyanate or hexamethylene diisocyanate (HDI-isocyanate). The polymers were prepared in a 2/1 ratio of NCO/OH in order to end up with terminal isocyanate groups. The occurrence of the synthesis reaction as well as the presence of NCO free groups on the prepolymers were confirmed by

ATR–FTIR. The urethanes exhibited low swelling ratio, so the effect of damaging the surrounding tissues because of the increase in its volume will not be considerable. Also the NCO groups were found to be stable enough to be kept under storage conditions as long as humidity is avoided. Even in water saturated conditions it took 6 days for the hydrolysis of all the NCO groups occurred.

The PCL + IPD polymer was able to promote adhesion between the aminated substrates since during the binding strength tests the gelatine pieces broke without compromising the glued section. The surface energies of the urethanes were proved to be low, which was consistent with the high thrombosis values induced by them. Considering that the application of the synthesised materials is focused on bleeding conditions, its haemostatic potential will constitute an advantage. When haemolysis tests were performed, it was concluded that although the urethanes that were not subjected to the extraction with PBS solution presented a haemolytic character, when the extraction was performed this haemolytic potential disappeared. This result does not mean, however that this material can not have a clinical application, once as already reported, we have to be certain that clinical benefits overcome the risks and that the values of haemolysis are within acceptable limits.

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