Polyurethane Based Materials for the Production of Biomedical Materials

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Summary: Polyurethanes (PUs) composed by hard and soft segments have been extensively used in the manufacturing of biocompatible prosthesis and medical devices. A broad variety of PUs can be obtained by modifying the balance between both segments. In the present work, different basically-flexible PUs have been prepared by employing different combinations of aliphatic hexamethylene diisocyanate, poly(ethylene glycol) (Mw 400 Da), poly(ε-caprolactone) diol (Mw 530 Da), and 1,4-butanediol. Thermal analysis of the synthesized PUs demonstrated high thermal stability and the assumption of glassy state well below room temperature, in agreement with their marked flexibility. Morphological characterization of PUs films indicated that films prepared by spin coating were smoother and more homogeneous than those obtained by casting. Biological assays performed by using 3T3/BALB-C mouse embryo fibroblast cell line confirmed the absence of toxicity and hence the biocompatibility of PU-films.

Keywords: block copolymer; hexamethylene diisocyanate; poly(ε-caprolactone) diol; poly(ethylene glycol); polyurethane

Introduction

The commercial development of polyurethanes dates back to 1937 when O. Bayer observed that the reaction between diisocyanates and glycols resulted in the formation of polyurethanes, susceptible to be converted to plastic and fiber items.

Diisocyanates and diols or polyols are the main raw materials employed in the preparations of PUs. The most common reactants used in the preparation of PUs comprise:[1]

- Diols or polyols: aliphatic diols (glycols), 1,4-butanediol being generally preferred.
- Diisocyanates: toluene diisocyanate (TDI); diphenylmethane diisocyanate (MDI); naphthylene 1,5- diisocyanate (NDI), and hexamethylene diisocyanate (HMDI).
- Polyethers: propylene oxide derivatives, such as glycol-initiated poly(propylene oxide) and

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block copolymers of propylene oxide-ethylene oxide (poloxamers); poly(oxytetramethylene) glycols.

- Polyesters: dihydroxy end-capped polyesters based on carboxylic diacids (adipic or sebatic) and either excess glycol (ethylene or diethylene glycols) or polyhydric alcohol (glycerol or trimethylolpropane).

In order to control the sequence of monomeric units in segmented linear polyurethanes, diisocyanate-terminated pre-polymers are usually prepared in a former stage of reaction; these products are successively reacted with a chain extender of choice, as sketched in Figure 1.

In the biomedical area, the use of PUs exceeds that of other polymeric materials including natural rubber, polyethylene, PVC, fluoropolymers and silicones, by virtue of the various options they offer to mimic the behavior of different tissues.

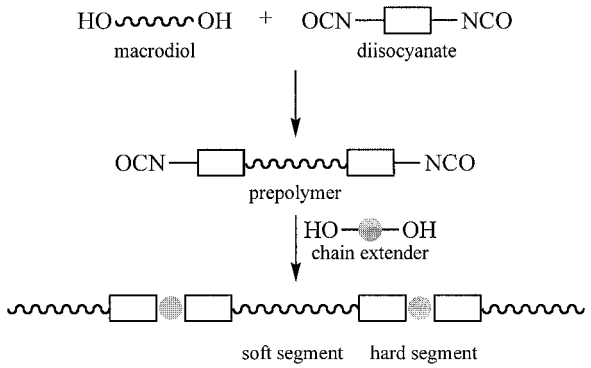

Fig. 1. Schematic representation of preparation of segmented linear polyuretanes.

Following our interest in the preparation of polymeric systems for the targeted delivery of proteic drugs and for tissue engineering applications,[2-7] in the present paper we report on the synthesis and characterization of semiflexible polyurethanes as attainable by reaction of a chain extender with diisocyanate-terminated prepolymers, consisting of polyether or polyester soft blocks coupled with hexamethylene segments via urethane moieties. Indeed, PUs based on aromatic diisocyanates are difficult to be metabolized and, when decomposed may produce aromatic amines, which are known be carcinogenic as documented in various applications.[8] On the other hand, it has been proved that aliphatic diisocyanates have lower reactivity than

aromatic ones and a catalyst is necessary to speed up the reaction with polyols or water. However, catalysts as amines or organometallic compounds may be highly toxic. Therefore, in the present work it was preferred to perform the polymerization reaction of the aliphatic hexamethylene diisocyanate [9] with oligomeric aliphatic diols under catalyst-free conditions. In view of future application of the synthesized PUs in the medical field, their *in vitro* biocompatibility was investigated by cell adhesion and proliferation tests by using the 3T3/BALB-C mouse embryo fibroblast cell line.

Experimental

Materials

The reagents used in the preparation of segmented urethanes were aliphatic hexamethylene diisocyanate (HMDI, Aldrich), poly(ethylene glycol) with molecular weight of 400 Da (PEG400, Fluka), poly(ε-caprolactone) diol with molecular weight of 530 Da (PCL530, Fluka), and 1,4-butanediol (BD, Fluka). HMDI was used without further purification. PEG400 and PCL530 were freed of moisture by azeotropic distillation with benzene prior to use. BD was distilled under vacuum.

Procedure of Polymer Synthesis

A given amount of HMDI was introduced into a flask equipped with stirrer, thermometer, dropping funnel, gas inlet and condenser connected to a drying tube. The system was purged with nitrogen and heated at 80 °C then a polyol was slowly added into the flask. The resulting mixture was stirred for 5 h at 80 °C under nitrogen flow, until the reaction between hydroxyl and isocyanate groups was completed. The reaction products were dried under vacuum at 40 °C for 24 h. The resulted products were used as pre-polymers, which were reacted with a chain extender selected between 1,4-butanediol and poly(ε-caprolactone) diol. Typically, the latter reactions were carried out in the melt, at 80 °C for 18 h, under dry nitrogen flow.

Preparation of Polymer Films

The films of synthesized PUs were prepared by solvent-casting or by spin coating of 0.5 and 2.5% (w/v) chloroform solutions on glass plates having 16 mm diameter. The resulting films were dried overnight at room temperature.

Polymer Characterization

FT–IR spectra were recorded on polymer films with a 410 Jasco spectrophotometer. ^{1}H-NMR spectra were recorded on 5-10% sample solutions in perdeuterated solvents with a Varian Gemini 200 spectrometer operating at 200 MHz. Differential scanning calorimetry analyses were performed between –110 and 160 °C at 10 °C/min heating and cooling rate with Mettler TC11 TA/DSC–30. Glass transition temperatures were recorded during the second heating cycle. Thermal gravimetric analyses were performed between 30 and 600 °C at 10 °C/min heating rate under nitrogen flow with a Mettler TG50 thermobalance. Scanning Electron microscopy was performed on polymer films with a JEOL-LSM5600LV microscope.

Biological Assays[10]

Cell adhesion and proliferation assays were carried out using the 3T3/BALB-C mouse embryo fibroblast cell line. Cells were grown in Dulbecco's modified eagles medium (DMEM) containing 10% of fetal bovine serum (FBS), 2 mM of glutamine, 100 U/mL of penicillin, and 100 μg/mL of streptomycin (complete DMEM).

Results and Discussion

Synthesis and Characterization of PU Materials

All polyurethanes were synthesized in bulk, except for polymers containing PCL segments, which were prepared in chloroform solution. A two-step process was used for the PU synthesis: in the first step pre-polymers were prepared by reacting hexamethylene diisocyanate (HMDI) with either poly(ethylene glycol) (PEG400) or poly(ε-caprolactone) (PCL530) at diisocyanate/diol ratios included between 0.7 and 2.0. Then, 1,4-butanediol (BD) or PCL530 were used as chain extenders. The pre-polymers based on PEG-diol and PCL-diol, were identified as HG and HC, respectively. ^{1}H-NMR spectra of PCL-containing pre-polymers presented signals related to –C**H$_2$**NCO groups from HMDI and CH$_2$C**H$_2$**O groups from PEG-diol or PCL–diol. A FT-IR band at 2275 cm^{-1} typical of the stretching of terminal-isocyanate groups was also detected.

The obtained pre-polymers were submitted to TGA and DSC thermal analyses. Temperatures corresponding to 5% weight loss (T_d) and the various decomposition steps (T_{d1}- T_{d3}), the

corresponding weight losses (Δ_{w1} - Δ_{w3}) and the residue at 590 °C (MR_{590}) are reported in Table 1. The glass transition (T_g), melting (T_m), and crystallization (T_c) temperatures are summarized in Table 2. The feed composition and the yield of the extension reactions of diisocyanate–terminated prepolymers are collected in Table 3.

The FT-IR spectra recorded on the diisocyanate terminated prepolymers and the corresponding polyurethanes as obtained after reaction with chain extenders are compared in Figure 2. In all spectra, a strong band at 1685–1720 cm^{-1} attributable to the stretching of urethane and ester carbonyl groups was present; the amide II band characteristic of the urethane functionalities was detected at 1530 cm^{-1}. The typical stretching absorption of isocyanate groups at 2270 cm^{-1} was present in the pre-polymer spectra whereas it completely disappeared in the extended PU spectra.

Table 1. TGA analysis of HG and HC pre-polymers.

Sample	T_d	T_{d1}	T_{d2}	T_{d3}	Δw_1	Δw_2	Δw_3	MR_{590}
	°C	°C	°C	°C	%	%	%	%
HG	297	209	385	473	8.5	81.8	7.6	2.0
HC	284	193	382	469	5.5	83.5	11.0	0

Table 2. DSC analysis of HG and HC pre-polymers.

Pre-polymer	T_g	T_m	T_c
	°C	°C	°C
HG	-58	–	–
HC	-63	17	-24

Table 3. Reaction of diisocyanate terminated prepolymers with diol chain extenders.

Run	Pre-polymer		BD	PCL530	NCO/OH	Duration	Yield
	type	mmol	mmol	mmol	mol/mol	h	wt-%
HGBu1	HG	3	3	–	1.0	21	68
HGBu2	HG	4	2	–	2.0	18	74
HGC1.2	HG	50	–	43	1.2	18	53
HGC1.9	HG	46	–	24	1.9	18	76
HCBu0.7	HC	3	4	–	0.7	72	61
HCBu1	HC	4	4	–	1.0	18	nd
HCBu2	HC	4	2	–	2.0	18	74

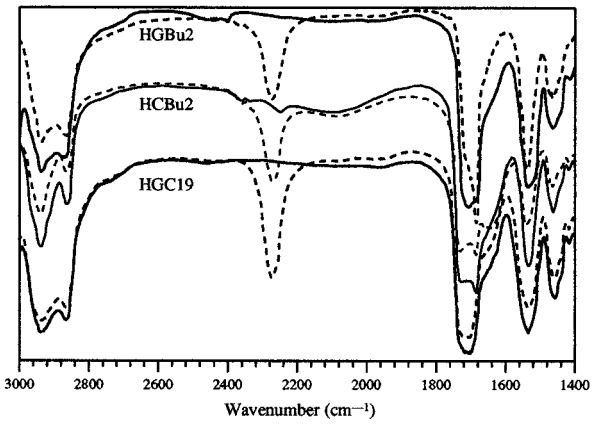

Fig. 2. FT-IR spectra in the interval between 3000 and 1400 cm^{-1} of diisocyanate-terminated pre-polymers (---) and extended PU-polymers (—).

^{1}H-NMR spectra further substantiate the information previously achieved by FT-IR analysis. A typical ^{1}H-NMR spectrum is shown in Figure 3.

TGA analysis of the synthesized polyurethanes, as performed under nitrogen atmosphere in the 30–600 °C temperature interval, provided the information collected in Table 4. Independent of the starting NCO/OH ratio, the T_d of all polymers in the same series resulted almost identical. Only HCBu1 presented slightly larger T_d showing a different decomposition curve, probably because of some crosslinking reaction. In all cases, the residue at 590 °C (MR_{590}) was lower than 5 %, suggesting that the degradation of the polymers is complete under oxygen-free conditions. By comparing the TGA data reported in Table 1 for the prepolymers with those reported in Table 4, a greater stability of the former with respect to the relevant extended systems can be stated. It looks like that the end-capping isocyanate groups some how prevent the thermal decomposition by unzipping process.

DSC measurements were performed in three heating cycles from –110 to 160 °C at 10 °C/min scanning rate, with 100 °C/min quenching between the 1st and 2nd cycle and 10 °C/min cooling between the 2nd and the 3rd cycle. The information collected in the second cooling (T_c) and in the third heating cycle are reported in Table 5.

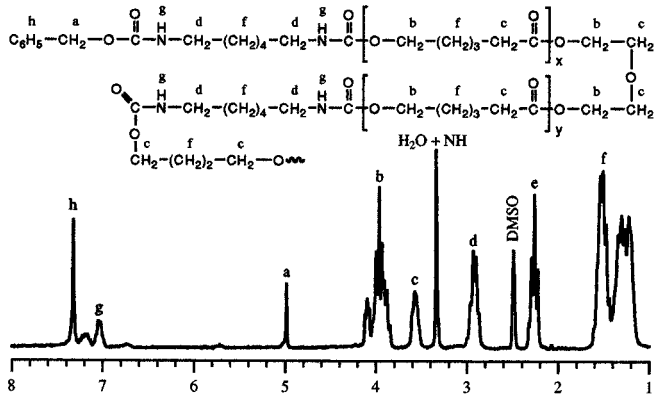

Fig. 3. ^{1}H-NMR spectrum of HCBu2 polymer sample.

Table 4. Thermogravimetric data of the prepared PUs.

Sample	T_d	T_{d1}	T_{d2}	Δw_1	Δw_2	Δw_3	MR_{590}
	°C	°C	°C	%	%	%	%
HGBu 1	282	371	–	1	90	7	2
HGBu 2	282	385	462	1	92	5	2
HGC 1.2	301	354	459	57	32	10	1
HGC 1.9	294	378	–	–	92	7	1
HCBu 0.7	263	361	455	–	96	3	1
HCBu 1	313	380	452	4	71	23	2
HCBu 2	269	366	462	–	97	3	0

All polymer samples revealed DSC traces with only glass and endothermic transitions. HGC1.9 and HCBu0.7 showed also a cold crystallization peak (T_{cc}) at –8 °C, indicating that these samples probably require longer times for reorganization. The HGC1.2 sample showed only glass transitions and resulted completely amorphous. The glass transition (T_{g1}) present at about –95 (HG series) and –45 °C (HC series) can be attributed to soft segments whereas the higher temperature glass transition (T_{g2}) can be associated with polyurethane hard segments. The endothermic transitions comprised between 9 and 23 °C (T_{m1}) were associated to the melting of crystalline zones from soft segments, while the transitions between 66 and 112 °C (T_{m2}) were

attributed to the melting and rearrangement of hard domains and to the dissociation of the diverse types of hydrogen bonds.

Table 5. DSC data of synthesized polyurethanes.

Sample	T_{g1}	T_{g2}	T_{m1}	T_{m2}	T_{cc}	T_c
	°C	°C	°C	°C	°C	°C
HGBu1	–93	–32	–	112	–	92
HGBu2	–93	–34	9	109	–	96
HGC1,2	–93	–37	–	–	–	–
HGC1,9	–95	–39	20	–	–8	9
HCBu0,7	–47	–	23	66	–8	–8
HCBu1	–48	–40	–	91	–	73
HCBu2	–38	12	–	112	–	98

Biological Assays

Biocompatibility tests were performed by recording the adhesion and spreading of mouse embryo fibroblast cells (3T3/BALB-C) on polyurethane films in order to evaluate the polymer citotoxicity. The films, as obtained by either casting or spin coating of chloroform solutions were carefully dried, incubated in PBS solution, and then UV sterilized for 10-15 min. Morphological analyses of PU-films were carried out by scanning electron microscopy (SEM). Samples prepared by spin coating were homogeneous and smooth, whereas those prepared by casting appeared much rougher (Figure 4), very likely because of the different solvent evaporation rate. Cells were seeded at appropriate density on polymer films and their adhesion was checked two hours later. The culture medium was then removed, thus getting rid also of not-adhered cells and replaced with fresh complete DMEM incubation medium. The recorded images (Figure 5) showed that the cells adhered and spread safe, thus indicating the good compatibility of the polymer matrices.

According to the results of the adopted protocol, the synthesized polyurethanes can be classified as biocompatible and hence suited for further exploitation as matrices for the formulation of bioerodible/biodegradable nanoparticle systems and scaffolds for tissue engineering applications. In this perspective, PEG-based PUs will be used for the preparation of proteic drug-loaded nanoparticles by the emulsion-evaporation technique, whereas those containing PCL will be tested in preparation of biodegradable surgical adhesives, according literature

indications. [8,9, 11-13] Microstructured films of both PUs types will be tested *in vitro* as substrates for cell adhesion and proliferation.[6]

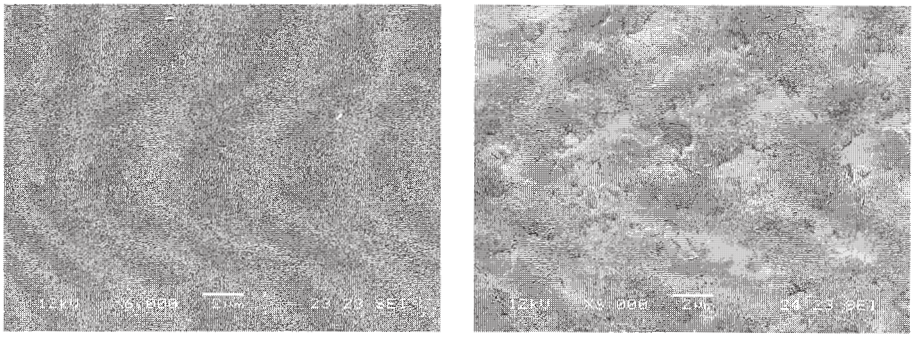

Fig. 4. SEM photomicrographs of HCBu 0.7 films obtained by spin-coating (left) and by casting (right) methods.

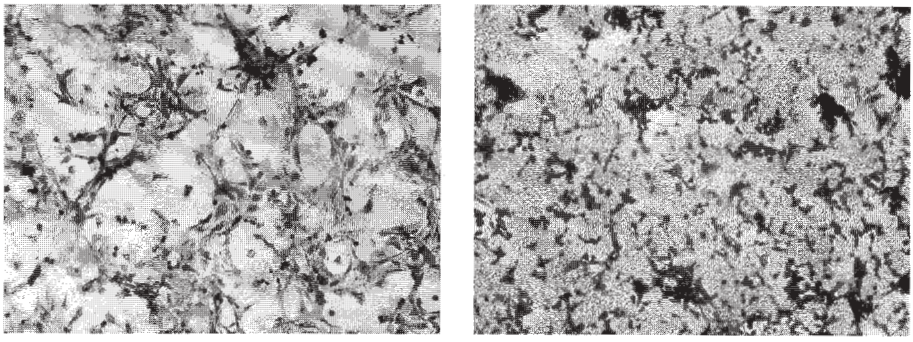

Fig. 5. Optical micrographs of 3T3 cells grown on HCBu0,7 films obtained by spin coating (left) and by casting (right).

Conclusions

The reported results indicate that flexible segmented polyurethanes (PUs) containing soft polyether or polyester segments and hard semiflexible hexamethylene segments can be conveniently prepared by using 1,4-butanediol or di-hydroxy terminated poly(ε-caprolactone) as chain extender. By taking into account their solubility, melting temperature, and rather high thermal stability these materials appear promising for the processing from the melt. *In vitro*

biological assays demonstrated the biocompatibility of the prepared PU samples. This last information and the rather low glass transition temperature indicate that the synthesized polyurethanes can be conveniently tested as biocompatible materials for the design of devices of pharmaceutical and biomedical interest.

Acknowledgments

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