Materials Science & Engineering C 93 (2018) 483–494

Contents lists available at ScienceDirect

Materials Science & Engineering C

journal homepage: www.elsevier.com/locate/msec

Study of chemical, physico-mechanical and biological properties of 4,4′-methylenebis(cyclohexyl isocyanate)-based polyurethane films

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A R T I C L E I N F O

Keywords:
Polyurethane films
H_{12}MDI
Chemical structure
Thermal properties
Surface properties
Cytotoxicity

A B S T R A C T

Polyurethane films were obtained in the solvent-free cycloaliphatic polyaddition process of 4,4′-methylenebis (cyclohexyl isocyanate), poly(ε-caprolactone) diol or poly(oxytetramethylene) glycol and 1,4-butanediol. Chemical structures of the polymers were confirmed by FTIR, NMR and GPC methods. Their surface, thermal and mechanical properties have been evaluated. Results of biological studies with polyurethane films as potential biomaterials for medical applications revealed their mild cytotoxicity against normal human fibroblasts (BJ) and immortalized keratinocytes (HaCaT).

Statement of significance: The research is relevant for the potential uses of polyurethane films made from commercial raw materials as general medical supplies.

1. Introduction

Polyurethanes (PUs) are traditionally used as foams, elastomers, films, coatings, adhesives and varnishes, and in recent years they have also been used as biomaterials [1,2]. This is due to their good mechanical and surface properties, hydrolytic resistance to bacterial enzymes, low cytotoxicity and good hemocompatibility [3]. These features are essential for PU applications as orthopedic scaffolds, coatings used to regenerate soft tissues and vascular prosthetics, and nerve regeneration [4,5]. Nevertheless, technical application of polyurethanes, where cytotoxicity issues are not crucial is also known. Examples include medical equipment requiring sterilization, protective coatings of medical devices, polyurethane coatings coming into contact with food and controlled dosing drugs [6–8].

It is already known, that the type of isocyanate raw material from which PUs are produced is crucial for tissue biocompatibility, due to toxic action of amine derivatives of isocyanates formed even in trace amounts during their spontaneous hydrolysis in the biological environment. Therefore, despite the poorer mechanical properties, PUs obtained from aliphatic diisocyanates such as HDI, H12MDI or TMDI are more favorable in biomedical applications than widely used PUs synthesized from the most popular aromatic diisocyanates as MDI and TDI [9,10].

For this reason, in recent years, polyurethane biomaterials have been produced using L-lysine diisocyanate, whose amine precursor and potential product of hydrolytic degradation is also important amino acid for protein biosynthesis [11,12]. However, poly (ε-caprolactone) diols are particularly important among many polyol raw materials. The reduced hydrolytic stability typical for polyester PUs is here compensated by the hydrophobic properties, resulting from the presence of numerous $-(CH_2)_5-$ groups [13].

PU synthesis conditions also play an important role. In order to reduce the formation of a low molecular weight products, solution polymerization and subsequent precipitation are preferred. The most commonly used bulk polymerization can lead to the formation of PUs with wide molecular weight distributions [14]. In order to separate the high molecular weight PU, it is preferred to its dissolve and isolate the high molecular weight fraction by precipitation from the solution a suitable non-solvent and isolation by centrifugation. It is necessary to remove small molecule substances formed in this process, which may have additionally toxic effects on the human body. For this reason, it is necessary to control the distribution of molecular weight.

The aim of this study was to evaluate the cytotoxicity of PUs with potential biomedical application, synthesized using diisocyanate H_{12}MDI, poly(ε-caprolactone) diol and/or poly(oxytetramethylene) glycol. PUs play an important role in many medical applications and their cytotoxicity must be examined in relation to their specific chemical composition and method of synthesis.

In this work we focused on determining the degree of toxicity of PUs against two different types of human skin cells - fibroblasts and

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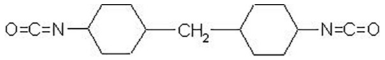
https://doi.org/10.1016/j.msec.2018.07.082
Received 20 November 2017; Received in revised form 5 July 2018; Accepted 30 July 2018

keratinocytes, which are considered as the one of most proper cell lines to this kind of tests. The obtained results can help to create new biocompatible polyurethane dressings.

2. Reagents and methods

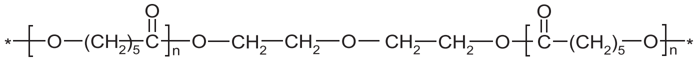
4,4′-Methylenebis(cyclohexyl isocyanate) ($H_{12}MDI$),



(1)

was used as obtained from Aldrich (Due to the high reactivity of this compound, it is not purified prior to use if stored in sealed packs opened immediately before use).

Poly(ε-caprolactone) diol, (PCL), (M_n = 2000) from Aldrich



$$*\!-\!\left[O-(CH_2)_5-\overset{O}{\overset{\|}{C}}\right]_n\!-O-CH_2-CH_2-O-CH_2-CH_2-O-\left[\overset{O}{\overset{\|}{C}}-(CH_2)_5-O\right]_n\!-*\qquad (2)$$

Poly(oxytetramethylene) glycol (PTMO 1000)

$$H\!-\!\left[O-CH_2-CH_2-CH_2-CH_2\right]_n\!-OH \qquad (3)$$

1,4-Butanediol (M = 90) (BD) from Aldrich

The low molecular weight volatiles including water were removed from PCL, PTMO and BD by heating under vacuum, at 120 °C, for 2–4 h.

Tetrahydrofurane (THF) and 1,4-Dioxane (POCh S.A. Poland, purified by distillation) Dibutyl, Dibutyl tin dilaurate, (DBTL) from Huntsman Performance Chemicals (used in very small quantities as catalyst of the polyaddition without purification).

Analytical reagents: dibutyloamine (Aldrich), HCl 0.1 m (POCh S.A. Poland), diiodomethane, formamide (Aldrich), Baxter physiological saline (used in purchased form) and redistilled water.

Normal human fibroblasts (ATCC BJ), Eagle's minimum essential medium (EMEM), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin solution were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Human immortalized keratinocytes were purchased from Cell Lines Service (CLS, Germany). Trypsin-EDTA solution, phosphate-buffered saline (PBS) with and without magnesium and calcium ions, 0.4% trypan blue solution and crystal violet (CV) (4-[(4-dimethylaminophenyl)-phenylmethyl]-*N*,*N*-dimethyl-aniline) were provided by Sigma–Aldrich (St Louis, MO, USA). All other cell culture materials were purchased from Corning Incorporated (Corning, NY, USA).

2.1. Cell culture

BJ human normal skin fibroblasts (ATCC, USA), doubling time 1.9 day, were cultured in EMEM supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. HaCaT human immortalized keratinocytes (CLS, Germany) doubling time 24 h, were propagated in a DMEM medium supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin. Cells were cultured at 37 °C in an atmosphere of 5% CO_2 and 95% humidity with growth medium changed every 2–3 days and passaged at 80% confluence, using 0.25% trypsin-0.03% ethylenediaminetetraacetic acid in PBS (calcium- and magnesium-free). Cell morphology was checked under the Nikon TE2000S inverted microscope (Tokyo, Japan) equipped with phase contrast. The number and viability of cells were estimated by the trypan blue exclusion test, using Automatic Cell Counter TC20™ (Bio-Rad Laboratories, Hercules, CA, USA).

2.2. Synthesis of polyurethanes

PU films were synthesized in a 1-stage polyaddition process, in a glass stand composed of: three-necked flask, heating bowl, mechanical agitator, dropping funnel, thermometer, reflux condenser and nitrogen supply nozzle. The quantities of substrates used to obtain PU samples are given in Table 1. Throughout the process the eqimolar ratio of the —NCO/—OH groups was maintained. DBTL catalyst was added to the polyol mixture in 0.1 wt% immediately before the synthesis. The process can be presented by the following reaction:

$$\frac{n}{2}(A_k + A_3) + nB \rightarrow (BA_q)_n-$$

where:

$$A_k = A_1 + A_2$$

$$A_q = \frac{1}{2}n(A_1 + A_2) + \frac{1}{2}nA_3$$

The structures of compounds used in the subsequent reactions are presented in Scheme 1.

After thorough mixing of ingredients at RT, the contents of the dishes were poured on the poly(tetrafluoroethylene) plate and dried in a vacuum drying at 105 °C for 8 h. Then the temperature was lowered to 70 °C and the samples were incubated for another 24 h. After removing from poly(tetrafluoroethylene) plate the obtained films were conditioned in the air for a min. 10 days. It was necessary to cure the PU films. At this time, the polyurethane succumbed additional crosslinking as a result of the reaction of —NCO groups with moisture from environment and obtained the required physicochemical properties.

2.3. Infrared spectroscopy

FT-IR spectra were recorded using a Nicolet iS10 (Thermo Scientific) Fourier transform spectrophotometer equipped with a diamond ATR unit. In all cases 16 scans at a resolution of 4 cm^{-1} were collected, to record the spectra in a range of 4000–650 cm^{-1}.

By measuring the peak area of the hydrogen bonded C═O group at ca. 1694–1712 cm^{-1} from urethanes and of the free urethane C═O group at ca. 1711–1730 cm^{-1} it is possible to estimate the degree of hydrogen bonding in the synthesized PU. The degree of the carbonyl groups participating in hydrogen bonding can be described by the carbonyl hydrogen bonding index R that is given in Eq. (4).

$$R = \frac{C_{bonded} \bullet fbonded}{C_{free} \bullet f_{free}} = \frac{A_{1694-1712}}{A_{1711-1730}} \qquad (4)$$

where C is the concentration, A is the area of absorption peak, and f is the respective extinction coefficient of bonded and free carbonyl groups. The ratio of f_{bonded}/f_{free} was taken as 1 according to Seymour et al. [15] The DPS was calculated using Eq. (5):

$$DPS = \frac{C_{bonded}}{C_{free} + C_{bonded}} = \frac{R}{1 + R} \qquad (5)$$

2.4. NMR spectrometry

^1H and ^{13}C NMR spectra of the obtained PUs were taken with the use of the spectrometer FT NMR Bruker Avance 500^{II}. The samples of