POLYURETHANE-HYDROXYPROPYL CELLULOSE MEMBRANES FOR SUSTAINED RELEASE OF NYSTATIN

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The characteristics of two series of polyurethane (PU) membranes with hydroxypropyl cellulose and butane diol were evaluated for sustained release of drug. The entrapping of the nystatin in a polymer matrix provides an approach to kill bacteria and to prevent infection. The polymers used for this study were synthesized from polycaprolactone diol (PCL) as polyol, 4,4'-diphenyl methane diisocyanate (MDI) or methylene-bis(4-cyclohexylisocyanate) (known as H_{12}MDI) as diisocyanate components and 1,4-butane diol (BD) or hydroxypropyl cellulose (HPC) as chain extender. The polymer-nystatin membranes with BD or HPC were obtained by the phase inversion method. The membranes were characterized by ATR-FTIR, DSC, UV-VIS and contact angle measurements. The sustained release of nystatin depends mainly on the diisocyanate nature of polyurethanes and also on the introduction of hydroxypropyl cellulose in the polyurethane matrix. An *in vitro* technique to determine the release of nystatin in buffer phosphate (pH=7.4) was used. The possibility of application of these polyurethane-hydroxypropyl cellulose membranes with nystatin was shown by biological test.

Keywords: polyurethane membrane, hydroxypropyl cellulose, nystatin, sustained release

INTRODUCTION

Polyurethanes have been extensively used for various biomedical applications, such as heart valves,[1] aortic grafts,[2] cardiac catheters[3] and intra-aortic balloons,[4] due to their good physical properties and good biocompatibility. Besides their conventional applications, the expansion of biodegradable polyurethanes for novel biomedical applications, including ligament reconstruction prostheses,[5] scaffolds,[6–8] controlled release systems of active principles,[9,10] has been investigated extensively.

The release of drugs from polyurethane matrices was gradually developed for therapeutic research and depends on the kind of polymer matrix, drug solubility in the polymer matrix and its interaction with the polymer structure.[11–13] One method to control the release of drug is the use of membranes as release system.[14] The membrane can be microporous or non-porous and have different applications as patches or implants.[15]

Polyurethane membranes are prepared as controlled sustained release delivery systems of antifungal agents incorporated into a polymeric matrix.

By introduction of natural compounds,[16-18] like cellulose and its derivatives, in a synthetic polyurethane matrix, biomaterials for drug delivery can be obtained.[19] Therefore, non-ionic cellulose derivatives, such as hydroxypropyl cellulose (HPC), have been used in controlled release formulations[20,21,12] and as film coatings.[22] The polyurethane-hydroxypropyl cellulose has improved properties, such as specific biodegradability or biostability.[24] On the other hand, the polymers based on cellulose derivatives are frequently used in the construction of medical devices[24] or in the release of drugs, such as nystatin[25] and sertaconazole nitrate,[26] to enhance solubility and therefore its feasibility for local fungal infections.

To deliver drugs to the patient in a controlled rate, making possible an appropriate drug concentration and prolonged effectiveness, new types of matrices with high performance properties were prepared.[27]

Nystatin is an antifungal polyene characterized by a potent broad-spectrum antifungal action, including a wide range of pathogenic and non-pathogenic yeasts and fungi.[11] *Candida albicans*, the most common oral fungal organism, has a greater level of pathogenicity and adherence than other *Candida* species to 65% of healthy adult mouths and is the fourth most commonly encountered nosocomial bloodstream pathogen.[11] Most patients with *Candida* species respond well to the treatment with nystatin.[28]

In this paper, we proposed novel segmented polyurethane based on polycaprolactone diol and HPC, to enhance the solubility of nystatin from the microporous polyurethane membranes and to study the influence of chemical structures in the release of nystatin. The biocompatibility of the polyurethanes based on polycaprolactone has been demonstrated in biomedical applications.[29]

EXPERIMENTAL

Materials

In this study, the following materials were used: polycaprolactone diol (PCL, Aldrich), average M_n~2000; 4,4'-methylene diphenyl diisocyanate (MDI-Fluka); methylene-bis(4-cyclohexylisocyanate) (H_{12}MDI, Aldrich); 1,4-butanediol (BD, Sigma Aldrich); hydroxypropyl cellulose (HPC, Aldrich); dibutyltin dilaurat (DBTL, Fluka); 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. Polyols and chain extender were checked for moisture and if necessary, it was lowered to 0.3%. The other chemicals were used as received, without further purification.

Characterization

FTIR spectra

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 angle measurements

Contact angle measurements were performed 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 were kept constant during the experiment (25 °C and 65%, respectively).

DSC analysis

Differential scanning calorimetry (DSC) measurements were performed by means of a Pyris Diamond (Perkin Elmer) instrument. The samples with a mass of 6-8 mg were placed in aluminum foil pans. DSC curves were recorded in nitrogen atmosphere (20 mL/min flow) with a heating rate of 5 °C/min in the temperature range from −100 to −40 °C. The inflexion point of DSC curve was taken as glass transition temperature (*Tg*). Two runs were performed for each sample. As reference, high purity (98%) indium was used, with a melting temperature of 156.68 °C and melting enthalpy of 28.4 J/g.

UV-VIS spectra

The UV-VIS determinations were performed with a JENWAY type 6505 spectrophotometer (Bibby Scientific Ltd., England).

Synthesis procedure of polyurethane and polyurethane with HPC

Polyurethane and polyurethane with HPC synthesis

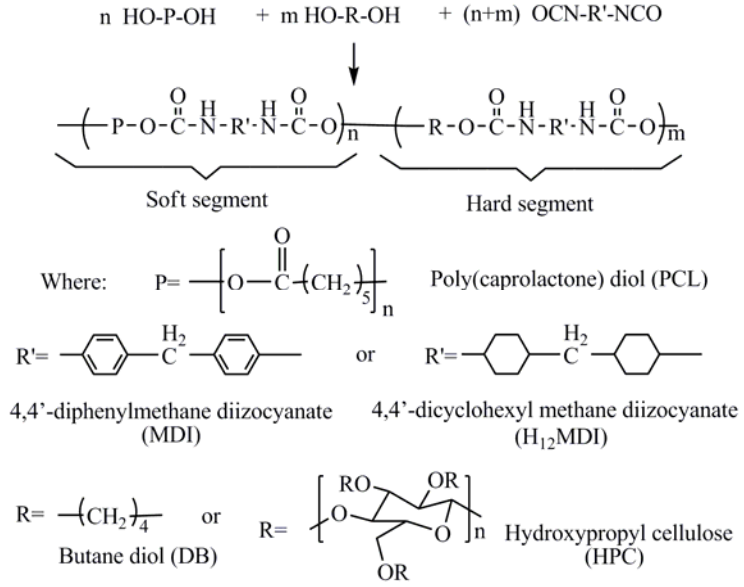
The polyurethanes used in this study were synthesized from PCL as polyol, MDI or H_{12}MDI as diisocyanate component and butane diol (BD) or hydroxy propyl cellulose (HPC) as chain extender, in DMF solution, according to a previously published method.[30] The PCL was dried in vacuum 1 mm Hg at 80 °C for 3 h. The reaction was carried out under stirring with H_{12}MDI/MDI at the temperature of 80 °C for 1 h, and then the DMF solution and BD or HPC were added and the mixture was kept under stirring at 60 °C for 6 h. The polyaddition was stopped with a solution of 10 mL ethyl alcohol:DMF 1:1 (v/v) at the viscosity of ~7000 cP. The synthesis route is shown in Scheme 1.

Preparation of polyurethanes and polyurethanes with HPC and solutions of nystatin

Nystatin (45 mg) was dissolved in 1.5 mL DMF. After complete dissolution of the drug, the homogenous solution was poured into 5 g polyurethane solution (30% w/w PU in DMF with BD or HPC). The solution was stirred vigorously for 3 h and then poured on Petri dishes (Ø=110 mm). In this way, all polyurethane solutions were obtained and were left on Petri dishes for 24 h. Two types of polyurethane solutions with nystatin were obtained.

Preparation of polyurethane membranes with nystatin

Polyurethane solutions with nystatin (Ny) were processed as membranes by using the phase inversion method initiated by precipitation in non-solvent.[31] The deionized water at 45 °C was poured over polyurethane solution with nystatin and kept under these conditions until the membrane separated entirely from the Petri dish. Then the membranes were dried in a vacuum oven at 25 °C and 0.2 mbar to a constant weight. The percentage of nystatin released from the membranes in distilled water was calculated using the slope and the intercept obtained from the standard nystatin curve, in bi-distilled water. The concentrations of the nystatin left in the membranes were the following: 2.7% for PCL-MDI-Ny and 2.9% for PCL-H_{12}MDI-Ny, and respectively 2.6% for PCL-H_{12}MDI-HPC-Ny and 2.8% for PCL-MDI-HPC-Ny.



Scheme 1: Synthesis route for preparation of polyurethane membranes with HPC

RESULTS AND DISCUSSION

ATR-FTIR analysis

Figure 1 and Table 1 present the ATR-FTIR spectra of the synthesized polyurethanes, the polyurethanes with HPC. The absorption bands with peaks at 3328 and 3339 cm^{-1}, respectively, are assigned to ν(N-H) bond stretching, the peaks at 2944, 2929 cm^{-1} and respectively 2858, 2866 cm^{-1} are the asymmetric and symmetric stretching vibration $\nu(CH_2)_{asym}$, $\nu(CH_2)_{sym}$ of the polyurethane.[30] The spectra of the polyurethane samples are characterized by the free carbonyl stretch ν(C=O) at 1729, 1727 cm^{-1} and bonded carbonyl stretch ν(C=O) at 1701 cm^{-1}, the stretching vibration ν(C=C) (MDI aromatic ring) at 1596 cm^{-1} and also by the band amide II at 1529, 1526 cm^{-1} respectively, consisting mainly in the deformation vibrations δ(N-H) associated with the stretching vibrations ν(C-N).[19] The peaks at 1230 and 1221 cm^{-1} are assigned to amide III with the same type of vibrations: the deformations δ(N-H) and the stretching vibrations ν(C-N), respectively. Finally, the peak of CH_2 rocking vibration appears at 781 cm^{-1}.[11]

On the other hand, hydroxypropyl cellulose is a water soluble polymer, which exhibits relatively numerous hydroxyl functional groups active in hydrogen bonding. The ATR-FTIR absorbance of the hydroxypropyl cellulose is related by the stretching vibrations ν (O-H) groups located at 3500 cm^{-1}, by the stretching vibrations ν(C-O-C) groups located at 1100 cm^{-1} and the bands corresponding to aliphatic groups located at 2800-2950 cm^{-1}.[12]

The typical ATR-FTIR absorbance bands in the spectrum of nystatin displays broad intense absorption bands with maxima at 3399 cm^{-1} – due to the stretching vibration of bonded hydrogen; at 2924 and 2824 cm^{-1} – due to the asymmetric and symmetric stretching vibration of CH_2 group, 1720 cm^{-1} – due to the stretching vibration of carbonyl from ester and carboxylic acids, 1403 cm^{-1} – due to the bonding vibration ν(C-H) and at 1064 cm^{-1} – due to the hydroxyl groups from nystatin.[32-34] In the ATR-FTIR of the polyurethanes with HPC (PCL MDI HPC) and polyurethane with HPC and nystatin (PCL MDI HPC Ny) the stretching vibration ν(N-H) peak

migrated from 3328 to 3316 cm^{-1}, which means stronger hydrogen bonded urethanes.

The analysis of the carbonyl absorption region in the polyurethane spectra can provide information related to the formation of the hydrogen bonds.

The relation ratio ($A_{bonded\ C=O}$:$A_{C=O}$) between the area of the bonded carbonyl absorption band to all areas of the carbonyl absorption band (free carbonyl + bonded carbonyl) was calculated in the carbonyl region of 1800-1630 cm^{-1}. The obtained results are presented in Table 2.

Table 1
The main IR bands and their assignment for polyurethanes, polyurethanes with nystatin, polyurethane with HPC and polyurethane with HPC and nystatin

Wave numbers, (cm^{-1})				Assignment	Intensity
PCL-H_{12}MDI/ PCL-H_{12}MDI-Ny	PCL-H_{12}MDI-HPC /PCL-H_{12}MDI-HPC-Ny	PCL-MDI-HPC /PCL-MDI-HPC-Ny	PCL-MDI-HPC /PCL-MDI-HPC-Ny		
3328/3334	3337/3335	3339/3332	3329/3330	ν(N-H), hydrogen-bonded	m,m
2929/2928	2928/2927	2944/2942	2939/2940	$\nu\ (CH_2)_{asym}$	m
2858/2856	2857/2855	2866/2864	2864/2863	$\nu\ (CH_2)_{sym}$	m
1726/1725	1729/1725	1723/1727	1725	ν(C=O) carbonyl free of urethane and polyester chain	vs,s
1700/1701	1730/1728	1700/1702	1729/1728	ν(C=O) hydrogen bonded urethane carbonyl	w,m
-	-	1596/1596	1596/1594	ν(C=C) aromatic ring	m
1526/1527	1526/1528	1529/1530	1528/1529	δ(N-H)+ν(C-N), amide II	s
1452/1451	1452/1453	1464/1413	1465/1414	δ (C-H) scissoring and bending	w
1230/1230	1229/1230	1221/1221	1221/1220	δ(N-H) + ν(C-N), amide III	s
1161/1161	1162/1163	1188/1168	1171/1172	ν(C-O) in ether group	s
1097/1096	1098/1099	1066/1077	1066/1068	ν(C-O-C) from aliphatic ether and ν(OC-O-) ester group	m
781/781	781/782	848.58/771	848/865	CH_2 rocking vibration	w

w, weak; m, medium; s, strong; vs, very strong

Table 2
Area ratio of bands assigned to bonded and total carbonyl groups (free + bonded carbonyl)

Sample	C=O[a] bonded/C=O[b]	Sample	C=O[a] bonded/C=O[b]
PCL-H_{12}MDI	0.182	PCL-H_{12}MDI-HPC	0.196
PCL-H_{12}MDI-Nyst	0.196	PCL-H_{12}MDI-HPC-Nys	0.220
PCL-MDI	0.721	PCL-MDI-HPC	0.821
PCL-MDI-Nyst	0.780	PCL-MDI-HPC-Nyst	0.854

[a] Area (1716-1630 cm^{-1}); [b] Area (1800-1630 cm^{-1})

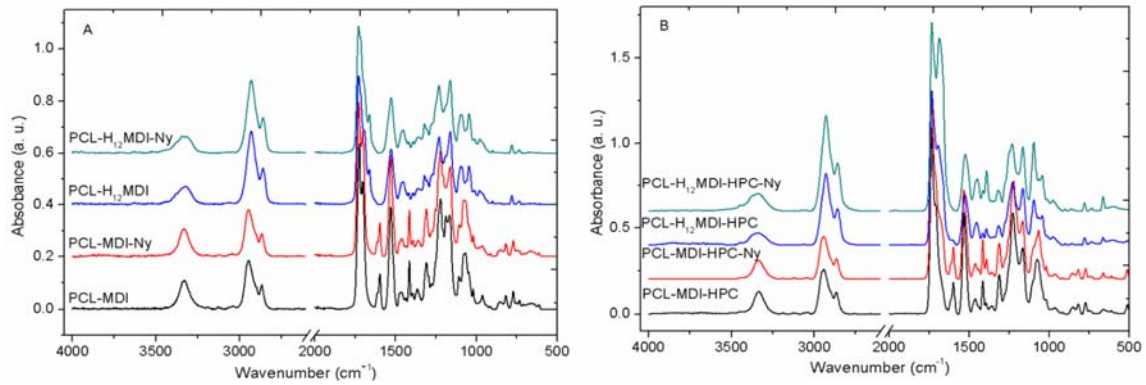


Figure 1: ATR-FTIR of (A) PCL-H_{12}MDI, PCL-MDI, PCL-H_{12}MDI-Ny, PCL-MDI-Ny (B) PCL-H_{12}MDI-HPC, PCL-MDI-HPC-Ny, PCL-H_{12}MDI-HPC-Ny

The changes in the area of the polyurethane structure with nystatin can be attributed to the presence of hydrogen bonds.

DSC analysis

Figure 2 presents the DSC curves of the polyurethanes synthesised with nystatin and the values of glass transition temperature (T_g) for PCL-H_{12}MDI at -53.9 °C and for PCL-MDI at -46.28 °C. In the case of PCL-H_{12}MDI-HPC, T_g is -50.70 °C and for PCL-MDI-HPC, it is -44.44 °C. The glass transition temperature of PCL-MDI and PCL-MDI-HPC is lower by comparison to PCL-H_{12}MDI and PCL-H_{12}MDI-HPC due to the crystalline-amorphous phase separation, which is specific to polyurethanes, when the concentration in urethane groups is higher than 2 mmol/g.[30] The incorporation of the hydroxypropyl cellulose and nystatin into the polyurethane matrix increased the values of the glass transition temperature of polyurethanes. The phenomenon may be attributed to the interactions between hydroxypropyl cellulose and polyurethanes and also between nystatin and synthesised polyurethanes. This is in accordance with the ability of nystatin to form hydrogen bonds, also reported by for other polyurethane systems.[11] The presence of drug shows that nystatin reorganizes the polyurethane matrix.

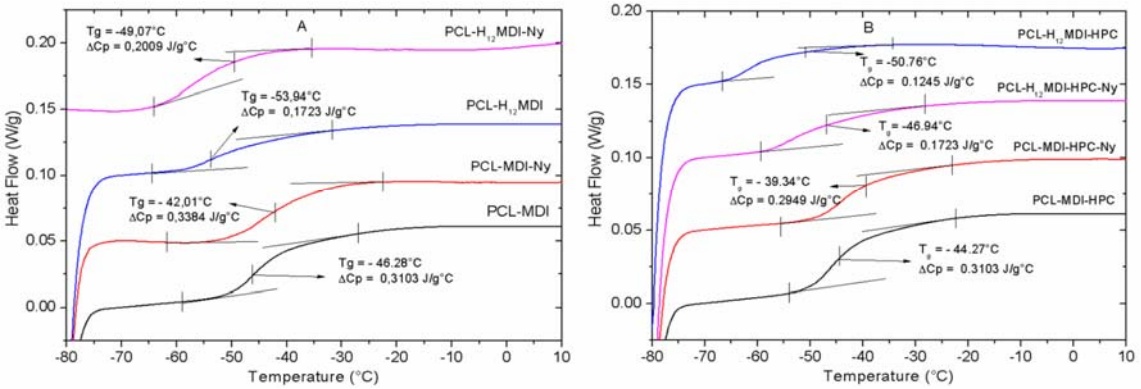


Figure 2: Differential scanning calorimetry for PCL-MDI, PCL-H_{12}MDI, PCL-MDI-Ny, PCL-H_{12}MDI-Ny

Contact angles and surface free energy

Biomaterial is any synthetic or natural material, which in contact with living tissue and biological fluids, in protein utilizations, diagnosis, therapeutic uses and storing of blood, does not affect the biological compounds and the whole living organism.[12] The analysis of the surface properties of polyurethanes is very important in establishing the biocompatibility of polymeric materials.[24] These may be controlled by the nature of the hard segment in the polyurethane structure. The contact angle has different values for each type of polyurethane and is determined by means of the interaction between the three interfaces described by Young's equation:

$$\gamma_{LV}\cos\theta = \gamma_{SV} - \gamma_{SL} \quad (1)$$

where γ_{SV} is the energy of the surface, γ_{SL} is the interfacial tension between the solid and the drop, γ_{LV} is the liquid-vapour surface tension and *cos* θ is the contact angle of the drop with the surface.[35]

Also, according to the method of Owens-Wendt-Rabel and Kaelbe,[35,36] the surface energy (γ_{SV}) results from the polar and disperse interactions:

$$\gamma_{SV} = \gamma_{SV}^{p} + \gamma_{SV}^{d} \quad (2)$$

$$W_a = 2\left(\sqrt{\gamma_{SV}^{d}\gamma_{LV}^{d}} + \sqrt{\gamma_{SV}^{p}\gamma_{LV}^{p}}\right) \quad (3)$$

Polar Coulomb interactions are formed between permanent dipoles, as well as between permanent and induced dipoles. The dispersive interactions are caused by fluctuations in time of the charge distribution within the molecules.[37] A contact angle below 90° indicates that the test liquid readily wets the surfaces, while an angle over 90° shows that the surface will resist wetting.[12]

The values of the contact angle for the polyurethane membranes are presented in Table 3. The hydrophilic character of the polyurethanes (PCL MDI BD and PCL MDI HPC) can be explained by the differences in the chemical structure and in the surface configuration.[38] It is well known that any increasing in the crystallinity of polymer determines an increase in the

hydrophobic character of the polymers.[29] As shown in Table 3, the values for the contact angle decrease gradually along with nystatin concentration, obviously due to the drug introduced. The work of adhesion (W_a) and superficial tension (γ_{SV}) are also presented in Table 3. The increase in the surface energy can be explained by the presence of the functional groups of HPC and nystatin. The value of the contact angle for PCL-MDI is 104° and for PCL-H_{12}MDI – 87.10°. In the case of PCL-H_{12}MD- HPC the value of the contact angle is 79.80° and for PCL MDI-HPC – 86.15°. The values of the contact angle for PCL-MDI, PCL-H_{12}MDI, PCL-H_{12}MD-HPC and PCL MDI-HPC are provided, showing a decrease depending on composition. This phenomenon could explain the higher release rate of nystatin from PCL-H_{12}MDI-HPC, compared to the other polyurethanes synthesised.

***In vitro* release study**

Figure 3 presents the cumulative release of nystatin from the polyurethane matrix in phosphate buffer (pH=7.4) at 37 °C. The release profiles of PCL-H_{12}MDI-Ny, PCL-H_{12}MDI-HPC-Ny and PCL-MDI-Ny, PCL-MDI-HPC-Ny show an initial "burst" followed by a sustained release of nystatin from the polyurethane membranes. The release mainly depends on the type of diisocyanate utilised and also by the introduction of hydroxypropyl cellulose in the polyurethane structure of the polyurethane membranes: it is significantly higher for the PCL-H_{12}MDI-Ny and PCL-H_{12}MDI-HPC-Ny polyurethane than for the PCL-MDI-Ny and PCL-H_{12}MDI-HPC-Ny (Figure 3). The quantities released are higher than the minimum inhibitory concentration (MIC) of *Candida Albicans* (4-6 μg/mL) after 24 h.[34]

Table 3
Water contact angle, work of adhesion, surface free energy and polar and dispersive components of surface free energy measurements

Sample	θ (°)	Wa, mN/m	γ_{SV}, mN/m	γ^p_{SV}, mN/m	γ^d_{SV}, mN/m
PCL-MDI	104.07	55.10	19.95	1.027	18.92
PCL-MDI-Ny	89.43	73.52	18.73	11.41	7.317
PCL-MDI-HPC	86.15	77.68	29.70	4.68	25.09
PCL-MDI-HPC-Ny	82.00	82.92	23.62	16.21	7.40
PCL-H_{12}MDI	87.10	76.48	25.15	6.34	18.80
PCL-H_{12}MDI-Ny	81.33	83.77	26.07	23.79	2.28
PCL-H_{12}MDI-HPC	79.80	85.69	28.29	26.60	1.63
PCL-H_{12}MDI-HPC-Ny	76.00	90.41	29.73	26.41	3.31

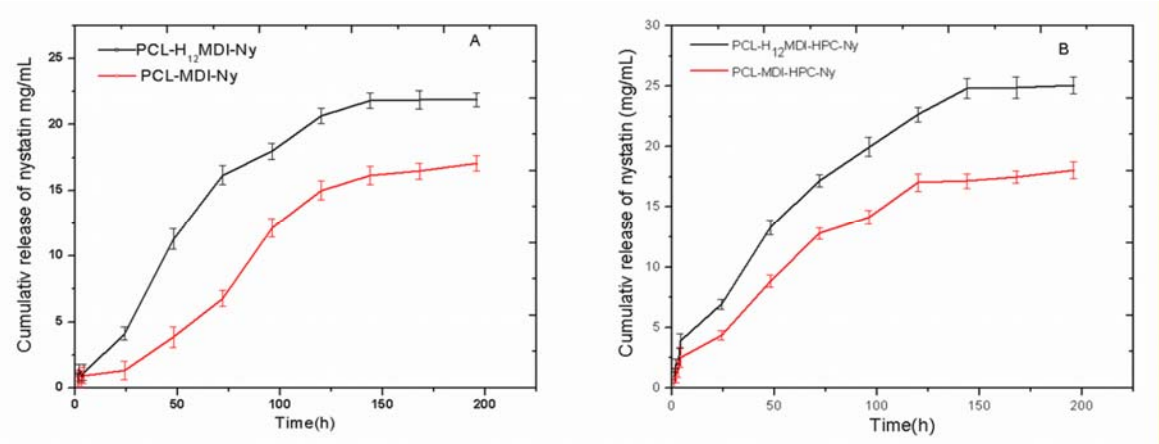


Figure 3: Release curve of (A) PCL-H_{12}MDI-Ny and PCL-MDI-Ny; (B) PCL-H_{12}MDI-HPC-Ny and PCL-MDI-HPC-Ny

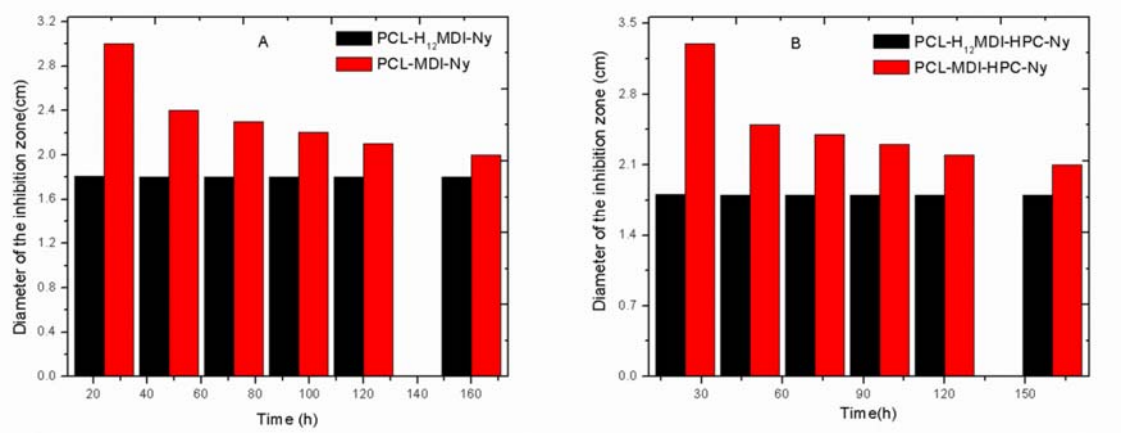


Figure 4: Diameter of the inhibition zone for (A) PCL-H_{12}MDI-Ny and PCL-MDI-Ny; and (B) PCL-H_{12}MDI-HPC-Ny and PCL-MDI-HPC-Ny

Antifungal activity test

The antifungal test was made with *C. Albicans ATCC 90028*, a bacterium that causes skin and catheter-related infections. All materials studied in this work show antifungal activity, with an inhibition zone ranging from 1.9 to 2.5 cm (Figure 4). However, the compounds differ significantly in their activity against the bacteria studied; by increasing the concentration in urethane the inhibition zone of the material decreased. Thus, the variation of the inhibition zone did not increase linearly with the release of the nystatin from the polyurethane membranes, because of the diffusion of nystatin in the agar medium or the sensitivity of the Kirby-Bauer test.[13]

CONCLUSION

The obtained results show that the synthesized polyurethane could be used as sustained release membranes of nystatin. The introduction of the natural polymer into the polyurethane matrix, the hydroxypropyl cellulose water soluble polymer, improves the property of the biomaterials and enhances the release of the drug. The physical interaction between nystatin and PCL-H_{12}MDI-Ny, PCL-MDI-Ny, PCL-H_{12}MDI-HPC-Ny, PCL-MDI-HPC-Ny was confirmed by ATR-FTIR, DSC analysis and contact angle measurements. The experiments show that the drug reorganizes the polyurethane matrix. The nystatin release mainly depends on the type of the hard segment and the introduction of hydroxypropyl cellulose in the polyurethane. It was found that the release from the polyurethane with aliphatic diisocyanate is significantly higher than that from the polyurethane with aromatic diisocyanate.

The nystatin-polyurethane systems could be advantageously adapted in applications in which a sustained release of this antifungal is necessary to prevent or reduce infections. The obtained membranes have a promising potential in matrix drug delivery and in application as wound dressing material.

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