Polymer Bulletin 59, 73–81 (2007)
DOI 10.1007/s00289-007-0744-2

Polymer Bulletin

Water flux, DSC, and cytotoxicity characterization of membranes of cellulose acetate produced from sugar cane bagasse, using PEG 600

Guimes Rodrigues Filho[1] (✉), Leandra Cardoso Toledo[1], Daniel Alves Cerqueira[1], Rosana Maria Nascimento de Assunção[1], Carla da Silva Meireles[1], Harumi Otaguro[2], Sizue Ota Rogero[2], Ademar Benévolo Lugão[2]

[1]Instituto de Química da Universidade Federal de Uberlândia, Av. João Naves de Ávila, 2121 Cx. P. 593 CEP: 38400-902 Uberlândia-Minas Gerais, Brasil
[2]Instituto de Pesquisas Energéticas e Nucleares, Av. Prof. Lineu Prestes, 2242 - Laboratório de Polímeros, Cidade Universitária Cx.P. 11049 CEP 05508-000 - São Paulo, SP – Brasil

E-mail: guimes@ufu.br; Fax: +55 (34) 3239-4208

Received: 27 October 2006 / Revised version: 22 January 2007 / Accepted: 9 February 2007
Published online: 28 February 2007 –

Summary

In this article, cellulose acetate produced through the homogeneous acetylation of sugar cane bagasse cellulose was used to produce membranes, using poly(ethyleneglycol) 600 (PEG 600) as an admixture. The membranes were characterized using water flux measurements (Payne's cup), differential scanning calorimetry (DSC) and neutral red uptake (cytotoxicity). The results showed that PEG 600 acts as a crystallinity inductor and/or pore former in the cellulose acetate matrix. The induction of crystallinity is important for this system since it had not been reported on the literature yet. The results also demonstrated that the studied membranes present a nontoxic behavior.

Keywords

cellulose acetate, membranes, PEG 600, sugar cane bagasse, crystallinity

1. Introduction

According to the literature [1-6], cellulose acetate membranes are used as matrices for systems of controlled release of drugs. Arthanareeswaran et al. showed that, in order to improve the performance of cellulose acetate membranes in ultrafiltration experiments, it is necessary to use poly(ethyleneglycol) 600 (PEG 600) as an admixture. PEG 600, according to those authors, is used as pore former and plasticizer. Furthermore, PEG has been used for many clinical applications due to some of its properties, as for example its hydrophilic character, solubility in water and in organic solvents, and absence of antigenicity and immunogenicity [7]. Another important characteristic of this material, when used for medical purposes, is that PEG of low molecular weight can be excreted through the kidney [6].

So far, the literature concentrates its focus on commercial polymers [1-8], and there is still no study concerning the reuse of sugar cane bagasse (SCB) for producing membranes of cellulose acetate with PEG 600 as an admixture. Furthermore, according to Idris et al. there is not enough material reporting the relationship between the membrane making process and the performance caused by different dialysis membrane development. For example, the effect of additives in the production of dialysis membranes has not been systematically investigated. Thus, the aim of our paper is to elucidate the role of PEG 600 in the production of membranes of cellulose acetate, which was produced using homogeneous acetylation of SCB cellulose, which results in a more biodegradable material than the product of heterogeneous acetylation [9]. Using SCB is justified since Brazil is one of the most important producers of sugar and alcohol from sugar cane, and a huge amount of this residue results from this activity. Besides, we have already been studying the possibility of recycling SCB in order to aggregate value to this residue, as well as looking forward into preserving the environment [10-12].
The produced membranes were characterized using water vapor flux measurements, utilizing Payne's cup technique [11]; and by differential scanning calorimetry, DSC. Through the DSC, we noticed that PEG 600 acts inducing crystallinity on cellulose acetate membranes. A similar phenomenon (induction of crystallinity by PEG) has already been demonstrated for PPDO/PEG blends [7]. Nevertheless this feature had not been reported yet for the system cellulose acetate/PEG 600. The cytotoxicity of the membranes was also evaluated, using the neutral red uptake assay, which showed that neither of the membranes presented cytotoxic effects.

2. Experimental

2.1. SCB Purification [12]

SCB was provided by Usina Alvorada de Açúcar e Álcool, from the 1999 harvest in Araporã-MG. For the purification, 4.0 g of ground dry bagasse was immersed in 76.0 mL of distilled water for 24 h to remove the water-soluble extractives. Then, it was filtered and immersed in 76.0 mL NaOH (0.25 M) for 18 h, after which, this mix was vacuum filtered. Next, bagasse was put in reflux with three successive portions of a mixture containing 20% (v/v) of nitric acid in ethanol, replacing the mixture after each hour. After the reflux, the mixture was filtered and washed with distilled water. The bagasse was dried at 105^{o} C for 3 h, and then ground in a blender.

2.2. Characterization of SCB and purified SCB cellulose

The characterization of SCB and SCB cellulose was performed in accordance to Vieira et al. [13].

2.3. Membrane preparation [10]

Cellulose acetate was produced through homogenous acetylation reaction, in which acetic anhydride, acetic acid and sulfuric acid are used. In order to produce the membranes, it was used cellulose acetate containing a degree of substitution of 2.83. Cellulose acetate was mixed with PEG 600, dissolved in dichloromethane and stirred for 24 hours. The solution was cast onto a glass plate, using a casting knife with a 200 μm gap, and the time of solvent evaporation was 2.5 minutes. In order to detach the membranes from the

glass plates, they were immersed into a bath containing distilled water and ice. The water bath also eliminates the PEG 600 from the membranes. After detaching from the glass plate, the membrane was removed from the bath and dried.

2.4. DSC

DSC experiments were performed in a DSC SP Rheometric Scientific. The scan rate was 10 ºCmin^{-1} and the nitrogen flow was 20 cm^3min^{-1}.

2.5. Water flow

The water flow was measured using the Payne's cup technique in the same conditions described elsewhere [11, 12].

2.6. Cytotoxicity Assay [14]

The cytotoxicity assay was carried out with the exposure of NCTC clone 929 cells to the eluate obtained from the membrane samples, which stayed in contact for 24 h with culture medium MEM (minimum Eagle's medium, Sigma Co., São Paulo, Brazil) at 37ºC. The cell line was acquired from American Type Culture Collection (ATCC) bank. The cytotoxic effect was evaluated using neutral red uptake (NRU), according to Ciapetti et al. (Ciapetti, Granchi, Verri, Savarino, Cavedagna & Pizzoferrato, 1996) and ISO tests [15].

The cells were maintained in MEM containing 10% fetal calf serum and 1% non-essential amino acids (MEM-FFCS) in a humidified incubator with 5% CO_2 at 37ºC. The cells were detached by 0.2% trypsin (Difco, São Paulo, Brazil) and 0.2 mL of the cell suspension, about 2.5 x 10^5 cell/ml, were seeded in flat-tottomed 96 microplate wells (Costar, Cambridge, MA, U.S.A.). The microplate was incubated for 24h at 37ºC in a CO_2 humidified incubator. After this period, the medium was discarded and replaced with 0.2 mL of serially diluted extract of each sample (50, 25, 12.5, 6.25 %). Control of cell culture was replaced with MEM-FCS. In the same assay with samples, a positive control (0.02% Phenol solution) and negative control (nontoxic tin stabilized polyvinyl chloride, Dacarto SA Indústria de Plásticos, Osasco, São Paulo, Brazil) were run. Samples and controls were tested in triplicate. The plate was incubated again for 24 h under the same conditions.

After the incubation period, the medium and extracts were discarded and replaced with 0.2 mL of neutral red solution (50µg/mL) diluted 1:100 in MEM-FCS. After incubation at 37ºC for 3h, the dye medium was discarded and the microplate was washed twice with phosphate buffered saline. The cells were washed with a solution of 1% $CaCl_2$ in 0.5% formaldehyde. The rupture of cells and neutral red release was obtained by addition of 0.2 mL/well of extracting solution containing 50% ethanol in 1% acetic acid. Absorbance was read on an Organon spectrophotometer for microplates with 540 nm filter. Average optical density was calculated after blank subtraction.

3. Results and Discussion

3.1. Klason Lignin, α-cellulose

SCB, used as raw material for obtaining cellulose, was chemically characterized, and its α-cellulose and lignin content were, respectively, 44.9% and 23.8%. After the

purification of sugar cane bagasse, the extracted cellulose has a lignin content of 3.84%.

3.2. DSC

Figure 1 shows a typical first scan thermogram of a cellulose triacetate (CTA) membrane in which we may observe the main features of a cellulose acetate thermogram:
an endotherm due to the loss of water (ΔH_w), having its maximum located around (T_{water}) 115 ºC;
the T_g located around 170 ºC;
the exotherm of crystallization, having its maximum located around 185 ºC and enthalpy (ΔH_c) of 8.00 J/g; and
the endotherm of fusion (ΔH_f), having 19.9 J/g of enthalpy, with two maxima located around (T_f) 216 ºC and 228 ºC representing two distinct kinds of crystals. The crystals melting at the higher temperature are the most perfect ones.
The real enthalpy of fusion (ΔH_r) would be given by the difference between the values of the enthalpies of fusion and crystallization. Thus this value would be approximately 11.9 J/g.

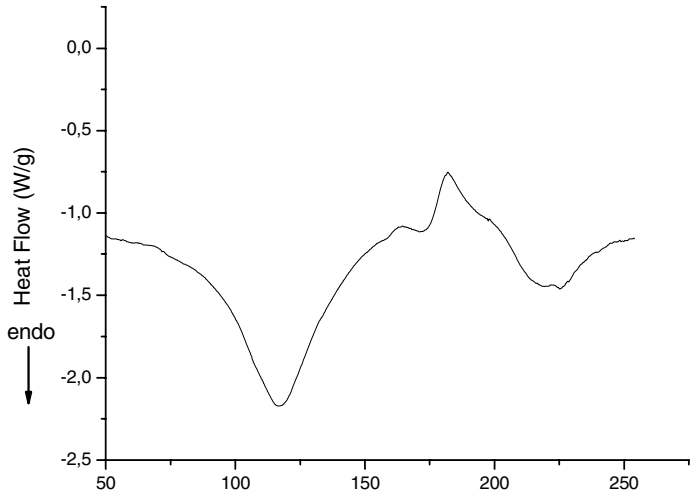

Figure 1. First scan DSC thermogram of CTA membrane.

Figure 2 shows a typical first scan thermogram of a membrane made with 10% PEG 600. Fundamentally, figures 1 and 2 present similar characteristics. However:
in relation to figure 1, there was a significant increase on the values of the enthalpy of crystallization (ΔH_c = 19.6 J/g) and of the enthalpy of fusion(ΔH_f = 32.5 J/g); and the T_f was higher, 284 ºC.
Despite that, the increase on the value of the real enthalpy of fusion (ΔH_r) was not significant, i.e., from 11.9 J/g in figure 1 to 12.9 J/g on figure 2, what indicates that both samples (CTA and CTA10PEG) present basically the same crystallinity. These results can be interpreted in a way to indicate an improvement in the perfection of the

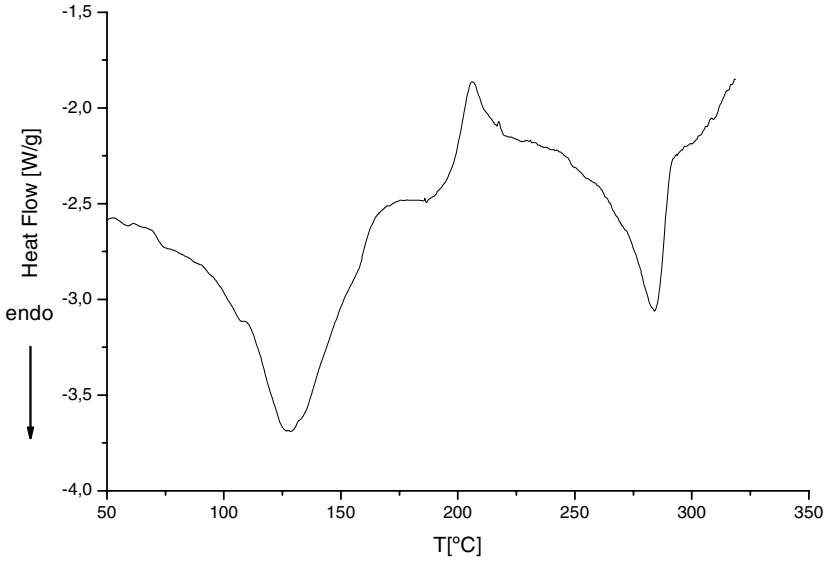

Figure 2. First scan DSC thermogram of CTA membrane made with 10% PEG 600.

crystals, which was induced by the PEG 600 content (10%) that was used to prepare the cellulose acetate membrane. This behavior is similar to that found by Zheng et al. for PPO/PEG blends, since when PEG content was higher than 5%, crystalline behavior of blends had no more significant changes.

Figure 3 presents a superposition of the first scan DSC thermograms of cellulose acetate membranes made with several PEG 600 compositions.

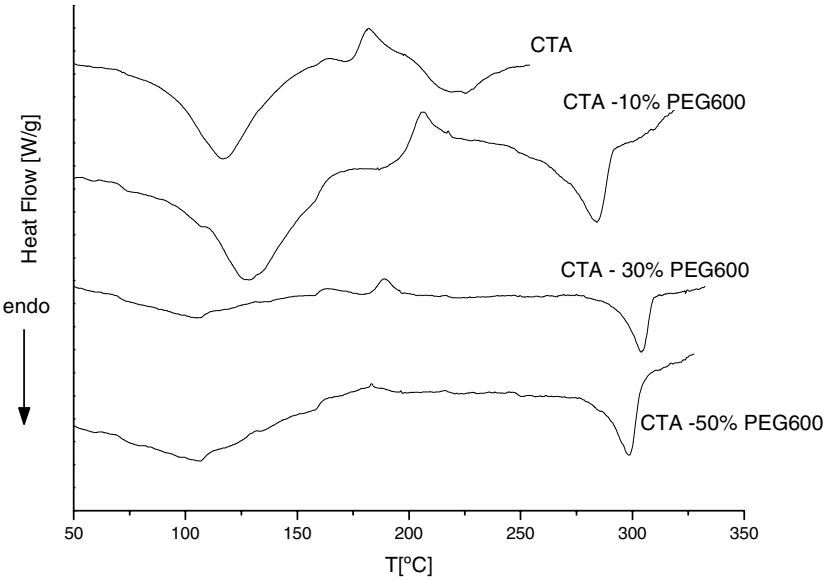

Figure 3. First scan DSC thermogram of CTA membranes made with different PEG 600 content.

The main features of these thermograms are presented on table 1.

Table 1. Values obtained from the DSC thermograms of the CTA membranes made with distinct PEG 600 compositions.

%PEG	T_{water} (ºC)	ΔH_{water} (J/g)	T_c (ºC)	ΔH_c (J/g)	T_f (ºC)	ΔH_f (J/g)	ΔH_r (J/g)
0	116.7	83.99	175.33	8.00	216.13	19.92	11.91
10	126.8	78.92	185.65	19.75	284.07	32.51	12.75
30	74.0	47.48	189.19	4.86	303.95	23.41	18.55
50	80.4	55.83	183.23	3.91	298.27	30.88	26.98

In figure 4 we may observe that the temperature of crystallization (T_c) (a) almost does not change with the increase of PEG 600. However, with 10% PEG 600, the temperature of fusion (b) is near to its maximum value. This result indicates that for membranes containing 10% PEG 600 the produced crystals were already more perfect than those of the original membrane (CTA). This is reinforced by the fact that the enthalpy of crystallization reaches its maximum value also with 10% PEG (c). This result is very important because Idris et al. found that the best performance of membranes produced with PEG as an admixture in dialysis experiments occurs with 10% PEG.

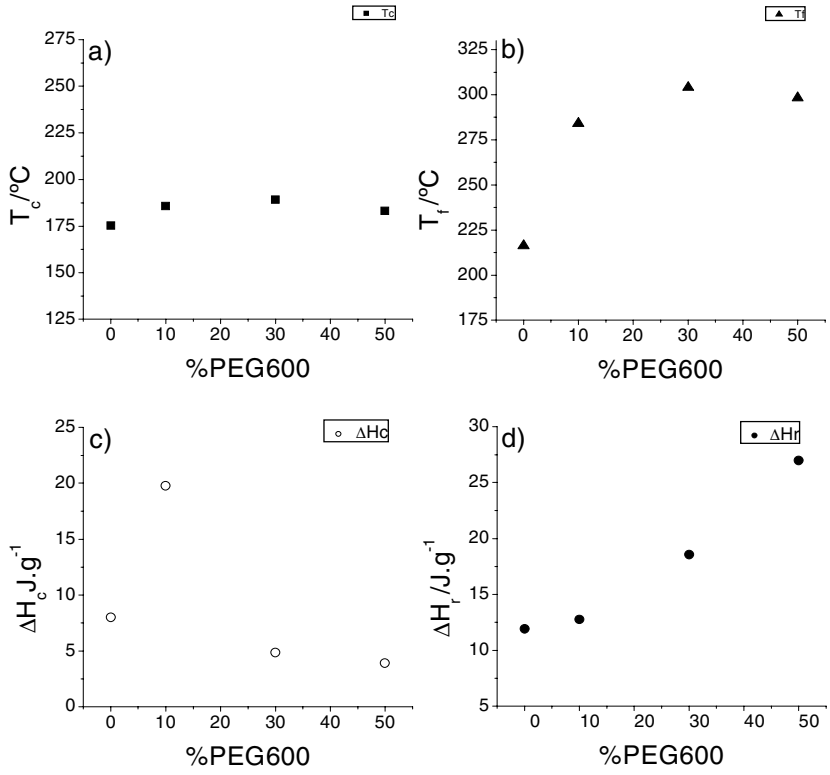

Figure 4. a) Tc versus % PEG 600; b) T_f versus % PEG 600; c) ΔH_c versus % PEG 600; d) ΔH_{fv} versus % PEG 600.

Another important aspect is the increase on the enthalpy of fusion value (ΔH_f), (d). These changes may be explained as follows: when 10% PEG 600 is added to the membranes, PEG 600 gives more mobility to the system, allowing morphological changes with the consequent increase of crystallinity, resulting in higher ΔH_c and ΔH_f, even though the increase on ΔH_r is not very significant. When even more PEG 600 is added to the system (30% and 50% PEG 600), this increase of PEG 600 content leads to a consequently higher increase in the mobility of cellulose acetate chains, which makes possible the reorganization of this material as crystals already at the production stage of the membranes. This leads to an increase of ΔH_r, since the crystalline content of the membranes increases at the same time as ΔH_c decreases. Figure 5 presents the DSC thermogram for a cellulose acetate membrane made with 50% PEG 600, 230 μm thick, which was prepared in a Petri dish. In this thermogram, the same characteristics of the previous samples are observed.

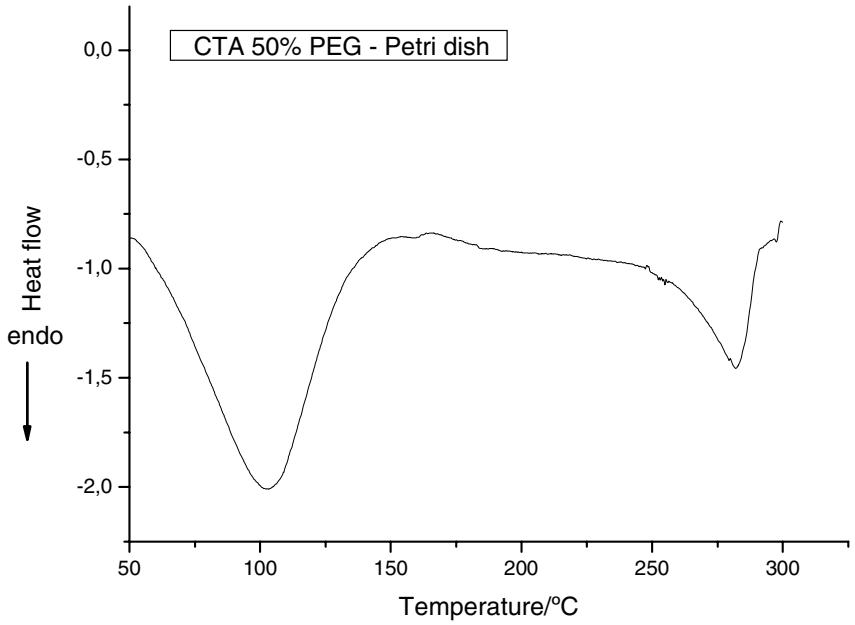

Figure 5. CTA membrane containing 50% PEG 600 (Petri dish).

3.3. Water vapor flux

Table 2 presents the water flux results, normalized according to their thickness, of water vapor through the membranes.

The results in table 2 show that, for membranes produced by solution casting, the flux of water vapor is nearly independent of the PEG 600 content. That indicates that transport occurs preferentially through noncrystalline regions of the membranes. As observed by DSC, the phenomenon of crystallinity induction occurs for all samples made with PEG 600. Nevertheless, it seems according to DSC that the morphology is not enough altered to produce considerable changes in the water vapor flux.

For membranes produced by pouring solution in Petri dishes, we observed that the normalized flux is ten times higher than for membranes produced by casting of

solution. The longer time of solvent evaporation due to the higher thickness for these membranes allows PEG 600 to act as pore former, changing the membrane morphology, and consequently, increasing the water flux. In this case, the results of water flux are comparable with that of the membranes for nanofiltration (SG®) produced by Osmonix (Rodrigues Filho et al., 2005).

Table 2. Results of water flow through the membranes, normalized by their thicknesses

PEG 600 content (%)	J.L ($g.s^{-1}.cm^{-2}.\mu m$)
0*	2.52×10^{-5}
10*	2.58×10^{-5}
10**	5.99×10^{-5}
30*	2.40×10^{-5}
50*	2.42×10^{-5}
50***	2.90×10^{-4}

* L = 20 μm, membranes produced by solution casting
** L = 61 μm
*** L = 230 μm, membranes produced by pouring the solution into a glass Petri dish

3.4. Cytotoxicity Assay

Figure 6 presents the cellular viability of the membranes in function of extract concentration. All samples exhibited behavior similar to the negative control, attesting that the studied membranes are nontoxic.

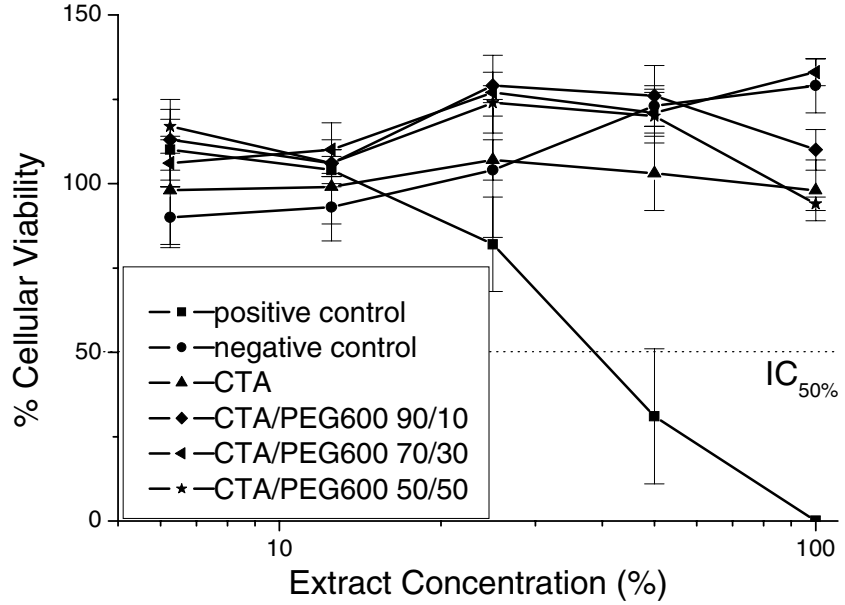

Figure 6. Cytotoxicity test results. Cellular viability curves using neutral red uptake cytotoxicity assay, with NCTC clone 929 cells for different membrane samples.

4. Conclusion

It was possible to demonstrate the viability to produce cellulose acetate membranes from sugar cane bagasse, using PEG 600 as admixture. The action of the admixture depends on how the membrane was produced (solution casting or deposit of solution on a Petri dish, both with free evaporation of solvent). In both of the methodologies, PEG 600 acts by inducing crystallinity on the membranes, but only for the membranes produced by deposit on Petri dishes the admixture acted as a pore former. It was also possible to attest the nontoxic behavior of the studied membranes.

Acknowledgements. The authors thank to FAPEMIG (CEX 1803-98, CEX2629-05); to CAPES for the use of "Portal Periódicos" and for PROAP; Toledo thanks to UFU for her PIBIC scholarship (A-007/2005), Cerqueira and Meireles thanks to CAPES for their doctor and master's scholarship, respectively.

References

1. Meier MM, Kanis LA, Soldi V (2004) Int J Pharm 278:99
2. Wang FJ, Yang YY, Zhang XZ, Zhu X, Chung TS, Moochhala S (2002) Mat Sci Eng C, 20:93
3. Kim JH, Lee KH (1998) J Memb Sci, 138:153
4. Rao PR, Diwan PV (1997) Pharm Acta Helv, 72:47
5. Makhija SN, Vavia PR (2003) J Control Rel, 89:5
6. Arthanareeswaran G, Thanikaivelan P, Srinivasn K, Mohan D, Rajendran M (2004) Eur Pol J, 40:2153
7. Zheng L, Wang YZ, Yang KK, Wang XL, Chen SC, Li J (2005) Eur Pol J, 41:1243
8. Idris A, Yet LK (2006) J Memb Sci, 280:920
9. Samios E, Dart RK, Dawkins JV (1997) Polymer, 38:3045
10. Toledo LC, Rodrigues Filho G, Assunção RMN, (2005) Proceedings of the 8º CBPol – Congresso Brasileiro de Polímeros, 438, Águas de Lindóia-SP, Brazil
11. Rodrigues Filho G, Silva RC, Meireles CS, Assunção RMN, Otaguro H (2005) J Appl Pol Sci, 96:516
12. Rodrigues Filho G, Cruz SF, Pasquini D, Cerqueira DA, Prado VS, Assunção RMN (2000) J Memb Sci, 177:225
13. Viera RGP, Rodrigues Filho G, Assunção RMN, Meireles CS,Vieira JG, Oliveira GS (2006) Carb Pol, in press, available on line in 07/21/06
14. Rogero SO, Malmonge SM, Lugão AB, Ikeda TI, Miyamaru L, Cruz AS (2003) Artif Organs, 27:424
15. Int Org Stand (1992) ISO 10993