

## Accepted Manuscript

Title: Supercritical Solvent Impregnation of Poly( $\epsilon$ -caprolactone/poly(oxyethylene-b-oxypropylene-b-oxyethylene) and Poly( $\epsilon$ -caprolactone/poly(ethylene- vinyl acetate) Blends for Controlled Release Applications

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PII: S0896-8446(08)00189-7  
DOI: doi:10.1016/j.supflu.2008.05.006  
Reference: SUPFLU 1591

To appear in: *J. of Supercritical Fluids*

Received date: 8-2-2008  
Revised date: 13-5-2008  
Accepted date: 29-5-2008

Please cite this article as: M.V. Natu, M.H. Gil, H.C. de Sousa, Supercritical Solvent Impregnation of Poly( $\epsilon$ -caprolactone/poly(oxyethylene-b-oxypropylene-b-oxyethylene) and Poly( $\epsilon$ -caprolactone/poly(ethylene- vinyl acetate) Blends for Controlled Release Applications, *The Journal of Supercritical Fluids* (2007), doi:10.1016/j.supflu.2008.05.006

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4 SUPERCRITICAL SOLVENT IMPREGNATION OF POLY( $\epsilon$ -  
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7 CAPROLACTONE/POLY(OXYETHYLENE-b-  
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10 OXYPROPYLENE-b-OXYETHYLENE) AND POLY( $\epsilon$ -  
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13 CAPROLACTONE/ POLY(ETHYLENE- VINYL ACETATE)  
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16 BLENDS FOR CONTROLLED RELEASE APPLICATIONS  
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36

37 **Abstract**  
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42 Poly( $\epsilon$ -caprolactone) blends were successfully impregnated with timolol maleate, an anti-  
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44 glaucoma drug, using a Supercritical Solvent Impregnation (SSI) technique. Supercritical  
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46 fluid impregnation efficiency results suggested that the best impregnating conditions were  
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48 obtained when a cosolvent was used and when specific drug-polymer interactions occurred  
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50 as a consequence of different chemical structures due to polymer blending. Pressure can be  
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52 either a favourable factor, when there is enough drug affinity for the polymers, or an  
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54 unfavourable factor when weaker bonding is involved. In order to determine the relative  
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4 hydrophilicity/hydrophobicity of the blends, contact angle analysis was performed, while  
5  
6 crystallinity determination was also useful to understand the obtained release profiles.

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8 Drug loading, heterogeneous/homogeneous dispersion of drug inside the matrix,  
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10 hydrophilicity, crystallinity, all seem to influence the obtained drug release rates. The “in  
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12 vitro” release results suggested that a sustained drug release rate can be obtained by  
13  
14 changing the SSI operational conditions and by modulating the composition of blends, as a  
15  
16 mean to control crystallinity, hydrophilicity and drug affinity for the polymer matrix. After  
17  
18 a first day burst release, all samples showed a sustained release profile (1.2-4 µg/ml/day,  
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20 corresponding to a mass of 3-10 µg/day) which is between the therapeutic and toxic levels  
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22 of timolol maleate, during a period of 1 month. These drug-loaded polymeric matrices can  
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24 be a feasible alternative treatment modality for the conventional repeated daily  
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26 administration of eye drops.  
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35 **Keywords:** Supercritical Solvent Impregnation, Poly( $\epsilon$ -caprolactone) blends, Ophthalmic  
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37 drug delivery systems.  
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## 49 **1. Introduction**

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53 The two main causes of blindness in adult population are age related macular degeneration  
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55 and primary open angle glaucoma, two diseases that affect the posterior segment of the eye  
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57 [1]. Glaucoma is frequently asymptomatic at the time of diagnosis, but it can result in  
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4 progressive visual field loss and, in extreme cases, in eventual blindness. Timolol maleate  
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6 (a beta blocker) is considered as the “golden standard” against which other glaucoma  
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8 medications are compared in terms of efficacy, side effects and cost. Although topically  
9  
10 administered timolol maleate is frequently recommended as first-line therapy, some  
11  
12 systemic side effects of this drug may limit its use. For example, timolol maleate and other  
13  
14 topically applied beta blockers have been associated to asthma exacerbation, worsening  
15  
16 congestive heart failure, heart block and, rarely, to sudden death [2].  
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20 Low drug bioavailability and systemic toxicity are usually caused by the relative  
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22 impermeability of the cornea, by tear dynamics and blinking and by nasolacrimal drug  
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24 drainage. In the case of eye drops medications, only around 5 % of the applied drug  
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26 actually penetrates through cornea [3]. The drug that is not absorbed by the cornea will  
27  
28 reach the bloodstream through the nasolacrimal duct causing some of the above mentioned  
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30 systemic side effects. To avoid low drug bioavailability, topical eye formulations normally  
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32 require high drug concentrations and frequent dosing treatments which also may increase  
33  
34 systemic side effects risks.  
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38 To overcome these issues, several efforts have been made in order to improve the ocular  
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40 delivery and bioavailability of topically applied ocular drugs and to reduce their adverse  
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42 effects. The most common approach is by developing ophthalmic polymeric-based  
43  
44 controlled drug delivery systems (CDDSs) such as bioadhesive and in situ forming  
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46 hydrogels, colloidal systems, ocular inserts and implantable devices [4-8].  
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50 Polymeric-based CDDSs can be prepared in numerous different ways. Dispersing a drug, or  
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52 therapeutic agent, in biocompatible and/or biodegradable polymeric matrices encompasses  
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54 the majority of all research in this field and there are several well-known methods to  
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56 incorporate and disperse drugs into polymeric matrices. However and in most cases, these  
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4 conventional methods present several disadvantages, like the potential use of toxic organic  
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6 solvents (specially for water insoluble drugs), drug/solvent dissolution and compatibility  
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8 issues, undesired drug reactions, drug photochemical and thermal degradation, low  
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10 incorporation yields and heterogeneous drug dispersion.

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13 Drugs may also be impregnated and dispersed in polymeric matrices by dissolving them in  
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15 compressed high volatile fluids (like carbon dioxide) at temperatures and pressures near or  
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17 above their critical temperatures and pressures, and contacting the resulting mixture with  
18  
19 the polymeric matrices to be infused. In these conditions, the compressed fluid can act also  
20  
21 as a swelling and plasticizer agent for polymers, dilating the matrices and helping drug  
22  
23 diffusion into them. This recent technique, known as Supercritical Solvent Impregnation  
24  
25 (SSI), already proved its advantages for the development of drug impregnated polymeric  
26  
27 materials which can be used as CDDSs for many biomedical applications [9-12]. SSI  
28  
29 allows the drug impregnation of most polymeric articles and, when properly employed,  
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31 without altering and/or damaging their physical, chemical, and mechanical properties and  
32  
33 without degrading their constituent drugs, additives and polymers. Furthermore, drug  
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35 loading and depth penetration can be easily controlled and drugs will be homogeneously  
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37 dispersed, in short treatment times and leaving no harmful solvent residues. Finally, SSI  
38  
39 also permits to have previously prepared polymeric articles and, later, impregnate them  
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41 with the desired drugs, according to the specific needs of the envisaged therapeutic  
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43 application, and without interfering with the established conventional method/procedure to  
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45 produce/process the original polymeric articles. This particular feature can lead to very  
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47 attractive and useful medical and commercial applications [13-14].  
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55 Although carbon dioxide is the most frequently employed supercritical fluid (SCF), it also  
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57 presents several limitations mainly due to its inability to dissolve high molecular weight  
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4 compounds and to its non-polarity and lack of several specific solvent-solute and solvent-  
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6 polymer interactions that would lead to high polymeric drug loading.

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8 A frequent strategy to increase drug solubility in supercritical carbon dioxide (scCO<sub>2</sub>) is the  
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10 addition of small amounts of specific cosolvents which can produce dramatic effects on its  
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12 solvent power, sometimes up to several hundred percent in terms of solubility enhancement  
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14 [15-16].

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17 Our long-term goal is to prepare an implantable (subconjunctival) system for continuing  
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19 drug delivery, with controlled release and degradation that could deliver timolol maleate  
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21 for up to 4-6 months, in an attempt to overcome the problems of low drug bioavailability  
22  
23 and the potential occurrence of systemic toxicity. The system would deliver only the  
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25 therapeutic drug amount [17] and would eliminate the problem of frequent administration  
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27 (timolol eye drops are applied twice daily), improving patient compliance.  
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31 For the present study, poly( $\epsilon$ -caprolactone) (PCL) was selected as the main blend  
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33 homopolymer for the preparation of the biodegradable CDDS due to its good  
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35 biocompatibility [18-20] and its known swelling ability in scCO<sub>2</sub> [21]. Poly(ethylene-co-  
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37 vinyl acetate) and poly(oxyethylene-b-oxypropylene-b-oxyethylene) are block copolymers  
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39 which have numerous and recognised applications in the development of CDDSs mainly  
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41 because of their biocompatibility, processability (e.g. extrusion) and proved long-term  
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43 release properties [22-26].  
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49 The aim of this work was to evaluate the effects of operational pressure, of blend chemical  
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51 nature and composition, as well as of cosolvent effects, on the supercritical solvent  
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53 impregnation process of different poly( $\epsilon$ -caprolactone) blends, in order to determine the  
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55 best operating conditions to achieve maximum drug loading and optimal drug release  
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57 profiles.  
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## 2. Experimental section

### 2.1. Materials

Timolol maleate, (99,6 % purity) was purchased from Cambrex Profarmaco Cork Ltd., Ireland. Poly( $\epsilon$ -caprolactone) pellets (PCL, average  $M_w$  65000 g/mol) were obtained from Sigma-Aldrich. Poly(ethylene-co-vinyl acetate), Luwax EVA 3 (Lw, 13-15 % vinylacetate content) and poly(oxyethylene-b-oxypropylene-b-oxyethylene), Lutrol F 127 (Lu, 9000-14000 g/mol, 70 % by weight of polyoxyethylene) were bought from BASF. It was not possible to obtain (from supplier) the average molecular weight of Luwax EVA 3. The chemical formulae of the employed copolymers are shown in Fig. 1. Tetrahydrofuran (HPLC grade) was obtained from Sigma-Aldrich. Phosphate buffer saline (PBS) tablets (pH 7.4, 10 mM phosphate, 137 mM sodium, 2.7 mM potassium) were used to prepare the drug release medium and were bought from Sigma-Aldrich. Carbon dioxide (99.998 % purity) was obtained from Praxair. All products were used without further purification.

### 2.2. Blends preparation

Several PCL-based blends were prepared by solvent casting and according to the procedure described below. The blends (Lutrol F 127/PCL: 25/75, 50/50 and Luwax EVA 3/PCL: 25/75, 50/50, 75/25, % w/w) were prepared by dissolution in tetrahydrofuran (10 % w/v total polymer solutions) at 40 °C and 60 °C, respectively. Blends films were obtained by solvent casting at room temperature in glass Petri dishes. Then, the obtained films were vacuum-dried at 37 °C, for 24 h, to ensure the complete removal of the solvent. After drying, the films were removed from the Petri dishes and cut in rectangular pieces of

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4 approximately 0.5 cm×0.5 cm and used as such in the subsequent supercritical  
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6 impregnation of the drug and characterization experiments.  
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### 10 **2.3. Supercritical fluid impregnation process**

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13 The supercritical solvent impregnation equipment is schematically presented in Fig. 2. The  
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15 equipment consists of a cylindrical high-pressure stainless steel cell (21.57 cm<sup>3</sup>) placed in a  
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17 controlled temperature water bath that maintains the temperature within ±1 °C. The water  
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19 bath temperature was measured by means of a digital thermometer. A magnetic stirring  
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21 plate (750-800 rpm) was used to homogenise cell-containing high pressure mixtures (CO<sub>2</sub>,  
22  
23 timolol maleate and cosolvent). Carbon dioxide was liquefied through a cooling unit and  
24  
25 compressed to the operating pressure with a high-pressure liquid pump. A one-way high  
26  
27 pressure valve (3) was introduced in the system. System pressure was measured with a  
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29 pressure transducer in-line with the impregnation cell.  
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35 The drug, or drug solution (in the cases when cosolvents were used), was loaded in the  
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37 bottom of the cell and the polymer films (with masses between 0.01-0.02 g) were separated  
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39 in a stainless steel grid, placed in the centre of the cell. The amount of drug was established  
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41 in order to obtain a saturated environment at the operational conditions. A cosolvent  
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43 concentration of 10 % (v/v), at PTN conditions, was used in order to increase drug  
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45 solubility in scCO<sub>2</sub> [27]. Then, carbon dioxide was allowed to flow through the cell to  
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47 remove all the air from the system. Then, valves 11 and 12 were closed and the cell was  
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49 loaded with CO<sub>2</sub> until the desired pressure and temperature conditions were attained. After  
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51 this, valve 6 was closed and the system was maintained static and under constant pressure  
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53 during the two hours of impregnation experiments.  
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4 At the end of the impregnation period, the system was depressurized (depressurization rate  
5 was 5 bar/min) in order not to alter or damage the polymeric samples. For this, two  
6 consecutive valves (11 and 12) were used in order to have a greater control over the  
7 depressurization rate. Impregnated samples were then recovered in a dry or soaked state  
8 (when cosolvent was used). Wet samples were dried in a vacuum oven at 37 °C for 2  
9 hours. Then, sample masses were registered in order to calculate the impregnation  
10 efficiency (Section 2.4).  
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20 A pressure of 200 bar and a temperature of 40 °C were chosen because scCO<sub>2</sub> has the  
21 highest solubility (3.2 g/g of PCL) in poly(ε-caprolactone) at these operational conditions  
22 [21]. At these conditions, PCL presents a maximum swelling degree which, supposedly,  
23 may help diffusion and increase drug loading yields. A second operational pressure (110  
24 bar) was chosen in order to study the possible pressure effects on the resulting polymer  
25 blends on which we didn't have any previous data regarding the solubility of scCO<sub>2</sub> in  
26 these polymeric matrices. The operational parameters for each of the performed  
27 experiments are summarised in Table 1.  
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#### 41 **2.4. Impregnation efficiency**

42 The impregnated timolol maleate mass ( $m_d$ ) was determined spectrophotometrically by  
43 UV-vis analysis (Jasco V-530 Spectrophotometer), at 299.5 nm, after dissolving the  
44 impregnated blends in tetrahydrofuran. The impregnation yield was calculated using Eq. 1.  
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$$\text{Impregnation efficiency (g drug/g blend)} = \frac{m_d}{m_p} \quad (1)$$

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4 In this equation,  $m_p$  is the polymer mass after the impregnation process. For each different  
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6 blend composition, the operating conditions leading to the highest impregnation were  
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8 selected and only these impregnated samples were tested for the in vitro kinetics drug  
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10 release experiments.  
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## 15 **2.5. Contact angle measurements**

17 The contact angle formed between a water droplet placed on the surface of a material and  
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19 the kinetics of spreading is related to the hydrophilicity/hydrophobicity of the material.  
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21 Water contact angles of the prepared polymer blend films were evaluated by static contact-  
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23 angle measurements using an OCA 20 from Dataphysics. The tests were performed on the  
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25 air-facing surfaces of the samples, using water and employing the sessile drop method.  
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28 Nine measurements on different sample points were performed to calculate the mean static  
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30 contact angle and its standard deviation.  
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## 36 **2.6. DSC - Crystallinity determination**

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38 Differential scanning calorimetry (DSC) was carried out using a SDT Q 600 calorimeter,  
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40 from TA Instruments. Films were heated under a constant nitrogen gas flow at a heating  
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42 rate of 10 °C/min. DSC results were calibrated using indium as the calibration standard.  
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45 The melting temperature of the blends was considered as the temperature at which the  
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47 endothermic peak occurred. The fusion enthalpy, for each blend, was determined  
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49 integrating the peaks from the melting endotherm using the TA Analysis software. The  
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51 relative crystallinity,  $X_{rel}$  of the blends was calculated using Eq. 2 [28-29]:  
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$$55 X_{rel} (\%) = \frac{\Delta H_f}{\Delta H_{f,100\%}} \times 100 \quad (2)$$

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4 In Eq. 2,  $\Delta H_f$  is the experimental fusion enthalpy of the blends. The value of  $\Delta H_{f,100\%}$  was  
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6 used considering the reported fusion enthalpy of 100 % crystalline polycaprolactone [30].  
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## 10 **2.7. In vitro kinetics of drug release studies**

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12 The kinetics of timolol maleate release from the prepared materials was studied in PBS  
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14 medium at 37 °C. The impregnated blend samples were compressed in a mould, using a  
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16 press, into discs of 6 mm diameter and 1 mm thickness. These discs were weighed and  
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18 introduced in vials containing 4 ml of PBS and maintained at 37 °C. At scheduled time  
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20 intervals (every 15 minutes during the first hour, then every hour during 6 hours, twice a  
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22 day during 2 days and finally once a day for the remaining time), half of the the PBS/drug  
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24 solution was removed from the vial and a fresh PBS solution of identical volume was  
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26 added to maintain sink conditions. The timolol maleate concentration in each of the  
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28 collected samples was measured at 299.5 nm using a Jasco V-530 Spectrophotometer. The  
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30 amount of timolol maleate released at time t ( $m_t$ ), was determined from a pre-determined  
31  
32 standard curve (with an absorption coefficient  $\epsilon_{299.5} = 20.97 \pm 1.51$  ml/mg cm). The total  
33  
34 amount ( $m_{total}$ ) of impregnated timolol maleate was determined after the release test ended,  
35  
36 by dissolving the blends in tetrahydrofuran and adding this residual mass to the  
37  
38 accumulated released mass. The percentage of released drug was calculated using Eq. 3.  
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40 Calculations of the amount of released drug took into account the drug removal and the  
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42 replacement with fresh medium at each sampling point.  
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$$52 \text{ Released drug (\%)} = \frac{m_t}{m_{total}} \times 100 \quad (3)$$

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56 In order to study the drug release mechanism, the Korsmeyer-Peppas equation (Eq. 4) was  
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58 used [31]:  
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$$\frac{m_t}{m_{total}} = k \cdot t^n \quad (4)$$

In this equation,  $m_t/m_{total}$  is the fractional release of the drug,  $k$  is the kinetic constant and  $n$  is the release exponent, which gives an indication of drug release type of mechanism. Following the Korsmeyer-Peppas equation, only the experimental drug release data that satisfied the relation  $m_t/m_{total} \leq 0.6$  were employed for the determination of the release exponent. Release exponents,  $n$ , were calculated as the slopes of the straight lines fitted to the release data using a least squares method.

### 3. Results and discussion

#### 3.1. Contact angle measurements

Contact angles are characteristic constants of liquid-solid systems and, when water or aqueous solutions are used as the liquids, may provide valuable information on the solid surface hydrophilicity or hydrophobicity. This information is of great importance for the development of polymeric CDDSs since water-promoted polymeric swelling will strongly influence drug diffusion through the polymeric network as well as polymeric erosion/dissolution and degradation [32]. The obtained water-polymeric blends contact angles are presented in Table 2. These results show that all the prepared blends, as well as the individual polymers and copolymers, are mainly hydrophilic (contact angles  $< 90^\circ$ ). But, and for the investigated individual samples, we can assume that Lu is the more hydrophilic sample, PCL has an intermediate hydrophilic character and Lw is the less hydrophilic sample. Moreover, and as expected, obtained blend contact angles are intermediate values of the constitutive polymers and copolymers. Thus, as PCL content is

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4 increased in Lu/PCL blends, the resulting blend samples become less hydrophilic. The  
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6 same behaviour is observed when Lw content is increased in Lw/PCL blends: contact angle  
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8 increased because the overall hydrophobic content of the blend was also increased. These  
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10 results were confirmed by water swelling experiments, which will be reported in due time,  
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12 together with other blend characterization data. Due to the specific interactions that may  
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14 occur between polymers/copolymers, resulting blends and the involved solvents, these  
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16 different relative hydrophobicity/hydrophilicity characters may have a strong influence on  
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18 the obtained kinetics of drug release results and on the impregnation efficiency results, as it  
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20 will be discussed later.  
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### 27 **3.2. DSC - Crystallinity determination**

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29 Polymer and copolymer crystallinity is known to play an important role in determining  
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31 degradability, erosion, water and drug permeability because the bulk crystalline phases that  
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33 may be present become more inaccessible to water diffusion. Moreover, scCO<sub>2</sub> induced  
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35 crystallization of polymeric substrates can also influence the overall supercritical solvent  
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37 impregnation process as well as the final relative crystallinity of the processed polymeric  
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39 materials [9, 12, 33-36].  
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44 PCL, Lu and Lw are semi-crystalline polymers and copolymers and, in their blends, the  
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46 overall final crystallinity degree may be strongly influenced by blend composition, by the  
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48 relative crystallinity of each component in the blend and by the specific interactions that  
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50 may occur between blend components or between specific blocks of the involved  
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52 copolymers in the blend. As shown in Table 3, the relative crystallinity,  $X_{rel}$  (%), calculated  
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54 using Eq. 2, increases with the PCL ratio in Lw/PCL blends and decreases with the PCL  
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56 ratio in Lu/PCL blends.  
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4 We did not find any previously reported values for the fusion enthalpy of 100% crystalline  
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6 Lu and Lw,  $\Delta H_{f,100\%}$ , and thus it was not possible to calculate the relative crystallinities for  
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8 these pure copolymers. However, the measured fusion enthalpies for both pure Lu and Lw  
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10 are higher than the corresponding value for PCL. Usually this is an indication that their  
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12 relative crystallinity values should also be higher than the corresponding value for PCL.  
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15 Therefore, it should be expected that blends with higher Lu and Lw contents should also  
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17 present higher relative crystallinities. This happens for Lu/PCL blends and this behaviour  
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19 was already found and discussed in other works involving, for example, polyethylene  
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21 oxide/PCL blends [37].  
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24 Despite this rule, some exceptions may occur especially when the co-crystallization of  
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26 blend components can take place with some crystallization restrictions of one component  
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28 due to the presence of the second one. For example, in the case of Lw/PCL blends, as the  
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30 proportion of Lw in the blend is increased, the expected higher crystallization ability of Lw  
31  
32 can be restricted and, as a consequence, the final relative crystallinity decreases.  
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34 Furthermore, and again in the case of Lw/PCL blends, it has been proposed that the  
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36 carbonyl groups from polyesters can interact favourably with the  $\alpha$ -hydrogens of the  
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38 poly(vinylacetate) (PVAc) block due to their proton accepting and proton donating  
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40 properties, respectively. Such favourable interactions between PCL and the PVAc block  
41  
42 can be responsible for the commonly found miscibility of PCL/PVAc blends [38]. It is also  
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44 accepted that the favourable interactions that are often established between two  
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46 constituents in miscible blends can contribute to slowing down of the formation rate of  
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48 crystallising species being drawn into (or diffusing to) the crystals [39]. Therefore, these  
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50 are other possible explanations of why, in the Lw/PCL series, the addition of Lw causes a  
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52 decrease in relative crystallinity in comparison with pure PCL. The obtained fusion  
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4 enthalpies of these blends are also smaller than that obtained for PCL alone, further  
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6 sustaining these hypotheses.  
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### 10 **3.3. Supercritical drug impregnation process**

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12 The 75/25 Lu/PCL blend was not studied because it was found that it dissolves in PBS, at  
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14 37 °C, and thus it is not a good material for the intended CDDS application. The 25/75  
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16 Lu/PCL, 50/50 Lu/PCL blends, as well as PCL samples, were not impregnated using 10%  
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18 of ethanol as cosolvent because the samples would dissolve completely at these operational  
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20 conditions.  
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25 It is important to notice that the employed cosolvent compositions are expressed in terms  
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27 of volume fractions (v/v) and are referred to PTN conditions. In the case of ethanol, at the  
28  
29 experimental conditions (40 °C/110 bar and 40 °C/200 bar), these compositions are slightly  
30  
31 different from this value but all ethanol was dissolved and the experiments were performed  
32  
33 employing a homogeneous supercritical fluid phase mixture (CO<sub>2</sub>+ethanol). However, in  
34  
35 the case of water, and because an excess of cosolvent was added to the cell, there are  
36  
37 always two immiscible phases inside the cell, with compositions determined by the high  
38  
39 pressure vapour-liquid equilibria of CO<sub>2</sub>+water mixtures, at those pressure and temperature  
40  
41 conditions: a high pressure fluid mixture (CO<sub>2</sub>+water), in contact with the polymeric  
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43 samples, and a high pressure liquid phase (water+CO<sub>2</sub>), at the bottom of the cell.  
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49 In general terms, the obtained results indicate that not just timolol maleate solubility  
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51 (which is highly dependent on the presence or absence of the cosolvent) in scCO<sub>2</sub> plays an  
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53 important role in the overall impregnation process efficiency, but also all the other specific  
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55 and complex interactions that may occur between all the involved components of the  
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57 system: scCO<sub>2</sub>-polymeric matrices-cosolvent interactions (which determine cosolvent and  
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4 scCO<sub>2</sub> solubility in the polymeric matrix and, consequently, swelling and plasticization  
5 effects) and drug-polymeric matrices-cosolvent interactions (which control the  
6 entrapment/deposition of the drug in the polymeric network).  
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10 In Fig. 3, a) and b), the effect of cosolvent on impregnation efficiency is illustrated for both  
11 employed impregnation pressures. It is clear that, for the Lw/PCL blends, the highest  
12 impregnation yields (0,018-0,033 g/g) were obtained when using ethanol (at both  
13 operational pressures) while for Lu/PCL blends, highest impregnation yields (0,012-0,018  
14 g/g) occurred in the presence of water as cosolvent (also at both employed pressures). For  
15 pure PCL samples, best results (0,009 g/g) were achieved when no cosolvent was used and,  
16 as observed, water addition decreased the amount of impregnated drug. As already referred,  
17 ethanol was not employed with pure PCL.  
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30 Therefore, the presence of the cosolvent and its inherent nature radically changed the  
31 impregnation results for these blends: ethanol visibly promoted Lw/PCL blends  
32 impregnation while water promoted Lu/PCL blends impregnation.  
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37 These results can be explained by the favourable specific interactions drug-CO<sub>2</sub>-cosolvent  
38 that may occur, i.e., by the timolol maleate (a water-soluble polar drug) solubility  
39 enhancement in the high pressure fluid phase, which was caused by the polarity increase of  
40 the mobile phase when the polar cosolvents (ethanol and water) were added [40]. As more  
41 drug can be dissolved, more drug can be carried out into the polymeric network by the  
42 mobile high pressure phase. In the case of timolol maleate, this ethanol induced solubility  
43 enhancement was already measured in our group [27].  
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53 For water, and to the best of our knowledge, there isn't any high pressure timolol maleate-  
54 CO<sub>2</sub>-water solubility data in the literature. However, and because timolol is a water soluble  
55 molecule and water is much more polar than ethanol, we should expect the same (or even  
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4 higher) solubility enhancement as the one observed for ethanol. However, when water was  
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6 employed as cosolvent, the impregnation efficiencies increased for Lu/PCL blends but for  
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8 Lw/PCL blends were drastically reduced. This suggests that other different phenomena  
9  
10 should also be involved in the impregnation process. A possible explanation can be the  
11  
12 occurrence of favourable specific timolol-maleate-polymeric matrix-ethanol interactions  
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14 for Lw/PCL blends and of specific timolol-maleate-polymeric matrix-water interactions for  
15  
16 Lu/PCL blends. Ethanol/blends contact angle measurements were not performed but  
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18 water/blends contact angle experiments indicated that Lu/PCL blends were more  
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20 hydrophilic than pure PCL and than Lw/PCL blends. Therefore, a high pressure mobile  
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22 phase containing water may interact more efficiently with Lu/PCL blends than with pure  
23  
24 PCL and with Lw/PCL blends, thus promoting a higher swelling degree and consequently  
25  
26 favouring diffusion. This effect seems to be increased at higher pressures (200 bar).  
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28 Furthermore, and if there is some water absorption in the hydrophilic portions of Lu/PCL  
29  
30 blends (as expected), this will also create the conditions for a water-soluble molecule, like  
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32 timolol maleate, to be deposited in these blends, instead of being removed with the mobile  
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34 phase during depressurization.  
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41 A recognized advantage of SCF polymeric processing is that SCFs can enhance the  
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43 diffusion of drugs+SCFs mixtures into some polymeric matrices because, in most cases,  
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45 they can increase the polymeric free volume and the side groups chain mobility.  
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47 Furthermore, this diffusion enhancement can be controlled (“tuned”) just by changing the  
48  
49 operational pressure and temperature. When polymer-SCF phase interactions are present  
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51 and are favourable, high pressures usually facilitate the diffusion process mostly because  
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53 they will allow more fluid absorption which will generate a higher swelling degree. This is  
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55 the case when higher operational pressure determines higher drug loading in the polymeric  
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4 matrix. On the other hand, when drug-SCF phase interactions are stronger than drug-  
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6 polymer interactions, pressure usually will be an unfavourable factor because higher  
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8 pressures will originate an increase in SCF phase density, thus leading to an increased  
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10 solvating power of the mobile phase. At the same time, and if the polymer-SCF phase  
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12 interactions are still appreciable, this increased density will also originate an increase in  
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14 polymer swelling. As a result of these two combined factors, more drug will “choose” to  
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16 diffuse out the polymeric matrix and stay in the mobile phase, originating a low polymeric  
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18 loading [41].  
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22 In Fig 4 a), b) and c), we present the explicit effect of pressure on the impregnation  
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24 efficiencies. Pressure effects complement the previous discussion about the cosolvent  
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26 effects on impregnation efficiencies and can also help to explain why impregnation  
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28 efficiencies are higher at 200 bar for Lu/PCL blends, while Lw/PCL blends and PCL have  
29  
30 higher impregnation efficiencies at 110 bar. More effective drug-polymer interactions are  
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32 expected to take place for Lu/PCL blends because of Lu/PCL blends higher  
33  
34 hydrophilicities. Thus, higher pressures will favour drug deposition. For Lw/PCL blends  
35  
36 and for PCL samples, drug diffusion into the polymeric samples also takes place but,  
37  
38 during depressurization, more drug comes out with the mobile phase, due to the weaker  
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40 drug-polymer interactions (when compared to the drug-SCF phase interactions). This is  
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42 also in agreement with other works in which the efficiency of the impregnation decreases at  
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44 higher pressures [42].  
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51 As already referred, copolymer/polymer chemical structures can strongly affect drug-  
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53 polymer and polymer-SCF phase interactions, thus controlling the overall impregnation  
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55 process. These complex interactions can be understood, for example, through the  
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57 supercritical surfactants theory. Generally, surfactants for use with carbon dioxide are  
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4 amphiphilic molecules containing both a CO<sub>2</sub>-phobic and a CO<sub>2</sub>-philic portion [43]. For  
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6 Lutrol F 127 (which is a non-ionic surfactant), the ethylene oxide segment is the  
7  
8 hydrophilic portion of the block copolymer but it also is less CO<sub>2</sub>-philic than the  
9  
10 polypropylene oxide segment. However, it still interacts with CO<sub>2</sub> thus still having some  
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12 swelling degree in scCO<sub>2</sub> media. The polypropylene oxide segment has superior CO<sub>2</sub>-  
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14 philicity (when compared to the polyethylene oxide block) mainly because of the pending  
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16 methyl groups along its backbone). Luwax is a copolymer containing a hydrophobic part  
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18 (polyethylene) and a slightly hydrophilic one (polyvinylacetate). In terms of CO<sub>2</sub>  
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20 interactions, we can assume than the polyethylene block will interact in a stronger way with  
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22 CO<sub>2</sub> than the polyvinylacetate block, thus being more CO<sub>2</sub>-philic than the PVAc segment.  
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24 Finally, PCL is a homopolymer that is known to interact strongly with CO<sub>2</sub> [21]. This  
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26 happens because of the methylene groups present on its backbone as well of the specific  
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28 interactions that can occur between CO<sub>2</sub> and carbonyl groups.  
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34 Therefore, a hydrophilic drug (like timolol maleate) when is transported by a SCF, or by a  
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36 SCF-cosolvent mixture, will have a tendency to specifically interact and deposit on the  
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38 hydrophilic portions of the employed polymeric matrices. However, and because CO<sub>2</sub> is  
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40 also interacting in a strong way with the hydrophobic (CO<sub>2</sub>-philic) portions of the  
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42 polymeric matrix, a hydrophilic drug can also be deposited (in a lower extent) in these  
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44 hydrophobic portions. The use of a hydrophilic cosolvent (like water and ethanol, as  
45  
46 already discussed), will yet increase these interactions with the more hydrophilic parts of  
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48 the polymeric matrices thus increasing impregnation efficiency. If a hydrophobic drug is  
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50 used, we should expect that these effects will influence impregnation efficiency in an  
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52 opposite way.  
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4 Consequently, we should expect that more timolol maleate would be impregnated in  
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6 Lu/PCL blends as the composition, in terms of the more hydrophilic blend compound (Lu),  
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8 is increased. In Fig. 5 a), this is verified but only when water is used as the cosolvent. For  
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10 Lw/PCL blends, Fig. 5 b), the same effect is observed and as the Lw content is increased  
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12 (the more hydrophobic component), the impregnation efficiency decreases, but only in the  
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14 case when ethanol is employed. In the case of Lw/PCL blends, and as already discussed in  
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16 terms of water-samples contact angles and relative hydrophilicity, water seems to not affect  
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18 greatly the impregnation efficiency.  
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22 Finally and as already mentioned, the scCO<sub>2</sub> induced crystallization of some polymeric  
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24 substrates can occur during the impregnation experiments, decreasing the overall chain  
25  
26 mobility of the involved polymeric materials. This effect is contrary to the favourable  
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28 plasticization effect and can increase the final relative crystallinity of the processed  
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30 polymeric materials, thus influencing negatively the overall impregnation efficiency [9, 12,  
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32 33-36]. However, we did not measure the relative crystallinity of the employed materials  
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34 after scCO<sub>2</sub> and scCO<sub>2</sub>+cosolvent processing and therefore we did not know if crystallinity  
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36 increased or decreased. This work is still being performed and results will be presented in  
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38 due time.  
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### 46 **3.4. In vitro kinetics of drug release studies**

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48 In vitro kinetics of drug release studies were performed for selected impregnated samples.  
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50 This selection was made taking in consideration the best impregnation conditions, in terms  
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52 of impregnation efficiency, for each set of blends or sample: 200 bar/ 10 % water for  
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54 Lu/PCL blends, 110 bar/ 10 % ethanol for Lw/PCL blends and 110 bar/ 0 % cosolvent for  
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56 PCL samples. Results are presented in Fig. 6 a) and b). The cumulative percentages of  
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4 released timolol maleate are shown in Fig. 6 a). A magnification of the initial 8 hour  
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6 release period is also shown. After 32 days of release studies, the cumulative released  
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8 percentages were found to be higher for the Lw/PCL blends, followed by the Lu/PCL  
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10 blends and, finally, by PCL (84.6-92.3 %, 79.2-79.9 % and 77.2 %, respectively). All the  
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12 impregnated samples presented almost the same drug release profile, i.e., a biphasic release  
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14 pattern: an initial burst period exhibiting a very rapid release rate (probably caused by the  
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16 drug deposited on/near the polymeric surface), followed by a polymeric swelling and/or  
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18 erosion period, exhibiting an almost constant release rate (3-10  $\mu\text{g}/\text{day}$  after the first day).  
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20 We must remember that Lutrol F 127 is soluble in water and poly( $\epsilon$ -caprolactone)  
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22 undergoes hydrolytic degradation.  
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27 The obtained results seem to indicate that the initial drug loading of the supercritical  
28  
29 impregnated samples somehow influenced drug release kinetics results: the higher  
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31 cumulative percentages of released drug were obtained for the samples which were  
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33 impregnated with higher drug amounts (Lw/PCL blends impregnated with 10% ethanol).  
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35 However this could not be the true reason for these observations and a possible explanation  
36  
37 may be that timolol maleate was released faster in Lw/PCL blends because most part of  
38  
39 impregnated drug was probably deposited very close to the surface. This will be confirmed  
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41 further on when the kinetics modelling results will be discussed.  
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46 Lu/PCL blends and PCL were impregnated in a lesser extent but show more sustained drug  
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48 release profiles. These results are probably due to the fact that these lower impregnated  
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50 amounts of drug (when compared to Lw/PCL blends) were deposited more deeply in the  
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52 polymeric structure (thus more homogeneously dispersed throughout all the polymeric  
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54 samples). And, this was the result of the favourable specific interactions that were  
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56 established between timolol maleate, water (cosolvent) and the highly hydrophilic  
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4 segments of the Lu/PCL blends (as discussed in section 3.3.). In addition, sample  
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6 crystallinity may also control the drug release rates (higher crystallinity degrees usually  
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8 lead to slower release rates) and Lu/PCL blends and PCL present the highest percentage of  
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10 crystalline phases (see Table 3).

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13 Cumulative released drug concentration results are presented in Fig. 6 b). It can be seen  
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15 that, after the initial first day burst release, timolol maleate concentration becomes almost  
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17 constant (1.2-4  $\mu\text{g/ml/day}$  corresponding to a mass of 3-10  $\mu\text{g/day}$ ), which is located above  
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19 the therapeutic limit of timolol maleate (5  $\mu\text{g/day}$ ) [44] and below the maximum  
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21 recommended human ophthalmic dose (0,42 mg/day, considering a patient weight of 60  
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23 kg) [45]. The burst dose, released by the systems during the first day is below the  
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25 maximum recommended human ophthalmic dose, with two formulations surpassing this  
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27 value (0.53 mg for 50/50 Lw/PCL and 0.78 mg for 75/25 Lw/PCL). Even these values are  
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29 well below the maximum recommended daily oral dose, which is 60 mg/day (considering a  
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31 patient weight of 60 kg) [46]. The knowledge of these values is essential for the  
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33 development of efficient and safe controlled drug release systems because the released drug  
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35 concentrations must always be kept between the therapeutic and toxic levels.  
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41 The Korsmeyer-Peppas model (Eq. 4) is usually employed to analyse kinetics of drug  
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43 release from systems where the release mechanism is not well-known or when more than  
44  
45 one type of release phenomena (diffusion-, swelling- or erosion-controlled) are involved.  
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47 For cylindrical systems, release profiles having a release exponent,  $n$ , around 0.45, exhibit  
48  
49 a drug release mechanism controlled by classical/Fickian diffusion. When  $n \sim 0.89$ , the drug  
50  
51 release rate is controlled by a polymer relaxation mechanism (or Case II transport).  
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53 Systems having release exponents,  $n < 0.45$ , account for pseudo-Fickian behaviour, while  
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4 when  $0.54 < n < 0.89$  are an indication of the superposition of both the above referred  
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6 phenomena. In this case, the release mechanism is termed anomalous transport [46-47].  
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9 The value of the release exponent,  $n$ , was calculated as the slope of the straight lines fitted  
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11 to the release data using the least squares method (Fig. 7). The obtained values are  
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13 presented in Table 4 and, for all systems,  $n < 0.45$ , accounting for pseudo-Fickian release  
14  
15 behaviour. Steeper slopes were obtained for Lw/PCL blends which confirmed the already  
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17 discussed higher initial burst release behaviour observed for these systems. As expected,  
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19 and in general terms, results suggest that the release mechanism is quite complex, as drug  
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21 diffusion, polymeric swelling, crystallinity and polymer erosion are all likely to contribute  
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23 to the overall release phenomenon.  
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#### 29 30 **4. Conclusions**

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34 Lu/PCL, Lw/PCL blends and PCL samples were successfully impregnated with timolol  
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36 maleate, an anti-glaucoma drug, in order to, as a final objective, prepare polymeric  
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38 implantable (subconjunctival) systems for long-term timolol delivery, with controlled  
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40 release and degradation.  
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44 Different blends (with distinct blend components and compositions) were prepared and  
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46 characterized in terms of water-sample contact angle measurements and sample relative  
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48 crystallinity. Several SSI experimental conditions were tested: blend composition,  
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50 impregnation pressure and different cosolvents (water and ethanol).  
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54 Impregnation efficiency was determined and the obtained showed indicated that, and in  
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56 general terms, the overall impregnation process and its efficiency is the result of the  
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58 relative specific interactions that may be established between all the involved components  
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4 of this complex system: scCO<sub>2</sub>-cosolvent-drug interactions (which control drug solubility  
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6 in the high pressure mobile phase and its overall polar character), scCO<sub>2</sub>-polymeric  
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8 matrices-cosolvent interactions (which determine cosolvent and scCO<sub>2</sub> solubility in the  
9  
10 polymeric matrix and, consequently, swelling and plasticization effects) and drug-  
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12 polymeric matrices-cosolvent interactions (which control the entrapment/deposition of the  
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14 drug in the polymeric network). In addition, the employed polymeric samples, with the  
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16 exception of PCL, are copolymer blends and each one of these copolymers has  
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18 blocks/segments with different hydrophobic/hydrophilic characters, thus increasing even  
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20 more the system complexity.  
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25 However, in specific impregnation conditions, the addition of a cosolvent (water and  
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27 ethanol for Lu/PCL and Lw/PCL, respectively) promotes higher drug loading. This  
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29 happened because, in these conditions, drug solubility is increased and higher drug  
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31 amounts can be transported by the mobile phase. Moreover, the relative  
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33 hydrophilicity/hydrophobicity of prepared blends, together with the cosolvent addition, also  
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35 seemed to affect favourably the impregnation process (because of the specific favourable  
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37 interactions that are formed between the drug, cosolvent and the more hydrophilic blend  
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39 segments). Higher pressures were either a favourable factor (for Lu/PCL blends) or an  
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41 unfavourable factor (for Lw/PCL blends and for PCL samples).  
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46 Selected impregnated samples (the ones that presented higher impregnation efficiencies)  
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48 were employed in kinetics of drug release studies and the obtained results indicated that the  
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50 higher cumulative percentages of released drug were obtained for the samples which were  
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52 impregnated with higher drug amounts (Lw/PCL blends impregnated with 10% ethanol).  
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54 However, these systems presented high initial drug burst release profiles. Lu/PCL blends  
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56 and PCL were impregnated in a lesser extent but they showed more sustained/controlled  
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4 drug release profiles. These results are probably due to the fact that timolol maleate was  
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6 more homogeneously dispersed throughout all the polymeric samples, as the result of the  
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8 favourable specific interactions that were established between timolol maleate, water  
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10 (cosolvent) and the highly hydrophilic segments of Lu/PCL blends. In addition, and for  
11  
12 these blends, sample crystallinity may have also influenced drug release rates because of  
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14 the Lu/PCL blends and PCL higher percentages of crystalline phases. These results were  
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16 confirmed by the application of Korsmeyer-Peppas model, that accounted for pseudo-  
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18 Fickian release behaviour for all the tested samples, which indicates that the release  
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20 mechanism is quite complex, as drug diffusion, polymeric swelling, crystallinity and  
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22 polymer erosion are all expected to contribute to the global release phenomenon.  
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27 After the first release day, all samples showed a sustained release of 1.2-4  $\mu\text{g/ml/day}$ ,  
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29 corresponding to a mass of 3-10  $\mu\text{g/day}$ , during a period of 1 month. These values are  
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31 between the therapeutic and toxic levels of timolol maleate.  
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35 The obtained impregnation efficiencies and drug release results suggested that a desired  
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37 final sustained drug release rate can be achieved by changing several operational  
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39 impregnation conditions and by modulating blend compositions, i.e., as a way to control  
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41 crystallinity, hydrophilicity and drug affinity for the polymer matrix.  
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45 As final conclusions, the prepared timolol maleate-loaded polymeric matrices can be a  
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47 feasible and promising alternative to the conventional repeated daily administration of  
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49 timolol maleate eye drops for glaucoma treatment. Moreover, the SSI method proved to be  
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51 a good choice and a “tunable” method for the preparation of these long-term controlled  
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53 release systems.  
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## 56 57 58 **5. Acknowledgements** 59 60

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6 This work was financially supported by FCT-MCTES, FEDER, Portugal, under contract  
7  
8 POCTI/FCB/38213/2001. Mădălina V. Natu acknowledges FCT-MCTES for PhD grant  
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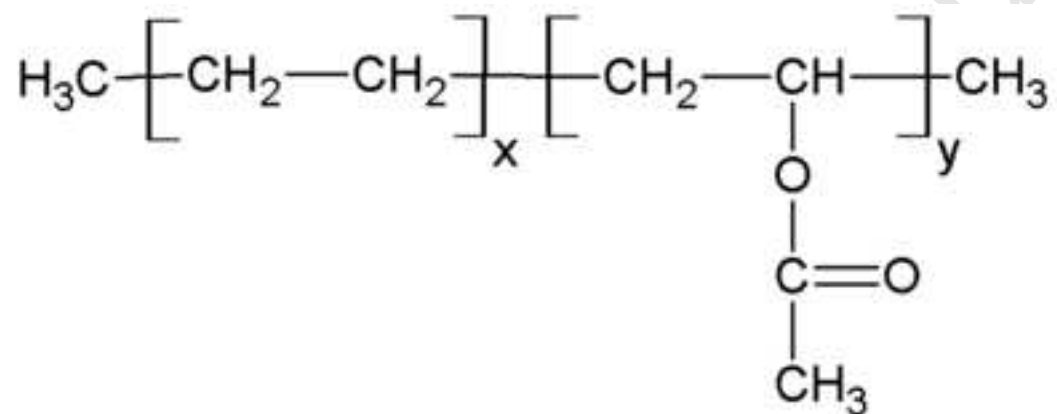
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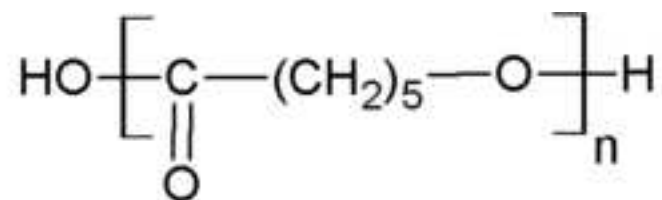
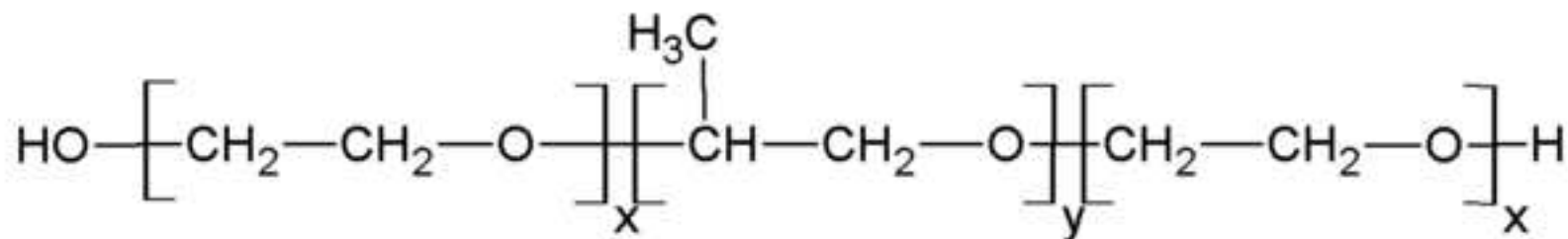
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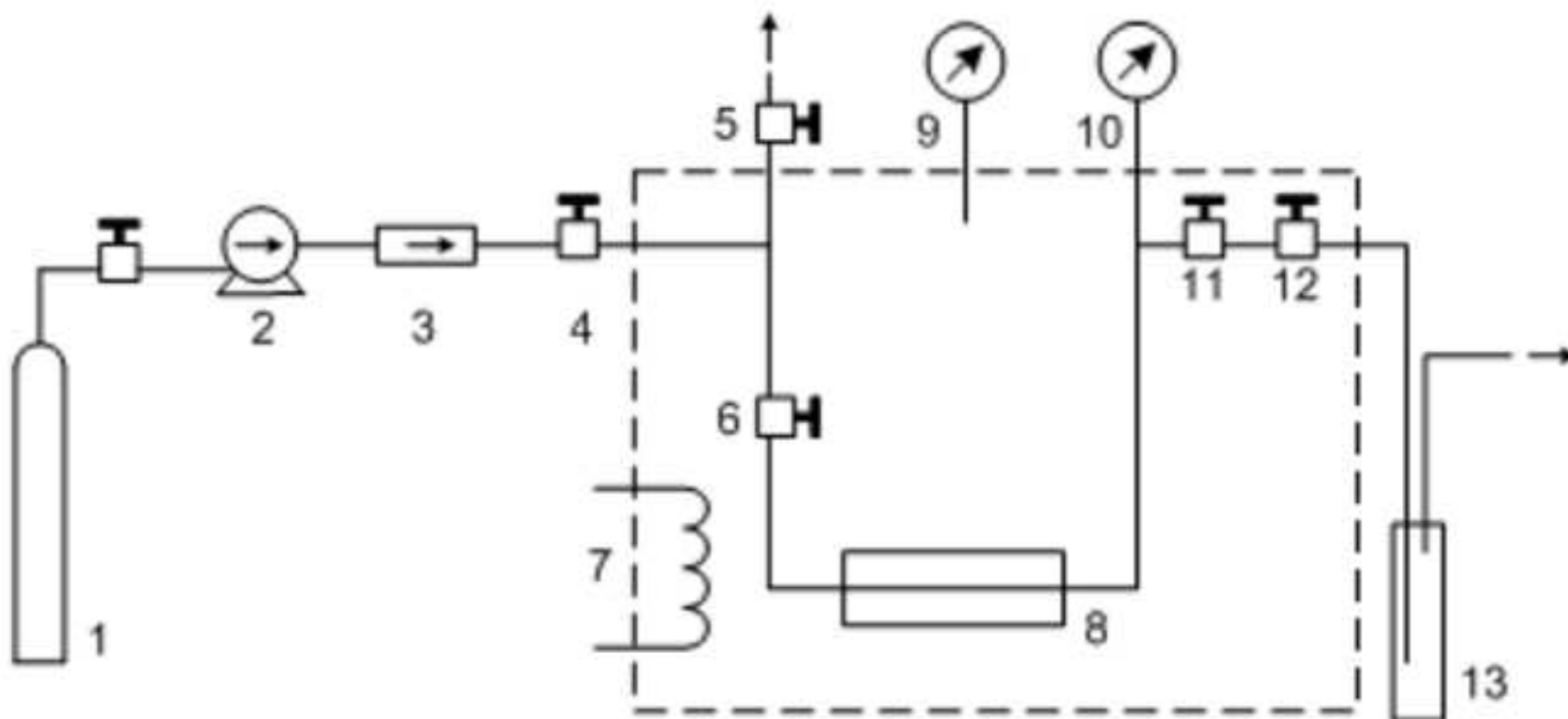
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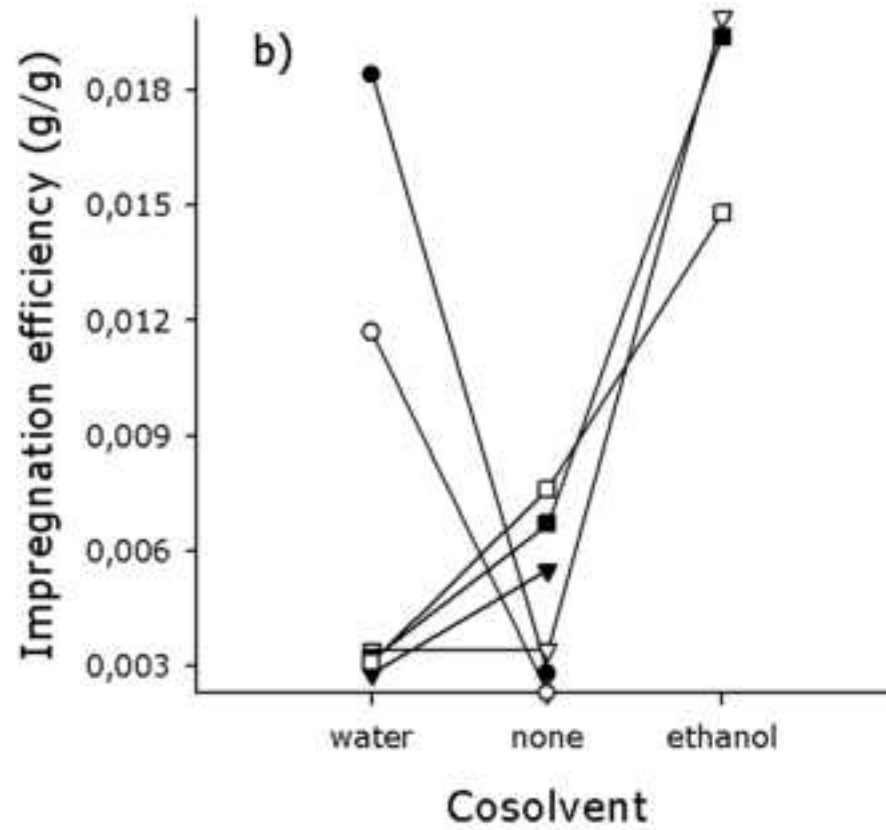
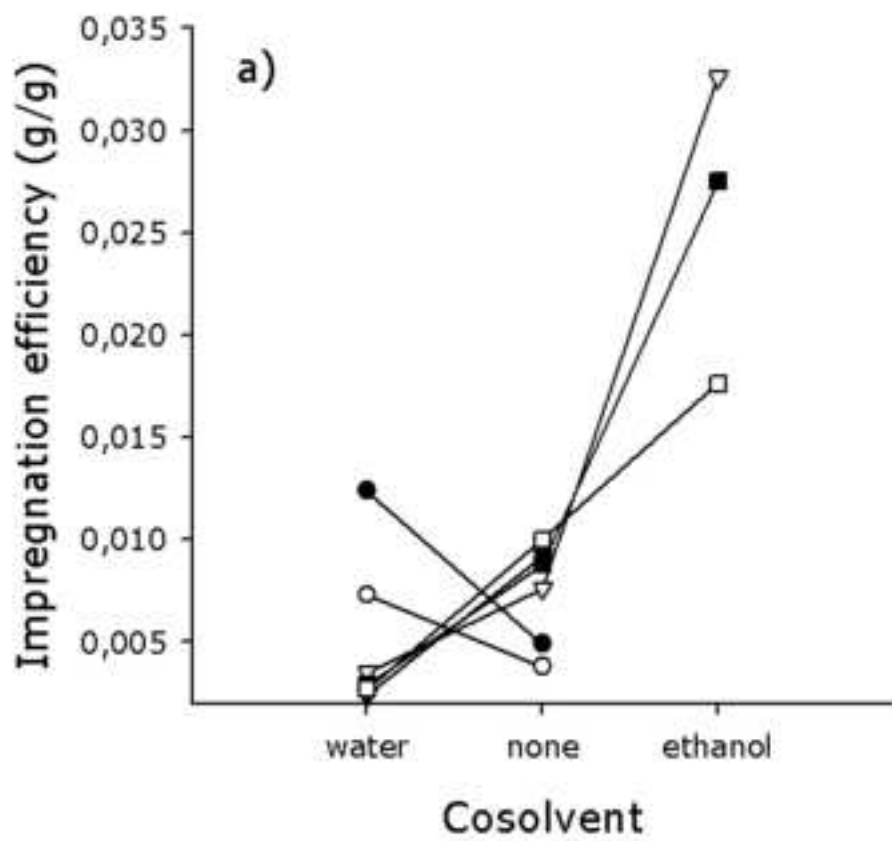
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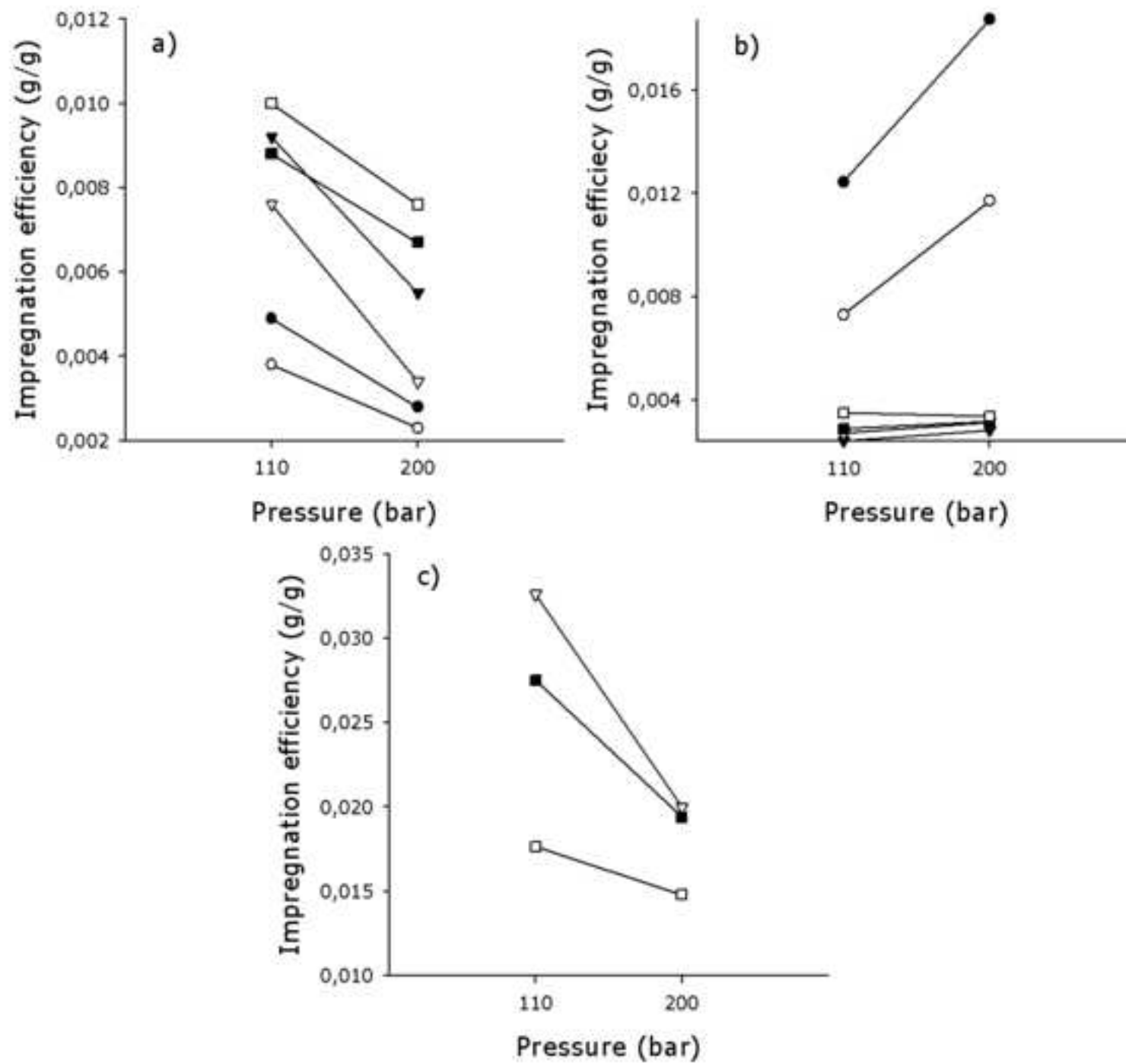


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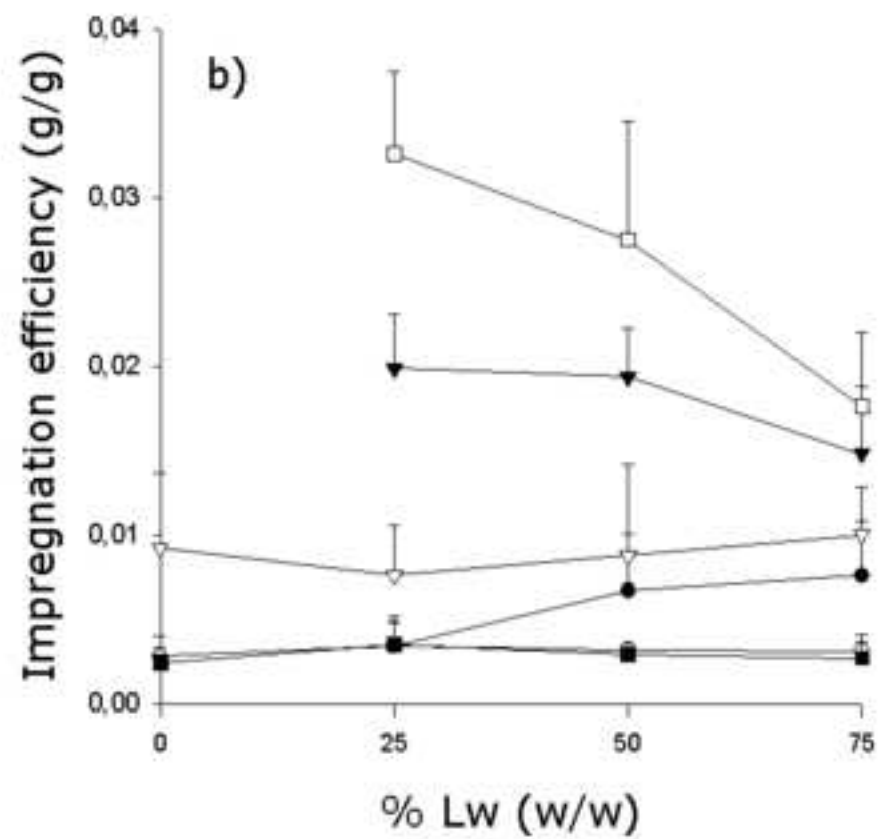
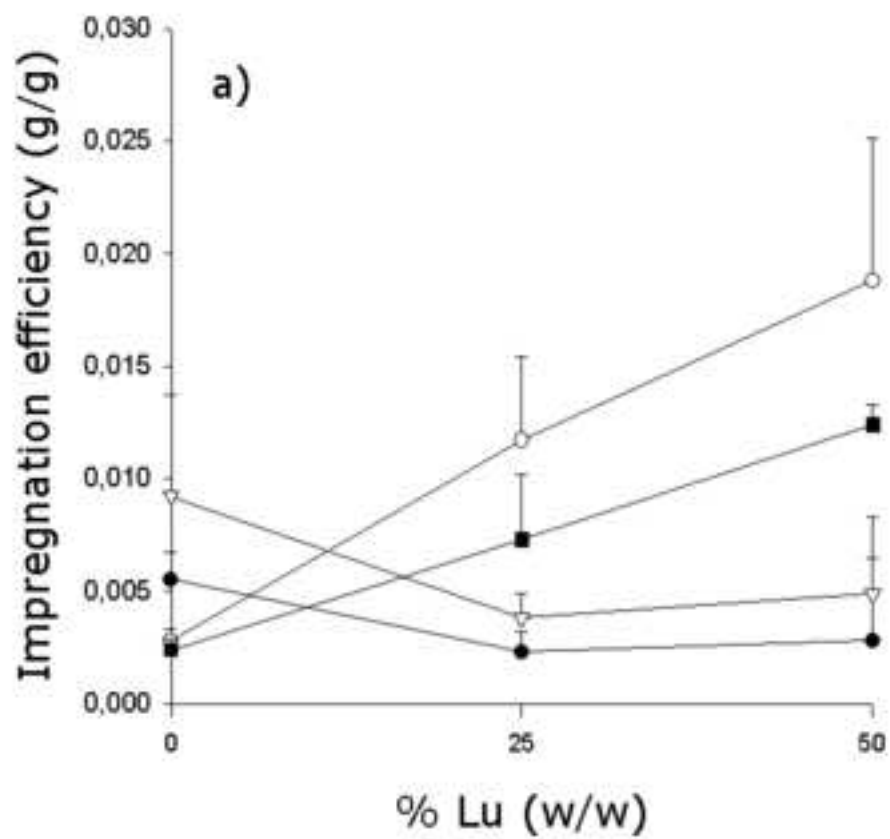


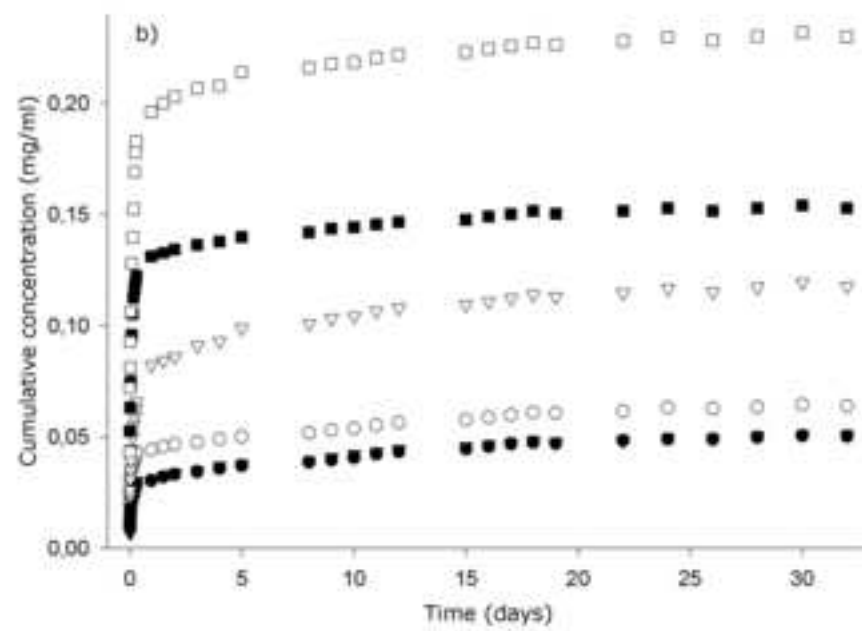
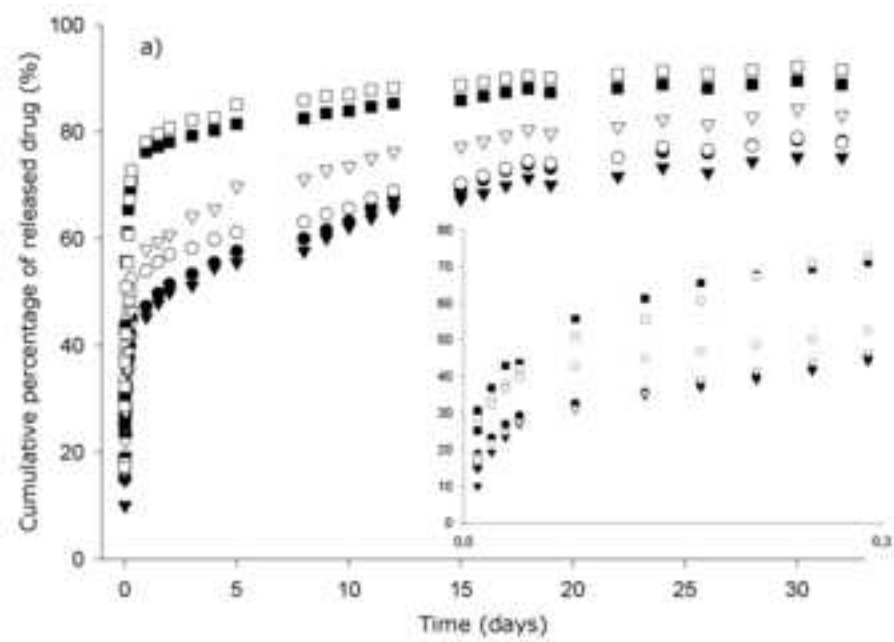
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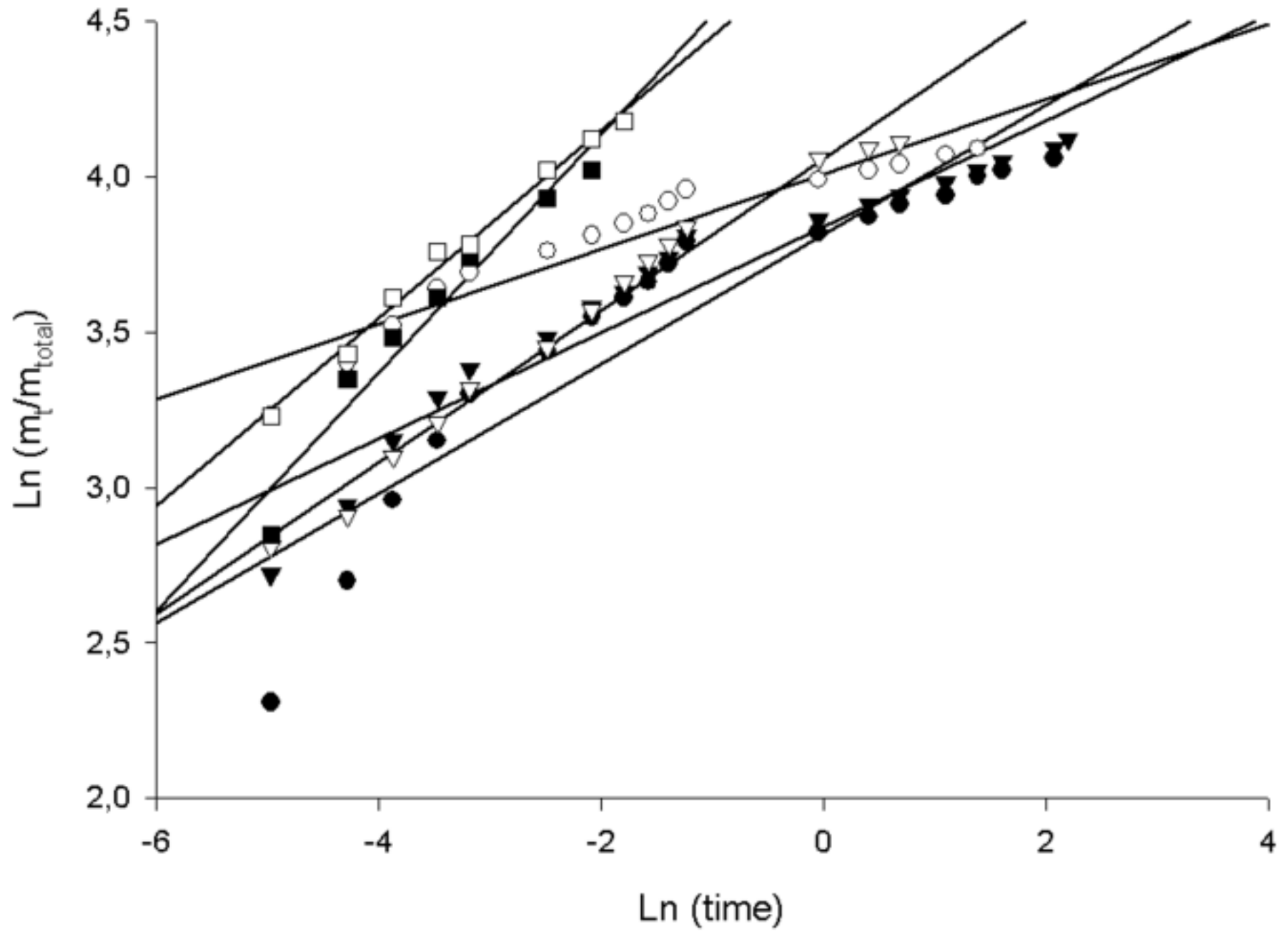




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## CAPTIONS TO FIGURES

**Fig 1.** Chemical structures of the employed polymers and copolymers.

**Fig 2.** Schematic diagram of the experimental supercritical solvent impregnation apparatus: (1) CO<sub>2</sub> reservoir; (2) High-pressure CO<sub>2</sub> pump; (3) One-way valve; (4, 5, 6, 11, 12) Valves; (7) Water bath heater/controller; (8) High-pressure stainless steel impregnation cell; (9) Digital thermometer; (10) Pressure transducer; (13) Glass trap.

**Fig 3.** Cosolvent (water and ethanol) effects on impregnated samples. a) 110 bar; b) 200 bar, (●) 50/50 Lu/PCL, (○) 25/75 Lu/PCL, (▼) PCL, (▽) 25/75 Lw/PCL, (■) 50/50 Lw/PCL, (□) 75/25 Lw/PCL.

**Fig 4.** Pressure effect on impregnated samples. a) No cosolvent; b) 10% water; c) 10% ethanol. (●) 50/50 Lu/PCL, (○) 25/75 Lu/PCL, (▼) PCL, (▽) 25/75 Lw/PCL, (■) 50/50 Lw/PCL, (□) 75/25 Lw/PCL.

**Fig 5.** Effects of blends compositions. a) Lu/PCL blends; b) Lw/PCL blends. (●) 0 % cosolvent, 200 bar, (○) 10 % water, 200 bar, (▼) 10 % ethanol, 200 bar, (▽) 0 % cosolvent, 110 bar, (■) 10 % water, 110 bar, (□) 10 % ethanol, 110 bar.

**Fig 6.** Kinetics of drug release studies. a) Cumulative percentages of released timolol maleate; b) Cumulative concentrations of released timolol maleate. (●) 50/50 Lu/PCL (200 bar, 10% water), (○) 25/75 Lu/PCL, (200 bar, 10% water), (▼) PCL, (110 bar, 0% cosolvent), (▽) 25/75 Lw/PCL, (110 bar, 10% ethanol), (■) 50/50 Lw/PCL, (110 bar, 10% ethanol), (□) 75/25 Lw/PCL, (110 bar, 10% ethanol).

**Fig 7.** Linear regressions to calculate kinetic constants and release exponents. (●) 50/50 Lu/PCL, (○) 25/75 Lu/PCL, (▼) PCL, (▽) 25/75 Lw/PCL, (■) 50/50 Lw/PCL, (□) 75/25 Lw/PCL.

**Table 1.** Employed impregnation experiments operational conditions.

Experiments	1	2	3	4	5	6
Pressure (bar)	200	200	200	110	110	110
Temperature (°C)	40	40	40	40	40	40
Time (hours)	2	2	2	2	2	2
Cosolvent concentration (v/v), PTN	None	Water (10%)	Ethanol (10%)	None	Water (10%)	Ethanol (10%)
Depressurisation rate (bars/min)	5	5	5	5	5	5

**Table 2.** Obtained contact angle for the employed homo- and copolymers and for prepared blends.

Samples	Lu	50/50 Lu/PCL	25/75 Lu/PCL	PCL	25/75 Lw/PCL	50/50 Lw/PCL	75/25 Lw/PCL	Lw
Contact angle (°)	48.3±0.8	50.1±1.2	55.9±1.3	61.8±1.8	63.6±1.0	66.1±0.9	70.9±1.3	72.5±1.4

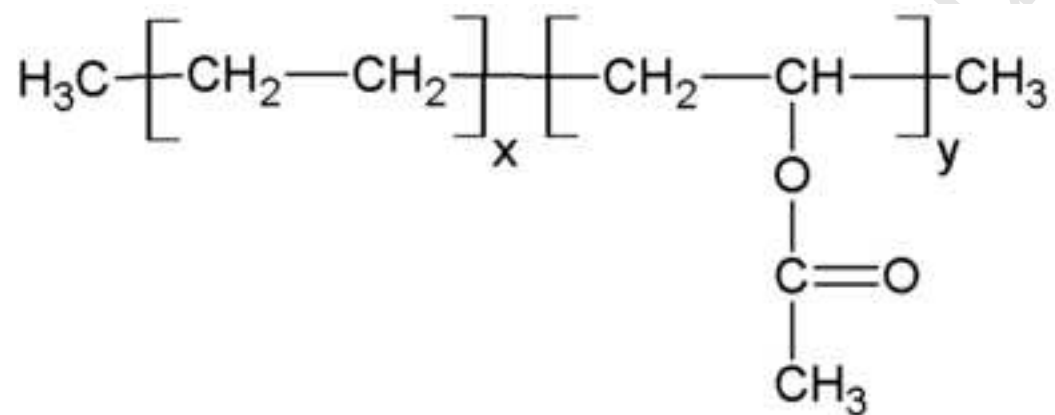
**Table 3.** Fusion enthalpies and relative crystallinities of the prepared blends.

Samples	$\Delta H_f$ (J/g)	$X_{rel}$ (%)
Lu	109.6	-----
50/50 Lu/PCL	85.7	60.4
25/75 Lu/PCL	80.9	57.0
PCL	72.8	51.3
25/75 Lw/PCL	70.4	49.6
50/50 Lw/PCL	62.1	43.7
75/25 Lw/PCL	57.4	40.4
Lw	116.8	-----

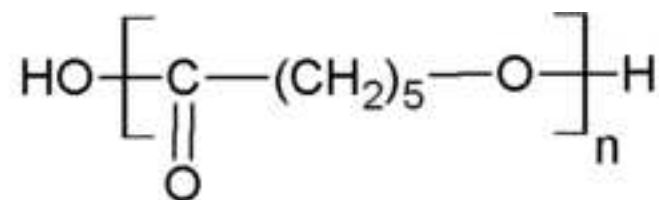
**Table 4.** Obtained kinetic parameters for kinetic drug release studies: release exponents ( $n$ ) and kinetic constants ( $k$ )

Samples	$k$ (days <sup>-n</sup> )	$n$	$R^2$
50/50 Lu/PCL	45.22	0.21	0.88
25/75 Lu/PCL	54.93	0.12	0.91
PCL	46.48	0.17	0.94
25/75 Lw/PCL	57.65	0.24	0.96
50/50 Lw/PCL	134.71	0.38	0.96
75/25 Lw/PCL	115.97	0.30	0.99

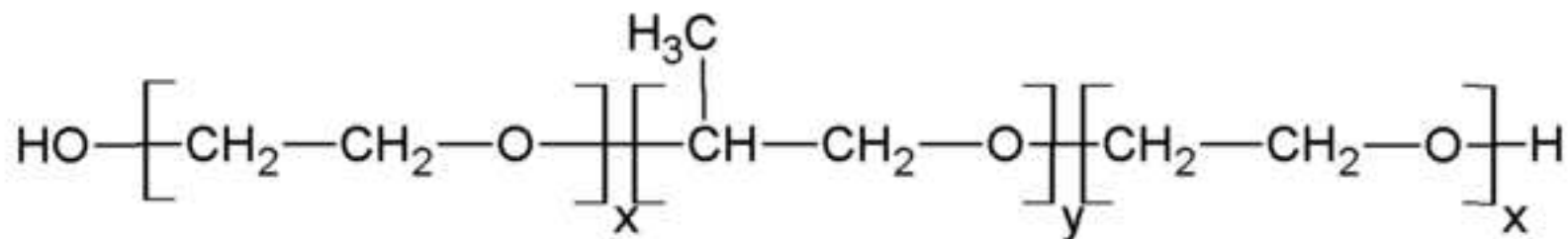




Luwax EVA 3



Poly( $\epsilon$ -caprolactone)



Lutrol F 127