Improving the Efficiency of the Supercritical Impregnation of Poly(D,L-lactide-co-glycolide) with Indomethacin

Cabezas, L. I., Fernández, V., Gracia, I., de Lucas, A., Rodríguez, J. F.*
Department of Chemical Engineering, Faculty of Chemistry,
University of Castilla–La Mancha,
Avda. Camilo José Cela, 12, C.P. 13071 Ciudad Real, Spain
*E-mail: juan.rromero@uclm.es
Fax: +34 926 295 318

The use of biocompatible polymeric foams impregnated with drugs is very interesting in tissue engineering and surgery from the point of view of treatments associated with cellular scaffolds besides tissular regeneration. In this work, the impregnation of polymeric supports with indomethacin (a non-steroidal anti-inflammatory drug) in supercritical media has been studied. The experimental planning has allowed understanding the influence of the copolymer composition (PLA/PLGA), the stirring rate and the depressurization time on the properties of the drug impregnated foams.

It has been confirmed that the copolymer exhibits enhanced mechanical strength with respect to that of the homopolymer, preventing its collapse and favouring the subsequent drug release. It has been also observed that fast stirring rate favours the impregnation process and a slow rate of depressurization is also desirable because promotes the formation of small size pores that retards the release of the indomethacin from the polymeric support.

INTRODUCTION

In the last years, the interest on the synthesis of biocompatible and biodegradable foamed supports for biomedical use is increasing greatly. This kind of foamed materials can be applied as scaffolds in tissue engineering, dressings and also in other implantable devices for healing injures and tissue regeneration. These foams are mainly prepared with polymeric materials and need to have an open cell microstructure to be invaded by the cells of the surrounding tissues –or degraded more or less easily by the body fluids.

When these polymers are used for controlled release of drugs the control of the impregnation and the properties of the support is an important issue because it will determine the polymer degradation rate and the drug release. In their application as scaffolds or for tissue repairing commonly these polymeric materials have to be impregnated with drug like antibiotics, anti inflammatories or antineoplasics. In any instance, the biomaterial is invaded or degraded at the same time that the controlled release of these substances takes place getting its specific target or spreaded in a gentle way all around the body by diffusion.

The synthesis and impregnation of these foams requires an exhaustive post synthesis elimination of any kind of organic solvent used in the preparation steps. A much better option is to employ a green process using $scCO_2$ for the foaming and impregnation of the polymeric support, which generates foams completely free of solvents since the beginning.

In this work, the foaming and impregnation procedure employed was a two steps method called the “Pressure Quench” [1]. The first one consists of placing a certain amount of the polymer in the high pressure vessel and saturating it with supercritical CO_2 (since it is inert

and highly soluble in the majority of the polymers). After an extended exposure of the polymer and CO_2 at high pressure, the polymer absorbs enough gas to decrease its Tg below the process temperature, and as consequence, a solution polymer-gas is generated. The second step is a rapid depressurization until ambient pressure. This fact, decrease the solubility of CO_2 in the polymer and causes the nucleation of bubbles due to the supersaturation reached. As the bubbles grow up, the concentration in the polymer decreases until its effective Tg is above vessel temperature. Furthermore, the rapid depressurization also causes the cooling of the polymer, possibly limiting the pore growth.

The more commonly used polymers in medical applications are those derived from lactic and glycolic acids (both homopolymers and copolymers). They are highly desirable since they are relatively harmless for cellular growth and their use in humans is widely extended and approved by the FDA. Furthermore, the degradation rate of PLGA can be modified varying the ratio of its comonomers [2].

The drug chosen in this study was the indomethacin (IDMC), a non-steroidal anti-inflammatory drug (NSAID) used in the treatment of disorders like several kind of arthritis, pericarditis, bursitis, tendinitis or spondylitis.

The influence of biopolymer composition, stirring rate and time for depressurization on the efficiency of the impregnation and foam porosity has been studied in this work.

MATERIALS AND METHODS

PLA (Mw = 28000 g/mol) and PLGA (74% D,L-lactide : 26% glycolide, Mw = 18000 g/mol) where synthesized previously using D,L-lactide and glycolide from *Purac.* Indomethacin was purchased from *Fagron* and used as received. The experimental setup used in the foaming and drug impregnation studies in $scCO_2$ is shown in Figure 1. It consists of a 300 ml high-pressure stainless steel reactor equipped with magnetic stirring. Heating was provided by an electric heating coat and the temperature was measured and controlled.

In a typical foaming and drug impregnation experiment, polymer (0.5 g) and 20 wt% of indomethacin (0.1 g) were placed in a high-pressure reactor and carbon dioxide was charged into the cell after its seal using the syringe pump until the pressure reached 180 bar at 40ºC. Zero time was considered when temperature was 40ºC. After 60 minutes of stirring, the CO_2 was vented off at different rates, and resulted polymer-drug porous monolith composites were collected.

It was checked that impregnation had really taken place using differential scanning calorimetry (TA Instruments, DSC Q1000) in which the sample was heated until 200 or 250ºC (PLA or PLGA respectively) followed by a cooling until -50 ºC and, finally, was heated until 200 or 250ºC again. Heating and cooling were carried out with a heating rate of 10 ºC min^{-1}.

The morphology of the composites was analyzed by optical microscopy (Carl Zeiss, Axio Imager A1) and the efficiency of the polymer impregnation was studied using UV-visible spectrophotometry (Shimadzu, UV-1603) using THF (purchased by *SDS*) as solvent.

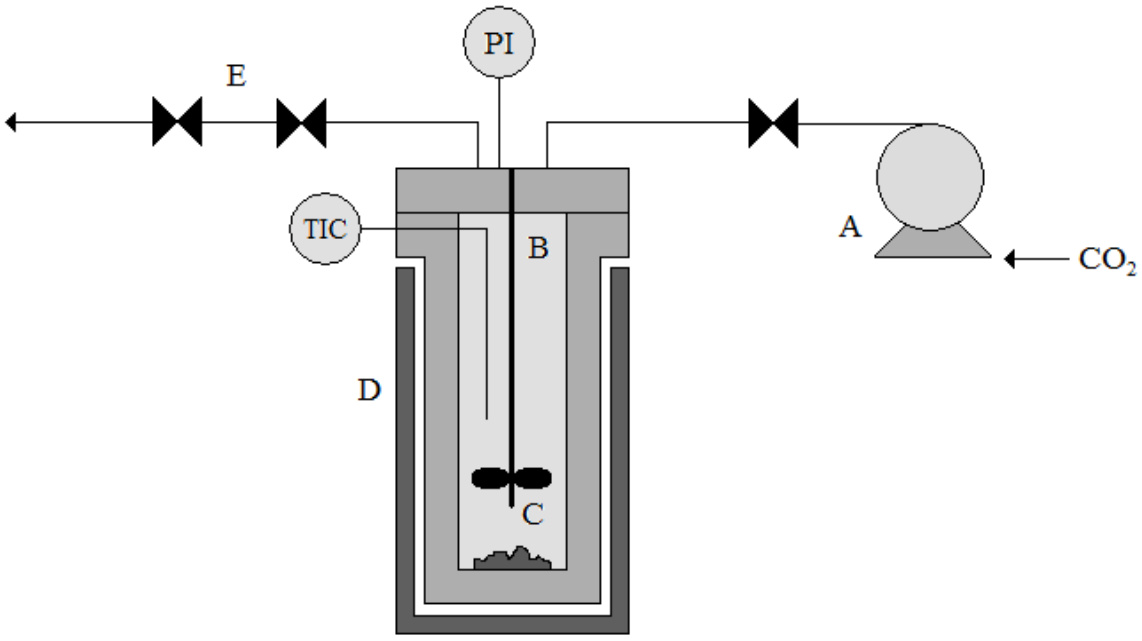

Figure 1. Equipment used to produce porous PLGA foams using the solvent-free encapsulation procedure. It consists of: (A) miniPump metering pump to pressurize the vessel; (B) stainless steel 316 high pressure vessel of 300 ml of volumen; (C) magnetic stirred; (D) electric heating coat; (E) vent valves.

RESULTS

In order to study the process of foaming and impregnation of biopolymers with indomethacin in supercritical CO_2, different values for polymer composition, stirring rate and depressurization time were chosen as it is shown in Table 1. The evaluated parameters were cell size, drug loading and impregnation efficiency.

Table 1. Experimental planning of impregnation and foaming experiments.

Variable	Value 1	Value 2
Molar composition of copolymer	L:G (%) = 74:26 (PLGA)	L:G (%) = 100:0 (PLA)
Stirring rate	500 rpm	1000 rpm
Depressurization time	3 minutes	90 minutes

In first instance, it was observed that the foams based on PLA resulted more crumbly than the based on PLGA.

Also as can be seen in Figure 2, the homopolymer (PLA) exhibits a larger cell size than the copolymer (PLGA). The larger cell size of the PLA foams can explain its higher fragility [3].

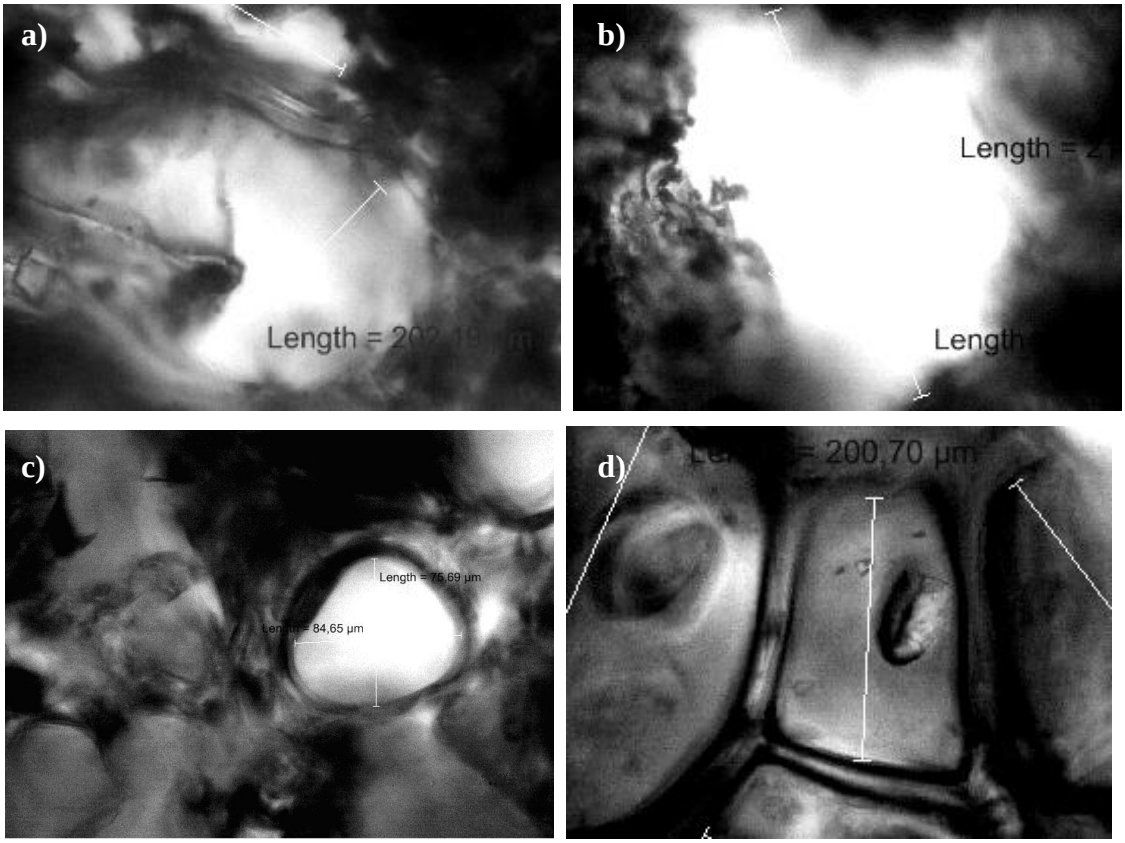

Figure 2. Microphotographies of polymer foams obtained at different depressurization times and their pore sizes. **a)** PLGA, N: 500 rpm, t: 3 min. **b)** PLA, N: 500 rpm, t: 3 min. **c)** PLGA, N: 500 rpm, t: 90 min. **d)** PLA, N: 500 rpm, t: 90 min.

In Figure 3, the influence of stirring rate and type of polymer on the average cell size of the foam is shown. It can be seen that the stirring rate seems not to have a significant influence on the pore size of the polymer foams independently of the type of polymer. This is due to that the stirring rate is fast enough to get a proper dispersion of CO_2 on the polymer that retains it homogeneously distributed inside during and after the depressurization process.

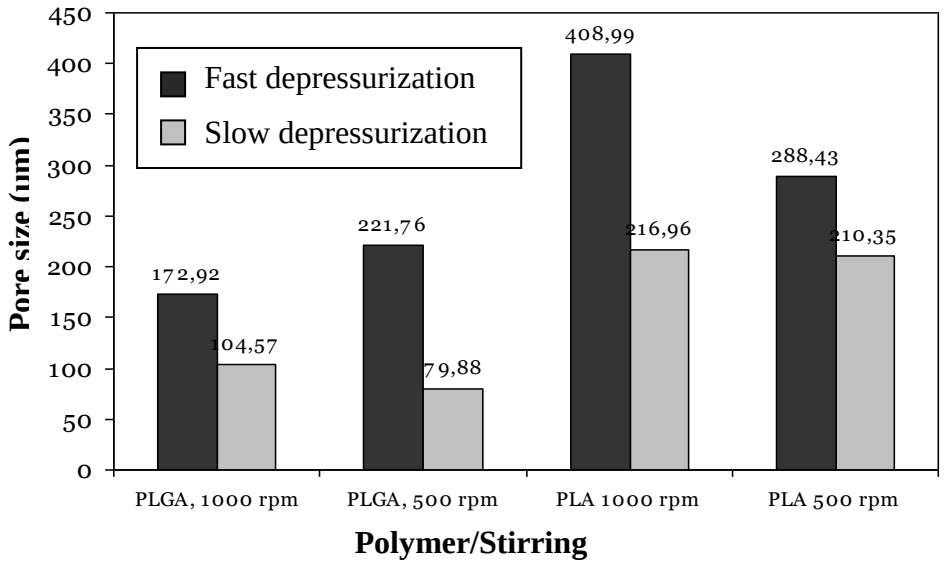

Figure 3. Comparison between average values of pore size in foams synthesized in processes with fast (dark grey) or slow (clear grey) depressurization for the same polymer composition and stirring rate.

Obviously, the faster is the depressurization rate, the greater is the size of the foam cells. According to the literature [4], in supercritical foaming processes with polymers based on lactic and glycolic acids, the higher cell sizes are obtained by the collapse of little ones due to the big force generated in rapid depressurizations. This can be appreciated in Figures 2.a and 2.b, in which the limits of the cells are irregular due to this crumbling. So because smaller cell sizes are preferred [5], slow vent is desirable.

This fact, in addition to the capacity to regulate the degradation rate of the copolymer varying its composition in both monomers [2], suggests that the best choice to prepare the foams is the PLGA.

To determine the efficacy in the impregnation of the process, the quantity of drug loaded in the monoliths in each case was analyzed and two characteristic parameters (drug loading –DL– and impregnation efficiency –IE–) were calculated with Equations (1) and (2) as is usually evaluated in literature [6]:

$$\text{Drug Loading} = \frac{\text{the mass of IDMC impregnated in the monoliths}}{\text{the total mass of monolith}} \cdot 100\% \quad (1)$$

$$\text{Impregnation Efficiency} = \frac{\text{the mass of IDMC impregnated in the monoliths}}{\text{the total mass of IDMC used in the process}} \cdot 100\% \quad (2)$$

The results obtained are summarized in Table 2. Although both of them were calculated, according to these definitions the more interesting parameter is the drug loading since it supposes the mass percentage of drug in the polymeric probe, directly related with the intensity of the effect of the drug in the body. Usually, both parameters follow the same trend but in the case the importance of drug loading prevails over impregnation efficiency.

Table 2. Experimental values of "Drug Loading" and "Impregnation Efficiency"

Polymer	N (rpm)	Depressurization (min.)	DL (%)	IE (%)
PLGA	1000	Fast (3')	13.61	2.26
PLGA	500	Fast (3')	16.71	1.76
PLA	1000	Fast (3')	16.86	0.69
PLA	500	Fast (3')	16.71	1.71
PLGA	1000	Slow (90')	16.80	1.78
PLGA	500	Slow (90')	2.96	0.30
PLA	1000	Slow (90')	21.67	2.34
PLA	500	Slow (90')	7.60	0.86

It seems clear that although the dispersion of the drug in those experiments with more than 10 % of DL is good a slow depressurization rate together with slow stirring rate produces first an inefficient dispersion of the drug into the polymer mass and subsequently the greater time employed for the depressurization of the system allows the loss of the drug with the CO_2 vented.

This effect is not observed in those experiments with low stirring rate but fast depressurization rate in which the values of DL remain high (greater than 10%). Probably at

low stirring rates the dispersion of the drug into the polymer is not as good as at 1000 rpm and the drug remains mainly in the outer part of the polymer mass. When the venting of CO_2 is fast the drug has not time to be released from the polymer but when the depressurization is slower the time is enough to allow the diffusion and further release of a significant part of the IDMC previously dispersed.

It was checked that the indomethacin was dispersed homogeneously into the foamed polymer and not deposited onto the surface in the experiment using PLGA, high stirring rate and slow venting. The thermal analysis by DSC of the impregnated polymer and the pure drug revealed that the peak corresponding to the crystalline structure of the indomethacin does not appear when it is impregnated into the polymer foams, Figure 4.

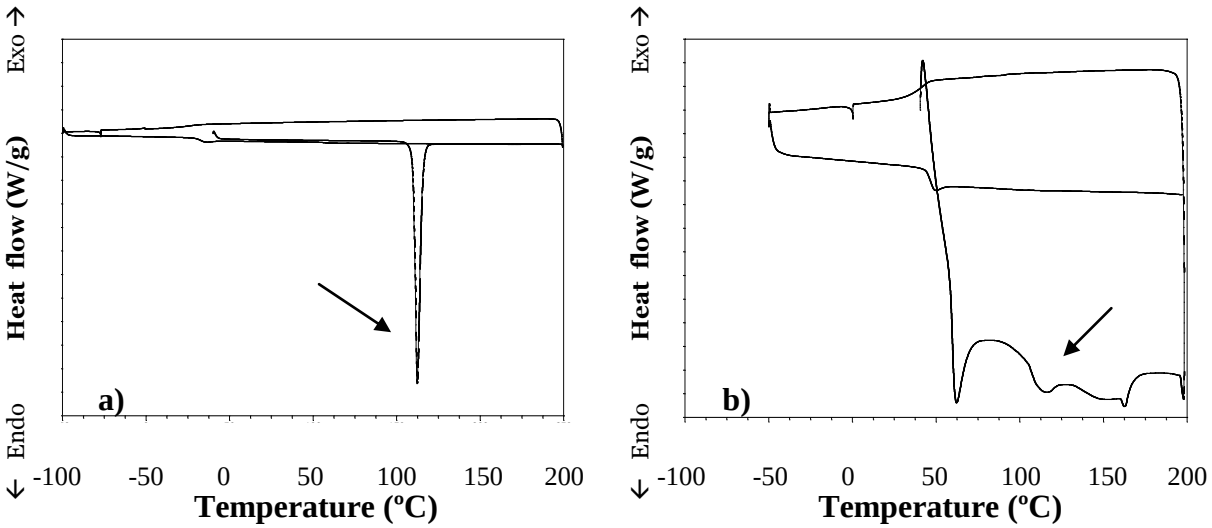

Figure 4. Thermograms: (a) pure IDMC; (b) sample impregnated on PLA at 1000 rpm and depressurizated during 90'.

The indomethacin (IDMC) is crystalline, as it is shown in Figure 4.a (well defined endothermic peak at 162.3ºC). The evidence that the impregnation takes place properly and homogeneously, avoiding the surface deposition, is shown in the change of character of the drug, initially crystalline, but becoming amorphous when the correct process occurs in a correct way in $scCO_2$ [6]. This fact is shown in the Figure 4.b where the peak corresponding to the drug is overlapped with the polymer peak becoming a shoulder in this one and indicating the drug is not in its crystalline form in the polymer blend.

Both polymers exhibit similar impregnation capacities and performance when similar operating conditions are used. It means that both polymers are good carriers for the drug when impregnated in the proper conditions.

It seems that the best results of drug loading are the consequence of using high values of stirring rate together with a slow venting rate, as is expected since a rapid stirring rate favours the dispersion of the indomethacin into the polymer mass. Furthermore, a slow depressurization rate promotes a more homogeneous cell size distribution of the monoliths and also favours the release of the impurities as monomers and the rests of not impregnated but superficial deposited drug [7].

Summarizing both polymers are suitable for the impregnation of IDMC in $scCO_2$ media with impregnation efficiencies around 17% or even greater. High stirring rates and slow

depressurization rates favours the homogeneous dispersion of the drug into the polymeric foam matrix and the formation of more homogeneous and smaller cells.

CONCLUSION

It was found that better values of drug loading and impregnation efficiency were obtained with highest stirring rates (1000 rpm) because favour the dispersion of the drug into the polymer matrix. Little pore sizes are desirable, something obtained at low depressurization times because confer to the foam a greater degradation resistance. The composition of the polymer had some influence on the pore size, producing more fragile PLA foams with larger cells, so the use of the copolymer, PLGA, seems to be the best option to produce biodegradable and biocompatible foams that will allow the regulation of the degradation rate varying the molar ratio lactide:glycolide in its composition.

REFERENCES

[1] SINGH, L., KUMAR, V., RATNER B. D. Biomaterials, Vol. 25, **2004**, p. 2611

[2] MILLER, R. A., BRADY, J. M., CUTRIGHT, D. E. Journal of Biomedical Materials Research, Vol. 11 (5), **1977**, 711

[3] HOWDLE, S. M., WATSON, M. S., WHITAKER, M. J., POPOV, V. K., DAVIES, M. C., MANDEL, F. S., WANG, J. D., SHAKESHEFF, D. M. Chemical Communications, Vol. 1, **2001**, p. 109

[4] LERAY, J. L., CHABOT, F., CHRISTEL, P., VERT, M. Transactions of the Annual Meeting of the Society for Biomaterials in conjuction with the Interna, Vol. 4, **1981**, p. 37

[5] HILE, D. D., PISHKO, M. V. Drug Delivery, Vol. 11, **2004**, p. 287

[6] KANG, Y., WU, J., YIN, G., HUANG, Z., YAO, Y., LIAO, X., CHEN, A., PU, X., LIAO, L. European Journal of Pharmaceutics and Biopharmaceutics, Vol., 70, **2008**, p. 85

[7] MIHAI, M., HUNEAULT, M. A., FAVIS, B. D., LI, H. Macromolecular Bioscience, Vol. 7, **2007**, p. 907