[Lipid-Chitosan] Hybrid Systems, Produced By PGSS®, For Oral Targeted Drug Delivery To Colon

Sara.L.Nunes[a,b], A.A. Matias[a,b], Isabel D. Nogueira[c], Nenad Bundaleski[d], Orlando M.N.D Teodoro[d], A.L. Simplício[a,b], C.M.M. Duarte[a,b*]

[a] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Av. República, 2780-157 Oeiras, Portugal
[b] Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal
[c] Universidade Técnica de Lisboa, IST, Instituto de Ciências e Engenharia de Materiais e Superfícies, P-1096 Lisboa, Portugal
[d] Center for Physics and Technological Research − CEFITEC, Physics Department, Faculty of Sciences and Technology, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
*E-mail: cduarte@ibet.pt, Fax: (+351) 214 421 161

ABSTRACT

Inflammatory bowel diseases (IBD) are a group of chronic disorders that affect millions of individuals. IBD patients need multiple medications and long-term up to life-long treatment. Although the drug delivery systems (DDS) already developed significantly improved the IBD therapeutic, intensive research need to be done in order to design and produce multiple-functional DDS with suitable characteristics in order to target the inflammation site.

This work aimed the production of hybrid particles composed by two different lipids with different hydrophilic-lipophilic balance (HLB) and chitosan in order to achieve a conjugation of properties required in IBD therapy, namely high bioavailability, biocompatibility and controlled release of drug, and also mucoadhesion to retain the particle at colon.

Hybrid particles composed by [Gelucire 43/01 + Gelucire 50/13 + Chitosan] and loaded with ibuprofen sodium salt were successfully obtained using PGSS® method. Operational conditions shown to have influence in the particles morphology, thermal behavior, surface composition, drug load and drug release profiles.

INTRODUCTION

Inflammatory Bowel Diseases (IBD) result from deregulated immune responses against enteric microflora and include two major diseases: Ulcerative Colitis (UC) and Crohn's Disease (CD) [1,2]. Drug targeting to colon is a challenging task in the field of drug delivery, particularly in case of chronic intestinal inflammation, due to the accelerated colonic transit times and all pathophysiological alterations caused by these diseases [3,4]. The drug delivery systems (DDS) already developed include the formulation of nano- or microparticulate systems with pH dependent coating, polymers with colon specific enzymatic cleavage, time – dependent, sustained drug delivery devices, reduction of drug carrier size and with mucoadhesive properties. Although the IBD DDS that have been prepared showed greater therapeutic impacts as compared to their conventional delivery forms, there's still a need for further extensive investigations on this area of research [2]. In response to this problem, the use of hybrid nanoparticles, composed by different polymers or combinations of polymers/lipids that target DDS to the inflammation site, could represent a promising strategy for drug delivery in this field [1].

Furthermore, the upgrade on DDS efficiency is strongly dependent on technological improvements. Several techniques have been extensively used for producing hybrid micro or

nanoparticles such as spray-drying, freeze-drying, fluidization techniques and emulsion and solvent evaporation [5] however they required the use of organic solvents. Alternatively to conventional methods, Supercritical Fluid (SCF) technology has been shown to be a viable option with relevant advantages like the use of mild conditions for therapeutic agents processing, minimization of organic solvent and use of environmentally benign non-toxic materials, and production of smaller particles with controllable morphology and narrow size distribution. [6,7].

The main aim of the present work was to produce solid lipid particles modified with chitosan and loaded with an anti-inflammatory model drug (ibuprofen sodium salt), using PGSS® technique, in order to obtain a mucoadhesive system with the several advantages of solid lipid particles such as higher bioavailability, biocompatibility and controlled release of drug [8]. Materials used in this study were chosen due to their singular properties: Gelucire 43/01 has a low hidrophilic-lipophilic balance (HLB = 1) and it is associated with sustained release, high bioavailability and biocompatibility [9], while Gelucire 50/13 presents a HLB = 13 and it can increase water soluble drug load in lipid particles. Chitosan properties include biocompatibility, mucoadhesion, possibility of surface functionalization and also specific degradation by colonic microflora enzymes [10]..

The influence of operational condition on the morphology, thermal behavior, surface composition, drug load and drug release behavior was investigated.

MATERIALS AND METHODS

Materials

Gelucire 43/01^{TM} and Gelucire 50/13 ^{TM} were a gift from Gattefossé (France). Chitosan (deacetylation degree ≥ 95 %, 15KDa) was purchased from Golden-Shell Biochemical CO., LTD (Zhejiang, China) and Ibuprofen Sodium Salt was from Sigma-Aldrich (St. Louis, USA). CO_2 (99.95 mol% purity) was delivered by Air Liquide (Portugal).

Particles from Gas-Saturated Solutions (PGSS®)

[Gelucire 43/01 :Gelucire 50/13:Chitosan] particles loaded with 10% (w/w) of Ibuprofen sodium salt were produced using the PGSS process (Table 1).

Table 1: Summary of the PGSS® experiments performed

Sample	Pressure (MPa)	Temperature (K)	% Gelucire 43/01(w/w)	% Gelucire 50/13 (w/w)	% Chitosan (w/w)	% Ibuprofen (w/w)
A	20	328.15	67.5	0	22.5	10
B	20	328.15	63.0	4.5	22.5	10
C	20	328.15	58.5	9.0	22.5	10
D	10	328.15	67.5	0	22.5	10
E	10	328.15	63.0	4.5	22.5	10
F	10	328.15	58.5	9.0	22.5	10
G	20	313.15	67.5	0	22.5	10
H	20	313.15	63.0	4.5	22.5	10
I	20	313.15	58.5	9.0	22.5	10

Briefly, carbon dioxide was fed by a high-pressure piston pump to a 50 cm^3 electrically thermostated high-pressure stirred vessel, containing the chitosan powder and the lipids (Gelucire 43/01 and Gelucire 50/13), until the desired working pressure was reached. After 30

minutes of stirring (170 rpm), the mixture was depressurised by an automated depressurisation valve and atomised through a two fluid nozzle (711 μm) to a cyclone, using compressed air (7 bar) as auxiliary fluid (Figure 1). The particles produced were recovered in a 18 L collector vessel.

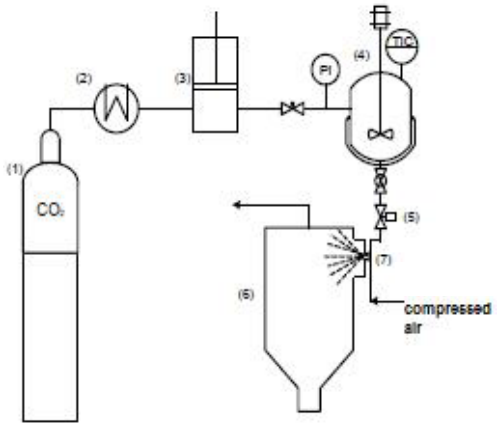

Figure 1: Experimental setup: (1) CO_2 cylinder (2) cryostate (3) pneumatic piston pump (4) stirred vessel (electrically thermostated) (5) automated depressurisation valve (6) recovery vessel (7) nozzle.

Field Emission Scanning Electron Microscopy (FE-SEM)

Particle size and morphology were analyzed visually by FE-SEM (Field Emission Scanning Microscopy) JEOL 7001F. Before the analysis, particles were covered with approximately 300 Å of a gold-platinum film with a sputter-coater in argon atmosphere (Polaron).

Differential Scanning Calorimetry (DSC)

DSC measurement was carried out on a DSC TA instruments Q200 with module MDSC.The samples were placed in an aluminium pan and sealed; the probes were heated from 273,15K to 623,15K at a rate of 10K/min under nitrogen atmosphere.

X-Ray Photoelectron Spectroscopy (XPS)

XPS measurements have been performed on a VSW XPS system with the Class 100 energy analyzer being a part of an experimental setup assembled for surface investigation. The samples have been analyzed using the non-monochromatic Mg Kα line (photon energy of 1256.3 eV). For the energy axis calibration Ag (110) and polycrystalline Au samples (previously cleaned by ion sputtering) were used. The energy was calibrated to the peak position of Ag 3d5/2 (binding energy of 368.22 eV) and Au 4f7/2 (binding energy of 83.96 eV) lines. Survey spectra were taken in the FAT 44 mode (0.5 eV energy step), while the detailed XPS lines were taken in FAT 22 mode with 0.1 eV of energy step.

Determination of Drug Load

The amount of ibuprofen loaded inside the particles was determined by HPLC analysis. Particles were dissolved in acetonitrile and centrifuged and filtered with 0.45 μm syringe filters in order to remove non-dissolved solids before analysis. The HPLC system is an Elite-LaChrom chromatograph, consisting of an Hitachi L-2130 pump, an UV-Hitachi L-2455 detector, and an Hitachi L-2200 autosampler. The separation was carried out in isocratic mode, with a mobile phase formed by 40:60 (%v/v) acetonitrile:water acidified with fosforic acid (pH 2.5), at a flow of 2 mL/min, and a constant temperature of 40°C. The column used

was a reverse phase column Phenomenex//Gemini-Nx-C18 (150 mm length, 4.6 mm diameter) with a mean size of particles of 5 μm and a mean size of pores of 110A˚. A UV/VIS detector set at 221 nm was used.

In vitro evaluation of drug release kinetics

A certain amount of particles was suspended in 25 mL of simulated intestinal fluid, consisting of a phosphate buffer solution with pancreatine (pH = 6.8), according to European Pharmacopeia. Samples were stirred at 150 rpm and maintained at a temperature of 37 ◦C. Aliquots (1mL) were withdrawn at predetermined time intervals (5 min, 15 min, 30 min, 60min, 90min, 120min and 180min)and the same volume of fresh medium was added to the suspension. The samples were filtered and analyzed by HPLC as previously described.

RESULTS

Ibuprofen loaded-[Lipid-Chitosan] hybrid particles (Table 1) were successfully precipitated using PGSS® methodology. Hybrid particles were produced at different pressures and temperatures in order to evaluate operational conditions impact on the physical characteristics of the final particles and also on their drug load and drug release profiles.

Morphology and thermal analyses of particles produced by PGSS®

In Figure 2 it is presented SEM pictures of all hybrid particles produced.

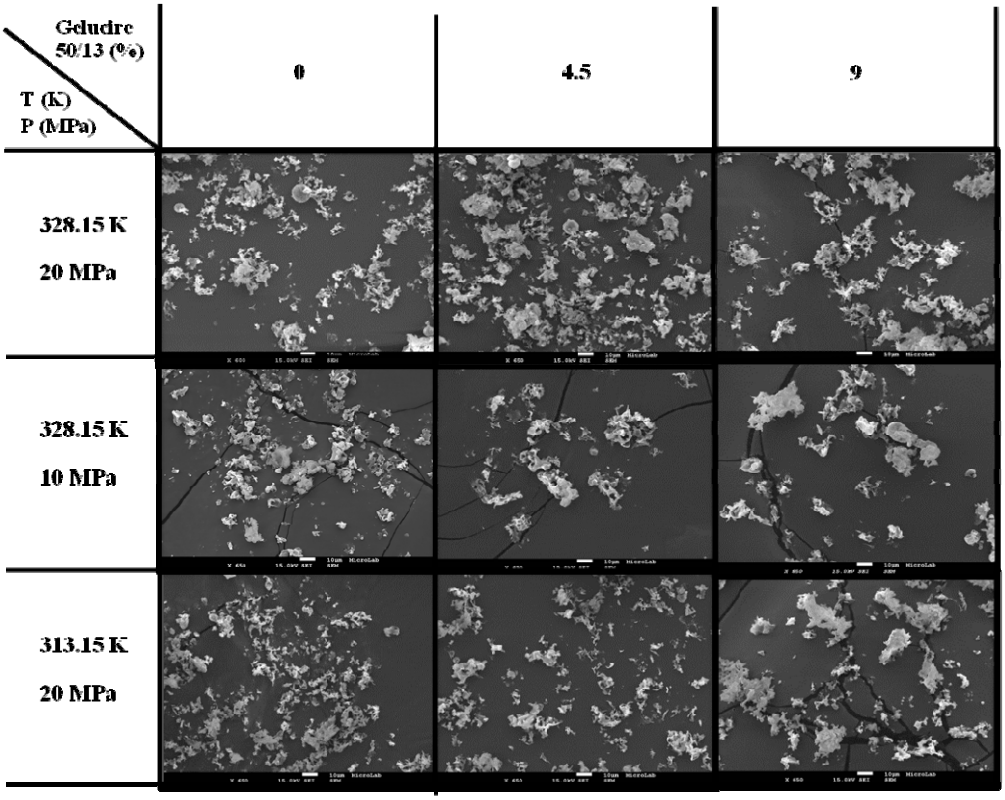

Figure 2 - SEM pictures (x650) of Ibuprofen loaded lipid-Chitosan particles at different operatory conditions.

In general, particles produced presented irregular shape and porosity due to the release of CO_2 from particles during the atomization process [11]. When produced at higher temperature (328.15K) and higher pressure (20MPa) particles tend to be more spheric. This fact can be

explained due to the lower viscosity of lipids with higher temperatures which turns the atomization step easier and also slower solidification of the droplets facilitating the diffusion of CO_2 out of the particles and originating more spheric structures [11].
When Gelucire 53/01 percentage increases in the particle, the particle size also increased which may be explained for the complex composition of Gelucire 53/01.

DSC analyses were carried out on non-processed components of hybrid particles (Table 2) and also on the particles produced (Figure 3).

Table 2: DSC peaks for non-processed pure compounds

DSC peaks	Gelucire 43/01	Gelucire 50/13	Ibuprofen sodium salt	Chitosan
T (K)	318.16	319.59	372.19	414.97
T (K)	-----	-----	469.13	584.27

Gelucire 43/01 presents a sharp endothermic peak at 318.16K corresponding to its melting point while in case of Gelucire 53/01 the endothermic peak appears at 319.59K. Concerning ibuprofen sodium salt, DSC thermogram present two peaks: one at 372.19K that corresponds to the water loss from the structure and other at 469.13K that refers to the melting point [12]. In the case of chitosan, the first peak is due do the water loss from the structure while the second peak is attributed to the decomposition of chitosan [13].
However, it is reported a depression on melting point caused by supercritical-CO_2 in both lipids [9] and in ibuprofen sodium salt [14], which means that in all experiments all component of the mixture should be liquefied, except chitosan that does not melt even in presence of SC-CO2 [15].

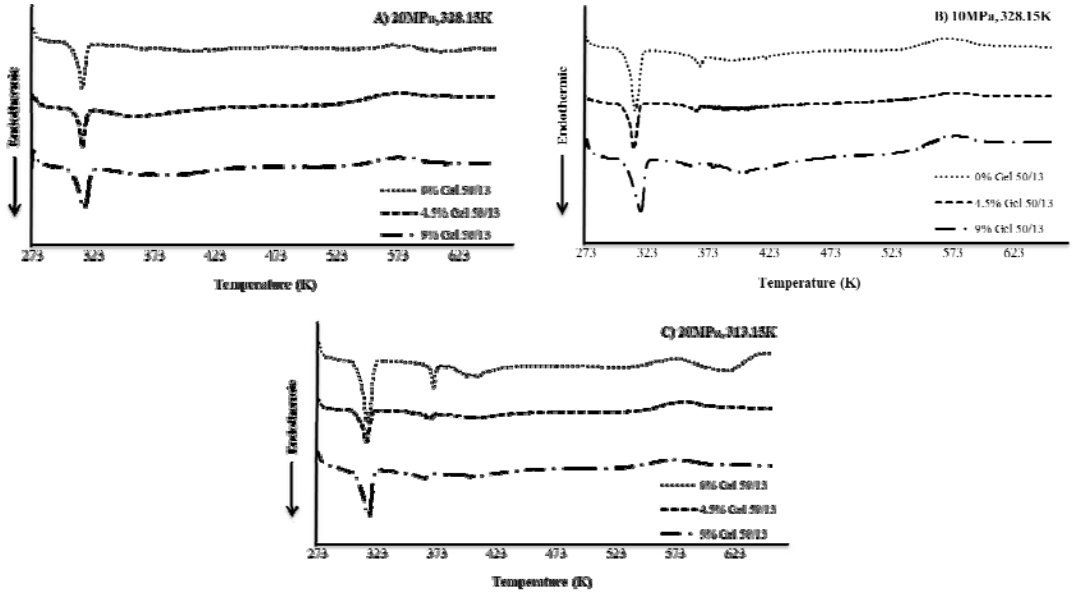

Figure 3: DSC thermograms of Ibuprofen loaded [Lipid- Chitosan] particles

As it is possible to see in figure 3, all particles present a broad exothermic peak around 570K that confirms the presence of chitosan in the particle. As chitosan does not melt, it is dispersed on the molten materials and it is not expected to produce a solid solution.

For particles produced at higher temperature (328.15K) and higher pressure (20MPa), besides chitosan peak, only one more peak can be identified which means that solid solution have been formed between the other components of the particle. However in particles produced without the presence of Gelucire 50/13 in the other two conditions studied (10MPa, 328.15K and 20MPa, 313.15K), it is possible to observe three other peaks, two of them corresponding to the ibuprofen sodium salt and that peak disappears with the addition of Gelucire 50/13. This fact might be due to the higher affinity of ibuprofen sodium salt to hydrophilic carriers.

Drug load and surface analysis

Hybrid particles produced were analyzed for drug load. Particles were destroyed and ibuprofen sodium salt was quantified using HPLC (Table 3).

Table 3: Drug load (%) and Encapsulation Efficiency (%) of ibuprofen-loaded [Lipid-Chitosan] hybrid particles produced by PGSS®

Sample	P (MPa), T(K)	% Gelucire 50/13	Drug load (%)	Encapsulation Efficiency (%)
A	20, 328.15	0	7.75±0.39	77.5±3.9
B		4.5	8.99±0.46	89.9±4.6
C		9	8.67±0.51	86.7±5.1
D	10, 328.15	0	7.85±0.37	78.5±3.7
E		4.5	8.98±0.45	89.9±4.5
F		9	7.93±0.50	79.3±5.0
G	20, 313.15	0	6.51±0.35	65.1±3.5
H		4.5	8.49±0.52	84.9±5.2
I		9	7.93±0.55	79.3±5.5

When Gelucire 50/13 was not present, the drug load is lower, in all conditions processed, which is expectable since ibuprofen sodium salt has higher affinity for hydrophilic materials than for lipophilic ones. Indeed, the addiction of the higher HLB lipid leads to an increase in drug load (Table 3). However, the increase from 4.5% to 9% in Gelucire 50/13 in the particle did not increase the drug load.
The particles with higher drug load (samples B, E and H) were further analysed by XPS in order to determined surface composition (Table 4).

Table 4: Atomic percentage of elements on the surface of different Ibuprofen-loaded [Lipid-Chitosan] particles determined by XPS

XPS analysis of pure chitosan showed the presence of C, O and N elements. From the detailed peak analysis it was determined the composition ratio as nC:nO:nN = 60.0:32.7:7.3, which is

Sample	Relative amount (%)				
	C	O	N	Na	C/O
Gelucire 43/01	89.6	10.4	-	-	8.6
Gelucire 50/13	86.6	13.3	-	-	6.5
Chitosan	60.0	32.7	7.3	-	1.8
Ibuprofen Sodium salt	79.2	16.2	-	4.6	4.9
B	77.5	22.5	0	0	3.4
E	77.9	19.8	0.5	1.9	3.9
H	83.6	15.4	0	1.0	5.4

in line with the result given by Matienzo and Winnacker [16] (nC:nO:nN = 61:31:6). In case of Gelucire 43/01 and in Gelucire 50/13, it was only observed the presence of carbon and oxygen in the spectrum and the mC:mO ratio equal to = [89.6:10.4] and [86.6:13.3], respectively. The most significant difference between the Chitosan and both Gelucire is in the presence of nitrogen in the chitosan; however the sensibility of XPS to nitrogen is low. Additionally, the ratio between carbon and oxygen is significantly different between lipids and chitosan, which can be also used to differ between them and identify the chitosan at the particles surface. In the case of pure ibuprofen sodium salt, it was verified the presence of C, O and Na with the following stoichiometry mC:mO:mNa = 79.2:16.2:4.6.
Analyses of hybrid particles surface suggest the presence of chitosan at the surface of all particles since C/O ratio is lower than both Gelucire C/O ratio. The higher content of chitosan at particle surface can be seen in the sample produced at 10MPa and 328.15K while the lower content is attributed to particles produced at 20MPa and 313.15K.
Regarding ibuprofen sodium salt distribution at particle surface, it can be seen that the condition that favors the encapsulation of the drug is at 20MPa and 328.15K. This fact might be due the spheric and less porous particles formed that efficiently entrapped the drug inside the structure.

In vitro ibuprofen release

The ibuprofen release from the hybrid particles was evaluated using simulated intestinal fluid (pH = 6.8) with pancreatin, according to European Pharmacopeia (Figure 4).

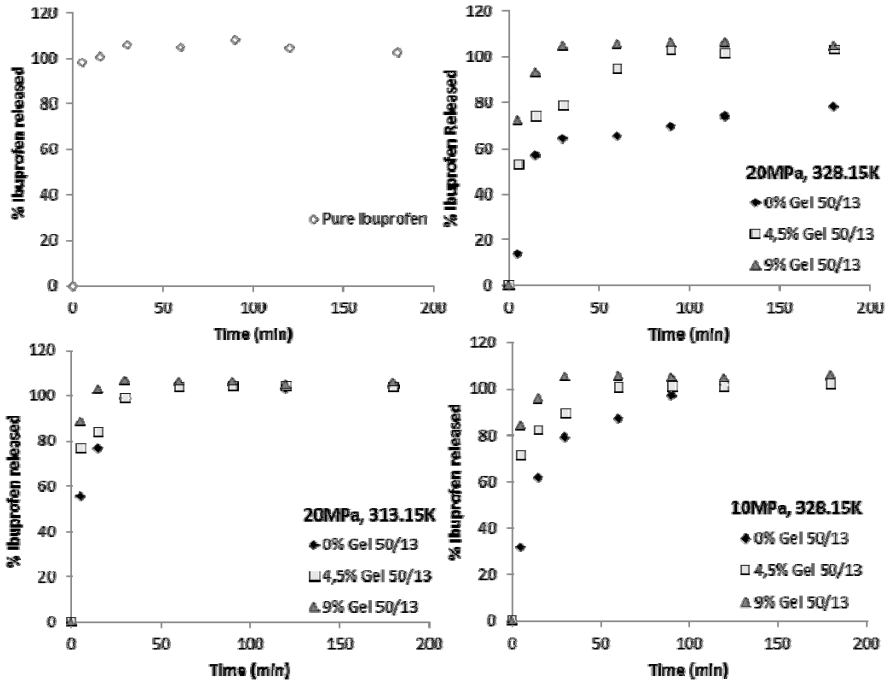

Figure 4: *In vitro* drug release profiles in simulated intestinal fluid

When Gelucire 50/13 is present in the hybrid particles it promotes a burst release due to the dissolution of the carrier in the media. The only condition where a sustained release was attained occurs without the presence of Gelucire 50/13 and at 20MPa, 328.15K. This fact

might be due to the more spheric and less porous structures. Also XPS results suggest that this condition promote an efficient entrapment of ibuprofen sodium salt.

CONCLUSION

Ibuprofen sodium salt loaded [Lipid-Chitosan hybrid] particles were successfully produced by PGSS®. Depending on the operatory conditions it is possible to obtain particles with distinct surface composition, morphology and drug release profile.
Further work will include the evaluation of the mucoadhesive properties of the particles produced and also the study of different lipids and lipids combinations.

REFERENCES

[1] COLLNOT, E.M., ALI, H., LEHR, C.M. World Journal of Controlled Release, vol. 161,**2012**, p.235.
[2] PICHAI, M.V.A., FERGUNSON, L.R. World Journal of Gastroenterology, vol. 18, **2012,** p.2895.
[3] ULBRICH, W., LAMPRECHT, A, Journal of The Royal Society Interface, vol. 7, **2010**, p. S55.
[4] PARMAR, P., WASNIK, S., International Journal of Pharmaceutical Sciences Review and Research, vol. 6, p.167.
[5] ZHANG, L., ZHANG, L., Nano Life, vol. 1, **2010**, p. 163.
[6] SHARIATI, A., PETERS, C.J., Current Opinion in Solid State and Materials Science, vol.7., **2003**, p. 371.
[7] REVERCHON, E., ADAMI, R., CARDEA, S., DELLA PORTA, G., Journal of Supercritical Fluids, vol. 47, **2009**, p. 484.
[8] MUELLER, R.H., MAEDER, K.., GOHLA, S., European Journal of Pharmaceutics and Biopharmaceutics, vol. 50, **2000**, p. 161.
[9] DE SOUSA, A.R.S., CALDERONE, M., RODIER, E., FAGES, J., DUARTE, C.M.M, Journal of Supercritical Fluids, vol.39, **2006**, p.13.
[10] PATEL, M.P., PATEL, R.R, PATEL, J.K., Journal of Pharmacy & Pharmaceutical Sciences, vol.13, **2010**, p. 536.
[11] KAPPLER, P., LEINER, W., PETERMANN, M., WEIDNER, E. Proceedings of the Sixth International Symposium on Supercritical Fluids, Vol. 3, **2003**, p. 1891.
[12] LEE, T., CHEN, Y.H., ZHANG, C.W., Pharmaceutical Technology, vol. 31, **2007**, p.72.
[13] ZHANG, C., PING, Q., DING, Y., CHENG, Y., SHEN, J., Journal of Applied Polymer Science, vol. 91, **2004**, p. 659.
[14] RIZZI, M. Master Thesis, Pádova University, **2011**.
[15] CASETTARI, L., CASTAGNINO, E., STOLNIK, S., LEWIS, A., HOWDLE, S.M., ILLUM, L., Pharmaceutical Research, vol. 28, **2011**, p. 1668.
[16] MATIENZO, L.J, WINNACKER, S.K., Macromolecular Materials and Engineering, vol. 287, **2002**, p. 871.