# Supercritical Fluids (SCF) strategies to produce double-walled particles for drug delivery applications

*Soraya Rodriguez-Rojo*,[a] *Duarte Rego*,[b] *Ana V.M. Nunes*,[b,c] , *Isabel D. Nogueira*,[d] *Maria J. Cocero*,[a] *Catarina M.M. Duarte* [b,c]*

[a]High Pressure Processes Grp, Dept Chem Engn & Environm Technol, Universidad de Valladolid, Dr Mergelina s/n-47005 Valladolid, Spain
[b]Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal
[c]Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, Avenida da Republica, 2780-157 Oeiras, Portugal
[d]Universidade Tecnica de Lisboa, Instituto Superior Tecnico, Instituto de Ciencias e Engenharia de Materiais e Superficies, P-1096 Lisbon, Portugal

*cduarte@itqb.unl.pt

Core-shell particles are attracting much attention in the development of suitable delivery systems to overcome barriers to drug's usefulness. In this work, the possibility of producing structured hybrid polymer-lipid particles by supercritical fluid processing, using the PGSS® (Particles from Gas Saturated Solutions) technique, was explored. The selected system of carriers is composed by a hydrophilic polymer, PEG 4000, and a lipid with low hydrophilic- lipophilic balance (HLB), Gelucire ® 43/01. Different process variables such as pressure, temperature and polymer/ lipid ratio were experimented in order to evaluate the influence of operating conditions on the morphology of the particles produced by this method. More sphere-like particles were obtained with increasing temperature and polymer/lipid ratios whilst for the experimented pressure range, no significant effect on particles' morphology was observed. The core-shell structure of the prepared systems was evaluated by Differential Scanning Calorimetry (DSC), confococal microscopy and by the determination of drug release profiles using flufenamic acid as the model active principle.

## INTRODUCTION

Research on controlled delivery systems is essential to improve the therapeutic effect of drugs, especially for molecules with narrow therapeutic ranges or high toxicity (chemotherapy drugs) [1]. Traditional release systems made of a single substance (polymer or lipid) have several limitations such as high initial burst and low encapsulation efficiency [2]. Therefore much interest has centred on developing core/shell or double-walled particles as an effective way to encapsulate a large number of substances ranging from organic molecules to biological macromolecules [3]. Another important advantage of these systems is the possibility of delivering more than one drug in a sequential manner [4, 5]. These particles can be either polymer-polymer or polymer-lipid structures.

Focusing on hybrid polymer-lipid structures, a common structure is to get a lipid core surrounded by a hydrophilic polymer, such as polyethylene glycol (PEG), which reduces the biofouling of the particles, resulting in long circulation half-life [5]. The role of the hydrophobic core can be either the controlled release of highly water soluble drug or to carry

poorly water-soluble drugs. In the latter case, lipids with high HLB are preferred. Furthermore, lipid carries can also act as absorption enhancers [6]. Conventional processes to produce such particles are melt-solidification, extrusion-spheronization [7], hot-melting extrusion [8] and melt dispersion–coacervation [9] techniques. These techniques implies many post-processing steps, as filtering, washing, drying, that are time consuming and difficult the scale-up of the process. Besides the particle size range is frequently between 0.5 – 2 mm and the double-layered structure is not always reported. More recent techniques are spray-congealing [10] and self-assembly [11] or self- emulsifying [12] process in aqueous solution, which can be followed by freezing drying step to obtain dry particles. The latter is particularly successful in the preparation of core-shell nanoparticles [5].

The aim of this work was to evaluate the feasibility of using supercritical precipitation techniques as alternatives to the aforementioned processes in the production of structured polymer –lipids particles, in order to reduce the number of process steps and make easier the scale-up of the process. Also, the content of residual organic solvents in the final product will be eliminated.

Hybrid lipid – polymer systems are proposed to be processed by PGSS® (Particles from Gas Saturated Solutions) [13]. This technique has been reported to be suitable for successfully processing both, lipids [14, 15, 16] and semicrystalline polymers, such as PEG, [17,18]. Our approach is to form an emulsion of hydrophilic polymer with a lipid, both molten in SC-$CO_2$, which would be micronized afterwards by a sudden decrease in pressure. PEG 4000 has been chosen as a hydrophilic polymer. Gelucire 43/01, with low hydrophilic-lipophilic balance (HLB) value, has been selected as the lipid. Both are widely used in pharmaceutical formulations, the first as solubility enhancer of poor-water soluble drugs [19] and the second, mainly as protective agent against light, oxidation and moisture [6] and release retardant [20]. Process variables such as pressure, temperature, stirring rate, polymer to lipid ratios, and lipid HLB were also studied in order to optimize operating conditions.

Flufenamic Acid (FA), a non-steriodal anti-inflammatory drug (NSAID) from the family of fenametes, was used as model drug and the determination of the release profiles was carried out to evaluate the distribution of the carriers in the particles.

## MATERIAL AND METHODS

PEG 4000, PEG 20000, Flufenamic Acid and Fluorescein Sodium Salt were purchased from Sigma. Lipids were kindly supplied by Gattefosé (Gelucire ® 43/01 and Gelucire ® 50/13) and Lambent Technologies (GMS-K). $CO_2$ with 99.95% purity was delivered by Air Liquide.

Particles were produced by PGSS (Particles from Gas Satured Solutions) in batch mode. The experimental setup (Fig. 1) consists on a high-pressure stirred vessel (4) where carbon dioxide is dissolved in the bulk of a melted mixture of substances, at certain pressure and temperature conditions. This gas saturated solution is further expanded through a 600 μm nozzle (7; Teejet TGSS, Spraying Systems Co.), into a collector (6) where particles are

recovered. Further details of the apparatus can be found in the work of Sampaio de Sousa [15].

The operating conditions used were based on data of temperature melting depression of the polymers and lipids in the presence of carbon dioxide from previous works [15, 16, 25] and literature [26, 27]. The minimum melting temperature data of each compound, and the corresponding pressure, are gathered in Table 1.

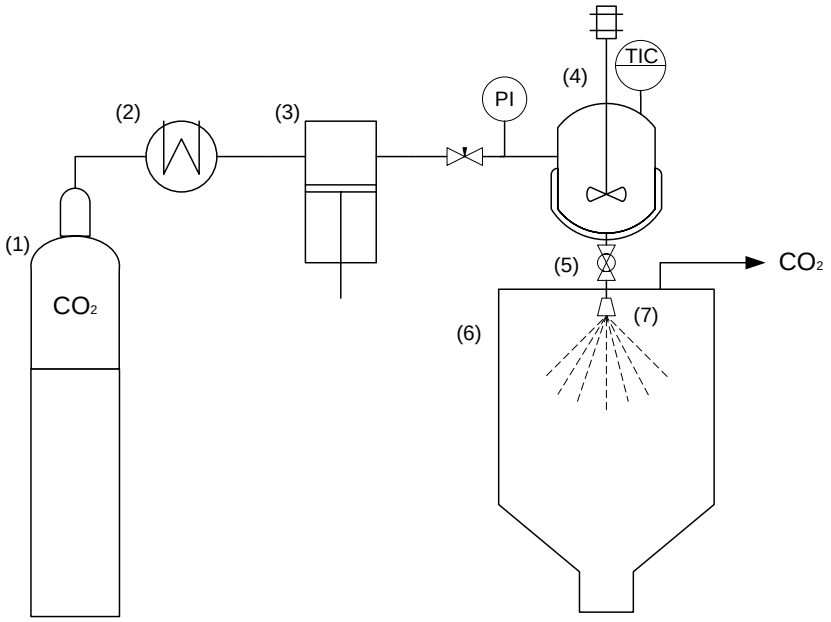


*Figure 1. Experimental setup. (1) Gas cylinder (2) cryostate (3) piston pump (4) stirred vessel (electrically thermostated) (5) depressurization valve (6) recovery vessel (7) nozzle*

Particle size and morphology were analyzed by FE-SEM (Field Emission Scanning Microscopy) JEOL 7001F. In order to check the core-shell structure, Confocal Fluorescence Microscopy was tested. A small amount (0.01 w.t.%) of a hydrophilic dye, fluorescein sodium salt, was added to the polymer-lipid mixture. Differential Scanning Calorymetry (DSC) anlysis (DSC 131 Setaram) were carried out, to check whether the two components of the particles were structured.

| **Component** | **$T^{o}_{m}$ (ºC)** | **$T_{m,\,min}$(ºC)** | **P (MPa) ≥** | **Literature** |
|---|---|---|---|---|
| PEG 4000 | 60 | 44 | 8 | [26][27] |
| PEG 20000 | 65 | 53 | 10 | [27] |
| Gelucire ® 43/01 | 40 | 37 | 9 | [25] |
| Gelucire ® 50/13 | 48 | 37 | 9 | [16] |
| GMS-K | 70 | 61 | 9 | [15] |

*Table 1. Physical properties of the carriers materials*

The loading of FA in particles was quantified by complete dissolution of a sample in chloroform and subsequent determination of UV absorption of the sample at 285 nm. The release rate of the drug from the different formulations in isotonic phosphate buffer pH 6.8 was measured, since FA solubility, at lower pH values (i.e. pH = 1.2), is very low (≈ 1ppm) [28]. A determined amount of sample, the necessary to achieve a maximum concentration of 100 ppm of FA, was placed in 500 mL of solution at 37ºC. The mixture was stirred at 100 rpm for 8h and 2 mL aliquots were taken at pre-defined intervals. The sample volume was

replaced with fresh buffer solution. The aliquot was filtered through a membrane filter (0.2 μm Sarstedt) and the filtrate was directly analysed by UV-spectrophotometry at the same wave length as in the loading quantification.

## RESULTS

Firstly, PEG 4000 and Gelucire ® 43/01 were processed by PGSS independently at 12 MPa and 50ºC. These operating conditions were chosen to assure melting state of the components (Table 1). The SEM analyses of these samples are shown in Figure 2. PEG particles are sphere-like with a broad particle size distribution (≈ 500 nm – 10 μm) and tendency to agglomerate; they are quite porous, as well, due to the release of solubilised $CO_2$ during depressurization. On the contrary, branched-polyp-shape Gelucire ® 43/01 particles do not show porosity particles but they also have a wide range of particle size.

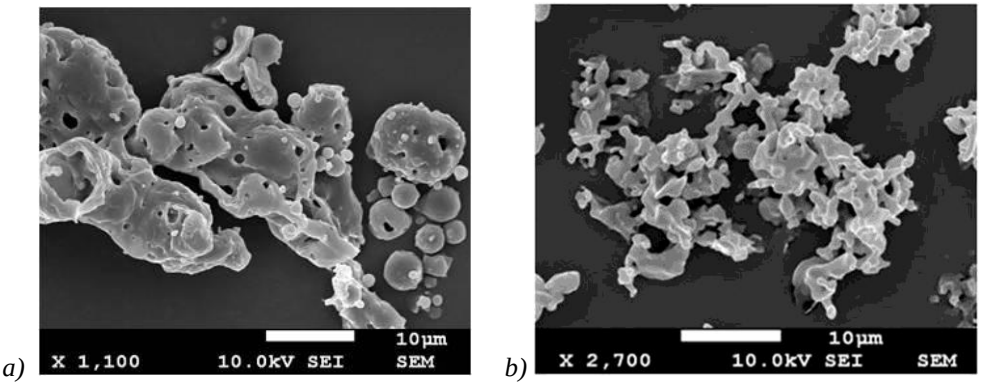


*Figure 2. a) PEG 4000 particles b) Gelucire® 43/01*

Next, PEG 4000 and Gelucire ® 43/01 were processed together at the same operating conditions. Both substances, in a ratio of 2:1, respectively, were mixed with $CO_2$ at 150 rpm for 20 min to assured the dissolution of $CO_2$. SEM photographs (Figure 3.a and b.) show that there is a great dispersion in size and shape, and also tendency to agglomeration. Confocal fluorescence microscopy image (Figure 3. c) shows that big particles are mainly formed by agglomeration of smaller ones with a core-shell-like structure; being PEG 4000 at the outer part of the particles, since it retains preferentially the hydrophilic fluorescence dye (green areas).

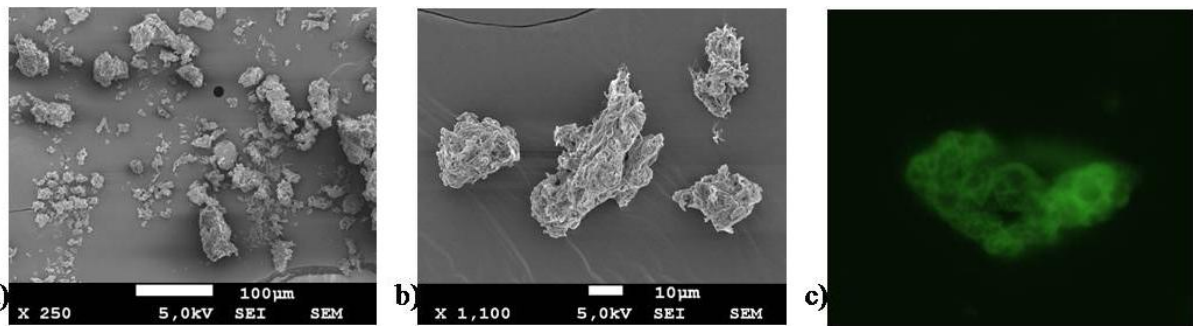


*Figure 3. Particles of PEG 4000:Gelucire® 43/01(2:1) at 50ºC and 12MPa, a) SEM photograph, general view b) SEM photograph, detail c) Confocal microscope of particles with hydrophilic dye*

The DSC analysis (Figure 4) also suggests structured core-shell particles, being the melting points of both components identified.

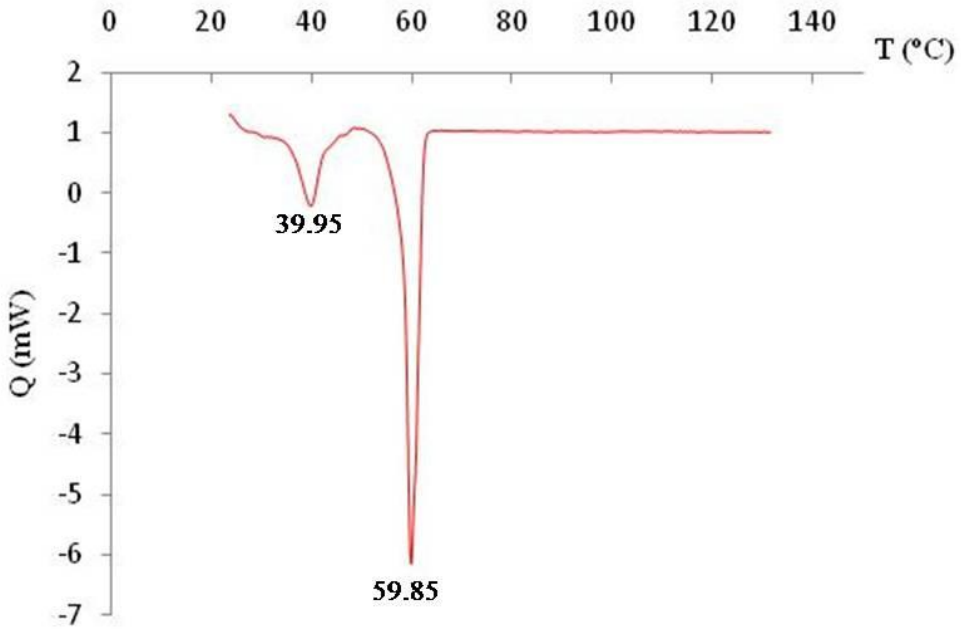


*Figure 4. DSC analysis of PEG 4000:Gelucire® 43/01(2:1) particles processed at 50ºC and 12MP, heating rate 2ºC/min*

The influence of the operating conditions was studied in the range from 12 to 15 MPa and 50 – 70ºC. The morphological analysis of the sample (Figure 6) shows that there is no clear influence of the pressure while the increase in temperature seems to favour the production of more sphere-like particles, in agreement with findings from Hao and co-workers [18] for particles of PEG 5000.

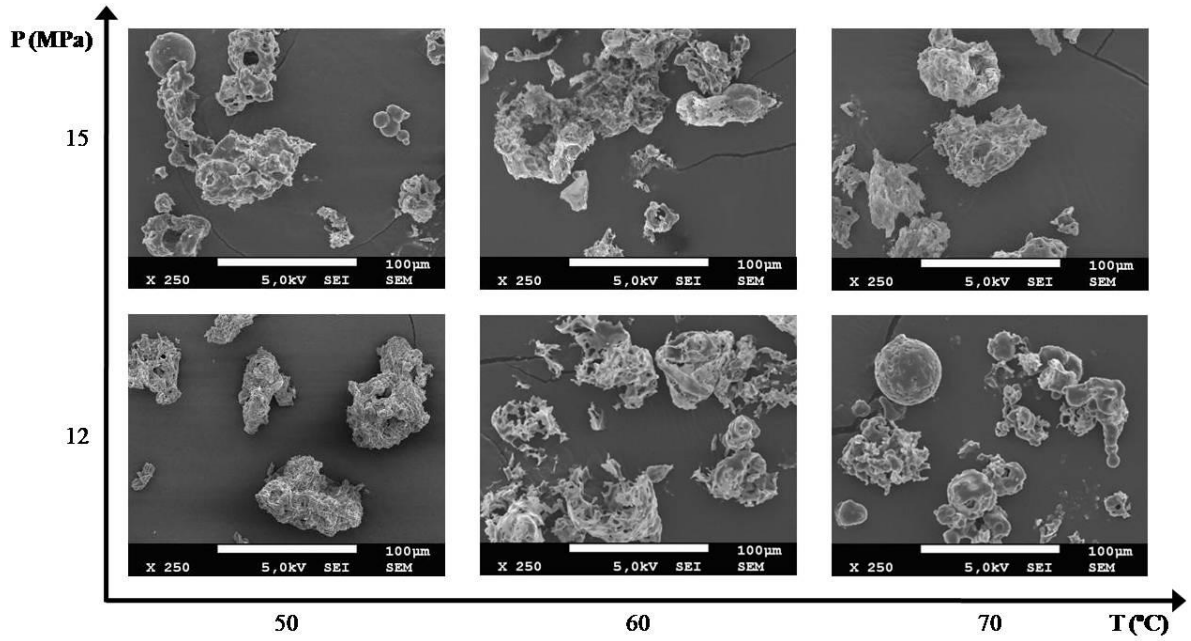


*Figure 4. Particles of PEG 4000:Gelucire® 43/01(2:1). Effect of operating conditions.*

A further increase on temperature would limit the application to thermo-labile drug, therefore the use of a nozzle with a smaller diameter (300 µm, Fullcone TGSS, Spraying Systems Co.) was tested to produce of more sphere-like particles, as pointed out in that work [18]. In our case the particles are no more sphere-like, but particle size is less broad.

In order to gain an insight into the structure of the particle, and regarding final application as drug deliver system, PEG 4000:Gelucire ® 43/01 particles were loaded with FA in the ratio of 14 (carrier) to 1 (drug). The operating conditions were kept constants (50ºC, 12 MPa) while the ratio PEG 4000: Gelucire ® 43/01 was varied to check its effect on the release profile. The drug load (mg FA/ 100 mg particles) and the release profile were determined following the procedure state in section 2, Material and Methods. The drug load shows (Table 2) that the FA, because of its hydrophilic character, is preferentially encapsulated in PEG 4000 formulations. Also, the hydrophilic character of PEG 4000 increase the dissolution rate of FA, and the maximum release is faster achieved although there is no complete dissolution (Figure 6). This behaviour is observed in all the formulations with a content of PEG 4000 higher or equal to that of Gelucire ® 43/01, which leads to assume that PEG 4000 forms the shell of the particles. In contrast, when the carrier is lipophilic, the loading is relatively low, is there is no initial burst but complete dissolution may be attained. In a system like the proposed - a hydrophilic polymer on the shell, a lipophilic carrier on core and a hydrophilic drug (FA) with no hindrance for dissolution – the release curve should show two differentiate profiles: firstly, an initial burst, corresponding to the progressive dissolution of the outer layer of PEG, which also holds the higher amount of drug, followed by a short plateau consequent to the complete dissolution of PEG; secondly and finally, a new increase in dissolution with a lower slope corresponding to the drug release from gelucire® core which eventually would reach a plateau when all the drug were released. This pattern is observed in the formulations with the PEG: Gelucire ratios of 1:1 and 1:2. Nevertheless the core-shell structure cannot be ruled out in the formulations of 2:1 and 4:1, whose DSC analyses suggests structured particles, because the stronger affinity of FA for PEG 4000, and the smaller amount of Gelucire ® in these samples can hide the role of the lipid core.

| PEG:GEL | Drug Load |
|---|---|
| 1:0 | 6.0 ± 0.5 |
| 1:1 | 6.0 ± 0.5 |
| 2:1 | 5.8 ± 0.7 |
| 4:1 | 6.4 ± 0.1 |
| 1:2 | 4.8 ± 0.5 |
| 0:1 | 3.8 ± 0.6 |

*Table 2. Drug load (mg FA/100 mg particles)*

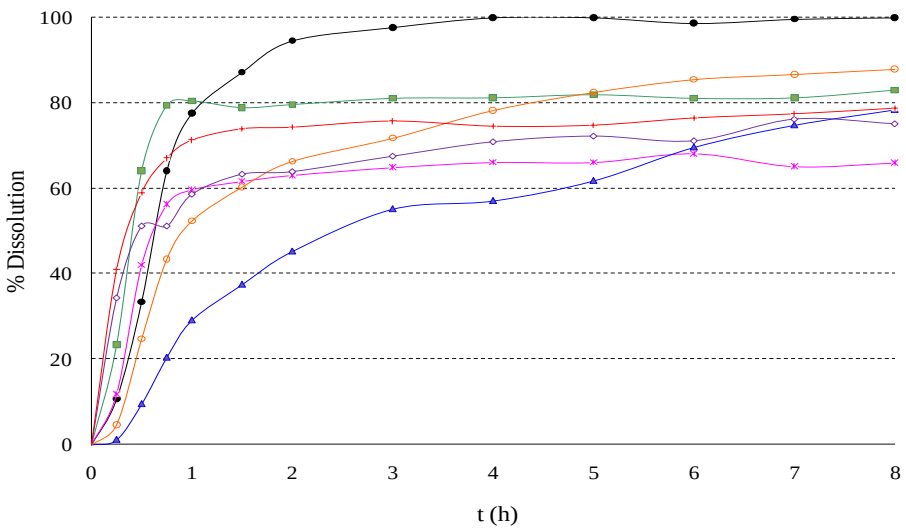


*Figure 6. Release profiles of Flufenamic acid (FA) in buffer solution pH 6.8 from particles of PEG 4000: Gelucire® 43/01 of different ratios. ● no carrier ■ 1:0; ▲0:1; ◊ 1:1; □ 2:1; + 4:1 and ○1:2.*

In other to get a better knowledge on the drug release performance of the particles, new release profiles with a lipophilic drug will be programmed.

To investigate the applicability of this procedure, lipids with different hydrophilic-lipophilic balance (HLB) were tested. The HLB was varied in the range from 1 (Gelucire ® 43/01) to 13 (Gelucire ® 50/13). The experimental conditions (12 MPa, 50ºC) were kept, except when processing GMS-K which melting temperature in SC-$CO_2$ atmosphere was above 50ºC. In this experiment the operating temperature was 61ºC and the pressure was set at 12,6 MPa. In the DSC thermogram (Figure 7), the mixture for components when increasing the HLB is quite clear. The picks for GMS-K ($T^o_m$ = 70ºC) and Gelucire ® 50/13 ($T^o_m$ = 46 - 51ºC) are not well defined and situated at lower temperatures (68.5ºC and 39.8ºC, respectively), while the pick associated to PEG at ca.60ºC is broader. The outer morphology of the particles is quite similar to those composed by PEG: Gelucire ® 43/01.

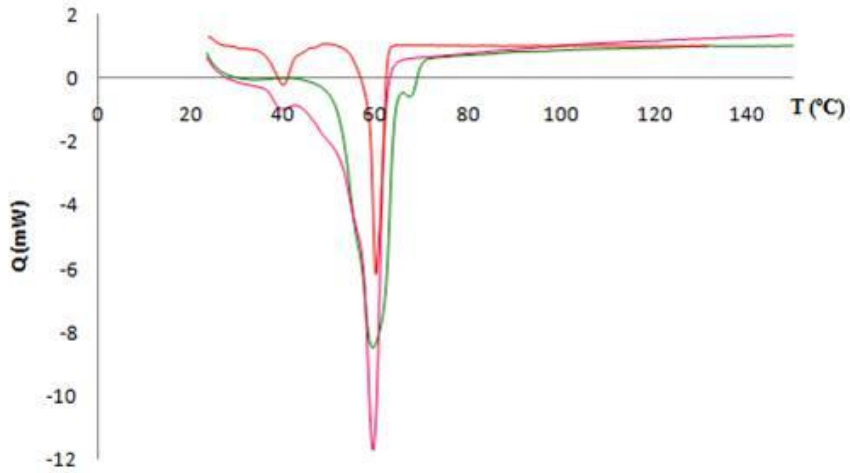


*Figure 7. DSC thermogram of particles of PEG with lipid of different HLB. Red line Gelucire® 43/01(HLB = 1); Green line GMS-K (HLB=4) and pink line, Gelucire® 50/13 (HLB = 13). In all the experiments the ratio PEG: lipid was 2:1.*

Also the molecular weight (MW) of the polymer was increased. A PEG with a mean MW 20,000 was chosen. Since its melting temperature for $CO_2$ pressures above 8 MPa, is slighty higher than 50ºC, the same operating conditions as for the processing of PEG 4000: GMS-K, were selected: 61ºC and 15 MPa. The particle shape (Figure 8) varies from tiny spheres to fibbers, including branched-polyp-shape particles. Fibbers are likely composed just by PEG 20,000.

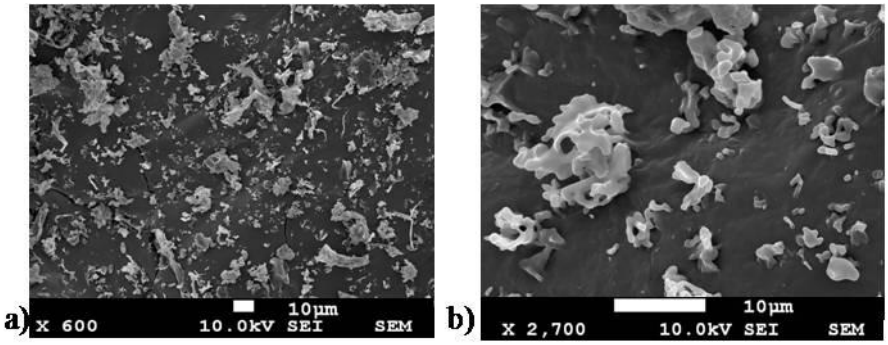


*Figure 8. Particles of PEG 20,000: Gelucire® 43/01 (2:1) a) General view b) detail. (scale bar: 10µm)*

## CONCLUSIONS AND FUTURE WORK

Hybrid polymer-lipid microparticles were prepared using the PGSS technique. The core-shell structure for the system PEG 4000: Gelucire ® 43/01 with different mass ratios has been confirmed through DSC analysis and release profiles of Flufenamic acid. The success of the process is related to the difference in the hydrophilic and lipophilic character of the components; PEG 4000 is a water soluble polymer and Gelucire ® 43/01 is highly hydrophobic, so in melted state, both phases are separated and an emulsion can be formed with the adequate mixing. If the hydrophilic to lipophilic balance (HLB) of the lipid is increased, part of the system is mixed-up, as evidence by DSC analysis.

The process should be improved to avoid particle agglomeration and to get a more uniform particle size distribution. Agglomeration may be caused by particle collision before completed solidification of the materials, so a faster solidification should be achieved.

## ACKNOWLEDGMENTS

The authors would like to thank the financial support from Conselho de Reitores das Universidades Portuguesas and the Spanish Ministerio de Ciencia e Innovación ( PT2009-0173) through the Integrated Actions Portugal – Spain, the Portuguese Fundacão para a Ciência e Tecnologia (PTDC/CTM/70513/2006) and Junta de Castilla y León (Spain), project GR11/ 2008.

## REFERENCES

[1] N.A. Rahman, E. Mathiowitz, Localization of bovine serum albumin in double-walled microspheres, Journal of Controlled Release 94 (2004) 163– 175

[2] T. H. Lee, J. Wang, C.-H. Wang, Double-walled microspheres for the sustained release of a highly water soluble drug: characterization and irradiation studies, Journal of Controlled Release 83 (2002) 437–452

[3] K.S. Oh, K. E. Lee, S. S. Han, S. H. Cho, D. Kim, S. H. Yuk, Formation of Core/Shell Nanoparticles with a Lipid Core and their Application as a Drug Delivery System, Biomacromolecules 2005, 6, 1062-1067

[4] Pekarek, K. J.; Jacob, J. S.; Mathiowitz, E., Double-Walled polymer microspheres for controlled drug-release. Nature 1994, 367 (6460), 258-260.

[5] Zhang, L. F.; Chan, J. M.; Gu, F. X.; Rhee, J. W.; Wang, A. Z.; Radovic-Moreno, A. F.; Alexis, F.; Langer, R.; Farokhzad, O. C., Self-assembled lipid-polymer hybrid nanoparticles: A robust drug delivery platform. Acs Nano 2008, 2 (8), 1696-1702.

[6] D.Q.M. Craig, Lipid matrices for sustained release—an academic overview, Bull. Tech. Gattefossé 97 (2004), pp. 9–19.

[7] F. Siepmann, S. Muschert, M.P. Flament, P. Leterme, A. Gayot, J. Siepmann, Controlled drug release from Gelucire-based matrix pellets: Experiment and theory, International Journal of Pharmaceutics 317 (2006) 136–143

[8] Dalpiaza, M. Mezzenaa, A. Scatturina and S. Scalia, Solid lipid microparticles for the stability enhancement of the polar drug N6-cyclopentyladenosine, International Journal of Pharmaceutics 355 (2008) 81-86

[9] F. Salaün, I.Vroman and C. Aubry, Preparation of double layered shell microparticles containing an acid dye by a melt dispersion–coacervation technique, Powder Technology 192 (2009) 375-383

[10] B. Albertini, N. Passerini, M. Di Sabatino, B. Vitali, P. Brigidi, L. Rodriguez, Polymer–lipid based mucoadhesive microspheres prepared by spray-congealing for the vaginal delivery of econazole nitrate, european journal of pharmaceutical sciences 36 (2009) 591–601

[11] K.S. Oh, K. E. Lee, S. S. Han, S. H. Cho, D. Kim, S. H. Yuk, Formation of Core/Shell Nanoparticles with a Lipid Core and their Application as a Drug Delivery System, Biomacromolecules 2005, 6, 1062-1067

[12] P. R. Patil, S. V. Biradar, A. R. Paradkar, Extended Release Felodipine Self-Nanoemulsifying System, AAPS PharmSciTech 10 (2009) 515-523

[13] E.Weidner, Z. Knez, Z. Novak, Process for preparing particles or powders, Patent WO95/21688 (1995).

[14] N. Elvassore, M. Flaibani, K. Vezzù, A. Bertucco, P. Caliceti, A. Semenzato, S. Salmaso, Lipid system micronization for pharmaceutical applications by PGSS techniques, 6th International Symposium on Supercritical Fluids, 28-30 April (2003) Versailles (France) April

[15] A.R. Sampaio de Sousa, A. L. Simplício, H. C. de Sousa, C. M.M. Duarte, Preparation of glyceryl monostearate-based particles by PGSS®—Application to caffeine, J. of Supercritical Fluids 43 (2007) 120–125

[16] A.R. Sampaio de Sousa, R. Silva, F. H. Tay, A.L. Simplício, S.G. Kazarian, C.M.M. Duarte, Solubility enhancement of trans-chalcone using lipid carriers and supercritical CO2 processing, J. of Supercritical Fluids 48 (2009) 120–125

[17] P. Senčar-Božič, S. Srčič, Ž. Knez, J. Kerč, Improvement of nifedipine dissolution characteristics using supercritical CO2, Int. J. Pharm. 148 (1997)

[18] J. Hao, M. J. Whitaker, G. Serhatkulu, K. M. Shakesheff , S. M. Howdle, Supercritical fluid assisted melting of poly(ethylene glycol): a new solvent free route to microparticles, Journal of Materials Chemistry 15 (2005) 1148–1153

[19] D.M.Q. Craig , Polyethylene glycols and drug release. Drug Dev. Ind. Pharm. 16 (1990) 2501–2526

[20] S. K. Jain, A. Gupta, Development of Gelucire 43/01 Beads of Metformin Hydrochloride for Floating Delivery, AAPS PharmSciTech 10 (2009) 1128-1136

[21] Lentjes EG, van Ginneken CA Pharmacokinetics of flufenamic acid in man. Int J Clin Pharmacol Ther Toxicol 25 (1987) 185–187

[22] C.-C. Tsai, M.-J. Lee, H.-M Lin, Micronization of Flufenamic Acid with Rapid Expansion of Supercritical Solution (RESS) Method, Proceddings of the 11th European Meeting on Supercritical Fluids, 4 -7 May (2008), Barcelona (Spain)

[23] M.-J. Lee, C.-C. Tsai, H.-M Lin, Solubilities of non-steroidal anti-inflammatory drugs in supercritical carbon dioxide, Proceddings of the 11th European Meeting on Supercritical Fluids, 4 -7 May (2008), Barcelona (Spain)

[24] Laitinen, O. Jauhiainen, and O. Aaltonen, Solubility of Fluorinated Pharmaceuticals in Dense Carbon Dioxide, Organic Process Research & Development 4 (2000) 353-356

[25] A.R. Sampaio de Sousa, M. Calderone, E. Rodier, J. Fages, C.M.M. Duarte, Solubility of carbon dioxide in three lipid-based biocarriers, The Journal of Supercritical Fluids 39 (2006) 13–19.

[26] I. Pasquali, L. Comi, F. Pucciarelli and R.Bettini, Swelling, melting point reduction and solubility of PEG 1500 in supercritical CO2, International Journal of Pharmaceutics Volume 356, Issues 1-2, 22 May 2008, Pages 76-81

[27] E. Weidner, V. Wiesmet, Z. Knez, M. Skerget, Phase equilibrium (solid-liquid-gas) in polyethyleneglycol - carbon dioxide systems, Journal of Supercritical Fluids 10 (1997) 139-147

[28] K. Nakanishi, Masukawa T., Nadai T., Yoshii K., Okada S., Miyajima K., Sustained release of flufenamic acid from a drug-triacetyl-β-cyclodextrin complex, Biological & Pharmaceutical Bulletin 20 (1997) 66-70