Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 338 (2007) 231–237
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Oil-in-oil microencapsulation technique with an external perfluorohexane phase

Zohra Mana, Yann Pellequer, Alf Lamprecht *

Laboratory of Pharmaceutical Engineering, Faculty of Medicine and Pharmacy, University of Franche-Comté, 25000 Besançon, France

Received 9 January 2007; received in revised form 6 February 2007; accepted 8 February 2007
Available online 13 February 2007

Abstract

Commonly, the microencapsulation of a lipophilic drug in a polymeric matrix via an ordinary oil/oil emulsification allows entrapping limited drug amounts due to its loss into the external phase. In this present paper, a new microencapsultion method describes the use of perfluorohexane as an external oil phase in order to produce microparticles of polyvinylpyrrolidon/vinylacetate (copovidone) and Eudragit® RS. Due to its highly non-solvent properties to most compounds, very limited miscibility with organic solvents, and very low toxicity, perfluorohexane (PFH) represents an excellent liquid for an external phase of the emulsion. Copovidone and Eudragit® RS microparticles were prepared by an oil/PFH method trapping ibuprofen as a lipophilic model drug and compared to results from conventional methods (oil/water and oil/oil). Morphological analyses of the obtained particles underlined the general matrix structure. The particle size varied between 75 μm (oil/oil) and 400 μm (oil/PFH) largely influenced by the stirring speed. Although drug release kinetics were principally similar for all preparation methods, it was generally found that encapsulation rates of oil/water and oil/PFH systems (oil/water: 74 ± 9%; oil/PFH: 86 ± 10%) were superior to ordinary oil/oil emulsification (3 ± 1%). The use of PFH was found to be a new promising tool for the preparation of microparticles. This modified emulsification method allowed the entrapment of lipophilic drugs into hydrophilic or lipophilic polymers in the absence of an aqueous phase.

Keywords: Microspheres; Copovidone; Eudragit® RS; Perfluorohexane; Solvent evaporation

1. Introduction

Microparticles can be designed for a large variety of therapeutic applications and for a nearly unlimited number of drugs. This has led to the creation of many different microencapsulation methods specifically adapted to the requirements of each drug's properties and of each production setup (Benita, 1996). The most widely applied methods for pharmaceuticals are based on the use of preformed polymers, employed to entrap the drug, for instance by an emulsification step followed by solvent evaporation solidifying the polymer with the drug entrapped. In such cases, microparticle preparation is achieved by an emulsification with mechanical stirring with the prerequisite that the inner phase solvent is highly volatile to ensure fast microparticle formation (Bodmeier and McGinity, 1988; Lamprecht et al., 2000). In most cases, strategies based on oil/water or oil/oil emulsifications are mainly related to two major decisive parameters: the solubility properties of the polymer and of the drug to be encapsulated. In particular, a low solubility of the drug in the external phase is required, otherwise low encapsulation rates may result due to drug leakage into the external phase (Nixon and Jalil, 1990). In consequence, for hydrophilic drugs to be entrapped in a water-insoluble polymer, the well-known double emulsion method has been developed (Alex and Bodmeier, 1989; Nihant et al., 1994; Hombreiro-Perez et al., 2003). In contrast, when oil/oil emulsification is applied for hydrophilic polymer matrices, the entrapment of lipophilic drugs still remains a problem due to leakage into the external oily phase providing sufficient solubility for the drug (Lamprecht et al., 2004).

An omnipotent and ideal external phase liquid to encapsulate hydrophilic as well as lipophilic drugs into various polymers independent of their hydrophilicity would have the following properties: high non-solvent properties to most compounds, and very limited miscibility with organic solvents, and a lower volatility then the internal phase solvent. Perfluorated hydrocarbons are potentially of interest in this context since some of

* Corresponding author. Tel.: +33 3 81 66 55 48; fax: +33 3 81 66 52 90.
E-mail address: alf.lamprecht@univ-fcomte.fr (A. Lamprecht).

0378-5173/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2007.02.010

them possess such limited solubility and miscibility properties. Perfluorohexane (PFH) represents an excellent example of such a compound. Moreover, PFH is considered to be 'practically non-toxic' which is the lowest rating of toxicity an equivalent to more than 1 kg for a 70 kg human (Knovel Library, 2006). Thus, it is used for a variety of pharmaceutical purposes (Krafft and Riess, 1998) such as in aerosol technologies (von der Hardt et al., 2002) or in medical diagnostics, especially in ultra-sonic imaging and magnetic resonance imaging. Intravenously injected PFH micrometer sized bubbles provide an efficient tool for magnetic resonance imaging (Riess, 2001; Pisani et al., 2006). PFH is also able to dissolve gases, such as oxygen and consequently such formulations can be used to deliver oxygen to several tissues (Riess, 2001). All these applications prove that perfluorohexane is an adequate excipient in terms to pharmaceutical requirements to formulate microspheres (MS).

The objectives of this study were firstly to evaluate the use of PFH for the microencapsulation of drugs by an emulsification method and secondly to characterize the MS with respect to particle size and morphology, drug loading, and release kinetics. Ibuprofen was encapsulated as a lipophilic model drug. The characteristics of the developed MS were compared with particles obtained from oil/oil or oil/water emulsification methods, which were used as a standard.

2. Materials and methods

2.1. Materials

Perfluorohexane and perfluoropolyester were generous gifts from H. Moebius & fils (Allschwil, Switzerland), Copovidone (vinylpyrrolidone–vinylacetate copolymer 60/40) was obtained from BASF (Ludwigshafen, Germany), and Eudragit® RS PO was a kind gift from Degussa/Röhm Pharma Polymers (Darmstadt, Germany). Polyvinyl alcohol, Polycaprolactone, ibuprofen, dichloromethane (DCM), heptadecyl-fluoro-1-decanol were purchased from Sigma–Aldrich (Deisenhofen, Germany). 1-Hexadecanol, 2-octyl-1-dodecanol, ethoxylated monoglycerides, ethoxylated C16–C18 alkyl alcohols, glycol P stearate, and magnesium stearate were obtained from Cooper (Melun, France), sorbitan monododecanoate, poly(oxy-1,2-ethanediyl) sorbitan monododecanoate, and sorbitan monooleate obtained from Seppic (Paris, France), mono-, di- and triglycerides (C8-10), 2-octyldodecyl-myristate, 1,2,3-propanetriol hexadecanoate octadecanoate, and 1,2,3-propanetriol isooctodecanoate were obtained from Gattefossé (Gennevilliers, France). Krytox® (synthetic fluorated oil) was purchased from IKV Tribologie (Saint-Donat, France). All other chemicals were purchased from Sigma–Aldrich (Deisenhofen, Germany) and were of analytical grade.

2.2. Methods

2.2.1. Preliminary emulsion stability tests

A stock solution of 30 mg surfactant in 10 ml DCM was prepared. 0.5 ml of this solution was mixed with 2.5 ml PFH and vortexed. Emulsion stability was determined qualitatively by measuring the time until complete phase separation was observed.

2.2.2. Preparation of the microspheres

Principally, the preparation of the MS was performed by three different methods: an oil/water, an oil/oil and the new oil/PFH emulsification-solvent evaporation. For all different techniques a fixed amount of polymer (200 mg) and drug (20 mg) were used.

2.2.2.1. Oil/oil emulsification. The polymer (Copovidone or Eudragit® RS) and ibuprofen were dissolved in a mixture of 3 ml acetone and 2 ml ethanol. This solution was poured into 80 ml of triglycerides (Miglyol® 812) containing 1%, w/w sorbitan monooleate and an oil/oil-emulsion was formed by stirring with a three-blade propeller at 500 rpm. The emulsion was stirred under vacuum until solvents were removed. MS were collected by filtration and washing steps were performed with *n*-hexane before drying at room temperature.

2.2.2.2. Oil/water emulsification. Since Copovidone is soluble in water, this experiment concerned only Eudragit® RS formulations: The polymer was dissolved in 3 ml of dichloromethane (DCM) together with the drug. This solution was then poured into 200 ml of water containing 0.1% PVA. The emulsion was formed by stirring with a three-blade propeller at 400 or 500 rpm. Once DCM was completely evaporated, the suspension was filtered and MS were dried at room temperature.

2.2.2.3. Oil/PFH emulsification. The polymer (Copovidone or Eudragit® RS) was dissolved in 3 ml of dichloromethane with the drug. This solution was poured into 25 ml of perfluorohexane, containing 48 mg/ml fluorated polyester. The emulsion was stirred with a three-blade propeller at 500 rpm. After approximately 15 min, when all the solvent of the internal phase was evaporated, the suspension in PFH was filtered and the MS were dried at ambient conditions.

2.2.3. Scanning electron microscopy (SEM)

The external and internal morphology of the resulting MS was analyzed by scanning electron microscopy. MS were fixed on supports with carbon-glue, and coated with gold using a gold sputter module in a high-vacuum evaporator. Samples were then analyzed under a JEOL JSM-T330A scanning microscope (Tokyo, Japan) at 10 or 24 kV.

2.2.4. Particle size analysis

The size of MS prepared with Eudragit® RS was measured in distilled water medium using Mastersizer II (Malvern Instruments, UK). Copovidone-based MS were analyzed for their size distribution in a particle/oil suspension by dispersing MS in 11 Isopar™ G containing 0.5% soy lecithin. Volume distribution was plotted using a computer program supplied by the manufacturer.

2.2.5. Determination of the drug content and in vitro drug release

Drug loaded MS (20 mg) were dissolved in 6 ml of dichloromethane, and the drug content was evaluated with an UV/vis spectrophotometer at 264 nm against a calibration curve.

For in vitro drug release experiments drug loaded MS (20 mg) were suspended in 10 ml phosphate buffer (pH 7.4) and the dissolution medium was kept under magnetic stirring at 100 rpm. All the experiments were carried out at 37 °C for 24 h. Aliquots of the dissolution medium (300 μl) were withdrawn at predetermined time intervals. Samples were filtered with a 0.22 μm Millipore® filter and drug concentrations were directly analyzed by UV/vis spectrophotometry after dilution with the phosphate buffer.

3. Results and discussion

The perfluorohexane is a very dense liquid ($d \approx 1.68$ g/cm^3), which exhibited a very low, non-determinable solubility of Eudragit® RS, Copovidone or ibuprofen (data not shown). Although other studies mentioned a miscibility of PFH in DCM, where mass fraction of PFH was around 1% (Pisani et al., 2006), PFH exhibited a large non-miscibility range in the DCM/PFH ratios applied in this study. This was advantageous for the emulsification step, but rather delicate in terms of finding an appropriate surfactant, allowing the necessary stabilisation of the oil/oil interface in order to ensure MS formation. In a preliminary study, the stabilizing effect of a variety of surfactants was tested on the DCM/PFH emulsion system (Table 1). In general, pharmaceutically relevant surfactants were more or less inefficient for the interface stabilization, and only fluorinated surfactants were suitable for the interface stabilization. This is apparently due to repulsion forces between non-fluorinated surfactants and the PFH phase. Among fluorinated products polyester were found to be most efficient while fluorinated surfactants with a head-tail structure did not sufficiently stabilize the DCM/PFH interface. The stabilizing mechanism in this context might be similar to that of polyvinyl alcohol in oil/water emulsion where mushroom conformations have been postulated (Philip et al., 2002).

Table 1
Qualitative stability of a DCM/PFH emulsion after the addition of various surfactants

Surfactant	Stability
1-Hexadecanol	+
2-Octyl-1-dodecanol	−
Ethoxylated monoglycerides	+
Sorbitan monododecanoate	−
Poly(oxy-1,2-ethanediyl) sorbitan monododecanoate	−
Ethoxylated C16–C18 alkyl alcohols	+
Glycol P stearate	++
Sorbitan monooleate	−
Mono-, di-, triglycerides (C8-10)	++
Polycaprolactone	++
Magnesium stearate	−
2-Octyldodecyl-myristate	++
1,2,3-Propanetriol hexadecanoate octadecanoate	−
1,2,3-Propanetriol isooctodecanoate	−
Heptadecyl-fluoro-1-decanol	++
Krytox® (synthetic fluorated oil)	−
Perfluoropolyester	+++

3.1. Eudragit® RS

Eudragit® RS belongs to the lipophilic non-pH-sensitive Eudragit® group of polyacrylates together with RL, mainly applied for diffusion controlled drug release after polymer swelling (Malamataris and Avgerinos, 1990). This is one reason combined with the property that Eudragit® RS is non-soluble in water but soluble in DCM and acetone which turned it into a very good model polymer for the characterization of the new microencapsulation process.

As a standard procedure, MS were prepared by the oil/oil emulsification based on the technique reported earlier (Lorenzo-Lamosa et al., 1998; Rodriguez et al., 1998; Jeong et al., 2001). The particles appeared spherical and their surface was smooth (Fig. 1A and B), but since ibuprofen displayed a relatively high

Table 2
Diameter and efficiency entrapment of ibuprofen of Eudragit® RS or copovidone microspheres prepared either by an oil/water (DCM/H_2O), oil/oil (ACE/TRI) or oil/PFH (DCM/PFH) emulsification (ER = encapsulation rate), selected for in vitro drug release experiments

	DCM/H_2O		DCM/PFH		ACE/TRI	
	Ø (μm)	ER (%)	Ø (μm)	ER (%)	Ø (μm)	ER (%)
RS (500 rpm)	135 ± 10[A] 197 ± 13[B] 367 ± 12[C]	74 ± 9	124 ± 8[A] 217 ± 17[B] 381 ± 21[C]	86 ± 10	46 ± 5[A] 75 ± 13[B] 168 ± 19[C]	3 ± 1
RS (400 rpm)	223 ± 15[A] 309 ± 24[B] 550 ± 36[C]	76 ± 12	272 ± 22[A] 399 ± 55[B] 553 ± 26[C]	92 ± 12		
Copovidone (500 rpm)			173 ± 14[A] 238 ± 35[B] 319 ± 24[C]	98 ± 11	86 ± 11[A] 155 ± 53[B] 231 ± 23[C]	5 ± 2

[A] $D(0.1)$.
[B] $D(0.5)$.
[C] $D(0.9)$.

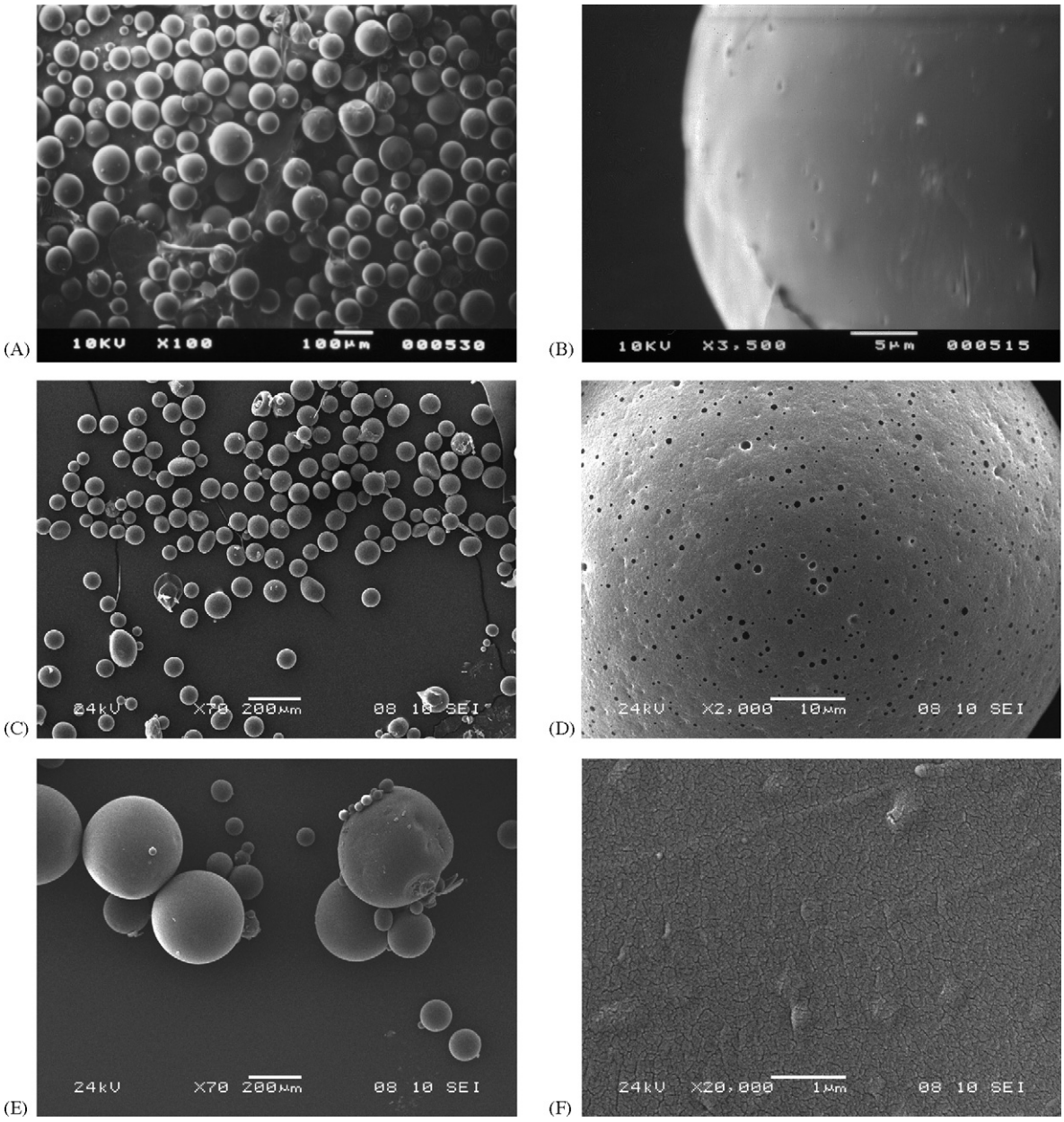

Fig. 1. Scanning electron microscopic images of Eudragit® RS MS prepared either by a DCM/TRI emulsification (A and B) or a DCM/H_2O emulsification (C and D) or an DCM/PFH emulsification (E and F).

solubility in the external triglyceride phase, the encapsulation rate was found to be very low (Table 2). It has been shown similarly that this preparation method might not be suitable for the encapsulation of rather lipophilic drugs into MS since high drug load might not be achieved (Lamprecht et al., 2004). Earlier reports about the microencapsulation describe mainly the entrapment of more hydrophilic compounds (Lorenzo-Lamosa et al., 1998; Rodriguez et al., 1998). Despite the low encapsulation rate observed, release kinetics were sustained with a 50% release after around 6 h and 80% ibuprofen release after 24 h (Fig. 2).

When applying oil/water emulsification, MS solidification was obtained by solvent evaporation. Again particles had a spherical appearance, showing small pores on the surface (Fig. 1C and D). Due to the low solubility in the outer phase, high entrapment rates were achieved. The drug release was faster than for the oil/oil method with a 50% release at $t < 4$ h and a drug release of around 90% after 24 h. Although oil/water phase systems also permit the application of solvent extraction procedures, in our study only solvent evaporation appeared to be a comparable preparation procedure.

MS obtained by the DCM/PFH emulsification were spherical and presented a smooth surface with nearly no pores (Fig. 1E and F). This underlines the impact of the external phase on the morphology of the final MS where the mechanisms of diffusion during the solvent evaporation are generally playing an important role (Dash, 1997; Lamprecht et al., 2000).

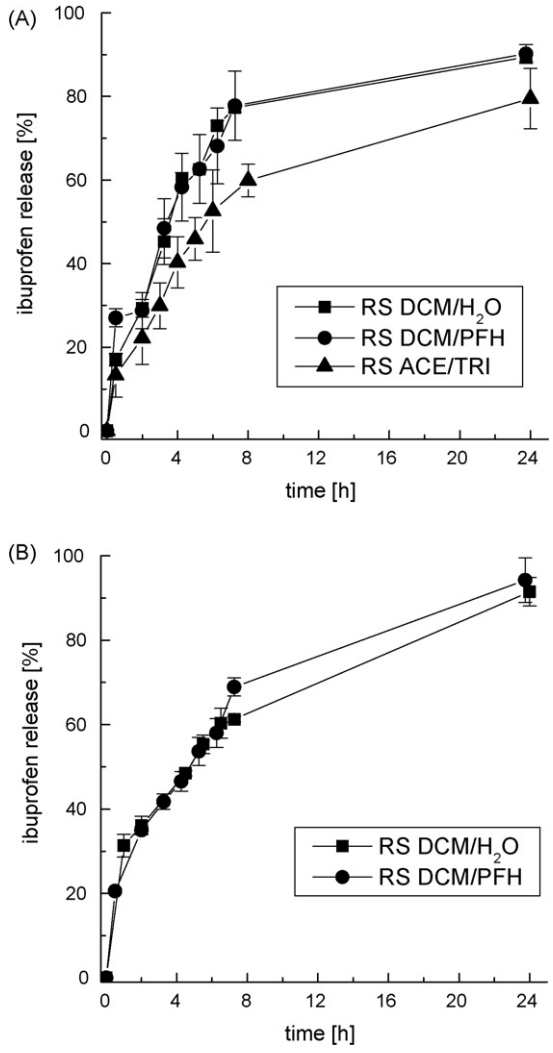

Fig. 2. Cumulated ibuprofen release vs. time for different Eudragit® RS MS formulations: DCM/H_2O, DCM/PFH, ACE/TRI at 500 rpm (A), and DCM/H_2O, DCM/PFH at 400 rpm (B). Data are given as mean ± S.D. for $n = 3$.

The encapsulation rates were higher with the DCM/PFH method compared to both other preparation methods. In both cases the essential parameter is the drug solubility in the external phase. While the solubility of ibuprofen in triglycerides is clearly visible, the presence of polyvinyl alcohol in the external aqueous phase might be responsible for lower entrapment efficiency based on its drug solubilizing effect in the external phase (data not shown).

Influences from principal preparation parameters for the oil/PFH method were not essentially different from ordinary oil/water emulsification methods. While increased polymer concentration led to only slightly larger MS and the increase of the external phase volume showed minimal influences on the MS properties, the surfactant concentration and the stirring speed were observed to be decisive fabrication parameters. Particle size increased distinctly with lower surfactant concentrations and reduced stirring speed, however, encapsulation rates remained constant. While in aqueous systems the solubilizing effect of the surfactants is considered to deteriorate the drug entrapment, this is visibly not the case with the oil/PFH system, due to the negligible drug solubility in the external phase.

Drug release kinetics of particles made by the DCM/PFH method at 500 rpm, showed a 50%-release after 4 h and 90% of the drug were released after 24 h (Fig. 2A). They were similar to those obtained from oil/water particles, while release from oil/oil was considered to be different as showed the f2 test (data not shown). In the case of the oil/oil method, a slight retention of drug release was observed in comparison with the oil/water and oil/PFH methods although the particle diameter was distinctly smaller. This may have two reasons, such as the generally lower drug content influencing the release velocity as well as the protecting layer of the sorbitan monooleate which might not be completely removed by the different washing steps. Thus, the surfactant still orients its lipophilic ends towards the external phase. The easy access of the aqueous phosphate buffer system to the particle surface may be reduced subsequently delaying the release kinetic. This phenomenon was observed similarly with other MS (Lamprecht et al., 2004).

When particles are prepared at lower stirring speed, MS prepared by oil/PHF and oil/water exhibited similar drug release kinetics (Fig. 2B). Since size, drug load, and matrix polymer are equyivalent, comparable drug release kinetics might be expected. The porosity which was variable in dependency of the preparation method, although not tremendously different, was considered to have only a slight impact on the drug release.

3.2. Copovidone

Hydrophilic polymers such as copovidone (weight ratio 60/40), are used for the enhancement of the dissolution rate of lipophilic drugs and increase the bioavailability (Vojnovic et al., 1993; Moneghini et al., 2000). The drug has to be dispersed in the polymer matrix as a “solid solution” in order to allow an immediate dissolution for an improved bioavailability. In a precedent study, MS were prepared by an oil/oil emulsion using as external phase triglycerides or paraffin oil due to the hydrophilic properties of the polymer refraining from the use of an oil/water emulsification. Subsequently, the percentage of encapsulation of the drug is very weak due to its loss in the external phase (Vojnovic et al., 1993).

Particles prepared by the oil/oil emulsification had a spherical shape despite the high viscosity of both, internal and external phase (Fig. 3A). The MS’ surface was again smooth and due to the solubility of ibuprofen in the external phase encapsulation rate was found to be very low (Table 2). Similar to observations with Eudragit® particles we observed a slight retardation effect potentially due to the higher hydrophobicity of these MS owing to the residual lipophilic surfactant (Fig. 4).

MS obtained from the DCM/PFH method had a spherical shape and a smooth surface (Fig. 3B and C). The internal morphology demonstrated in some cases the presence of internal cavities which were concluded to the consequences from the droplet coalescence trapping small amounts of PFH (Fig. 3D). Another possibility is the slight miscibility of small PFH amounts in DCM as reported elsewhere (Pisani et al., 2006) which may lead to the entrapment of PFH bubbles

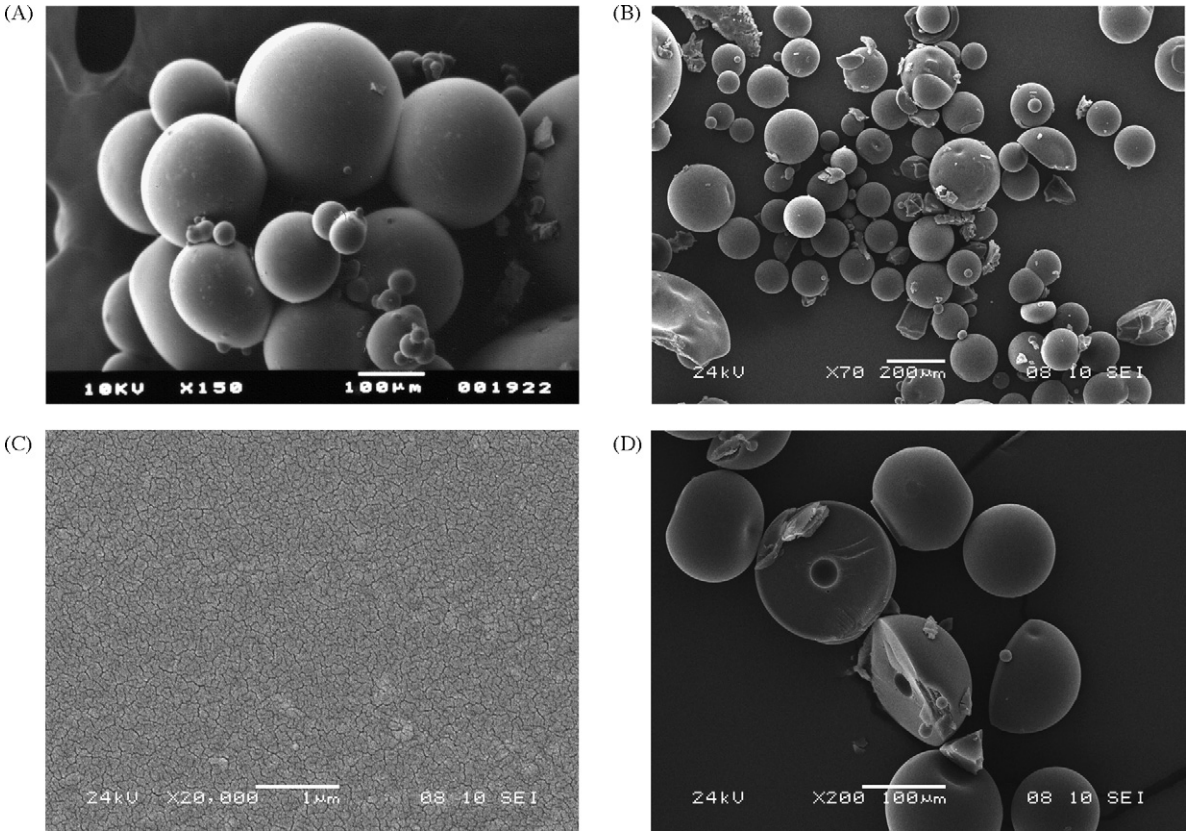

Fig. 3. Scanning electron microscopic images of copovidone MS prepared either by a DCM/TRI emulsification (A) or DCM/PFH emulsification (B–D) showing their surface morphology (C) and internal structure from cross-sections (D).

during the DCM evaporation inside the solidified polymer matrix.

The DCM/PFH interface stabilizing perfluoropolyester in the external phase was apparently limited in efficiency since persistently relative polydisperse MS were obtained irrespectively the surfactant concentration. Encapsulation rates in copovidone MS were again very high where values of around 95% entrapped ibuprofen were similar to observations with Eudragit® RS.

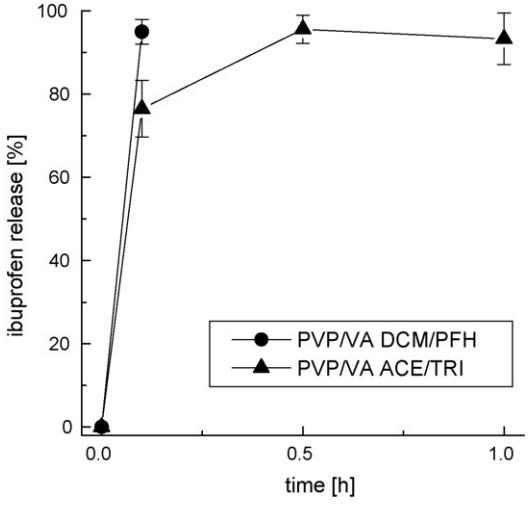

Fig. 4. Cumulated ibuprofen release versus time for different copovidone MS formulations: DMC/PHF or ACE/TRI emulsification. Data are given as mean ± S.D. for $n = 3$.

As copovidone is soluble in water, a very rapid particle dissolution was observed, consequently leading to a nearly immediate drug release (Fig. 4).

Generally, this microencapsulation method allows the entrapment of lipophilic drugs into hydrophilic polymers by an emulsification method. Although knowing that also other methods are available to solve this problem, however, they mostly require large apparatus, e.g. nebulizer while this method allows the fabrication of preliminary batches at the laboratory scale.

4. Conclusion

The use of PFH was found to be a new promising tool for the preparation of MS. A modified emulsification method allowed the entrapment of lipophilic drugs into hydrophilic or lipophilic polymers in the absence of an external aqueous phase. This method turns the parameter of drug's solubility in the external phase to a factor of negligible importance and could tremendously facilitate the design of drug loaded MS. The obtained MS differ only slightly from properties found with particles obtained from ordinary preparation processes. This method appears to cover numerous applications in the broad field of microencapsulation in pharmaceutics.

References

Alex, R., Bodmeier, R., 1989. Encapsulation of water-soluble drugs by a modified solvent evaporation method. I. Effect of process and

formulation variables on drug entrapment. J. Microencaps. 7, 347–355.
Benita, S., 1996. Microencapsulation–Methods and Industrial Applications, Drugs and the Pharmaceutical Sciences, vol. 73. Marcel Dekker, New York.
Bodmeier, R., McGinity, J.W., 1988. Solvent selection in the preparation of poly(DL-lactide) microspheres prepared by the solvent evaporation method. Int. J. Pharm. 43, 179–186.
Dash, A.K., 1997. Determination of the physical state of drug in microcapsule and microsphere formulations. J. Microencaps. 14, 101–112.
Hombreiro-Perez, M., Siepmann, J., Zinutti, C., Lamprecht, A., Ubrich, N., Hoffman, M., Bodmeier, R., Maincent, P., 2003. Non-degradable microparticles containing a hydrophilic and/or a lipophilic drug: preparation, characterization and drug release modelling. J. Controlled Release 88, 413–428.
Jeong, Y.I., Prasad, Y.R., Ohno, T., Yoshikawa, Y., Shibata, N., Kato, S., Takeuchi, K., Takada, K., 2001. Application of Eudragit P-4135F for the delivery of ellagic acid to the rat lower small intestine. J. Pharm. Pharmacol. 53, 1079–1085.
Knovel® Library - a properties database, http://www.knovel.com/knovel2/library/default.jsp.
Krafft, M.P., Riess, J.G., 1998. Highly fluorinated amphiphiles and colloidal systems, and their applications in the biomedical field. Biochimie 80, 489–514.
Lamprecht, A., Rodero Torres, H., Schäfer, U., Lehr, C.M., 2000. Biodegradable microparticles as a two-drug controlled release formulation: a potential treatment of inflammatory bowel disease. J. Controlled Release 69, 445–454.
Lamprecht, A., Yamamoto, H., Takeuchi, H., Kawashima, Y., 2004. Design of pH-sensitive microspheres for the colonic delivery of the immunosuppressive drug tacrolimus. Eur. J. Pharm. Biopharm. 58, 37–43.
Lorenzo-Lamosa, M.L., Remunan-Lopez, C., Vila-Jato, J.L., Alonso, M.J., 1998. Design of microencapsulated chitosan microspheres for colonic drug delivery. J. Controlled Release 52, 109–118.
Malamataris, S., Avgerinos, A., 1990. Controlled release indomethacin microspheres prepared by using an emulsion solvent-diffusion technique. Int. J. Pharm. 62, 105–111.
Moneghini, M., Voinovich, D., Princivalle, F., Magarotto, L., 2000. Formulation and evaluation of vinylpyrrolidone/vinylacetate copolymer microspheres with carbamazepine. Pharm. Dev. Technol. 5, 347–353.
Nihant, N., Schugens, C., Grandfils, C., Jérôme, R., Teyssié, P., 1994. Polylactide microparticles prepared by double emulsion:evaporation technique. I. Effect of primary emulsion stability. Pharm. Res. 11, 1479–1484.
Nixon, J.R., Jalil, R., 1990. Biodegradable poly(lactic acid) and poly (lactide-co-glycolide) microcapsules: problems associated with preparative techniques and release properties. J. Microencaps. 7, 297–325.
Philip, J., Jaykumar, T., Kalyanasundaram, P., Raj, B., Mondain-Monval, O., 2002. Effect of polymer-surfactant association on colloidal force. Phys. Rev. E. Stat. Nonlin. Soft Matter Phys. 66, 1–8.
Pisani, E., Tsapis, N., Paris, J., Nicolas, V., Cattel, L., Fattal, E., 2006. Polymeric nano/microcapsules of liquid perfluorocarbons for ultrasonic imaging: physical characterization. Langmuir 22, 4397–4402.
Riess, J.G., 2001. Oxygen carriers ("blood substitutes")-raison d'etre, chemistry, and some physiology. Chem. Rev. 101, 2797–2920.
Rodriguez, M., Vila-Jato, J.L., Torres, D., 1998. Design of a new multiparticulate system for potential site-specific and controlled drug delivery to the colonic region. J. Controlled Release 55, 67–77.
Vojnovic, D., Rubessa, F., Bogataj, M., Mrhar, A., 1993. Formulation and evaluation of vinylpyrrolidone/vinylacetate copolymer microspheres with griseofulvin. J. Microencaps. 10, 89–99.
von der Hardt, K., Schoff, E., Kandler, M.A., Dötsch, J., Rascher, W., 2002. Aerosolized perfluorocarbon suppresses early pulmonary inflammatory response in a surfactant-depleted Piglet model. Pediatr. Res. 51, 177–182.