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Effects of process parameters on the properties of biocompatible Ibuprofen-loaded microcapsules

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ABSTRACT

The objective of this study was to obtain an optimum formulation for microencapsulating Ibuprofen. This was achieved by investigating various factors which influenced the microcapsule size. Considering Ibuprofen as a lipophilic model drug, biocompatible Ibuprofen-loaded microcapsules in the size range of 20–60 μm were prepared by the water in oil emulsion-solvent evaporation method. An aqueous surfactant phase was used as the continuous external phase (W), a biocompatible organic solvent dissolving Ibuprofen was used as oil phase (O), in addition with a low boiling solvent. The biocompatible polymeric microcapsule membrane was composed of Eudragit RSPO or Ethylcellulose. The influence of various process parameters, such as the volatile organic solvent, the oily core, the stirring rate, on the characteristics of microcapsules was investigated. The encapsulation yield of Ibuprofen close to 100%, whatever the polymer type, was determined by UV–vis experiments, in accordance with the results obtained by ^{13}C NMR spectroscopy. An innovative technique, DSC-based thermoporosimetry, was used for the estimation of the loading rate of Ibuprofen. The results indicated that this developed analytical method had to be improved since DSC-transitions accounted to free and enclosed Ibuprofen were observed and altered the accuracy of the results.

1. Introduction

The widespread interest for microencapsulated drugs can be explained by the aim to reduce drug oral administration gastric secondary effects. The short biological half-life of 1–3 h following oral dosing makes Ibuprofen, a non-steroidal anti-inflammatory drug (NSAId), an ideal candidate for microencapsulation (Saravanan et al., 2003). Most of Ibuprofen encapsulation processes reported in the literature lead to the formation of microspheres with a matrix morphology (Perumal, 2001). The most common method of producing microcapsules was probably the interfacial step-growth polymerization, whereby a monomer in the continuous phase of an emulsion reacts with a second monomer in the dispersed phase. Unfortunately, unreacted monomer may remain present in the core, that limits their use in some applications.

Even if the emulsion-solvent evaporation was a commonly employed procedure for the preparation of polymeric microspheres, it seems to be a suitable method for preparing biocompatible microcapsules. Microcapsules offer greater versatility for encapsulation and more triggered release applications than microspheres (Atkin et al., 2004). The emulsion-solvent evaporation method relies on the removal of a low boiling solvent from the emulsion droplets under reduced pressure or by increasing temperature, inducing the separation of small liquid droplets rich in solvent and polymer ("coacervate phase") in the emulsion droplets. These coacervate droplets are mobile and migrate to the oil/water interface where they fuse and spread to engulf the original oil droplet if the wetting conditions are correct. This method involves the emulsification of a solution containing drug and polymer into another medium in which the drug and polymer cannot be dissolved, by using a suitable dispersing agent (Giunchedi et al., 1998; Jalil and Nixon, 1990; Lavergne et al., 2007).

The degree of drug partitioning into the aqueous phase will influence the efficiency of drug incorporation (Yuksel et al., 2000). Because of its poor solubility in water and hence low partitioning into the external phase during the microcapsule formation, Ibuprofen was a good candidate to the encapsulation by the emulsion-solvent evaporation.

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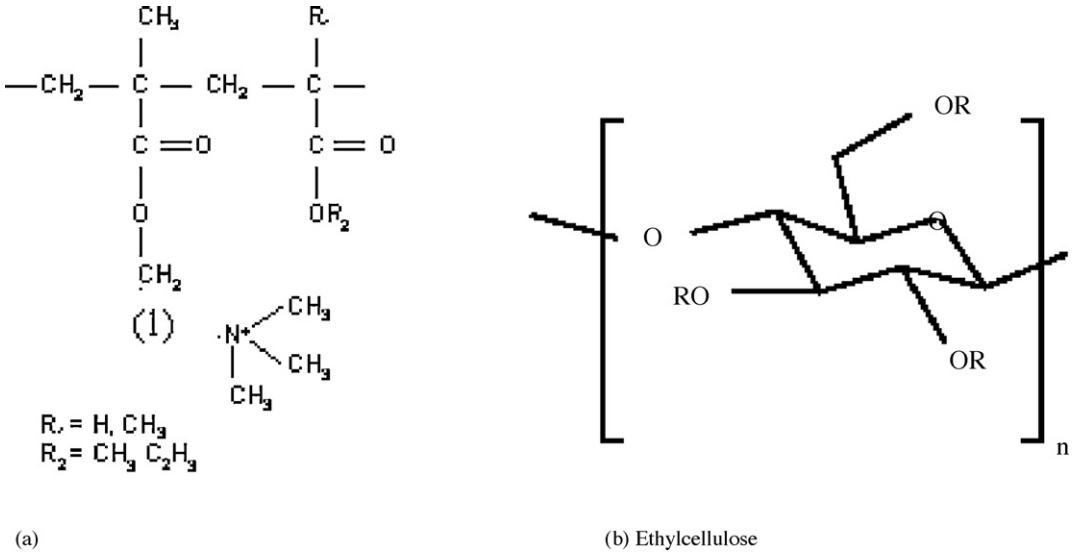

Scheme 1. Chemical structures of Eudragit RSPO (a) and Ethylcellulose (b).

Several process parameters involved in the emulsion-solvent evaporation method, like nature and concentration of dispersing agents, aqueous and organic phase volumes, stirring rate of emulsion system, affect the final microcapsule properties (Pongpaibul et al., 1988; Sansdrap and Moes, 1993; Sanchez et al., 1996). Dispersing agents have an important role in the production of microcapsules. They decrease the interfacial tension between the lipophilic and hydrophilic phases of the emulsion (Lambert et al., 2000). During the solvent evaporation process, the gradual removal of the solvent from the polymer droplets is accompanied by a corresponding decrease of the volume and the increase of the viscosity of the individual droplets. Highly viscous droplets particularly coalesce must faster than they can divide. Droplet coalescence and particle coagulation can usually be overcome by the use of a small amount of a suitable dispersing agent (Giunchedi et al., 1998; Jalil and Nixon, 1990). It provides a thin protective layer around the droplets and hence reduces the extent of their collision and coalescence. Dispersing agents can be various polymeric materials (Bezemer et al., 2000), proteins (Lambert et al., 2000) and surfactants. Poly(vinyl alcohol) is commonly used as a polymeric stabilizer and provides the formation of particles with relatively small size and uniform size distribution (Scholes et al., 1993).

The method presented here leads to microcapsules composed of a biocompatible inert oily core containing Ibuprofen, a non-steroidal anti-inflammatory drug (NSAId), and a biocompatible polymer such as Eudragit RSPO and Ethylcellulose. The applicability of the emulsion-solvent evaporation process depends on the successful entrapment of the active agent within the microparticles. The presence of an inert organic oil dissolving Ibuprofen during the process, enables the formation of core–shell systems. Furthermore, it could prevent the drug from crystallizing at the microcapsule surface, implying a better efficiency of the drug delivery system.

The influence of the nature of the low boiling good solvent of the polymer, the high boiling poor solvent (inert oil), the surfactant concentration, the stirring rate, the dispersed and continuous phase volumes on the diameter and the size distribution of microcapsules were studied. The determination of the encapsulation yield and the loading rate of Ibuprofen was investigated by various methods such as UV–vis spectroscopy, ^{13}C NMR spectrometry and DSC-based thermoporosimetry.

2. Experimental

2.1. Materials and methods

2.1.1. Materials

Ibuprofen was kindly supplied by Gibaud Pharma (Saint-Etienne, France). Eudragit RSPO (Mw = 33 800 g/mol; batch no. G031038154), N-22 Pharm Ethylcellulose (137 500 g/mol; batch no. 40588) (Scheme 1) and Miglyol 812 (Mi) were kindly purchased from Röhm Pharma Polymers (Germany), Hercules (France) and SASOL (Germany), respectively. Poly(vinyl alcohol) (PVA) 88% hydrolyzed and 22 000 g/mol (ACROS ORGANICS), poly(vinylpyrrolidone) (PVP, Mw = 29 000 g/mol, SIGMA–ALDRICH), benzyl benzoate (BB, ALDRICH 99%), D-alpha-tocopherol (Vitamin E, ACROS ORGANICS, 99%), chloroform (SIGMA–ALDRICH, 99.8%), methylene chloride (SIGMA–ALDRICH, 99.9%) were used as received.

2.1.2. Preparation of Ibuprofen-loaded microcapsules by emulsion-solvent evaporation method: standard procedure

Ibuprofen (1 g) was dissolved in the high boiling organic solvent (4 g), Eudragit RSPO (4 g) was dissolved in dichloromethane (80 mL). The solutions were then magnetically stirred and added dropwise to the aqueous PVA solution (800 mL), and the oil–water emulsion was obtained under mechanical stirring at 600 rpm at room temperature during 5 h and the low boiling solvent (dichloromethane) was removed under pressure (20 Torr). The microcapsules were centrifuged at 800 rpm and washed three times with distilled water, and then dried at room temperature.

2.2. Particle characterization

The average particle diameter and size distribution of microcapsules were determined by laser diffractometry using a Mastersizer 2000 (Malvern instruments, UK).

Scanning electron microscopy (SEM) experiments were performed on a Hitachi S800 (10 kV electron beam).

^{13} C NMR spectra of microcapsules collected after drying were recorded on a Bruker DRX 400 spectrometer (100.25 MHz for ^{13}C) in DMSO-*d*6 at 298 K. The pulse angle was 70° and the delay time was 10 s for the spectra recorded in quantitative conditions.

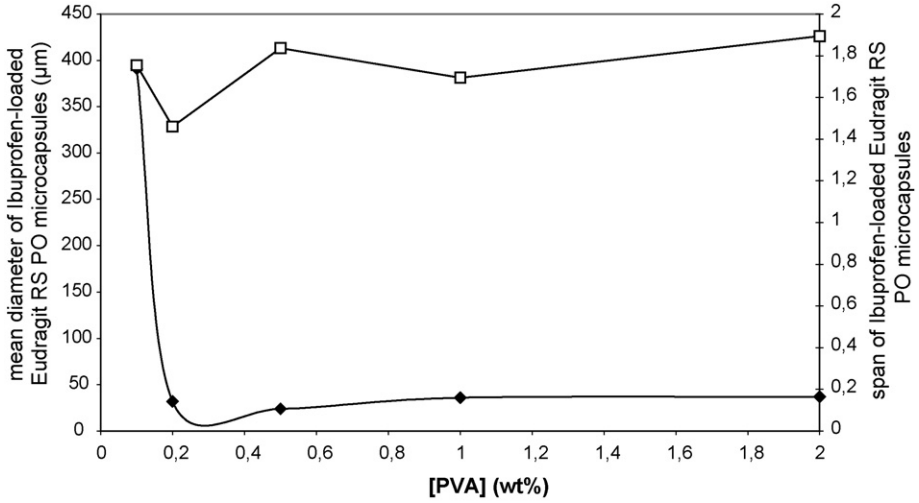

Fig. 1. Mean diameter (♦) and span value (□) of Ibuprofen-loaded Eudragit RSPO microcapsules obtained with benzyl benzoate as high boiling organic core versus PVA concentration (wt%).

The UV-vis spectra were obtained with a Lambda 35 Perkin Elmer spectrophotometer in the range 190–600 nm wavelength range. Ibuprofen concentration was determined from the absorbance at $\lambda = 263$ nm. All measurements were performed in triplicate.

A Mettler Toledo DSC 30 was used for the thermoporosimetry experiments under nitrogen atmosphere. The stainless steel pans were not sealed and the sample was first equilibrated at 0 °C. Then a temperature ramp from 0 °C to −100 °C was applied at a cooling rate of 0.7 °C/min and at a flow rate of 25 mL/min.

3. Results and discussion

3.1. Effects of process parameters on mean diameter and size distribution of microcapsules

The initial conditions of the microcapsule preparation were based on the use of PVA as surfactant, dichloromethane as low boiling solvent (Tb = 40 °C), and benzyl benzoate as the biocompatible inert oil (or high boiling poor solvent of the polymer (Tb = 323 °C)). The polymer shell was composed of Eudragit RSPO. The initial experimental conditions were based on experimental conditions reported in several works (Perumal, 2001; André-Abrant et al., 2001; Bodmeier and Chen, 1989; Kristmundsdottir and Ingvarsdottir, 1994; Carrio et al., 1995).

The first step was the study of the effect of the surfactant concentration on the mean diameter, the size distribution and the physical aspect of dried microcapsules observed after centrifugation and drying at room temperature.

3.1.1. Influence of the surfactant concentration

Microcapsules were prepared with various PVA 88% hydrolyzed concentrations ranging from 0.1 wt% to 2 wt%. The mean diameter of the microcapsules ($\emptyset = 33$ µm) was not influenced by the surfactant concentration above 0.2 wt%, whereas it significantly increased below 0.2 wt% (Fig. 1). The variation suggested that the droplet cover was insufficient at this level, and could not prevent the droplet coalescence.

The size distribution also depends on the surfactant concentration. Even if all the size distributions were bimodal, only the size distribution of microcapsules obtained with a surfactant concentration equal to 0.2 wt% was relatively narrow. The broadness of the size distribution observed at higher PVA concentrations could be due to the higher viscosity of the continuous phase which disperses the stirring energy (Song et al., 2008).

The microcapsules collected after centrifugation and drying were particularly sticky. The stickiness decreased when the surfactant concentration decreased. It is well known that a fraction of PVA, used as surfactant, remains associated to particles despite repeated washings, because PVA forms an interconnected network with the polymeric membrane at the interface (Carrio et al., 1995; Sahoo et al., 2002; Boury et al., 1994). The adsorption of the polymeric surfactant at the surface of the microcapsules should be improved at high PVA concentrations, and then PVA macromolecules could not be completely removed from the surface even after several washings, leading to the sticky behaviour of the microcapsules (Zambaux et al., 1998). Lee et al. observed the irreversible nature of PVA binding on the PLGA particle surface (Lee et al., 1999). The interpenetration of PVA and polymer molecules occurs during the encapsulation process when the organic solvent is removed from the interface. As a conclusion, the surfactant concentration must be sufficient in order to prevent the coalescence of the droplets but the surfactant must be removed relatively easily. We finally concluded that a PVA concentration equal to 0.2 wt% was efficient for the formation of Eudragit RSPO microcapsules with a mean diameter around 25 µm, and reduced the stickiness of the microcapsules after drying.

3.1.2. Influence of the biocompatible core oil type

The physical chemical properties of the high boiling organic solvent play also an important role in the formation of the microcapsules. As reported by Atkin et al. (2004) and Loxley and Vincent (1998), the formation of core–shell systems is favoured if the non-volatile solvent is a non-solvent of the polymer. Furthermore, the affinity of the high boiling solvent for the external phase can also influence the drug partitioning, then decreasing the encapsulation efficiency.

The surfactant can also play a role on the degree of the drug partitioning, since the addition of PVA decreasing the polarity of the aqueous continuous phase favours the hydrophobic drug partition in water (Giunchedi et al., 1998).

Although the solubility of benzyl benzoate in water is relatively low (0.0154 mg mL^{-1} at $T = 25$ °C), the presence of PVA probably increases its solubility in the aqueous phase and favours its diffusion to the external phase, via the diffusion of dichloromethane during the process of microcapsule formation. Then benzyl benzoate can be entrapped in the polymeric membrane of

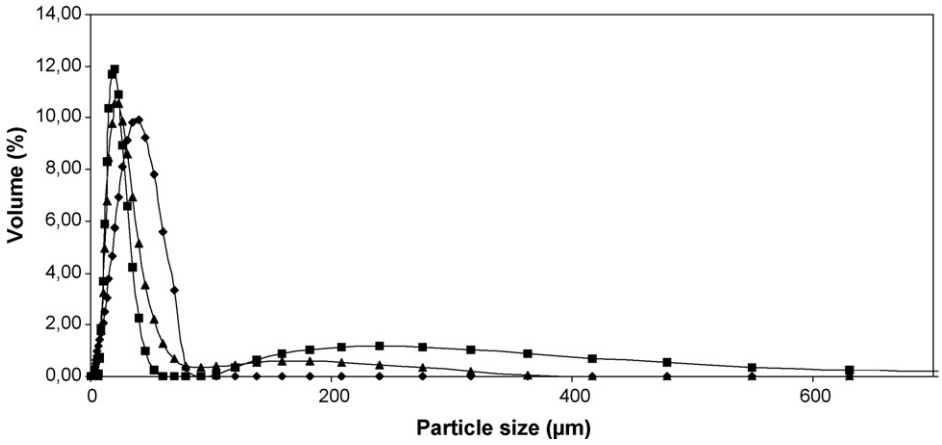

Fig. 2. Size distributions of Ibuprofen-loaded Eudragit RSPO microcapsules obtained with benzyl benzoate (♦), Vitamin E (▲) and Miglyol 812 (■) as high boiling organic core with PVA concentration equal to 0.2 wt%.

the microcapsules, close to the surface leading to their sticky behaviour.

Since PVA modified the hydrophilicity of the external phase and then the diffusion of the high boiling organic solvent and the drug, we suggested that the PVA concentration equal to 0.2 wt% was an optimal surfactant concentration allowing the emulsion stability without significantly modifying the solubility in water of the benzyl benzoate and Ibuprofen.

Beside benzyl benzoate (Tb = 323 °C), Vitamin E (Tb = 210 °C) and Miglyol 812 (Tb = 240 °C) were also used as high boiling organic solvents, and the physical aspect of the corresponding dried microcapsules was examined. In the both cases, Ibuprofen was loaded at its maximal solubility in the oil, i.e. 25 wt%. The size distribution of microcapsules obtained with Vitamin E and Miglyol 812 appeared narrower than those obtained with benzyl benzoate, even if they present two populations with the major size distribution with a $d(0.5)$ diameter around 24 μm (Fig. 2).

That was confirmed by the values of the span index characterizing the distribution broadness as follows:

$$\text{span} = \frac{d(0.9) - d(0.1)}{d(0.5)} \quad (1)$$

The size distribution of the microcapsules prepared with Miglyol 812 (span = 1.10) was narrower than with Vitamin E (span = 1.55). Even with the presence of a minor distribution corresponding to the $d(0.1)$ diameter equal to 13 μm, we considered that the size distribution almost monomodal. Furthermore, the microcapsules obtained with Miglyol 812 were much less sticky than the others and formed a powder after drying. The change of the sticky aspect of the microcapsules versus the high boiling organic solvent nature, can be attributed to their solubility in water and also to their affinity towards the volatile solvent, i.e. dichloromethane. The solubility in water of Miglyol 812 and Vitamin E respectively equal to 0.003 mg mL^{-1} and 0.029 mg mL^{-1} is very low compared to benzyl benzoate (15.4 mg mL^{-1}). The solubility parameters of each oil calculated according to Hoftyzer-van Krevelen method, and equal to 21.7 J$^{1/2}$ cm$^{-3/2}$, 21.6 J$^{1/2}$ cm$^{-3/2}$ and 19.1 J$^{1/2}$ cm$^{-3/2}$ for benzyl benzoate, Vitamin E and Miglyol 812 respectively, are close to the solubility parameter of dichloromethane (δ = 20.2 J$^{1/2}$ cm$^{-3/2}$), they present almost the same affinity with dichloromethane. The difficult collection of the microcapsules as powder-like substance with benzyl benzoate is essentially due to its higher solubility in water, in addition to its high solubility in dichloromethane, carrying it to the microcapsule surface and inducing the collapse during the process drying.

As Miglyol 812 enabled the formation of around 24 μm narrow size distribution microcapsules, collected as powder, it appeared the most adapted high boiling solvent. Then Miglyol 812 was used as inert oil to prepare the microcapsules.

3.1.3. *Influence of the polymer solvent*

Although their reported toxicity, the most used volatile organic solvents are generally chlorinated such as dichloromethane or chloroform. As microcapsule formation corresponds to a phase separation process, chloroform and dichloromethane should impact various final characteristics of the microcapsules. Because of the higher water solubility of dichloromethane (1.961 wt% at 25 °C) compared to chloroform (0.815 wt% at 25 °C), it should diffuse more rapidly to the aqueous phase (Jeyanthi et al., 1996). Successful entrapment of drugs, as well as other properties of microspheres depends to a large extent on the selection of the organic solvent used to dissolve the polymer (O'Donnell and McGinity, 1997). Bodmeier and McGinity (1988) found that the rate of polymer precipitation from the organic solvent was strongly affected by the rate of diffusion of the organic solvent into the aqueous phase combined to its evaporation rate at the emulsion surface. Organic solvents of low water solubility, as chloroform, allows to reach a fission–fusion equilibrium of the droplets along the collisions before the precipitation of the polymer in the droplets. The transport parameters that control the physicochemical properties of the system also involve the polymer/solvent interactions and thus the solvation power of the solvent. It was difficult to estimate the difference of dichloromethane and chloroform solvation power on the mean diameter of Ethylcellulose and Eudragit RSPO. Indeed, even if Ethylcellulose and Eudragit RSPO present different solubility parameters between 20.2 J$^{1/2}$ cm$^{-3/2}$ and 24.5 J$^{1/2}$ cm$^{-3/2}$ and between 18.0 J$^{1/2}$ cm$^{-3/2}$ and 21.4 J$^{1/2}$ cm$^{-3/2}$ respectively, the solubility parameters of chloroform (δ = 19 J$^{1/2}$ cm$^{-3/2}$) and dichloromethane (δ = 20.2 J$^{1/2}$ cm$^{-3/2}$) are slightly close.

We observed that the mean diameter of the microcapsules was lower when chloroform was used as organic volatile solvent instead of dichloromethane, independently of the polymer (Scholes et al., 1993) (Table 1).

Nevertheless the size decrease was more important in the case of Eudragit RSPO ($\Delta\emptyset$ = 33%) than Ethylcellulose ($\Delta\emptyset$ = 15%) and the size distribution of Eudragit RSPO became bimodal with chloroform, with a minor size distribution around 2.5 μm. These results could be attributed due the combination of the solubility in water and the boiling point of each solvent. Dichloromethane through its higher solubility in water and its lower boiling point is favourable to

Table 1
Mean diameter of Eudragit RSPO and Ethylcellulose with PVA or PVP (0.2 wt%) as surfactant and dichloromethane or chloroform as volatile organic solvent of the dispersed phase.

Formulation	Mean diameter (μm)	Span
Eudragit RSPO, PVA, CH_2Cl_2	23.8 ± 0.5	1.10
Eudragit RSPO, PVP, CH_2Cl_2	51.1 ± 1.0	2.06
Eudragit RSPO, PVA, $CHCl_3$	14.0 ± 0.3	2.25
Ethylcellulose, PVA, CH_2Cl_2	64.0 ± 1.3	1.44
Ethylcellulose, PVP, CH_2Cl_2	226.0 ± 4.5	4.28
Ethylcellulose, PVA, $CHCl_3$	54.0 ± 1.1	1.67

the increase of the dispersed phase viscosity, the rapid precipitation of the polymer in the droplets and then the increase of microcapsule sizes (Malamataris and Avgerinos, 1990).

3.1.4. Influence of the volume of the dispersed phase

The volume ratio of the dispersed to continuous phases is a classically influent parameter on the microcapsule characteristics.

Three various formulations based on a dispersed phase volume equal to 40 mL, 80 mL and 160 mL corresponding to a volume ratio of 20, 10 and 5 respectively were prepared, while the continuous phase volume was kept equal to 800 mL. Consequently the polymer concentration in the droplets was decreased (0.1 g/mL; 0.05 g/mL and 0.025 g/mL) as well as Ibuprofen concentration, whereas the Ibuprofen/polymer ratio (1/4) was kept constant. The mean diameter of the microcapsules significantly decreased with the increase of the volume ratio (Fig. 3a and b), since it induced the increase of the emulsion viscosity (O'Donnell and McGinity, 1997; Bolourtchian et al., 2005; Sah, 1997; Kiliçarslan and Baykara, 2003).

This ratio influences also the diffusion rate of the volatile solvent to the continuous phase which is controlled by its solubility in the aqueous phase, and then induces the variation of the precipitation rate of the polymer as a function of its desolvation. The decrease of the volume ratio induces the decrease of the microcapsule size (Jalil and Nixon, 1990) since the polymer phase saturation occurs less rapidly, delaying the hardening of the polymer droplets (Lambert et al., 2000). The change in viscosity, concentration or a combination of the both could account for the modification of the diffusion rate of the organic solvent (Tamilvanan and Sa, 1999). The size decrease was more important in the case of Ethylcellulose ($\Delta\emptyset = -434\%$) compared to Eudragit RSPO ($\Delta\emptyset = -106\%$) due to the difference of their intrinsic viscosity in dichloromethane, respectively equal to 380 mL/g and 40 mL/g.

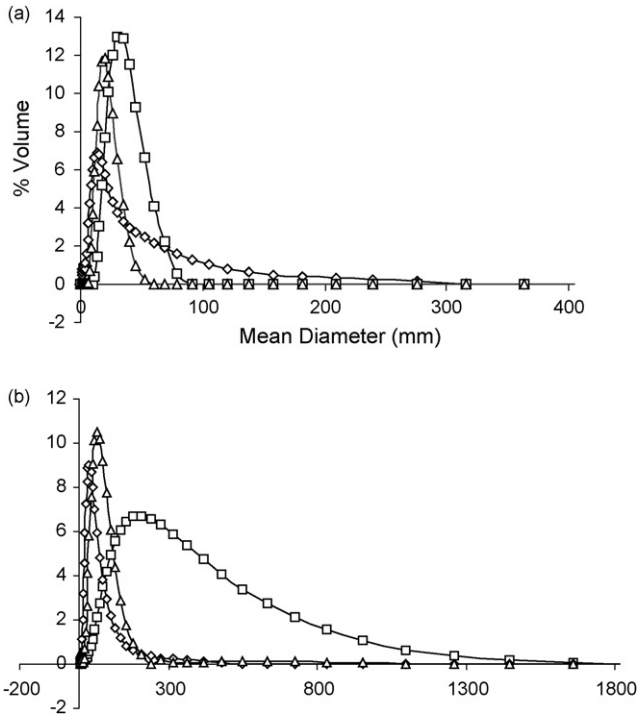

Fig. 3. (a) Size distributions of Ibuprofen-loaded Eudragit RSPO microcapsules obtained with a dispersed phase volume equal to 40 mL (□), 80 mL (△) and 160 mL (◊). (b) Size distributions of Ibuprofen-loaded Ethylcellulose microcapsules obtained with a dispersed phase volume equal to 40 mL (□), 80 mL (△) and 160 mL (◊).

Furthermore, we observed that the microcapsule size distribution also varied with the volume of the organic solvent. In the case of Ethylcellulose (Fig. 3b), the size distribution was particularly broader with the smallest volume of dichloromethane (40 mL) because spherical and uniform droplets did not have time to form before the polymer hardened. The broader size distribution of microspheres could be due to a bigger difference between the viscosities of the dispersed and the dispersing media (Kiliçarslan and Baykara, 2003).

The size distribution evolution of Eudragit RSPO microcapsules was different since the size distribution was broader and multimodal with three populations around 2 μm, 15 μm and 100 μm, when the organic phase volume was equal to 160 mL (Fig. 3a). This could be explained by the very low viscosity of the dispersed phase when its volume is equal to 160 mL. The mechanical stirring is efficient and enables the formation of very low diameter (1 μm) droplets, but concurrently the precipitation of the polymer rapidly occurs inducing the formation of large microcapsules (100 μm).

From these results, it appeared that the most appropriate dispersed phase volume to optimize our system in terms of size characteristics was equal to 80 mL.

3.1.5. Influence of the volume of the continuous phase

We previously showed that the volume of the organic dispersed phase was influent on the diameter of the microcapsules, as it influenced the viscosity of the dispersed phase and the diffusion rate of the organic solvent to the aqueous continuous phase. Concurrently the volume of the continuous phase can have an effect on the diffusion of the volatile organic solvent towards the aqueous external phase. The increase of the aqueous phase volume should increase the amount of dichloromethane that diffuses from the organic phase providing the faster precipitation of the polymer and then the increase of the mean diameter.

No significant difference in Eudragit RSPO microcapsule size was observed. Their mean diameter and their size distribution did not change when the external volume was increased from 800 mL to 1200 mL ($\phi_{V=800\,mL}$ = 21 μm and $\phi_{V=1200\,mL}$ =29 μm). The size distribution became broader with two minor populations when the external volume was equal to 400 mL centred on 3 μm and 100 μm. An external volume of 400 mL is probably too low to provide the homogeneous removal of the organic solvent and induces the formation of various size Eudragit RSPO microcapsules.

As regards Ethylcellulose microcapsules, a mean diameter equal to 64 μm was observed with a 800 mL external phase volume, whereas the microcapsule mean diameter was higher when this volume was equal to 400 mL and 1200 mL ($\phi_{V=400\,mL}$ = 90 μm and $\phi_{V=1200\,mL}$ = 92 μm). The high viscosity of the emulsion with an external phase volume of 400 mL combined to the high intrinsic viscosity of the internal Ethylcellulose solution may explain the increase of mean diameter of Ethylcellulose microcapsules. A relatively high external phase volume of 1200 mL induced a non-uniform turbulence in the reactor due to the subdimensional size of the propeller. Keeping organic phase volume (80 mL) constant, an optimal continuous phase volume of 800 mL was required.

3.1.6. Influence of the polymer concentration in the dispersed phase

With the dispersed (80 mL) and the continuous phase volumes (800 mL) constant, the effect of the polymer concentration on the size properties of the microcapsules was investigated.

Microcapsules with Eudragit RSPO and Ethylcellulose concentration in the dispersed phase equal to 37.5 g/L, 50 g/L and 62.5 g/L were prepared. A different effect of the polymer concentration on microcapsule size was observed according to the polymer. When Eudragit RSPO concentration varied from 37.5 g/L to 62.5 g/L, narrow size distributions were observed. The mean diameter centred on the major population equal to 10 μm, 28 μm and 20 μm for 37.5 g/L, 50 g/L and 62.5 g/L respectively, did not significantly vary in this range of polymer concentration, as the measurement error is about 5 μm in this size range (Perumal, 2001). The lowest mean diameter (10 μm) was logically observed for the lowest polymer concentration (37.5 g/L) in relation with the corresponding viscosity of the dispersed phase.

The effect was different with Ethylcellulose microcapsules. Standard formulation (50 g/L) implied the formation of the narrowest size distribution microcapsules centred on 64 μm. At lower and higher polymer concentrations, the size distributions were quite broader and sizing on higher diameter, especially with the polymer concentration equal to 62.5 g/L (Ø = 150 μm). It was explained that higher polymer concentration could result in the increased rate of polymer precipitation because of the higher viscosity of dispersed phase (Bodmeier and Chen, 1989).

The effect of the polymer concentration on Eudragit RSPO and Ethylcellulose microcapsules relies on the different intrinsic viscosity of the both polymers in dichloromethane (Kiliçarslan and Baykara, 2003), respectively equal to 40 mL/g and 380 mL/g at 25 °C.

As a conclusion, it appeared that the polymer concentration equal to 50 g/L for both polymers was adapted to the formation of microcapsules with the lowest mean diameter and the narrowest size distribution.

3.1.7. Influence of the stirring rate

Varying the stirring speed from 200 rpm to 1000 rpm, we observed, as it is classically reported, that the increase in stirring speed decreased the mean diameter of the microcapsules (Fig. 4) independently of the polymer (Perumal, 2001; Kiliçarslan and Baykara, 2003; Tamilvanan and Sa, 1999). Nevertheless the size decrease profiles were different according to the polymer and the organic solvent nature and especially the polymer–solvent interactions.

Three regions were observed on the Eudragit RSPO microcapsule size profiles. A first plateau around Ø = 40 μm between 200 rpm and 400 rpm, then the mean diameter significantly decreases between 400 rpm and 600 rpm and finally another plateau is observed around 20 μm. At an agitation rate below 600 rpm, the uniformity of the mixing force throughout the emulsion mixture may be decreased, resulting in a wider size distribution of the final microcapsules and a higher size. In contrast, agitation rates higher than 600 rpm are vigorous and uniform enough to form small and homogeneous size distribution microcapsules. The plateau at high stirring rates may suggest that PVA at a weight concentration of 0.2% is efficient for the droplet cover and the coalescence suppression. As the interfacial area occupied by a PVA molecule is equal to 95 $Å^2$/molecule (Boury et al., 1994), the number of PVA molecules required to the complete cover of the interfacial area developed by 80 mL of dispersed phase composed of droplets with a mean diameter of 17 μm is equal to 3×10^8. Since the number of PVA molecules contained in the dispersion medium is equal to 2.3×10^{12}, the complete cover of the emulsion droplets at this stirring speed guaranties the stability of the O/W emulsion.

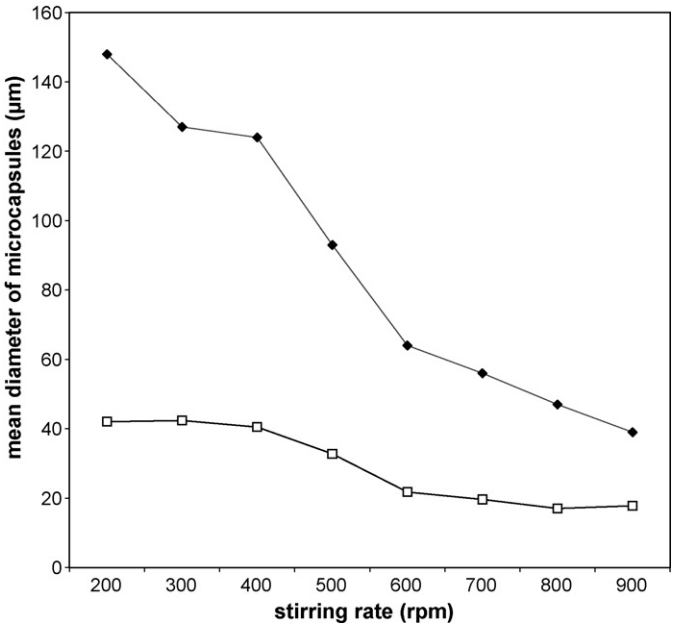

Fig. 4. Mean diameter of Ethylcellulose (□) and Eudragit RSPO (♦) and span values of Ethylcellulose (○) and Eudragit RSPO (▲) Ibuprofen-loaded microcapsules versus the stirring rate.

The variation of the mean diameter of Ethylcellulose microcapsules was not the same. Indeed, the mean diameter did not reach any plateau even at the highest stirring rate of 1000 rpm that is the security limit for the propeller agitator and the cylindrical reactor. Furthermore, except at 1000 rpm, the mean diameter of Ethylcellulose microcapsules was quite higher than Eudragit RSPO microcapsules. The size range of microcapsules obtained with Ethylcellulose is much larger than with Eudragit RSPO. At agitation rates below 400 rpm, the flocculation and the sedimentation of Ethylcellulose microcapsules were observed, attesting for the inefficiency of slow agitation rates to produce small and uniform size microcapsules. The high intrinsic viscosity may favour the coalescence of Ethylcellulose droplets. Then a much higher energy is required to produce small microcapsules. The higher size decrease from 130 μm to 60 μm was observed between 400 rpm and 600 rpm, indicating that this agitation rate range was the most efficient. Two simultaneous opposite effects of the increase of the agitation rate should occur during the formation of the Ethylcellulose microcapsules. While the increase of the shearing energy favoured the fission of the polymer droplets and the decrease of the droplet size, it also increased the interfacial surface between the dispersed and the aqueous phases, inducing the increase of diffusing dichloromethane amount towards the aqueous phase. Yamamura et al. (2002) clearly demonstrated the effect of the air velocity on solvent evaporation rate in the formation of polystyrene–polycarbonate films. The influence of the air velocity on polymer films can be transposed to the stirring rate in the case of microcapsule formation. We followed the evaporation kinetics of dichloromethane from Eudragit RSPO and Ethylcellulose solutions dispersed in aqueous phase containing PVA 0.2 wt% at 22 °C at atmospheric pressure, by the on-line controlled weight loss of the system (Fig. 5). The weight loss corresponding to the evaporation rate of dichloromethane was determined as follows:

$$\text{weight loss}\,(\%) = \frac{CH_2Cl_2 \text{ evaporated amount}}{CH_2Cl_2 \text{ theoretical amount}} \times 100 \qquad (2)$$

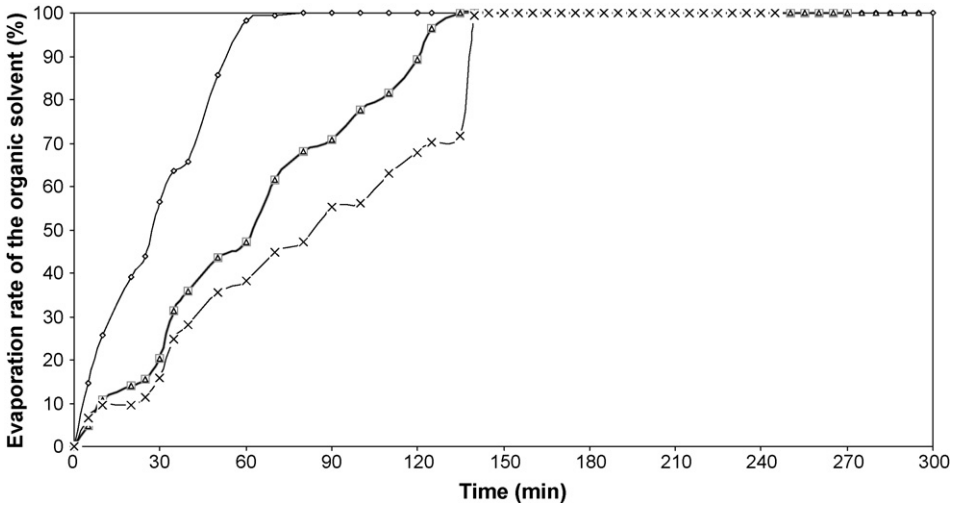

Fig. 5. Evaporation kinetics of dichloromethane during the formation of Eudragit RSPO (▲) and Ethylcellulose (♦) microcapsules and evaporation kinetics of chloroform during the formation of Eudragit RSPO (×) and Ethylcellulose (□) microcapsules with PVA (0.2 wt%) as surfactant at 22 °C and atmospheric pressure.

with

$$CH_2Cl_2 \text{ evaporated amount} = (\text{weight of the system})_0 - (\text{weight of the system})_t$$

where $(\text{weight of the system})_0$ corresponds to the initial weight of the system before the solvent evaporation beginning; $(\text{weight of the system})_t$ = weight of the system at time t during the dichloromethane evaporation. The same equation was used with chloroform as volatile organic solvent.

It clearly appeared that dichloromethane evaporated much rapidly from polymer solution with Ethylcellulose (80 min) than Eudragit RSPO (130 min). Furthermore, the profile of the weight loss is different depending on the polymer. The rate of the weight loss was relatively constant in the case of Ethylcellulose, whereas the dichloromethane evaporation from Eudragit RSPO droplets occurred with various rates versus time. The slope was very high at the beginning of the dichloromethane evaporation for Ethylcellulose, confirming a rapid precipitation of the polymer and then the higher mean diameter of the Ethylcellulose microcapsules. However, the evaporation rate is controlled by the solvent diffusion to the external phase. The total developed surface of Ethylcellulose droplets was lower, as the mean diameter was higher. Furthermore, the higher viscosity of the Ethylcellulose droplets should not favour the diffusion of dichloromethane from the polymer droplets as well. Thus, the measured weight loss of Ethylcellulose droplets was quite amazing and attested for the importance of the polymer–solvent interactions on the emulsion-solvent evaporation process and the final microcapsule characteristics.

On the basis on all the results, the optimized formulation of Ibuprofen-loaded microcapsules was the following: continuous phase/dispersed phase ratio = 10, dichloromethane as low boiling organic solvent, Miglyol 812 as inert high boiling oil, polymer amount = 50 g/L and stirring speed = 600 rpm.

3.2. Drug encapsulation efficiency of microcapsules

The efficiency of Ibuprofen-loaded microcapsules as delivery systems depends on the successful encapsulation. The loading efficiency or encapsulation yield (Y) depends on the encapsulation technique and experimental parameters. It can be defined as follows:

$$Y = \frac{L_{\text{Ibuprofen}}}{T_{\text{Ibuprofen}}} \times 100 \quad (3)$$

with $L_{\text{Ibuprofen}}$ corresponding to experimental-loaded amount of Ibuprofen and $T_{\text{Ibuprofen}}$ corresponding to the theoretical-loaded amount of Ibuprofen.

The loaded drug content can be classically determined by direct analysis of microparticles by differential scanning calorimetry (DSC) (Bodmeier and McGinity, 1988; Kosaraju et al., 2006) or after extraction of the active agent from the microparticles and quantitative analysis of the supernatant by chromatography techniques (Mendez et al., 2003; Bouchemal et al., 2003) or UV–vis spectrometry (Mendez et al., 2003; Amperiadou and Georgarakis, 1995).

3.2.1. UV–vis experiments

UV–vis spectroscopy is classically used in the determination of the encapsulation yield (Wang et al., 2007). This technique requires on one hand that the loaded active agent presents some chromophoric groups that can absorb UV–vis radiations, and on another hand, that the solvent dissolving the active agent does not absorb radiations. Furthermore, the solvent must not dissolve the polymeric membrane of the microcapsules. Heptane fills all these requirements (Bustamante et al., 1998) and was retained for UV–vis experiments. First of all, Ibuprofen (δ = 19.7 $MPa^{0.5}$) (Bustamante et al., 2000) was extracted from the microcapsules dispersed in heptane (δ = 15.3 $MPa^{0.5}$) (Bustamante et al., 1998). As Ibuprofen was enclosed inside the polymeric wall, the microcapsules were broken in order to release Ibuprofen in heptane. The breakage of the microcapsules was performed by sonication in heptane at various durations, in order to reach an extraction plateau. An extraction time of 10 min enabled the release of the total amount of Ibuprofen from Ethylcellulose, whereas only 43% of the total amount of Ibuprofen was extracted from Eudragit RSPO at the same extraction time. The plateau of the extracted amount of Ibuprofen from Eudragit RSPO microcapsules was reached after only 20 min. The different Ibuprofen extraction profiles obtained from Ethylcellulose and Eudragit RSPO microcapsules will be not commented here, it will be done in a forthcoming paper. Anyway, the amount of Ibuprofen extracted at the plateau corresponded to the total amount of loaded Ibuprofen. Then we found 99.5 ± 0.2% and 99.8 ± 0.2% of the

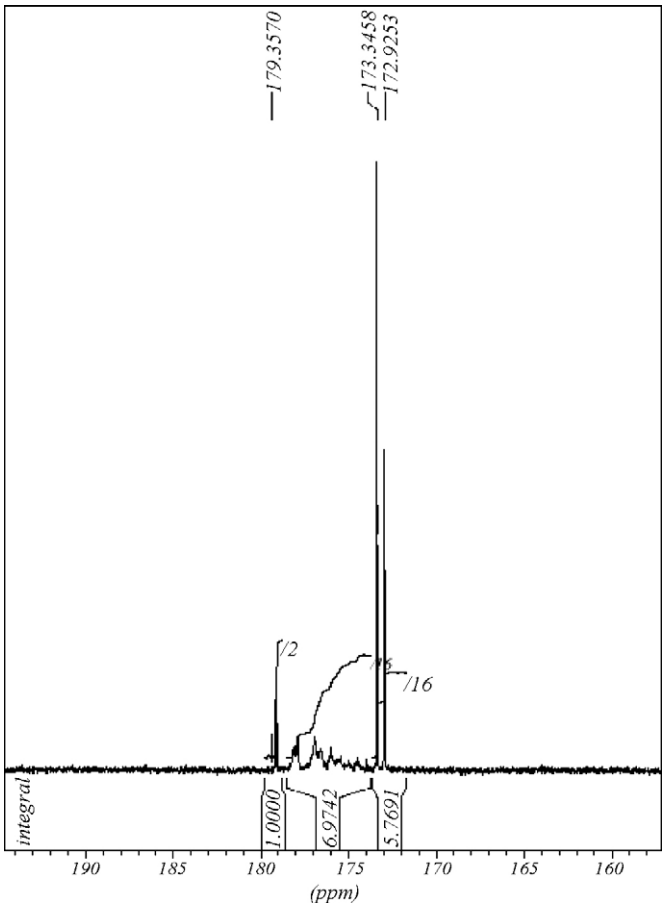

Fig. 6. Enlargement of quantitative ^{13}C NMR spectrum (carbonyl region) of Ibuprofen-loaded Eudragit RSPO microcapsules.

theoretical amount of Ibuprofen were extracted from Eudragit RSPO and Ethylcellulose microcapsules respectively.

We concluded that the encapsulation yield was close to 100% whatever the polymer type. UV–vis results confirmed that the standard formulation of Eudragit RSPO and Ethylcellulose led to the complete encapsulation of Ibuprofen inside the liquid Miglyol 812 core of the microcapsules.

3.2.2. ^{13}C NMR experiments

Even if the main drawback of ^{13}C NMR spectroscopy is its low sensitivity, that requires relatively high sample concentration in deuterated solvents, it is a powerful analytical tool for qualitative and quantitative characterizations of chemical structures. By this method, we expected to obtain information about the relative amount of Ibuprofen and Miglyol 812 enclosed in microcapsules, in order to verify that Miglyol 812 and Ibuprofen present the same encapsulation ratio. We tended to quantify the carbonyl resonance of Ibuprofen and Miglyol 812 (Fig. 6) comparing experimental molar ratio (R_{exp}) of Ibuprofen and Miglyol 812 to theoretical one (R_{th}) as follows:

$$R_{th} = \frac{\text{molar number of Ibuprofen}}{\text{molar number of Miglyol}} \quad (4)$$

with molar number of Ibuprofen corresponds to the Ibuprofen amount involved in the preparation of microcapsules (1 g; 4.8×10^{-3} mol) and molar number of Miglyol 812 corresponds to the Miglyol 812 amount involved in the preparation of microcapsules (4 g; 8×10^{-3} mol).

Then the theoretical ratio was equal to 0.60.

We observed the enlarged carbonyl region presenting three integrated resonances corresponding to the Ibuprofen carbonyl (δ = 180 ppm), Eudragit RSPO carbonyls (δ = 177 ppm), PVA carbonyls (δ = 174.5 ppm) and Miglyol 812 carbonyls (δ = 173 ppm). Carbonyl resonances of Ibuprofen and Miglyol 812 were not overlapped by the others and then their integration was not altered. The experimental molar ratio was calculated from the Ibuprofen ($I_{Ibu} = CO_{Ibu}$; δ = 180 ppm) and Miglyol 812 (δ = 173 ppm) carbonyl intensities (Eq. (5)). The calculation was based on the fact that the carbonyl intensity of Miglyol 812 (I_{Mi}) corresponds to three carbons due to its triglyceridic nature ($CO_{Mi} = I_{Mi}/3$):

$$R_{exp} = \frac{CO_{Ibu}}{CO_{Mi}} \quad (5)$$

R_{exp} was found equal to 0.61 and 0.60 for Eudragit RSPO and Ethylcellulose microcapsules respectively, whereas the theoretical value was equal to 0.60. Then the relative amount of Ibuprofen and Miglyol 812 enclosed in microcapsules was found to be close to the theoretical one, regardless of the polymer type. These results attested for the right amount of loaded Ibuprofen compared to Miglyol 812 inside the microcapsules for both polymer types, indicating that neither Ibuprofen nor Miglyol 812 was lost during the encapsulation procedure.

3.2.3. DSC experiments

DSC experiments, classically based on the observation and the quantification of the melting transition of the drug, do not stand for the determination of the loaded Ibuprofen content if the drug is molecularly dissolved in the melted polymeric matrix. In this case, the melting transition disappears and no quantitative analysis can be performed (Malamataris and Avgerinos, 1990; Ding et al., 2005). Since Ibuprofen was dissolved in the oily core of the microcapsules, classical DSC experiments could not be used to quantify the loaded Ibuprofen amount in the microcapsules. Instead of investigating Ibuprofen loading, we proposed to examine Miglyol 812 loading, by studying its crystallization and determining its crystallization enthalpy, and then the Miglyol 812 loading in the microcapsules. Finally the Ibuprofen loading was deduced from the Miglyol 812 loading. This method relied on the assumption that the encapsulation efficiency of Miglyol 812 and Ibuprofen were similar, that was proved by ^{13}C NMR results (cf. above).

The crystallization of Miglyol 812 was studied by decreasing the temperature from 0 °C down to −50 °C with a ramp of 0.7 °C/min. The method was developed from DSC-based thermoporosimetry experiments which classically permit the quantitative characterization of porous structures (<200 nm) based on the dependence of the crystallization and the melting points on the confinement state of the products (Nedelec and Baba, 2004; Laudone et al., 2004).

Our aim was then to use the potential various crystallization peaks of Miglyol 812 to have information about the total amount of loaded Miglyol 812 in the microcapsules by comparing the loaded Miglyol and free pure Miglyol 812 enthalpies. Furthermore, as DSC-based thermoporosimetry experiments should inform about the microcapsule porosity filled by the liquid core, i.e. Miglyol 812, they could then provide some information about the internal morphology of the microcapsules.

The crystallization temperature and the corresponding enthalpy of free Miglyol 812 are equal to −20 °C and 39.03 J/g respectively. The thermograms of Ethylcellulose and Eudragit RSPO microcapsules clearly showed the crystallization of Miglyol 812 (Fig. 7a and b).

Two peaks at −20 °C and −27 °C corresponding to respectively free and loaded Miglyol 812 in Eudragit RSPO microcapsules were observed (Fig. 7a). In contrary one large peak between −80 °C and −20 °C was only detected in the case of Ethylcellulose micro-

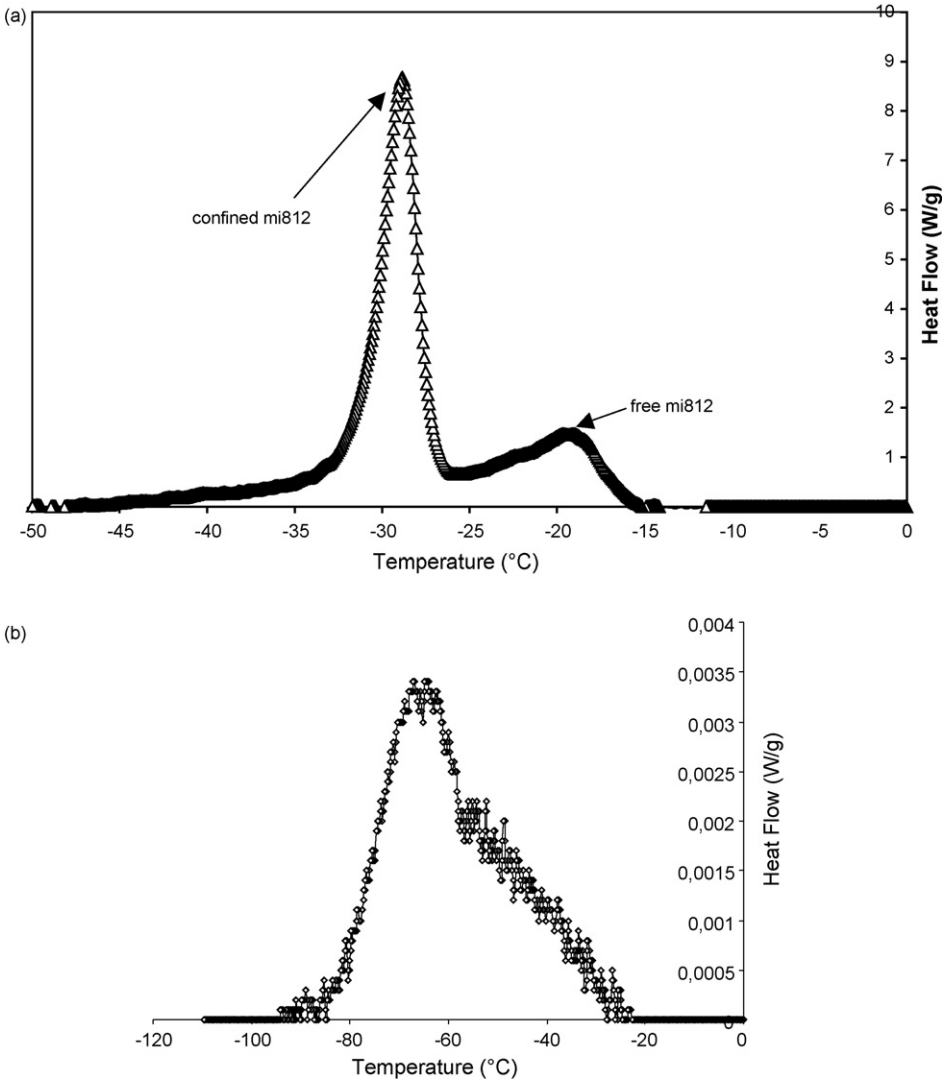

Fig. 7. (a) DSC thermogram of Eudragit RSPO microcapsules performed between 0 °C and −50 °C with a ramp of 0.7 °C/min. (b) DSC Thermogram of Ethylcellulose microcapsules performed between 0 °C and −50 °C with a ramp of 0.7 °C/min.

capsules (Fig. 7b). Free Miglyol 812 corresponds to Miglyol 812 that would be present at the extreme surface of microcapsules and not enclosed. Thus, the corresponding peak was not considered in the calculation of the loaded Miglyol 812 amount. The crystallization temperature of loaded Miglyol 812 was then different according the polymer type, suggesting that the internal morphology was also different. Furthermore, the relative intensity of free and confined Miglyol 812 also depended on the polymer type, suggesting a different external morphology of Ethylcellulose and Eudragit RSPO microcapsules. These results were confirmed by SEM micrographs. We observed that the external surface of Ethylcellulose was quite porous (Fig. 8a), whereas the external surface of Eudragit RSPO appeared definitely smooth and dense (Fig. 8b).

This various external morphology could be explained by the different behaviour of both polymers during the microcapsule formation, due to different polymer–solvent interactions. The particular behaviour of both polymers and the polymer–dichloromethane and polymer–Miglyol 812 will be studied in a forthcoming paper. These results were quite interesting as they suggested various behaviours of both microcapsules concerning drug delivery.

The crystallization enthalpy of loaded Miglyol 812 was finding to be equal to 16.5 J/g and 10.4 J/g for Eudragit RSPO and Ethylcellulose microcapsules respectively. Then the loading rate of Miglyol 812 in Eudragit RSPO and Ethylcellulose microcapsules was found equal to 42% and 27% respectively, according to Eq. (6):

$$L_{\mathrm{Mi}} = \frac{\Delta H_{\mathrm{exp}}}{\Delta H_{\mathrm{th}}} \times 100 \tag{6}$$

with ΔH_{exp} corresponds to the crystallization enthalpy of loaded Miglyol 812 and ΔH_{th} is equal to 39 J/g corresponding to pure Miglyol 812.

The encapsulation yield of Miglyol 812 (Y) for Eudragit RSPO and Ethylcellulose microcapsules was then calculated as follows:

$$Y = \frac{L_{\mathrm{Mi}}}{T_{\mathrm{Mi}}} \times 100 \tag{7}$$

with L_{Mi} corresponding to the experimental-loaded amount of Miglyol 812 determined by DSC and T_{Mi} corresponding to the theoretical-loaded amount of Miglyol 812, equal to 44.4%.

They were found equal to 95% and 60% for Eudragit RSPO and Ethylcellulose microcapsules respectively.

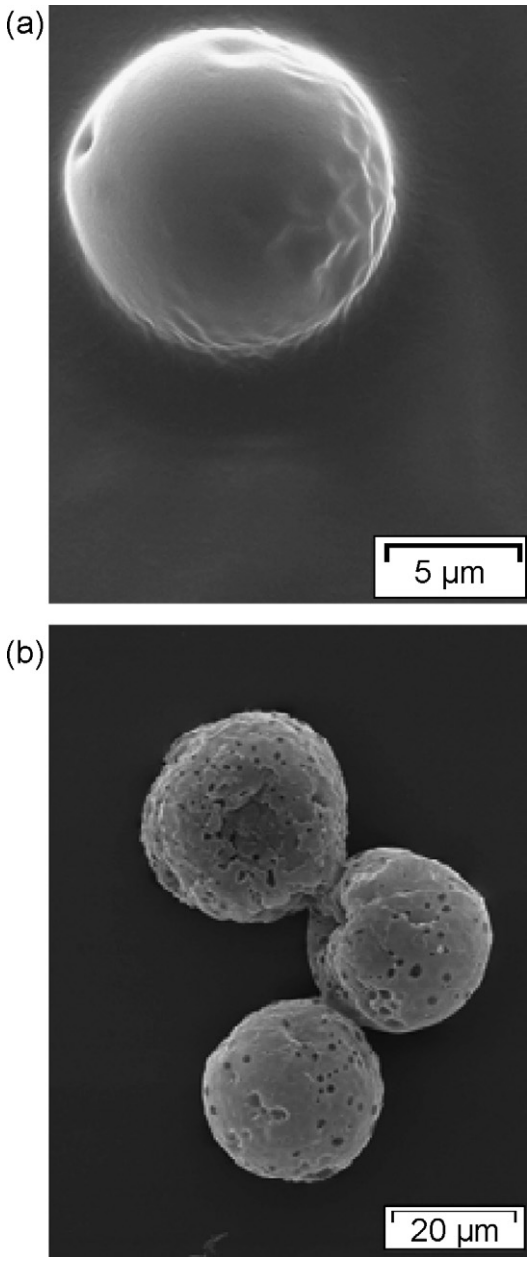

Fig. 8. (a) Scanning electron micrograph of Ibuprofen-loaded Eudragit RSPO microcapsules obtained by optimized formulation. (b) Scanning electron micrograph of Ibuprofen-loaded Ethylcellulose microcapsules obtained by optimized formulation.

Then the Ibuprofen encapsulation yield was deduced and found equal to 95 wt% and 60 wt% for Eudragit RSPO and Ethylcellulose microcapsules respectively. These values were quite different from the encapsulation yields determined by UV–vis spectroscopy, especially for Ethylcellulose microcapsules. As regards the respective accuracy of UV–vis spectroscopy and DSC-based thermoporosimetry for the determination of loaded agent amount, UV–vis spectroscopy appeared the most appropriate method. The inconvenient of DSC-based thermoporosimetry relied on the presence of Miglyol 812 at the surface of the microcapsules, that was particularly important in the case of Ethylcellulose and was not considered for the determination of the loading amount, whereas UV–vis method allowed the detection of the total amount of Miglyol independently of its confinement degree.

Thereof, some attempts were performed using FTIR based on Attenuated Total Reflection (PERKIN ELMER FTIR 1760-X spectrometer) in order to quantify Miglyol 812 expected to be present at the extreme surface of the microcapsules. Unfortunately the presence of PVA adsorbed at the surface and surrounded microcapsules interfered with the Miglyol 812 vibration bands, and hindered further information.

4. Conclusion

This paper describes the formation of Ibuprofen-loaded Eudragit RSPO and Ethylcellulose microcapsules with a liquid core containing Ibuprofen. The influence of various process parameters involved in the formulation of the microcapsules was studied, and a standard procedure based on the use of PVA 0.2 wt% as surfactant, Miglyol 812 as biocompatible oily core was developed. The effects of other parameters such as the stirring rate, the volume ratio of dispersed and continuous phases, the polymer concentration were also investigated. We underlined the variation of the mean diameter and the size distribution of microcapsules with the previous parameters, since these characteristics depend on the interactions between the polymer, the organic volatile solvent, the biocompatible oily core and water, as well as the physicochemical properties of each component. These studies gave rise to an optimized procedure.

Finally the encapsulation yield of Ibuprofen close to 100%, whatever the polymer type, was determined UV–vis experiments. DSC-based thermoporosimetry was used for the estimation of the loading rate of Ibuprofen in accordance with the results obtained by ^{13}C NMR spectroscopy. Nevertheless this original developed analytical method had to be improved as the observed DSC-transitions accounted to free and enclosed Ibuprofen altered the accuracy of the results. All findings reported in this article suggested that the morphology, key characteristic of microcapsules since it will influence Ibuprofen release, may be dependent on numerous parameters. The sustained release of the active agent is mainly controlled by the diffusion rate through the polymeric shell and hence determined by its thickness and its degree of swelling, or alternatively by the rupture of the microcapsule membrane depending of its mechanical strength.

On the basis of the results reported in this paper, Ibuprofen-loaded microcapsules were expected to be employed in biomedical textile applications. The investigation of the link between microcapsules morphologies and Ibuprofen release from these microcapsules will be described in a forthcoming paper.

Acknowledgements

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