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Synthesis of lidocaine-loaded PLGA microparticles by flow focusing Effects on drug loading and release properties

M.A. Holgado[a,*], J.L. Arias[b], M.J. Cózar[a], J. Alvarez-Fuentes[a], A.M. Gañán-Calvo[c], M. Fernández-Arévalo[a]

[a] Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, Spain
[b] Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Granada, Spain
[c] Departamento de Ingeniería Aeroespacial y Mecánica de Fluidos, Escuela Técnica Superior de Ingenieros, Universidad de Sevilla, Spain

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ABSTRACT

In the present work, two methods for the preparation of lidocaine-loaded PLGA microparticles are compared. The differences between the polymeric particles obtained by solvent evaporation (SEVM) or flow focusing (FF) were studied by means of scanning electron microscopy and surface thermodynamics determinations. A detailed investigation of the capabilities of the polymer particles to load this drug is described. The physical state of the drug in the polymeric particles and the existence of interactions between both entities were studied by differential scanning calorimetry. The main factors determining the lidocaine incorporation and the release kinetics were the synthesis procedure followed, the amount of drug dissolved in the organic phase during the synthesis routine, the type of polymer (molecular weight and end chemical groups) and the size and the hydrophobic/hydrophilic properties of the particles. The FF technology allowed higher drug incorporations and slower release kinetics. The release studies showed a biphasic profile probably due to diffusion-cum-degradation mediated processes.

1. Introduction

Local anaesthetics are widely used in the treatment of both acute and chronic pain, but their usefulness is limited by the short duration of the local anaesthetic effect that generally lasts for only few hours (Nurkeeva et al., 2002). Lidocaine is a local anaesthetic type amide, that can be considered as a model molecule for hydrophobic drug encapsulation because of its small molecular weight and low water solubility (Görner et al., 1999). Lidocaine has a faster onset of action and a higher length of action than amino ester anaesthetics. However, its therapeutic potential is restricted by its short plasma half-life (1.5–2 h). In order to increase the therapeutic index of this molecule in the treatment of pain, with respect to its effectiveness and safety, biodegradable microspheres have been used as drug delivery systems to achieve a localized and sustained drug release. This will reduce the dose needed to obtain a pharmacological effect and, therefore, the incidence of systemic effects (Chen et al., 2004). Moreover, a long-term drug delivery system will be an ideal candidate to improve drug adherence and to ensure continued optimum drug dosage levels that maximize the benefits of therapy.

Microencapsulation is a very common method for elaborating delivery systems for drugs and vaccines (Freitas et al., 2005). Microspheres can be prepared using different strategies, although most of them are modifications of three basic techniques: solvent extraction/evaporation, phase separation (coacervation) and spray-drying (Aftabrouchad and Doelker, 1992). However, traditional drug microencapsulation methods do not provide particles with the desired drug loading, and, moreover, a complementary treatment (filtration or sieve systems) is also needed to obtain particles with a monodisperse size distribution. Therefore, an easy methodology is needed for the preparation of particles with a suitable drug loading, a homogeneous shape and a narrow size distribution in the nanometer or micrometer range (Martín-Banderas et al., 2005).

Poly(lactic-co-glycolic) acid block copolymers (PLGA) are polyesters commonly used for the microencapsulation of therapeutics and antigens. Their use as drug delivery systems is due to their excellent biocompatibility and biodegradability properties. Moreover, PLGA-based microparticles offers many advantages in comparison to other materials used as drug carriers (Kumar et al., 2001). Several methods can be followed in the preparation of PLGA microparticles; however, the success of the technique is determined by many factors related to the drug (solubility, partition

* Corresponding author at: Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Sevilla, c/ Profesor García González, No. 2, 41012 Sevilla, Spain. Tel.: +34 95 4551624; fax: +34 95 4556085.
E-mail address: holgado@us.es (M.A. Holgado).

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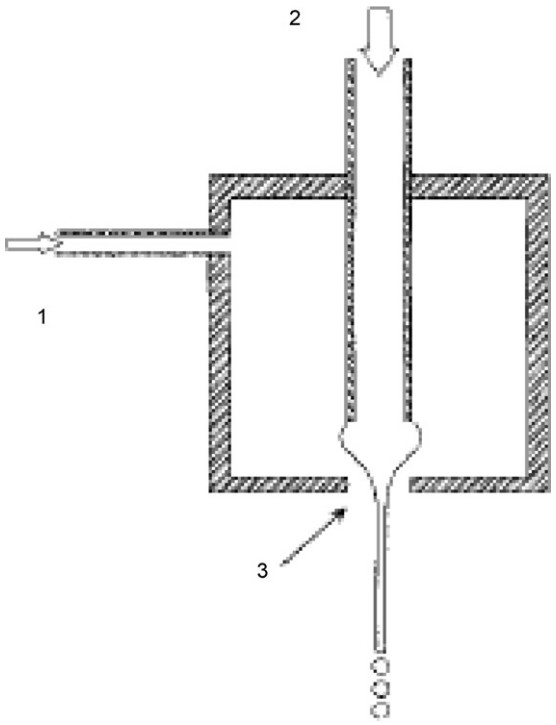

Fig. 1. Flow focusing atomizer: focusing fluid (1), focused fluid (2), and meniscus (3).

coefficient, etc.) and the polymer (composition, molecular weight, end chemical groups, etc.) (Fu et al., 2005).

The flow focusing (FF) technique (Gañán-Calvo, 1998) can efficiently control the production of monodisperse simple or encapsulated particles, in the micron or sub-micron range, in just one step and without additional purification procedures. The FF technology is based on a useful microfluidic concept resulting from the combination of hydrodynamic forces with a specific geometry. Briefly, a FF device (Fig. 1) consists of a pressurized chamber with a continuous focusing fluid (gas or liquid) provision. Inside, a hydrodynamic "funnel shaped lens" is created when the flowing focusing fluid undergoes a pressure drop across an orifice. By feeding an immiscible liquid flow into this hydrodynamic funnel made by the focusing fluid, a steady thin jet of immiscible liquid is created in the core of the co-flowing focusing stream, giving rise to a micro- or nanojet that leaves the chamber through the orifice together with the focusing fluid. The jet diameter is much smaller than the diameter of the exit orifice, thus precluding any contact. Capillary instability breaks up the stationary jet into droplets of homogeneous size.

This versatile technique allows the control of the size, the surface characteristics and the internal structure of the elaborated systems (Martín-Banderas et al., 2005), and presents several advantages in comparison to other traditional encapsulation technologies: (i) compatibility with different fluid mixtures (liquid–liquid, liquid–gas) using simple liquids, polymeric solutions, emulsions, suspensions or melted solids. (ii) Production, without external excitation sources and additional purification steps, of smaller particles with narrow size distribution, in just one step. (iii) Suitability for the encapsulation of labile compounds (proteins, cells, etc.), as because of the special flow geometry, the particle generating fluid is subjected to low stresses, this making the FF technique most adequate for the encapsulation of labile compounds (proteins, cells, and similar entities). (iv) Control of the particle design, involving freely chosen morphology, surface treatment, and composition (e.g., homogeneous particles, two-phase capsules, or hollow capsules). (v) High performance and applicability to industry large-scale production (Martín-Banderas et al., 2005).

In this work we describe the preparation of lidocaine-loaded PLGA microparticles with long-lasting effects, using two techniques, the FF technology and a traditional method based on solvent evaporation (SEVM). A comparative study is carried out to check the influence of both techniques on the: (i) size and morphology, and surface thermodynamics of the polymers; (ii) physical state of the drug in the PLGA particles; and (iii) drug loading and release kinetics of the particles. The release studies are performed with the aim of evaluating the dissolution behavior of these systems in the administration site. In the present paper, a parenteral route is intended for the local administration of these systems, and hence a physiological pH condition has been reproduced.

2. Materials and methods

2.1. Materials

Lidocaine, a model drug for hydrophobic encapsulation, was purchased from Sigma–Aldrich (Germany). Poly(lactic-co-glycolic) acid block copolymers (PLGA 50:50) Resomer® RG 502 (Mw: 12,000; inherent viscosity: 0.24 dl/g), Resomer® RG 502H (Mw: 12,000; inherent viscosity: 0.19 dl/g), Resomer® RG 504 (Mw: 48,000; inherent viscosity: 0.5 dl/g) and Resomer® RG 504H (Mw: 48,000; inherent viscosity: 0.53 dl/g) were obtained from Boehringer Ingelheim (Germany). All other chemicals used were of analytical quality from Panreac (Spain), except from formamide (Aldrich, USA) and polyvinyl alcohol [PVA, Mw: 16,000; Fluka (Germany)]. Water used in the experiments was deionized and filtered (Milli-Q Academic, Millipore, France).

2.2. Methods

2.2.1. Preparation of the lidocaine-loaded PLGA microparticles

The preparation of the microparticles by means of the solvent evaporation method involves the following procedure: 250 mg of PLGA was dissolved at room temperature in 10 ml of ethyl acetate. In the resulting solution, different amounts of lidocaine (6.25–50 mg) were dissolved. This organic phase was added to a 0.3% (w/v) PVA solution and homogenized at 8000 rpm during 8 min (Heidolph DIAX 900, Germany). The obtained emulsion was stirred at 300 rpm for at least 12 h with a magnetic stirrer hotplate SM6 (Jepson Bolton, UK), under room conditions, in order to evaporate the organic solvent. The obtained PLGA particles were collected by centrifugation at 3000 rpm during 25 min (Orto Alresa, mod. Digicen, Spain) and washed twice with 5 ml of water in order to remove the weakly adsorbed (or simply mechanically adhered) lidocaine. Finally, microparticles were frozen in liquid nitrogen and lyophilized (Telstar Cryodos, Spain).

The synthesis of the PLGA particles by means of the flow focusing technology involves the dissolution of lidocaine (6.25–50 mg) in a solution containing 250 mg of PLGA in 10 ml of ethyl acetate. The resulting solution was sprayed, using a standard FF nozzle fixed at 5 ml/h and 100 mbar, inside a chamber with an inlet temperature of $60 \pm 10\,^{\circ}C$. The formed particles were collected at the bottom of the chamber as a dry powder on a plate, freeze–dried and stored at $4\,^{\circ}C$.

The formulations used in the synthesis of the PLGA particles by both methods are collected in Table 1. Lidocaine-loaded PLGA copolymers (Resomer® RG 504 and Resomer® RG 504H) obtained by the FF method were discarded because of their high viscosity, useless for our drug delivery purposes. All the formulations were prepared in triplicate.

Table 1
Formulations of lidocaine-loaded PLGA microparticles obtained by means of the solvent evaporation method (SEVM) and the flow focusing technology (FF)

Formulation	Lidocaine (mg)	PLGA (250 mg)
1	50	502
2	25	502
3	12.5	502
4	6.25	502
5	50	502H
6	25	502H
7	12.5	502H
8	6.25	502H
9	50	504
10	12.5	504
11	50	504H
12	12.5	504H

2.2.2. Characterization methods

The size distribution of the microparticles was determined by means of an optical microscope (Olympus BH-2, USA) and an image-processing program (ImageJ 1.30v software, NIH, USA; Jillavenkatesa et al., 2001). The diameter determination and the statistical analysis were carried out from various microphotographs (sample size: 200 particles). The morphology and surface characteristics of the microparticles were studied by scanning electron microscopy (Philips XL-30, Philips Electron Optics, The Netherlands). Prior to observation, a dilute suspension of the particles (≈0.1%, w/v) was sonicated during 5 min, and drops of the suspension were placed on copper grids and coated with a palladium:gold alloy film, using a fine-coat sputter JFC1100 (Jeol Ltd., Japan). The grids were then dried at 35.0 ± 0.5 °C in a convection oven.

In order to determine the physical state of the drug in the polymeric particles and the possible existence of interactions between both entities, thermograms of PLGA, lidocaine, PLGA–lidocaine physical mixture, and lidocaine-loaded PLGA microparticles were obtained in an automatic differential scanning calorimetry (DSC) analyzer system (Mettler FP80 HT Central Processor and Mettler FP85 TA Cell, Spain). The data processing system (Mettler FP89 HT, Spain) was connected to the thermal analyzer. Temperature calibrations were performed with indium as a standard. An empty flask was used as a reference. All samples (5 mg) were run at a scanning rate of 10 °C/min, from 30 to 300 °C.

Finally, the differences between the surface properties of the PLGA microparticles obtained by both methods (SEVM and FF) were also investigated by performing a surface thermodynamic analysis. Our starting point is the model developed by van Oss and his group (van Oss et al., 1988; van Oss, 1994; Arias et al., 2006), according to which the total surface free energy of any material i is the sum of two contributions:

$$\gamma\mathrm{TOT}i = \gamma\mathrm{LW}i + \gamma\mathrm{AB}i = \gamma\mathrm{LW}i + 2\sqrt{\gamma+i\gamma-i} \quad (1)$$

one of which, γLWi, is the non-polar Lifshitz–van der Waals component, and the second one, γABi, is the acid–base component. This is related to the electron-donor ($\gamma-i$) and electron-acceptor ($\gamma+i$) characteristics of the material. Similarly, the interfacial solid/liquid free energy, γTOTSL, and its LW and AB components ($\gamma_{\mathrm{SL}}^{\mathrm{LW}}$, $\gamma_{\mathrm{SL}}^{\mathrm{AB}}$, respectively) are related to the surface free energies of both the solid (subscripts S) and the liquid (subscripts L):

$$\gamma\mathrm{TOTSL} = \gamma\mathrm{LWSL} + \gamma\mathrm{ABSL} = \gamma\mathrm{LWSL} + 2\sqrt{\gamma+\mathrm{S}\gamma-\mathrm{S}} + 2\sqrt{\gamma-\mathrm{S}\gamma+\mathrm{S}} - 2\sqrt{\gamma+\mathrm{S}\gamma-\mathrm{L}} - 2\sqrt{\gamma-\mathrm{S}\gamma+\mathrm{L}} \quad (2)$$

Using the Young's equation (Adamson, 1990), these quantities can be related to the contact angle θ between the liquid and the solid:

$$(1+\cos\theta)\,\gamma\mathrm{TOTL} = 2\sqrt{\gamma\mathrm{LWS}\,\gamma\mathrm{LWL}} + 2\sqrt{\gamma+\mathrm{S}\gamma-\mathrm{L}} + 2\sqrt{\gamma-\mathrm{S}\gamma+\mathrm{L}} \quad (3)$$

The three unknowns (γLWS, $\gamma+$S and $\gamma-$S) can be obtained by solving the resulting system of three equations if one measures the contact angles of three liquids of known γLWL, $\gamma+$L and $\gamma-$L. In our case, we used water (γLWL = 21.8, $\gamma+\mathrm{L} = \gamma-\mathrm{L} = 25.5\,\mathrm{mJ/m^2}$), formamide ($\gamma$LWL = 39.0, $\gamma+\mathrm{L} = 2.28$, $\gamma-\mathrm{L} = 39.6\,\mathrm{mJ/m^2}$) and α-bromonaphtalene (γLWL = 43.6, $\gamma+\mathrm{L} = \gamma-\mathrm{L} = 0\,\mathrm{mJ/m^2}$; all data taken from van Oss, 1994). The contact angles of the three liquids were determined at 25.0 ± 0.5 °C, using a Ramé-Hart 100-00 goniometer (USA), on pellets (radius: 1.3 cm) obtained by compressing the dry powders in a Spepac hydraulic press set to 10 ton during 5 min.

2.2.3. Determination of the lidocaine loading to PLGA microparticles

In order to determine the drug loading, 7 mg of lidocaine-loaded PLGA microparticles were dissolved in acetonitrile. The obtained solutions were filtered with 0.45 μm Millipore filters. The amount of lidocaine-loaded was determined at 254 nm using a HPLC system manager (Hitachi, Japan), composed by four units: isocratic pump L-7100, automatic injector L-7200, DAD UV–vis L-7455 and D-7000 interface, using a column LiChrospher 100 5 RP-18 μm (125 mm × 4 mm) from Merck (Germany). Acetonitrile:ammonium acetate (70:30, 0.0257 M, pH 4.85) was used as mobile phase with a flow rate of 0.6 ml/min.

The lidocaine content (%) of microparticles was calculated as

$$\text{Lidocaine content} = \frac{\text{incorporated lidocaine(mg)}}{\text{microparticles(mg)}} \times 100 \quad (4)$$

The normal distribution of each continuous variable was assessed by using the Shapiro–Wilk test. Levene's test is used to verify that variances are equal across groups or samples; it is called homogeneity of variance. Some statistical tests, for example the analysis of variance, assume that variances are equal across groups or samples.

A factorial analysis of variance was performed to determine the effect on drug loading of the polymer type used and the amount of drug dissolved in the organic phase during the synthesis routine. Snedecor's F distribution is most commonly used to test for statistical significance in tests of variance. The size of the F factor relates directly to the confidence at which the null hypothesis (no difference among treatments or interactions) can be rejected—the larger the factor the greater the confidence of the statistical inference and the smaller the significance level. The F-statistic is the mean square for the factor divided by the mean square for the error. This statistic follows an F distribution with $(k-1)$ and $(N-k)$ degrees of freedom where k is the number of levels for the given factor and N is the number of cases.

When comparing more than two means, an ANOVA F-test tells you whether the means are significantly different from each other, but it does not tell you which mean differs from which other means. Multiple comparison procedures (MCPs) give you more detailed information about the differences among the means; so the Tamhane test (for unequal variances) was used for post hoc comparisons between groups. All statistical tests were two-sided, and p values <0.05 were considered to be statistically significant.

2.2.4. In vitro lidocaine release from the PLGA microparticles

A dialysis method was used for the drug release determinations. Briefly, 30 mg of lidocaine-loaded PLGA microparticles were placed in a dialysis bag and immersed in 40 ml of a phosphate

buffer medium (pH 7.4 ± 0.1). The dialysis process was carried out using a cellulose acetate membrane (diameter: 20.4 mm) in a Spectra/por membrane MWCO: 12-14.000 (Spectrum Laboratories, USA), at 37 ± 0.5 °C and under magnetic stirring (50 rpm). This membrane was pretreated during 1 h with the same buffer solution (pH 7.4 ± 0.1), to ensure its wetting and sealing. At specified times aliquots of 1 ml were withdrawn and the amount of drug released was determined by means of a HPLC system manager, as previously described. An equal volume of buffer, maintained at the same temperature, was added after sampling to ensure sink conditions. All the experiments were carried out in triplicate.

3. Results and discussion

3.1. Particle geometry

As an example, Fig. 2 shows SEM picture of lidocaine-loaded PLGA microparticles obtained by SEVM (Fig. 2(a)) and by FF (Fig. 2(b)). As it can be observed, the microparticles obtained by SEVM are spherical with a broad size distribution and a smooth surface. The microparticles obtained by FF show also a spherical shape and a smooth surface. As it can be seen, the selected technique of microencapsulation does not seem to influence the final morphology and surface characteristics of the particles. (Similar pictures are not shown for the rest of PLGA formulations).

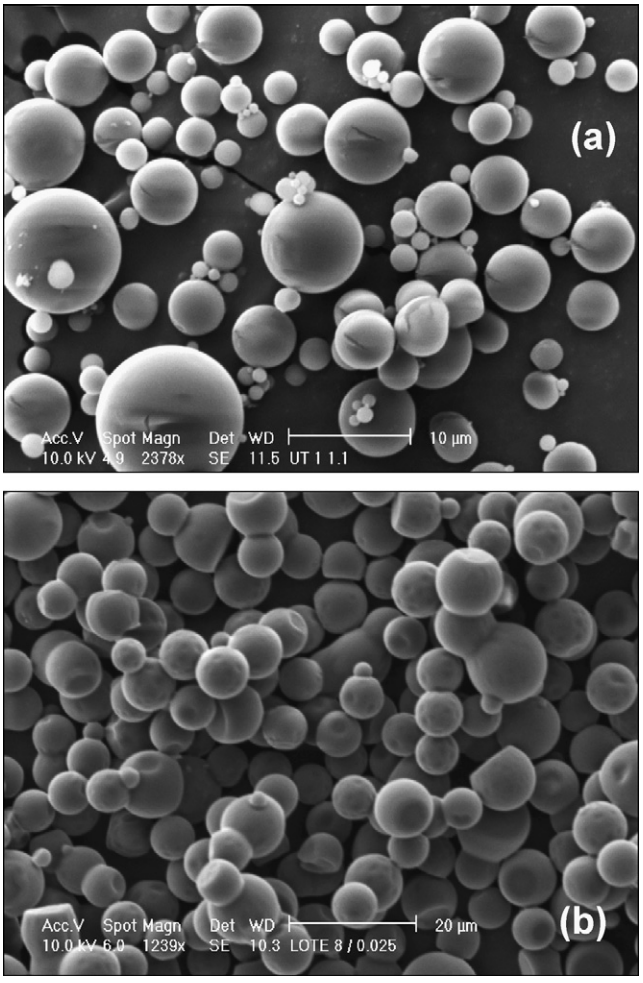

Fig. 2. Scanning electron microscopy photographs of lidocaine-loaded PLGA microparticles obtained by SEVM (a) and FF (b).

Table 2
Mean diameter of the lidocaine-loaded PLGA microparticles obtained by the SEVM and the FF techniques

Formulation	SEVM		FF	
	Size (μm)	CV (%)	Size (μm)	CV (%)
1	3.2 ± 1.6	48.8	8.4 ± 1.1	13.2
2	4.7 ± 1.7	36.4	7.9 ± 1.2	14.7
3	3.8 ± 1.7	44.6	8.5 ± 1.2	13.9
4	3.9 ± 2.0	51.0	7.6 ± 1.2	16.2
5	4.5 ± 1.9	42.2	7.9 ± 1.1	14.4
6	3.3 ± 1.5	45.8	8.3 ± 1.1	12.9
7	4.8 ± 1.8	37.7	8.0 ± 1.2	15.0
8	4.5 ± 2.0	44.2	8.1 ± 1.1	13.2
9	3.7 ± 1.4	38.5	–	–
10	3.9 ± 1.2	30.0	–	–
11	4.4 ± 1.5	34.5	–	–
12	4.3 ± 1.1	25.4	–	–

In relation to the microparticles mean size, the SEVM method allows to obtain little smaller particles than the FF technology in the experimental conditions assayed in this paper. On the other hand, important differences with respect to the size distribution of the microparticles have been detected (Table 2). The FF technique allows to obtain microparticles with a more narrow size distribution in comparison to the particles obtained by SEVM. This is especially interesting considering the biopharmaceutical influences that can be derived.

Finally, the morphology, surface and size of the microparticles did not vary significantly when loaded with different drug amounts, and they were also independent to the type of PLGA used. Pictures for different drug concentrations are not shown for brevity.

3.2. Thermal analysis

Fig. 3 shows DSC thermograms of lidocaine, PLGA 502 and a PLGA 502/lidocaine physical mixture. The presence of both the lidocaine endothermic peak (80.8 °C) and the PLGA glass transition temperature peak (57.2 °C), in the thermogram of their physical mixture, reveals the absence of interactions between both entities.

With respect to the physical state of the drug-loaded to the PLGA particles, the lidocaine endothermic peak is not observed in the thermograms of the lidocaine-loaded polymeric particles obtained by either SEVM (Fig. 4(a)) and FF (Fig. 4(b)) methods. Thus, the drug must be loaded in its amorphous form, rather than in crystalline state; it can be possibly thought of as a molecular dispersion or a solid solution (Zidan et al., 2006). This is confirmed by SEM pictures (Fig. 2): no drug crystals are observed on the PLGA surface. Let us finally mention that neither the amount of drug-loaded nor the type of PLGA used had any significant influence on the drug physical state

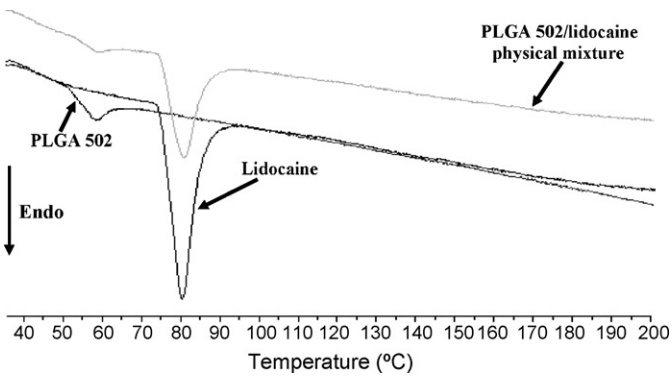

Fig. 3. Thermograms of lidocaine, PLGA 502 and PLGA 502/lidocaine physical mixture.

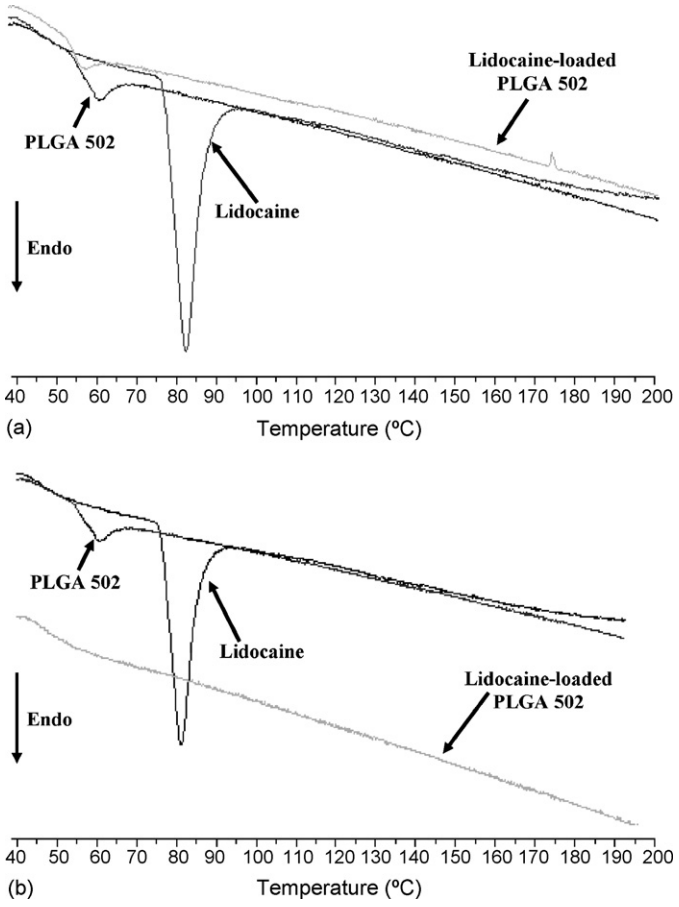

Fig. 4. Thermograms of lidocaine, PLGA 502 and lidocaine-loaded PLGA 502 microparticles (formulation 1) obtained by SEVM (a) and by FF (b).

or the drug–polymer interaction. This observation is based on the thermograms obtained in the different conditions described (data not shown).

3.3. Surface thermodynamics

The surface free energy components (γLWS, $\gamma+$S, $\gamma-$S) of the three types of particles constitute a set of physical quantities that can also be analyzed to ascertain the differences between both synthesis techniques. In order to obtain such quantities using Eq. (2), we measured the contact angles of water, formamide and α-bromonaphtalene on pellets obtained by compressing dry powders of the drug-loaded microparticles. The results are detailed in Table 3. Even these raw contact angle data already denote that there exist significant differences among the lidocaine-loaded PLGA microparticles obtained by SEVM and FF techniques. But it is the evaluation of the γ_S components, given in Table 4, that provides the true physical information about the thermodynamics of the lidocaine-loaded PLGA microparticles prepared by both techniques. Whatever the component considered, its values for particles obtained by both procedures are different. Thus the Lifshitz–van der Waals component is the least affected (as it is usually the case, see, e.g., Arias et al., 2001), although its value for the PLGA obtained by FF is slightly higher than the one for the particles obtained by SEVM. Concerning the electron-acceptor component, $\gamma+$S, although small in both cases, is close to zero for the particles obtained by SEVM. The electron-donor component $\gamma-$S shows large values in particles obtained by SEVM, in comparison to particles obtained by FF.

Obviously, these changes in the surface free energy manifest themselves in the hydrophobicity/hydrophilicity characteristics of the different materials. According to van Oss (1994), the following criterion may be used to check whether a solid can be considered hydrophobic or hydrophilic. The free energy of interaction (not considering the electrostatic component) between the solid phases immersed in the liquid can be written as follows in terms of the total interfacial tension between phases S and L (Eq. (2)):

$$\Delta G_{SLS} = -2\gamma_{SL}^{TOT} \quad (5)$$

This quantity gives a quantitative indication of the hydrophobic/hydrophilic nature of the solid: if it happens to be negative, interfacial interactions favour attraction of the particles to each other, and they are considered hydrophobic. Hydrophilicity will correspondingly be associated to positive values of ΔG_{SLS}^{TOT}. Table 5 shows the results for both kinds of lidocaine-loaded PLGA microparticles. As observed, lidocaine-loaded PLGA microparticles are hydrophobic materials, independently of the type of polymer used, the synthesis method followed and the amount of drug-loaded. However, the hydrophobic nature of the PLGA particles is incremented when they are obtained by the FF technique (more negative values of ΔG_{SLS}^{TOT}). This is probably due to the contribution of the adsorbed PVA to the surface thermodynamics of the PLGA microparticles obtained by SEVM. As PLGA microparticles prepared using PVA tends to strongly adsorb this emulsifier on their surface (Sahoo et al., 2002), this should induce a reduction in the hydrophobic nature, ΔG_{SLS}^{TOT} values, of the PLGA microparticles (Perrin and English, 1997; Gómez-Lopera et al., 2001; Gómez-Lopera, 2003).

Finally, the molecular weight of the polymer should also be taken into account as it is an indication of the polymer chain length and, therefore, of the degree of hydrophilicity/hydrophobicity of the polymer. It is clear that the higher the molecular weight the

Table 3
Contact angle θ (°) of the probe liquids indicated on the different formulations of lidocaine-loaded PLGA micropaticles obtained by SEVM and FF techniques

Solid	SEVM			FF		
	Water	Formamide	α-Bromo-naphtalene	Water	Formamide	α-Bromo-naphtalene
1	55.4 ± 2.7	21.4 ± 1.6	14 ± 5	73.4 ± 2.1	22.6 ± 1.4	7.9 ± 1.2
2	54.8 ± 1.0	24.0 ± 4.6	16 ± 4	75.5 ± 2.2	16.8 ± 1.9	9.2 ± 1.1
3	53.7 ± 4.2	23.3 ± 3.2	15.4 ± 1.0	82.0 ± 2.3	16.5 ± 1.5	7.2 ± 1.1
4	56.9 ± 1.0	28.2 ± 1.0	24.1 ± 1.0	72.2 ± 2.8	18.5 ± 2.1	8.7 ± 1.0
5	60.9 ± 2.9	28.9 ± 1.0	19.7 ± 1.0	76.9 ± 2.6	21.3 ± 0.6	8.3 ± 1.1
6	59.1 ± 1.8	31.3 ± 1.9	17.2 ± 2.3	79.3 ± 3.4	18.1 ± 1.5	7.5 ± 1.3
7	57.9 ± 2.7	28 ± 3	18 ± 5	78.8 ± 1.7	19.1 ± 1.7	9.2 ± 1.4
8	59 ± 3	26 ± 3	17.5 ± 2.4	82.6 ± 3.1	23.6 ± 1.3	7.6 ± 2.8
9	59.1 ± 1.8	31.3 ± 1.9	17.2 ± 2.3	–	–	–
10	57.7 ± 1.0	28.6 ± 2.8	20.2 ± 1.0	–	–	–
11	61 ± 4	20.1 ± 1.0	18.2 ± 1.0	–	–	–
12	62.0 ± 2.9	26 ± 6	19.8 ± 1.5	–	–	–

Table 4
Surface free energy components of the different formulations of lidocaine-loaded PLGA micropaticles obtained by SEVM and FF techniques

Solid	SEVM			FF		
	γLWS	$\gamma+$S	$\gamma-$S	γLWS	$\gamma+$S	$\gamma-$S
1	42.3 ± 0.9	2.4 ± 0.3	13.9 ± 2.3	43.2 ± 0.1	4.3 ± 0.1	1.2 ± 0.6
2	41.9 ± 0.9	2.1 ± 0.3	15.4 ± 0.4	43.1 ± 0.1	5.6 ± 0.2	0.3 ± 0.3
3	42.1 ± 0.2	2.1 ± 0.1	16.4 ± 3.5	43.3 ± 0.1	6.8 ± 0.3	0.2 ± 0.3
4	39.3 ± 0.3	2.2 ± 0.1	14.6 ± 0.7	43.1 ± 0.1	4.8 ± 0.2	1.3 ± 0.9
5	41.1 ± 0.2	2.2 ± 0.2	10.9 ± 2.4	43.1 ± 0.1	5.1 ± 0.4	0.3 ± 0.4
6	41.7 ± 0.5	1.6 ± 0.1	13.6 ± 1.1	43.2 ± 0.1	6.1 ± 0.5	0.1 ± 0.1
7	41.6 ± 1.1	2.1 ± 0.1	13.1 ± 1.7	43.1 ± 0.2	5.9 ± 0.1	0.1 ± 0.1
8	41.6 ± 0.5	2.3 ± 0.1	11.9 ± 1.9	43.2 ± 0.3	5.7 ± 0.4	0.1 ± 0.3
9	41.7 ± 0.5	1.6 ± 0.1	13.6 ± 1.1	–	–	–
10	40.9 ± 0.2	1.9 ± 0.2	13.9 ± 0.1	–	–	–
11	41.5 ± 0.2	3.4 ± 0.5	8.3 ± 3.5	–	–	–
12	41.1 ± 0.4	2.8 ± 0.4	8.9 ± 0.9	–	–	–

γLWS is the Lifshitz–van der Waals component; $\gamma+$S ($\gamma-$S) is the electron-acceptor (electron-donor) component. All values in mJ/m^2.

Table 5
ΔG_{SLS}^{TOT} (interfacial energy of interaction between solid particles, S, in a liquid medium, L, per unit area of solid/liquid interface) values and hydrophobicity/hydrophilicity of the different formulations of lidocaine-loaded PLGA micropaticles obtained by SEVM and FF techniques

Solid	ΔG_{SLS}^{TOT}	
	SEVM	FF
1	−25.1 ± 5.5	−54.3 ± 3.7
2	−22.7 ± 1.7	−55.5 ± 3.8
3	−21.1 ± 6.4	−51.6 ± 4.2
4	−22.9 ± 1.5	−51.8 ± 5.1
5	−31.1 ± 5.9	−57.5 ± 5.7
6	−27.2 ± 2.5	−56.1 ± 3.6
7	−27.2 ± 4.1	−56.8 ± 2.2
8	−28.8 ± 4.4	−57.5 ± 6.6
9	−27.1 ± 2.6	–
10	−25.2 ± 0.6	–
11	−33.9 ± 9.1	–
12	−33.8 ± 3.5	–

All values in mJ/m^2.

Table 6
Lidocaine loading (% w/w) to PLGA microparticles obtained by SEVM and FF techniques

Formulation	SEVM		FF	
	Drug content	CV (%)	Drug content	CV (%)
1	1.21 ± 0.02	1.9	16.91 ± 0.86	5.1
2	0.80 ± 0.06	7.1	8.98 ± 0.20	2.2
3	0.75 ± 0.11	14.5	4.18 ± 0.58	13.9
4	0.61 ± 0.03	4.8	2.58 ± 0.49	19.0
5	2.86 ± 0.49	17.4	17.74 ± 1.03	5.8
6	2.55 ± 0.49	19.1	9.69 ± 0.28	2.9
7	1.51 ± 0.05	3.2	5.93 ± 0.16	2.8
8	0.93 ± 0.04	4.2	2.78 ± 0.07	2.4
9	1.24 ± 0.05	4.0	–	–
10	0.69 ± 0.01	1.4	–	–
11	1.77 ± 0.09	5.1	–	–
12	0.87 ± 0.05	5.8	–	–

longer the chain and, hence, the higher the hydrophobicity of PLGA (Mittal et al., 2007). In summary, whatever the synthesis procedure followed, higher ΔG_{SLS}^{TOT} values (more hydrophobic solids) are obtained in PLGA particles of higher molecular weights (Resomer® RG 504 and 504H).

3.4. Lidocaine loading to PLGA microparticles

Table 6 shows that drug loading is clearly affected by the amount of drug dissolved in the organic phase during the synthesis routine, the type of polymer used and the synthesis technique followed. As determined with drugs of different nature, a positive effect of the increment in drug concentration is observed on the loading efficiency (McCarron et al., 2000; Prior et al., 2000; Dillen et al., 2004; Rivera et al., 2004; Vega et al., 2006). With respect to the effect of the type of PLGA used on drug loading, the influence of the polymeric end groups has been previously evaluated (Budhian et al., 2005). The use of PLGA particles carrying predominantly free carboxylic end groups (in our case, Resomer® RG 502H and Resomer® RG 504H) increases the lidocaine incorporation to the polymeric matrix, because of the formation of hydrogen bonds between these end groups and the drug molecule (Fig. 5). This chemical bonding determines a higher drug loading than that achieved with Resomer® RG 502 and Resomer® RG 504 copolymers, which carry predominantly alkyl ester end groups (Budhian et al., 2005). In addition, the hydrophobicity of the matrix must also be considered: the incorporation of the drug to the polymeric matrix is favoured in more hydrophobic polymers; in other words, it is thermodynamically preferred for the drug to get into the polymer matrix rather than stay in the aqueous solution.

A factorial analysis of variance was carried out in order to check the described effect on drug loading of both factors, the amount of drug dissolved in the organic phase during the synthesis routine and the type of polymer used. When the SEVM method was followed, results showed a significant effect of the type of PLGA used (different hydrophobicity and viscosity), $F_{(3,\,60)} = 114.94$, $p < 0.001$; and of the amount of drug dissolved in the organic phase, $F_{(3,\,60)} = 90.51$, $p < 0.001$. A significant interaction between both factors was detected ($F_{(5,\,60)} = 17.37$, $p < 0.001$), indicating that the effect of the amount of lidocaine dissolved was different depending on the type of PLGA used. The mean lidocaine loading was

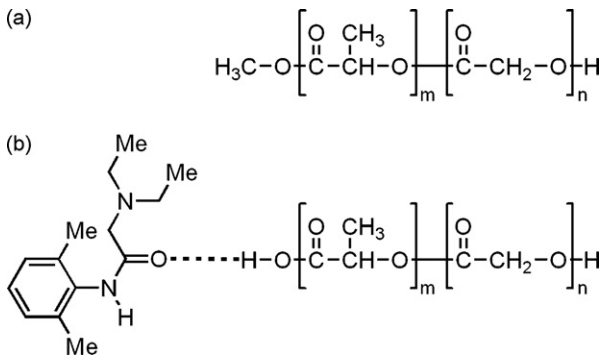

Fig. 5. Chemical structure of PLGA copolymers with an alkyl ester end group (a) and hydrogen bonding between PLGA with carboxylic end group and the lidocaine molecule (b).

more than three times higher when the most hydrophilic polymers were used (Resomer® 502H and Resomer® 504H; see Table 6) and only two times by using Resomer® 502 and Resomer® 504. The differences in variance values in the formulations studied were detected by Levene's test of homogeneity of variances, and the post hoc Tamhane test was performed to identify significantly different group means when the factorial ANOVA test was significant. This pairwise comparison procedures, which compare more than one pair of means at the same time, clearly showed significant differences ($p < 0.05$) between the lidocaine loading if the following polymers were used: Resomer® 502H and Resomer® 504 (mean difference = 1.12, $p < 0.001$), Resomer® 502 and Resomer® 504 (mean difference = 1.00, $p < 0.001$), Resomer® 504H and Resomer® 502 (mean difference = 4.73, $p = 0.036$), Resomer® 502H and Resomer® 504H (mean difference = 0.65, $p = 0.04$).

When a factorial analysis of variance was applied to the PLGA particles obtained by the FF method, results showed a significant effect of the amount of drug dissolved in the organic phase during the synthesis routine on the drug loading efficiency ($F_{(3, 32)} = 1302.16$, $p < 0.001$). Slightly higher lidocaine loading values were achieved when PLGA particles with free carboxylic end groups were used ($F_{(1, 32)} = 7.92$, $p = 0.008$).

Finally, the influence of the synthesis procedure followed can also be clearly observed. Whatever the amount of drug dissolved or the type of PLGA used, higher drug loadings are obtained if use is made of the FF technology. This is probably due to a stronger interaction between the hydrophobic drug and the PLGA matrix obtained by FF, as these polymeric particles are more hydrophobic than the ones obtained by SEVM (McCarron et al., 2000). Moreover, as PLGA particles are obtained in just one step by FF, the possibility of drug loss during the synthesis is minimal.

3.5. In vitro dissolution studies

Drug release profiles from PLGA particles reported in the literature are quite controversial and a general trend cannot be stated. Different PLGA properties, such as size, molecular weight, hydrophobicity/hydrophilicity and the nature of the polymer used, have been reported to influence the drug release (Luan and Bodmeier, 2006).

As can be seen in Figs. 6 and 7, lidocaine release follows a biphasic profile probably due to diffusion-cum-degradation mediated processes. During the rapid early phase, drug release occurs mainly by the loss of the surface-associated drug and by drug diffusion in the polymeric matrix. With respect to the release during the slower phase, it may result from polymer degradation, from drug diffusion through the polymeric matrix, or both (Panyam et al., 2003; Mittal et al., 2007). However, some differences are observed between the PLGA particles obtained by SEVM and FF (Fig. 6). Whatever the lidocaine concentration dissolved in the organic phase and the type of PLGA used, faster release rates are achieved by formulations obtained by SEVM: first, an early rapid release of around 75% takes place within ≈15 days, while the remaining 25% is slowly liberated during the next 35 days. With respect to the polymeric particles obtained by FF, the release rate is slower: around 75% is released within around 30 days, while the remaining 25% is liberated during the next 20 days.

These differences are due to a stronger interaction between the lipophilic drug and the more hydrophobic PLGA particles obtained by FF; this induces a slower drug release process (Gibaud et al., 1998; McCarron et al., 2000; Arias et al., 2007). Moreover, the hydrophobicity/hydrophilicity characteristics of the polymeric par-

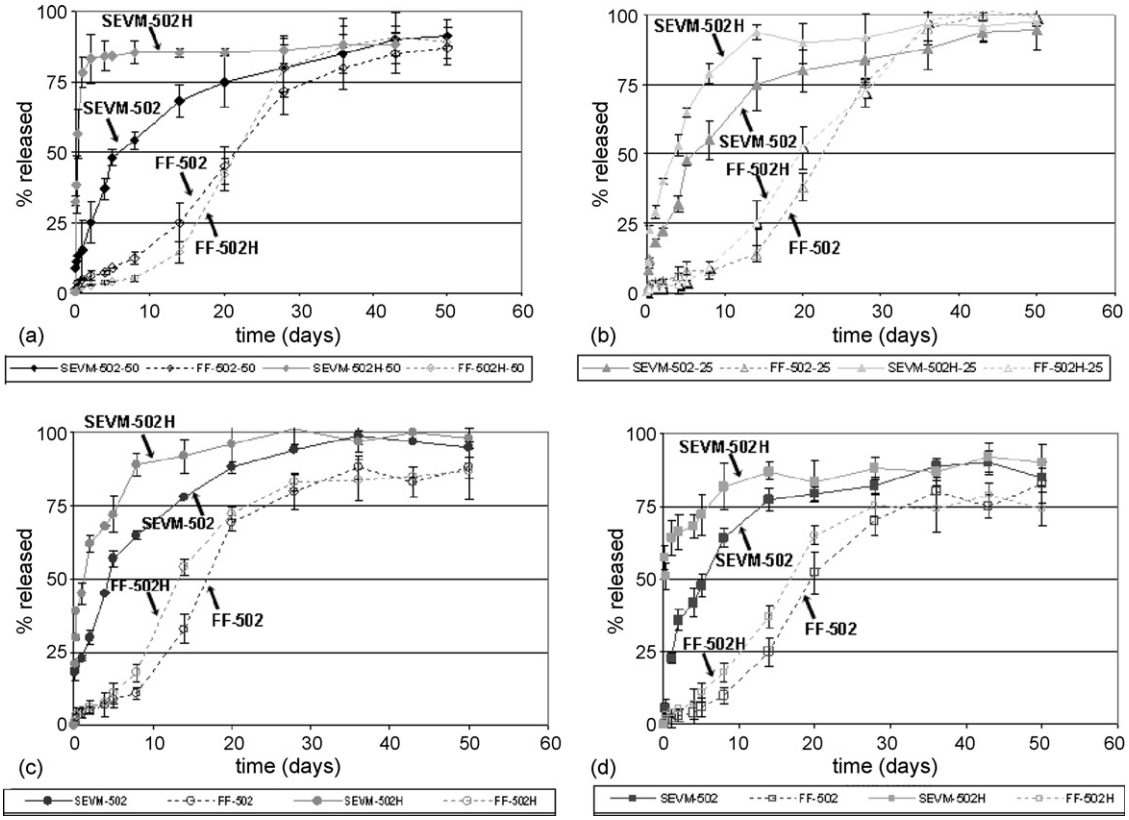

Fig. 6. Lidocaine release (%) from PLGA 502 and 502H particles obtained by SEVM and FF. The drug-loaded PLGA particles were obtained by varying the amount of lidocaine dissolved in the organic phase during the synthesis procedure: 50 mg (a), 25 mg (b), 12.5 mg (c), and 6.25 mg (d).

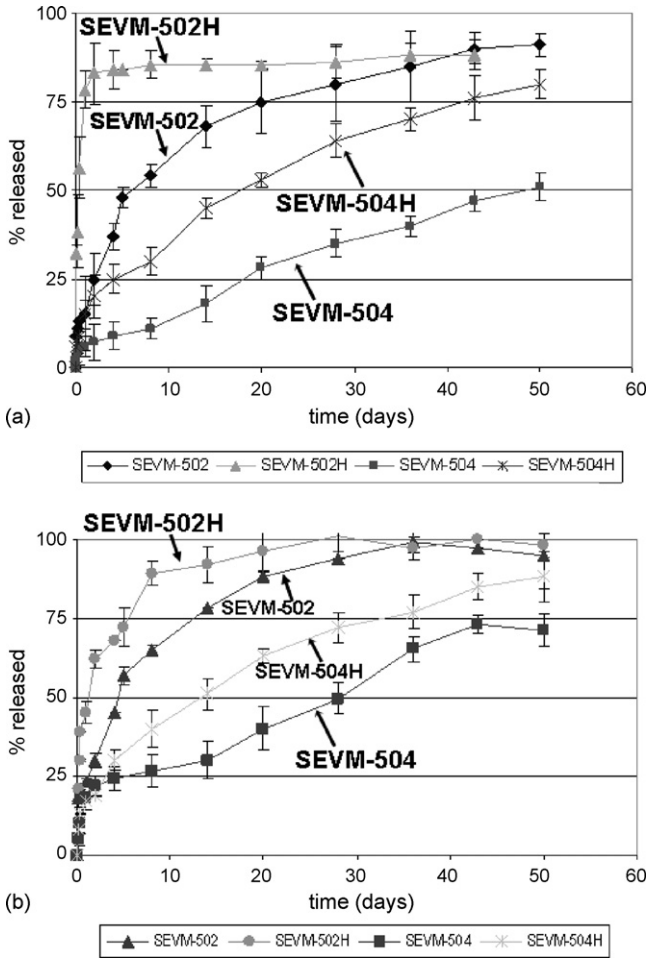

Fig. 7. Lidocaine release (%) from PLGA 502, 502H, 504 and 504H particles obtained by SEVM. The drug-loaded PLGA particles were obtained by varying the amount of lidocaine dissolved in the organic phase during the synthesis procedure: 50 mg (a) and 12.5 mg (b).

ticles also determine their degradation rate and, therefore, the drug release. Increases in hydrophobicity (recall that FF particles are more hydrophobic, as shown in Table 5) contribute to a decrease in the degradation rate and, hence, in drug release (Mittal et al., 2007). We should also take into account that particle size is an important parameter that could affect the degradation of the polymer matrix. An increase in particle size reduces the surface area/volume ratio of the polymer, leading to decreased buffer penetration in the particles and slower release of the drug. This may be another reason for the slower drug release from PLGA microparticles obtained by FF (Mittal et al., 2007).

The lidocaine loading and, therefore, the type of PLGA used, also determines the drug release rate. Figs. 6 and 7 show a slightly faster release from PLGA particles with free carboxylic acid end groups (Resomer® RG 502H and 504H) in comparison to copolymers with alkyl ester end groups (Resomer® RG 502 and 504), independently of the synthesis routine followed. As an increase in drug loading also enhances the cumulative drug release (Brasseur et al., 1991; Arias et al., 2007, 2008), a faster process will be observed in PLGA 502H and 504H particles, because of their higher loadings values (Table 6). Finally, Fig. 7 also allows studying the influence of the PLGA molecular weight on the lidocaine release. As can be seen, an increase in molecular weight decreases the drug release rate. This is due to the higher hydrophobicity of Resomer® RG 504 and 504H (see Table 5) determined by their higher molecular weight; more hydrophobic polymers will more easily retain the drug and, therefore, will lead to a slower drug release (Soppimath and Aminabhavi, 2002; Díez and Tros de Ilarduya, 2006; Mittal et al., 2007).

4. Conclusions

In this study, two synthesis procedures for the preparation of spherical PLGA microparticles loaded with the anaesthetic drug lidocaine, have been analyzed. The differences between the classical solvent evaporation method and the novel flow focusing technique, have been demonstrated by morphology and surface thermodynamic characterizations. It is found that the FF technology allows obtaining microparticles with a more narrow size distribution in comparison to those obtained by SEVM. Moreover, the microparticles obtained by the FF method show a higher loading and a slower drug release profile. These processes are determined by the synthesis procedure followed, the type of polymer (molecular weight and end chemical groups), the size and the hydrophobic/hydrophilic properties of the particles, and the amount of drug dissolved in the organic phase.

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