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Research paper

Solid lipid microparticles as a sustained release system for pulmonary drug delivery

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Abstract

The controlled release of drugs for pulmonary delivery is a research field which has been so far rather unexploited but is currently becoming increasingly attractive. The introduction part of this research article first details the potential advantages of solid lipid microparticles (SLMs) as drug carrier compared to liposomes and polymeric microspheres. The aim of this work is to use SLMs to impart a sustained release profile to a model drug, salbutamol acetonide (SA). SA was synthesized from salbutamol in order to increase the lipophilicity of this molecule and thereby to increase its incorporation efficiency into SLMs. SA-loaded SLMs were then produced by a hot emulsion technique followed by high-shear homogenisation and the manufacturing parameters were optimized using the experimental design methodology in order to reach a suitable particle size for pulmonary administration. Scanning electron micrographs showed that SLMs are spherical, have a smooth surface and that SA crystallises outside of the particles when the drug loading is higher than 20%. This was confirmed by X-ray diffraction. SA in vitro release study from SLMs showed that the release rate increased with SA loading but remained in every case lower than the dissolution rate of pure SA.

Keywords: Solid lipid microparticles; Pulmonary drug delivery; Sustained release; Salbutamol acetonide; Experimental design

1. Introduction

The pulmonary tract tends to be considered as a very promising and attractive route for the administration of active substances intended to treat local pulmonary (e.g., asthma, COBP, and microbial infections) as well as systemic diseases (e.g., diabetes). The pulmonary drug delivery presents many advantages compared to other administration routes. If a local effect is intended, the drug is concentrated at the action site, the amount of drug administered to patients is lower compared to the dose used with traditional administration routes (10–20% of the amount administered by the oral route), systemic undesirable effects decrease and the first pass hepatic and renal effects are avoided. When a systemic effect is aimed, the interest of pulmonary administration is based on the large lung surface area available (140 m^2 for the human adult lung), which is, thanks to its thin absorption mucosal membrane (0.1–0.2 μm), in intimate contact with the circulatory system [1–4].

Currently there is an increasing amount of drugs for pulmonary administration on the market. However, up to now, sustained release formulations for pulmonary delivery have not been marketed yet in spite of the increasing interest in this research field. Only long acting β_2-mimetics and corticosteroids have been developed by pharmacochemical modulation. But it is worth controlling the drug release rate and thereby achieving a sustained release dosage form by employing suitable carriers possessing appropriate drug release characteristics [5].

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In this purpose the liposomes have been the most extensively investigated carriers [6–8,3,9]. They can be prepared with lung endogenous phospholipids as surfactants, with a wide range of size and are able to incorporate both hydro- and lipophilic drugs [10]. Liposomes proved to be able to impart a sustained release profile to the incorporated substances [6] but they also present some disadvantages, i.e., a high production cost, a relative instability during storage [11] and nebulisation [12] that can lead to their disruption and to the premature loss of entrapped substances. Therefore liposomal dry powder formulations are currently getting more attractive [6,13,14].

Polymeric microspheres have also been successfully tested in vitro [15,16] as well as in vivo as sustained release drug delivery system [6,17–19]. They are more physicochemically stable than liposomes, both in vitro and in vivo, allowing thus a slower release of encapsulated drugs [1,10]. Their main disadvantage is that their safety still remains uncertain. Armstrong et al. [20] showed that pulmonary administration of PLA microspheres to rabbits led to histological damages assessed in terms of pulmonary haemorrhage, eosinophilia and neutrophil infiltration. Inflammation can, however, be avoided using large porous particles [21].

In this paper we decided to focus on solid lipid microparticles (SLMs), a carrier that has not been extensively studied up to now, especially for pulmonary administration.

However, this kind of drug carrier presents many advantages. SLMs can be considered as physicochemically stable, allowing a large-scale production at a relative low production cost [22,23] and, seeing their composition, physiologically compatible [24]. Sanna et al. [25] evaluated in vivo SLMs acute toxicity after intratracheal administration in rats. No significant inflammatory airway response was observed at the studied concentrations.

The aim of this study was to produce SLMs containing a bronchodilator agent and providing a sustained release of this active substance and thus a long acting protection against asthma attacks. SLMs carriers allow high entrapment yield for hydrophobic drugs [22]. Indeed, as asserted by Courrier et al. [1] for SLNs (solid lipid nanoparticles), the factors determining the loading capacity of a drug in lipidic excipients are, i.e., the solubility of the drug in melted lipid and the miscibility of melted drug and melted lipid. It must, however, be noticed that Cook et al. [13] recently showed that it was possible to incorporate hydrophilic molecules in SLMs by using a two-step manufacturing process consisting of entrapping hydrophilic nanoparticles in an hydrophobic matrix microsphere.

At the beginning of our research work, we first chose to work with salbutamol, a well known β_2-mimetic. But given that this molecule was relatively too hydrophilic to be efficiently incorporated into SLMs with usual production methods, we decided to synthesize a more hydrophobic derivative of salbutamol, i.e., salbutamol acetonide (SA). The pharmacological effects of this molecule are currently still under investigation but we chose to use it as model molecule to show the efficiency of SLMs as sustained release drug delivery system.

Since the produced SLMs are intended to be loaded with SA, a molecule that is currently studied for its potential β_2-mimetic activity, SLMs must reach the smooth muscle β-receptors of the smallest airways. It is generally considered that an optimal deep lung delivery is obtained with particles having an aerodynamic diameter between 0.5 and 5 μm. One of the main difficulties of this work was then to produce SLMs with a suitable particle size for pulmonary administration. Therefore we chose to use the methodology of experimental design in order to optimize the different manufacturing parameters. The produced SLMs are finally intended to be administered by inhalation as a dry powder.

So the aims of this paper are to produce SLMs, to optimize their size, to characterize the obtained SLMs, and especially to assess the in vitro efficiency of SLMs as sustained release delivery system.

2. Materials and methods

2.1. Materials

Glyceryl behenate (Compritol® 888 ATO) was received as free sample from Gattefossé (France). This excipient is a mixture of 13–21% mono-, 40–60% di- and 21–35% of tri-esters of glycerol and behenic acid (C_{22}) while other fatty acids than behenic acid account for less than 20%. Poloxamer 188, a block copolymer of ethylene oxide and propylene oxide (Lutrol® F68) was purchased from BASF (Germany). Acetonitrile of HPLC grade was from Merck. Salbutamol acetonide was synthesized by the the Natural and Synthetic Drugs Research Centre of the University of Liège. Salbutamol base used as reagent in the synthesis was obtained from Cambrex Profarmaco (Italy). All other solvents used in the synthesis and all other chemicals were of analytical grade.

2.2. Development of a high-performance liquid chromatography (HPLC) assay of SA

The used HPLC system consists of an L-7100 Merck-Hitachi pressure pump, an L-7200 Merck-Hitachi autosampler, an L-7350 Merck-Hitachi column oven, an L-7400 Merck-Hitachi UV/visible detector and a D-7000 Merck-Hitachi interface. The system is controlled by a computer running the "HPLC System Manager v 4.0" acquisition software developed by Merck-Hitachi. The assay method was inspired and adapted from the salbutamol HPLC assay described in the fifth edition of the European Pharmacopoeia [26].

Twenty microliter samples are injected on a Lichrocart column (125 × 4 mm i.d.) prepared with a Lichrospher RP-Select B 5 μm phase (Merck®). The temperature is maintained at 30 °C. The mobile phase is composed of a 65/35 (v/v) mixture of a 0.018 M potassium dihydrogen-

phosphate buffer (pH 3.65) and acetonitrile. The flow rate is adjusted to 1.0 ml/min. All samples are assayed in duplicate at 220 nm with 6 min run time. This method was successfully validated and showed good linearity, reproducibility and accuracy from 0.5 to 20 μg/ml.

The limits of detection (LOD) and quantification (LOQ) were both determined and found to be equal to 0.039 and 0.13 μg/ml, respectively.

2.3. Salbutamol acetonide synthesis

The synthesis of salbutamol acetonide was performed in the Natural and Synthetic Drugs Research Centre of the University of Liège as described by Caira et al. [27]. The structure of SA is represented in Fig. 1.

2.4. Evaluation of SA lipophilicity

SA has been synthesized from salbutamol in order to get a more lipophilic molecule, which could be more efficiently incorporated into SLMs. So, first of all, computed logP values were determined using the software CS Chem draw® Pro version 4.5. The obtained logP values were 0.061 ± 0.5 and 1.82 ± 0.5 for salbutamol and SA, respectively. Those theoretical values seem to confirm that SA is more lipophilic than salbutamol. But in this research work it appeared to be more important to evaluate experimentally and to compare the partition coefficients of both salbutamol and SA between the lipidic excipient used for SLMs manufacturing, i.e., glyceryl behenate, and the aqueous phase. So 40 mg of SA or salbutamol was added to tubes containing 4 g glyceryl behenate and 4 g purified water. The tubes were kept for 30 min under stirring at 80 °C to ensure melting of glyceryl behenate and thus to mimic the operating process of SLMs fabrication (see Section 2.3). After cooling at room temperature, centrifugation was performed and SA or salbutamol was assayed in the aqueous phase by the HPLC method. Each test was realized in triplicate.

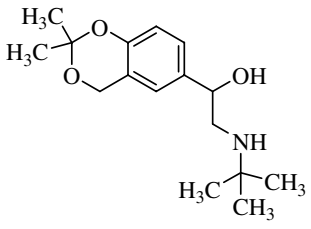

Fig. 1. Chemical structure of salbutamol acetonide.

2.5. Preparation of SA-loaded solid lipid microparticles

SLMs loaded with SA were prepared by a hot emulsion technique followed by a high-shear homogenization using Ultra-Turrax® T25 (Janke & Kunkel, IKA- Labortechnik, Germany). Glyceryl behenate and SA were brought together to 90 °C to allow the melting of glyceryl behenate and the dissolution of SA in the melted lipidic excipient. Poloxamer 188 and water were heated together at the same temperature. The hot aqueous phase is poured into the melted lipid under high-shear mixing using Ultra-Turrax® T25, but in view of Poloxamer 188 HLB value (which is equal to 29) and of the relative concentrations of lipidic and aqueous phases (see Section 2.6), an O/W emulsion is finally obtained by a phase-inversion process. It is preferable to pour the hot aqueous phase in the melted lipid rather than the contrary to avoid loss of lipidic excipient during the manufacturing. This process is illustrated in Fig. 2. The SLMs suspensions obtained after cooling at room temperature were then lyophilized using a Heto® drywinner freeze-dryer FD8-DW8 (Heto Holten, Denmark) in order to get water-free SLMs.

2.6. Optimization of SLMs size by experimental design

SLMs optimal size was studied with the help of the methodology of experimental design using the software MODDE® 6.0. First of all the critical manufacturing parameters and their lower and upper limits were determined. Two independent experimental designs were carried out, the first one using 2.5% of dispersed phase (SA + glyceryl behenate) and the second one using 5% of dispersed phase. The influence of SA concentration, of the surfactant concentration and of the mixing duration on SLMs size was studied within each experimental design. So the influence of SA concentration was tested from 5% to 25% (expressed as the weight percentage of the dispersed phase). The upper limit (25%) corresponds to the maximal concentration of SA that can be dissolved in melted glyceryl behenate. The influence of surfactant (Poloxamer 188)

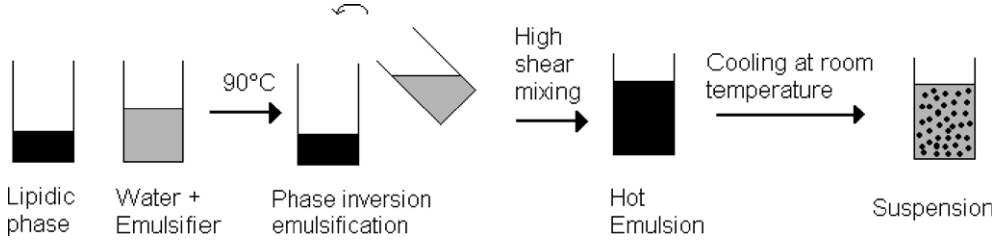

Fig. 2. Schematic representation of the manufacturing method of SLMs suspensions by hot emulsion technique followed by high-shear homogenization and cooling.

Table 1
Limits of variation of the critical manufacturing parameters in both experimental designs containing either 2.5% or 5% of dispersed phase

Dispersed phase (%)[a]	Poloxamer 188 conc. (%)[a]		Mixing duration (min)		SA conc. (%)[b]	
	Lower limit	Upper limit	Lower limit	Upper limit	Lower limit	Upper limit
2.5	0.1	1	1	10	5	25
5	0.1	1	1	10	5	25

[a] Percentage of the total suspension weight (w/w).
[b] Percentage of the dispersed phase (w/w).

concentration was studied between 0.1% and 1% (expressed as the weight percentage of SLMs suspension). The 0.1% value corresponds to the minimal poloxamer 188 concentration which is necessary to obtain a stable emulsion and 1% corresponds to the value above which much foam appears. The influence of mixing duration was studied between 1 and 10 min. The studied parameters and their limits of variation are summarized in Table 1. The mixing speed was studied in the screening step. Thus for a surfactant concentration of 0.5%, a SA concentration of 10% and a mixing time of 5 min, mixing speeds of 8000–13,500 and 24,000 rpm were tested. The best results in terms of SLMs size were obtained with the lowest speed. This value (8000 rpm) has been chosen as a fixed parameter for the experimental designs.

A central composite face-centered (CCF) design, which is a cubic response surface methodology design, was used to optimize manufacturing parameters. The factor endpoints define the vertices of the cube and the axial points are in the middle of all the cube faces.

2.7. SLMs characterization

2.7.1. Laser diffractometry

SLMs geometrical diameter was determined using the laser diffractometry principle of Mastersizer® 2000–Scirocco® 2000 (Malvern Instruments, Malvern, United Kingdom) which applies the Mie scattering measurement principle. 1.347 as real and 0.01 as imaginary refractive index were assumed for our particles. The geometrical diameter was expressed in terms of volume median diameter (D(v,0.5)). The percentage of particles (in terms of volume) whose diameter lies in a specific range of size was also determined.

2.7.2. Determination of SLMs true density and evaluation of SLMs aerodynamic diameter

For particles intended to be administered by inhalation, the geometric diameter is not enough to predict the deposition site. The aerodynamic diameter is actually the most important parameter influencing the particles deposition. This parameter is defined as the diameter of a unit-density sphere which has the same settling velocity in air as the particle. This notion takes particle shape, density and physical size into account, all of these parameters influencing the particle aerodynamic diameter. The theoretical aerodynamic diameter of particles can be calculated based on the following equation [28]:

$$d_{\mathrm{aer}} = \sqrt{\frac{\rho}{\rho_1}} d,$$

where d is the mass-mean geometric diameter, ρ is the particle mass density and $\rho_1 = 1\ \mathrm{g/cm^3}$.

The density ρ was evaluated in triplicate by weighing a known amount (m) of SLMs powder in a graduated cylinder, by melting them at 80 °C and by reading the final volume (V) after cooling at room temperature. The density ρ was assumed to be equal to: m (g)/V (ml).

2.7.3. Scanning electron microscopy

Morphology and surface of dried SLMs were observed by scanning electron microscopy (SEM) (Jeol JSM-840A). The samples were coated with platinum under an argon atmosphere and were examined under an accelerating voltage of 20 kV.

2.7.4. Differential scanning calorimetry

DSC measurements were carried out in order to determine the melting points of glyceryl behenate and of SA, but also to study the solid state of SA in SLMs loaded with increasing percentages of SA. Thermograms of 10 mg samples were recorded with a Mettler-Toledo® DSC25-TC15 TA Controller system, between 35 and 190 °C at a speed of 10 °C/min under a 20 ml/min nitrogen flux. DSC system was controlled by the software STAR System v 6.1 SW.

2.7.5. X-ray diffraction

X-ray diffraction measurements were performed in order to clearly elucidate the solid state of SA in SLMs, using a X-ray diffractometer Siemens® DX500 (Cu K_α source) working at a constant temperature of 25 °C.

2.8. Determination of the drug loading of SLMs powders

It appeared that it was really difficult to wash the obtained freeze-dried SLMs on a filter without either clogging the filter with the smallest particles or allowing the biggest ones to pass through the filter. None of the manifold tested filters allows SLMs filtration. So it has been decided to use the whole SLMs powder for further experiments. This powder contains mainly SA-loaded SLMs but also some possible SA crystals. In this case the drug load-

ing parameter just takes account of the possible loss of SA on the surface of the vessel during the cooling phase of the suspension manufacturing process.

A liquid-liquid extraction technique was developed and validated. A known amount of SLMs powder was weighed and dissolved in chloroform. Two consecutive extractions were carried out on the organic phase with a pH 1 hydrochloric acid diluted solution. At this pH SA is totally converted into salbutamol. In view of its relative hydrophilicity salbutamol went into the aqueous phase. Salbutamol was then assayed by the HPLC method described in the fifth edition of the European Pharmacopoeia [26]. The assayed salbutamol concentration was finally expressed in terms of SA concentration. The real drug loading in the powder was then calculated and compared to the theoretical drug loading. The entrapment efficiency (EE) of SA in SLMs powder was also determined [23] using the following equation:

$$\mathrm{EE}(\%) = (\text{Real drug loading}/\text{Theroretical drug loading}) \times 100.$$

2.9. Determination of SA in vitro release from SLMs

The in vitro drug release was studied using the rotating basket method described in the 4th edition of the European Pharmacopoeia [26] (Sotax AT7 dissolution apparatus coupled with an autosampler Sotax C615). The drug release study was carried out at 150 rpm in PBS, pH 7.4, at 37 °C. Seven replicates of each test were performed. Powder samples were wrapped up in Whatman® glass fiber filters of GF/F grade, the whole being closed with cyanoacrylate glue to prevent SLMs escaping to the medium while allowing them to immerse. A relative high speed of rotation has been chosen in order to access discriminancy. We have demonstrated that the hydrodynamic conditions of the test play an important role in the dissolution rate of SA. Indeed, by increasing the rotating speed, SA dissolves faster. Thereby the release rate of SA from SLMs becomes less dependent on SA intrinsic dissolution. The aim of this study was not to mimic the pulmonary conditions but rather to compare SA release from SLMs with different drug loadings.

3. Results and discussion

3.1. Evaluation of SA lipophilicity

The experimental partition coefficients between melted glyceryl behenate and water were found to be 46.88 ± 0.87 and 4.40 ± 0.15 for SA and salbutamol, respectively. These results confirm that SA is more hydrophobic than salbutamol and that its affinity for melted glyceryl behenate is about ten times higher than salbutamol. Given that the incorporation efficiency into SLMs increases with drug solubility in lipidic excipients, it can be assessed that SA incorporation into SLMs will certainly be higher than with salbutamol.

3.2. Optimization of SLMs size by experimental design

SLMs suspensions were realized following the experimental plan determined by the software. Dried SLMs obtained by freeze drying were measured by laser diffractometry. SLMs size was expressed in terms of percentage of particles (in volume) whose geometric diameter lies between 0.5 and 6 μm. This range of size has been chosen because it corresponds to the suitable size for pulmonary delivery taking the density and its influence on aerodynamic diameter into account. Indeed as explained previously, the optimal aerodynamic diameter for deep lung delivery is in the range of size 0.5–5 μm. Given that SLMs true density is lower than 1 (see Section 3.3.1) the corresponding geometric diameter limits have been defined at 0.5–6 μm.

The best results were obtained with the second experimental design which uses 5% of dispersed phase. Indeed the maximal percentages of particles having a median diameter between 0.5 and 6 μm are around 39.5% for the first experimental design and around 45% for the second one. Moreover the R^2 which expresses the percentage of variability that can be explained by the model is equal to 0.60 for the first experimental design using 2.5% of dispersed phase and is higher (0.94) for the second one using 5% of dispersed phase.The analysis of main effects has shown that SA concentration has no significant effect on the response in the studied limits and that poloxamer 188 concentration and mixing time exert a significant influence on the response. A quadratic effect of mixing time and an interaction between mixing time and poloxamer 188 concentration were also observed.

The optimal production parameters were thus calculated by the software and are summarized in Table 2. Since SA concentration does not influence the response and since drug loading is likely to influence drug release profiles it was decided to choose SLMs containing 5%, 10%, 15% and 20% SA for further studies. SLMs suspensions were produced in triplicate for each theoretical optimal manufacturing condition determined by the software. The obtained results were compared with the theoretical ones

Table 2
SLMs optimal manufacturing parameters determined by analysis of experimental designs and corresponding theoretical responses

Dispersed phase (%)[a]	Poloxamer 188 (%)[a]	Mixing duration (min)	SA (%)[b]	SLMs in range of size 0.5–6 μm (%)
5	0.1	10	11.5	45.31
5	0.1	10	5.5	45.00
5	0.1	10	20.2	44.73
5	0.1	10	16.5	44.53

[a] Expressed as the weight percentage of the total suspension (w/w).
[b] Expressed as the weight percentage of the dispersed phase (w/w).

Table 3
Comparison between theoretical and experimental percentages of SLMs whose geometric diameter lies between 0.5 and 6 μm

Dispersed phase (%)[a]	Poloxamer 188 (%)[a]	Mixing duration (min)	SA (%)[b]	SLMs in range of size 0.5–6 μm (%) ± SD ($n = 3$)	Theoretical responses calculated by the software (%)
5	0.1	10	5	44.52 ± 0.61	44.90
5	0.1	10	10	43.21 ± 1.42	45.30
5	0.1	10	15	44.47 ± 1.61	45.25
5	0.1	10	20	43.59 ± 0.87	44.76

[a] Expressed as the weight percentage of the total suspension (w/w).
[b] Expressed as the weight percentage of the dispersed phase (w/w).

and are presented in Table 3. These results showed that the experimental responses are well correlated with the theoretical ones.

3.3. SLMs characterization

3.3.1. SLMs true density and evaluation of aerodynamic diameter

Experimental value of true density is 0.752 ± 0.013 ($n = 3$). As explained in the introduction part, the optimal aerodynamic diameter range of size is considered to be approximatively 0.5–5 μm. So, by applying the equation quoted in Section 2.7.2 which takes account of the true density to determine the relation between geometric and aerodynamic diameters, we decided to consider the range of size 0.5–6 μm as acceptable geometric diameter for pulmonary administration.

3.3.2. Scanning electron microscopy

Fig. 3 shows SEM images of SA crystals, of drug-free SLMs, and of SLMs containing 1% and 25% SA. The crystals of SA are needle-shaped. Scanning electron micrographs of SLMs revealed a spherical shape and a smooth surface. Needle-shaped crystals identified as SA crystals have been observed on the pictures of SLMs containing 25% SA but not on those containing 1% SA. The crystallisation of SA outside of the particles increases with the drug loading.

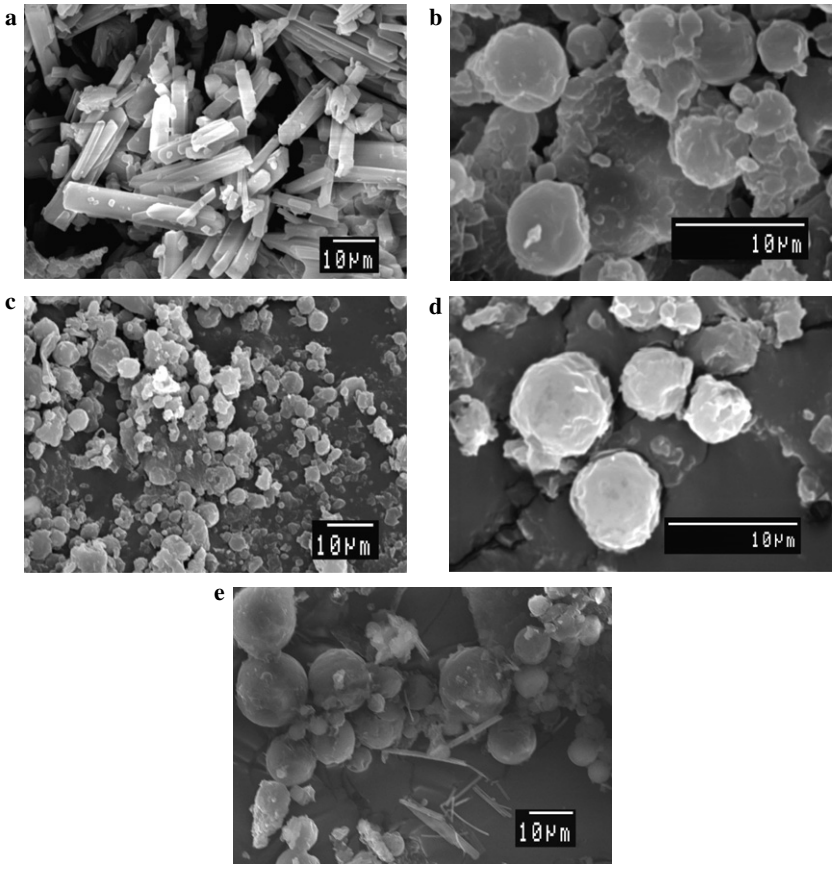

Fig. 3. Scanning electron micrographs of (a) Salbutamol Acetonide; (b) drug-free SLMs; (c) SLMs 1% SA: overall view (d) SLMs 1% SA: higher magnification; (e) SLMs 25% SA.

3.3.3. Differential scanning calorimetry

DSC profiles of glyceryl behenate, pure SA, drug-free SLMs and SLMs loaded with 1% and 20% SA, respectively, are represented in Fig. 4. Thermograms of glyceryl behenate showed a fusion peak at 74 °C while DSC trace of pure SA showed a single fusion peak at 97 °C. Thermograms of SLMs with 1% and 20% SA did not show any peak around 97 °C which could lead to the conclusion that SA does not exist at the crystalline state in SLMs. In order to confirm this hypothesis we compared thermograms of SLMs containing 1% and 20% SA with those of physical mixtures of glyceryl behenate and SA at different relative concentrations (see Fig. 5). DSC profiles of physical mixtures containing 70% glyceryl behenate and 30% SA showed no peak corresponding to SA melting point, but when the relative concentration of SA increased to 50% and 70%, a peak corresponding to SA melting appeared. This can be explained by the fact that SA can dissolve during the heating phase in melted glyceryl behenate because the latter melts at a lower temperature than SA. SA fusion peak appeared in physical mixtures when SA reached the saturation concentration in melted glyceryl behenate. This phenomenon was previously described by Passerini et al. [29,30]. Vippagunta et al. [31] also showed that DSC analysis was unsuitable to give information about the solid state of a drug when it is soluble in the excipients. This observation allowed us to determine the maximum solubility of SA in melted glyceryl behenate by integration of SA fusion peaks appearing in the thermograms of the physical mixtures containing 50% and 70% SA. The maximum solubility of SA in hot glyceryl behenate at the operating conditions proved to be equal to 0.309 ± 0.023 mg SA/mg glyceryl behenate ($n = 6$). The same experiment was carried out with salbutamol in order to determine its solubility in

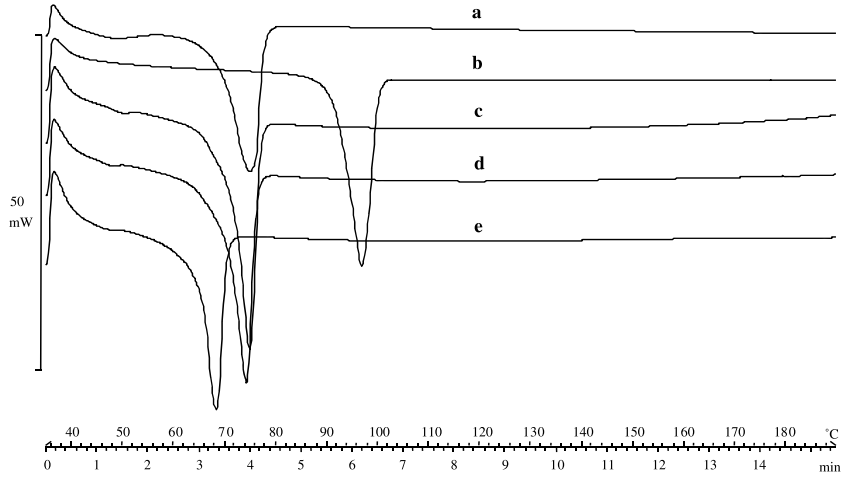

Fig. 4. DSC thermograms of (a) glyceryl behenate, (b) salbutamol acetonide, (c) drug-free SLMs and SLMs loaded with (d) 1% and (e) 20% SA.

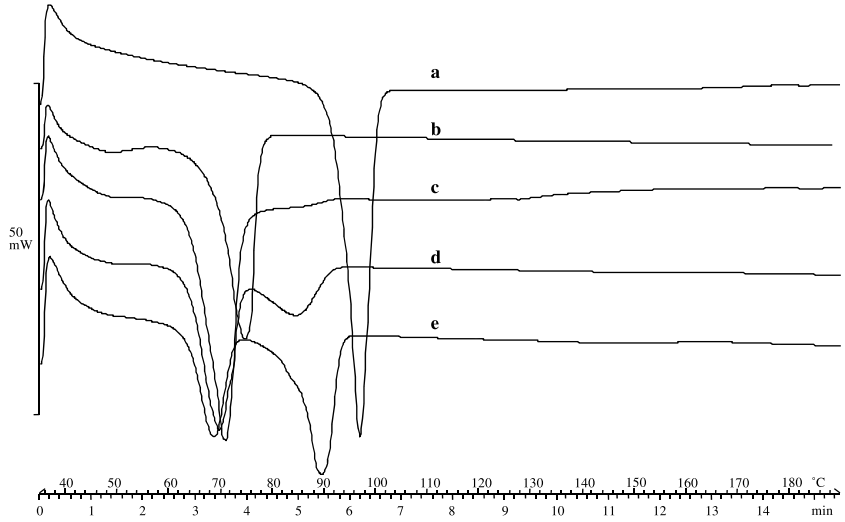

Fig. 5. DSC thermograms of (a) salbutamol acetonide, (b) glyceryl behenate and of physical mixtures containing (c) glyceryl behenate/SA (70/30), (d) glyceryl behenate/SA (50/50) and (e) glyceryl behenate/SA (30/70).

melted glyceryl behenate and to show that this latter is lower than SA solubility. The obtained thermograms are represented in Fig. 6. The maximum solubility of salbutamol in melted glyceryl behenate was found to be equal to 0.104 ± 0.003 mg salbutamol in glyceryl behenate ($n = 6$). Indeed the thermograms of the physical mixtures glyceryl behenate/salbutamol represented in Fig. 6 show that the melting peak of salbutamol disappears when its relative concentration is close to 10%.

However, DSC did not allow us to conclude about SA solid state in SLMs containing 1% and 20% SA, seeing that, at these concentrations, SA dissolves in glyceryl behenate when this latter melts. Therefore another investigation technique (X-ray diffraction) was used to elucidate SA solid state in SLMs.

It was also observed that a decrease of the glyceryl behenate melting point occurs on the DSC curves of all the tested physical mixtures of glyceryl behenate and SA (30/70; 50/50 and 70/30) and of SLMs containing 20% SA. This phenomenon was not observed with physical mixtures containing salbutamol even at high concentrations. Venkateswarlu and Manjunath [32] also observed the same characteristics with SLNs and assumed them to be influenced by the presence of surfactant [33]. In fact, owing to its chemical structure, SA molecule possesses an amphiphilic character with a hydrophilic part and another part which is more hydrophobic and proved, at the opposite of salbutamol, to act as a surfactant at high concentrations. This hypothesis could explain the depression of the glyceryl behenate fusion peak in presence of relatively high SA concentrations.

3.3.4. X-ray diffraction

The X-ray diffraction patterns of poloxamer 188, glyceryl behenate, salbutamol acetonide, drug-free SLMs, SLMs 5% SA, physical mixture SA/drug-free SLMs 5/95, SLMs 20% SA, and physical mixture SA/drug-free SLMs 20/80 are represented in Fig. 7. The patterns of SLMs with 5% and 20% SA were compared with those of physical mixtures containing the same proportions of SA. The characteristic peak of SA located at 18.4° 2θ increases with increasing concentration of SA in physical mixtures. This peak was also observed with SLMs containing 20% SA but not with SLMs containing 5% SA. This confirms that SA is at least partially at the crystalline state in SLMs containing 20% SA, but appears to be mainly amorphous in SLMs containing 5% SA.

3.3.5. Drug loading determination

Drug loading values of SLMs powders containing theoretically 5%, 10%, 15% or 20% SA and obtained according to the production conditions determined by the experimental design were found to be $4.36\% \pm 0.02$, $8.83\% \pm 0.46$, $13.06\% \pm 0.26$ and $18.03\% \pm 0.60$, respectively ($n = 3$). The SA encapsulation efficiencies (EE) in SLMs powders were $87.30\% \pm 0.44$, $88.27\% \pm 4.65$, $87.08\% \pm 1.74$ and $89.26\% \pm 3.03$ ($n = 3$) for SLMs containing 5–10–15 and 20% SA, respectively.

3.3.6. Drug release in vitro study

The dissolution rate of pure salbutamol acetonide was compared to the release rate of salbutamol acetonide from SLMs containing either 5%, 10%, 15% or 20% SA. The release of SA from physical mixtures of drug-free SLMs and SA at two different relative concentrations (5/95 and 20/80) was also studied. The aim was to compare the release profiles of SA when this drug is incorporated into SLMs and when it is only physically mixed with SLMs. The obtained drug release profiles are depicted in Fig. 8. This figure shows that dissolution of pure SA is complete after 2 hours and that the

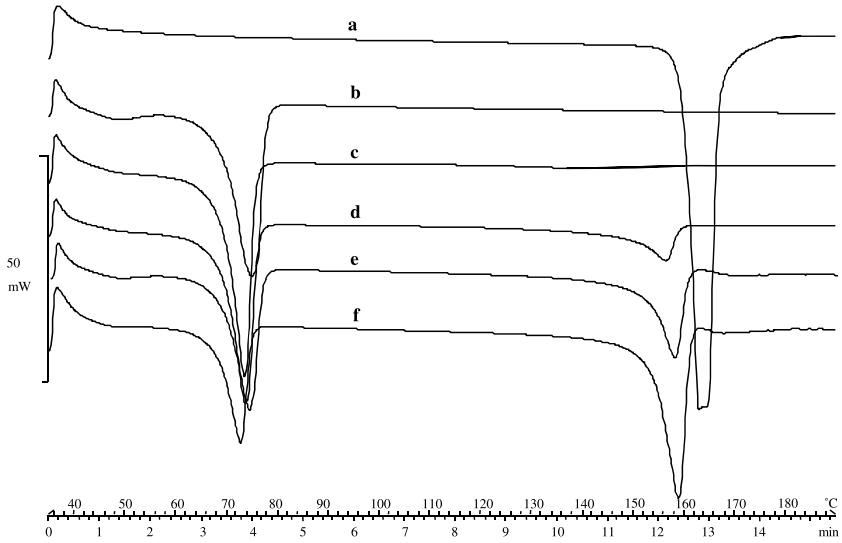

Fig. 6. DSC thermograms of (a) salbutamol, (b) glyceryl behenate and of physical mixtures containing (c) glyceryl behenate/salbutamol (90/10), (d) glyceryl behenate/salbutamol (80/20), (e) glyceryl behenate/salbutamol (70/30), (f) glyceryl behenate/salbutamol (50/50).

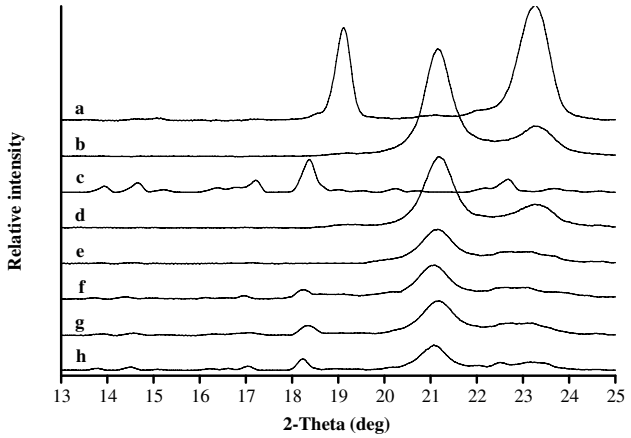

Fig. 7. X-ray diffraction patterns of (a) poloxamer 188, (b) glyceryl behenate, (c) salbutamol acetonide, (d) drug-free SLMs, (e) SLMs 5% SA, (f) physical mixture SA/drug-free SLMs 5/95, (g) SLMs 20% SA and (h) physical mixture SA/drug-free SLMs 20/80.

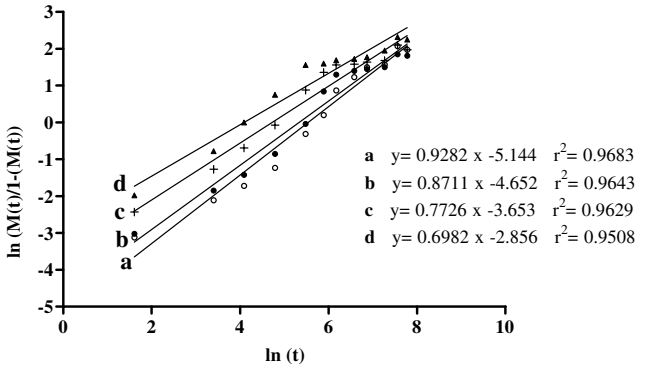

Fig. 9. Experimental and linearized release profiles of SA from (a) SLMs 5% SA (○), (b) SLMs 10% SA (●), (c) SLMs 15% SA(+) and (d) SLMs 20% SA (▲).

release rate is the slowest for SLMs containing 5% SA. The drug release rate increases with drug loading but is in every case lower than the dissolution rate of pure SA. It was also observed that SA release rates from physical mixtures are higher than the release rates of the corresponding SA-loaded SLMs. The obtained curves were linearized in order to determine for each tested sample the times corresponding to 25%, 50% and 75% of SA release, i.e., $t(25)$, $t(50)$ and $t(75)$. We succeeded in fitting the curves by converting the variable time t in ln (t) (min) for the curve of SA dissolution and by converting for the other curves, respectively, the variable time t in ln (t) min and SA release in Logit$(M(t))$, i.e., $\ln\left(\frac{M(t)}{1-M(t)}\right)$ where $M(t)$ represents the SA fraction released at time t considering that M_∞ is equal to 1. The linearized profiles are presented in Fig. 9 and the calculated $t(25)$, $t(50)$ and $t(75)$ values are summarized in Table 4.

These results tend to prove that a sustained release of SA can be obtained by incorporation of SA into SLMs but that physical mixtures of SA and drug-free SLMs are not able to confer a sustained release profile to SA.

Table 4
Theoretical times (min) after which SA release is equal to 25% ($t(25)$), 50% ($t(50)$) or 75% ($t(75)$) SA

	SA	SLMs 5% SA	SLMs 10% SA	SLMs 15% SA	SLMs 20% SA
$t(25)$	6.2	78.1	59.1	27.3	12.4
$t(50)$	16.1	255.0	208.6	113.2	59.8
$t(75)$	40.8	832.8	736.5	469.2	288.7

4. Conclusions

Solid lipid microparticles were successfully prepared in a size range suitable for pulmonary administration using a rather simple production technique. The methodology of experimental design proved to be an interesting and useful tool for optimizing particle size. In practice it has also been shown that it is possible to produce SLMs containing 20% SA (or even 25%) but it has also been observed by SEM and by X-ray diffraction that a high drug loading led to SA crystallisation outside of the particles. The in vitro drug release studies showed that SA release is slower when the drug loading is lower but is in any case slower than the dissolution rate of pure SA. This observation allows us to assess that the drug release can be sustained by using SLMs

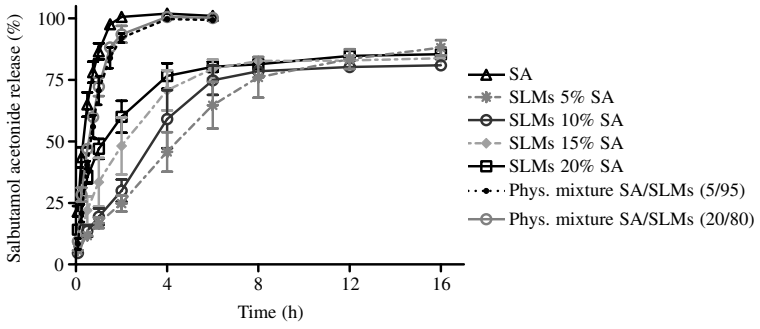

Fig. 8. SA dissolution profiles of pure SA compared with SA release profiles from SLMs containing 5–10–15 or 20% SA or from physical mixtures containing SA and drug-free SLMs (5/95 and 20/80) ($n = 7$).

as drug carrier in comparison with pure drug. It can be concluded that, given their numerous advantages and their ability to impart in vitro sustained release properties to the incorporated active substance, SLMs can reasonably be considered as a promising drug carrier system that could notably be used for pulmonary administration. However, thorough in vivo drug release studies of pulmonary administered SLMs is up to now still missing in the literature. As previously specified SLMs are intended to be administered by inhalation as a powder. Further researches will then consist in formulating inhalation powders containing SLMs with suitable excipients and determining respirable fractions of the tested powders.

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