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Solid lipid microparticles: formulation, preparation, characterisation, drug release and applications

Séverine Jaspart†, Géraldine Piel, Luc Delattre & Brigitte Evrard
†University of Liège, Laboratory of Pharmaceutical Technology, Tour 4, étage 2, Avenue de l'hôpital 1, 4000 Liège, Belgium

This review details the properties of solid lipid microparticles (SLMs): a promising drug carrier system that has been until now rather unexploited. First, the advantages of SLMs compared with other drug carrier systems are listed. Then an overview of SLM manufacturing compounds and techniques is presented. A detailed discussion of the characteristics of SLMs follows, and includes the determination of particle size distribution, the determination of SLM morphology, the solid-state analysis, the determination of SLM drug loading and the factors influencing it. The *in vitro* drug release studies that have been carried out so far and the parameters affecting them are also described. Some preliminary *in vivo* aspects (*in vivo* drug release studies, biocompatibility studies and *in vivo* fate) are also considered.

Keywords: administration routes, characterisation, drug loading, drug release, preparation, solid lipid microparticles

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1. Introduction

There is increasing need to develop suitable drug carrier systems in order to control, localise and improve drug delivery. Many different drug carriers can be used depending on the administration route, the chosen drug properties and the intended drug release profile. The carriers that have been the most often studied in the controlled release of the incorporated substances are:

- liposomes
- polymeric nano- and microparticles
- cyclodextrins
- solid lipid nanoparticles (SLNs)

Liposomes are spherical particles composed of one or more concentric phospholipid bilayers alternating with aqueous partition. This kind of structure makes it possible to incorporate lipophilic drugs into lipid bilayers as well as hydrophilic drugs into the aqueous compartment. Liposomes are one of the most investigated drug carrier systems. Drug release from liposomes, stability and pharmacokinetic profiles depend on liposome composition, size and surface charge, and drug solubility. Liposome formulations of many different drugs show a significant increase in therapeutic activity compared with nonliposomal formulations. Liposomes are biocompatible and biodegradable, but also have some disadvantages including low stability, low encapsulation efficiency, high cost and difficulties for scaling up production [1-5].

Polymeric nano- and microparticles are general terms that include nano- and microspheres (consisting of a polymeric matrix) as well as nano- and microcapsules (reservoir systems composed of a solid or liquid core which can contain either dispersed or dissolved drugs and which is surrounded by a thin polymer layer).

Table 1. Lipid excipients used in solid lipid microparticles.

Category	Description	References
Fatty alcohol	Cetyl alcohol	[20]
	Stearyl alcohol	[21,22]
Fatty acid	Stearic acid (C18 fatty acid)	[20,22,23]
Fatty acid esters of glycerol	Glyceryl monostearate	[20,22,24]
	Glyceryl monobehenate	[24]
	Glyceryl behenate	[20,23,25-28]
	Glyceryl palmitostearate	[28-30]
	Glyceryl ditristearate	[20,28]
	Glyceryl tripalmitate	[1,10,28,31-33]
	Glyceryl tristearate	[28,34]
Fatty acid esters of polyglycerol	Tetraglycerol pentastearate	[22]
	Tetraglycerol monostearate	[22]
Hydrogenated fatty acid ester	Hydrogenated hardened castor oil	[23]
Polar wax	Complex mixture containing, e.g., esters of acids and hydroxyacids	[20,22,23,29,30,35]
Others	Saturated polyglycolised glycerides	[26,29,30,36]
	Beeswax	[29,30,37,38]
	Paraffin wax	[24]
	Cholesterol	[24,39]
	Phospholipids	[39]
	Microcrystalline wax	[21]

Hydrophilic and lipophilic drugs can be incorporated or entrapped into polymeric nano- and microparticles with relatively high efficiency. These kinds of drug carrier systems have proved to be more physicochemically stable than liposomes both *in vivo* and during storage. Their main disadvantages are that preparation methods generally require organic solvents and that large-scale production is rather difficult. Moreover it is crucial to choose suitable polymers that have proved to be nontoxic, biodegradable and nonimmunogenic [2,3,6-9]. Synthetic polymer matrix materials have also been suspected to lead to detrimental effects on peptides and proteins incorporated during the manufacturing process [10].

Cyclodextrins have also been studied as a potential drug carrier system that could modulate drug release rate. Cyclodextrins are cyclic oligosaccharides composed of six (α-cyclodextrin), seven (β-cyclodextrin), eight (γ-cyclodextrin) or more glucopyranose units. They are known for being able to include apolar molecules inside their hydrophobic cavities and provide these guest molecules with better stability, higher water solubility and increased bioavailibility and/or decrease undesirable side effects. However, so far, no study has established the ability of cyclodextrins to induce a controlled release of the included drug *in vivo* [2,11-13].

SLNs were developed in the early 1990s and have since been considered to be promising drug carrier systems, especially with a view to giving the incorporated active substance a sustained-release profile. Compared with liquid lipid formulations, such as fat nanoemulsions, drug mobility is indeed lower in solid lipids than in liquid oils. SLNs are in the submicron size range (50 – 1000 nm) and are composed of a lipidic matrix that is in the solid state at room temperature. They seem to provide an alternative drug carrier system to liposomes and polymeric nanoparticles. SLNs indeed combine several of those carriers advantages while avoiding some of their disadvantages. The lipids used are similar to physiological lipids, so toxicity is reduced. SLNs are physicochemically stable and can be produced relatively easily on a large industrial scale. In addition, raw materials and production costs are relatively low. Their most important limitation is that the drugs that have to be incorporated into SLNs must be lipophilic enough so as to ensure high entrapment efficiency (EE). So far, SLNs have been studied for parenteral and oral administration, and topical application [1,14-19].

This review describes solid lipid microparticles (SLMs), production methods, characterising methods, the ability of SLMs to be used as drug carrier systems, and applications of SLMs. The composition of SLMs is equivalent to SLNs, but in the micrometre size range. Given the similar compositions of SLNs and SLMs, SLMs may also be considered as physiologically compatible, physicochemically stable and allowing a large-scale production. The difference between SLNs and SLMs lies in their respective size ranges, meaning that their application domains and administration routes can be different. Nevertheless, SLMs as well as SLNs, in their respective application fields, can both be considered as promising drug delivery systems. However, so far, SLMs have remained rather untapped.

2. Solid lipid microparticle preparation techniques

2.1 Materials

Commonly used materials for SLM manufacturing are:

- Lipids, including fatty alcohols, fatty acids, fatty acid esters of glycerol (mono-, di- and/or triglycerides), waxes, cholesterol etc. A summary of regularly used materials is presented in **Table 1**.
- Surfactants: many different surfactants can be used, as presented in **Table 2**.
- Water

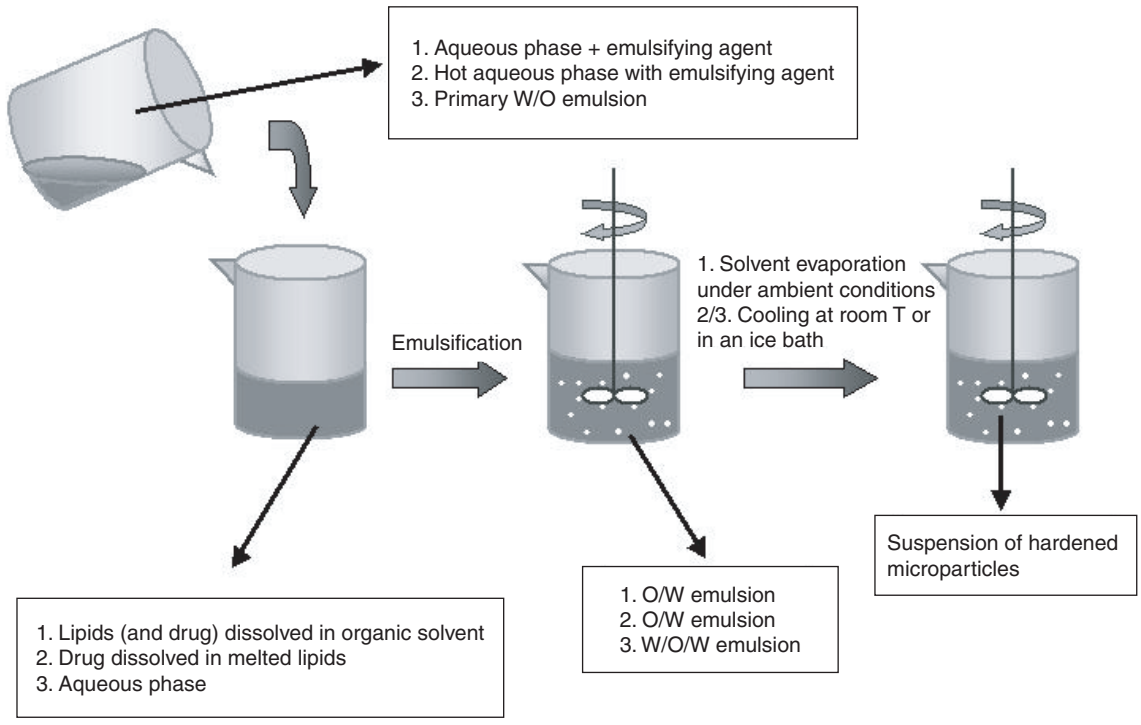

Figure 1. Schematic representation of solid lipid microparticles production by: (1) solvent evaporation method; (2) O/W melt dispersion technique; and (3) W/O/W double emulsion technique. Modified from CORTESI R, ESPOSITO E, LUCA G *et al.*: Production of lipospheres as carriers for bioactive compounds. *Biomaterials* (2002) **23**(11): 2283-2294 [42].

Table 2. Emulsifiers used for solid lipid microparticles manufacturing.

Description	References
Poloxamer 188	[25,27,30]
Poloxamer 407	[20]
Polysorbate 40	[1]
Polysorbate 80	[26,37]
Sorbitane monopalmitate	[1]
Sodium dodecyl sulphate	[1,25,29,30]
Polyvinyl alcohol	[10,25,29]
Soya lecithin	[1,21]
Egg phosphatidyl choline	[34]

2.2 Preparation techniques

Studies have shown that simply mixing the ingredients is not sufficient to ensure controlled-release SLMs formulation [40,41]. Drug release cannot be prolonged, based on a solid matrix where drug and lipids are just physically mixed. The production technique must allow the drug to dissolve or to disperse into lipids.

2.2.1 Solvent evaporation method

A similar method is widely used for the preparation of polymer microparticles. The classical solvent evaporation method regularly used is described in **Figure 1**. Lipids are first dissolved in an organic solvent (most often chlorinated solvents) and are then emulsified in an aqueous phase containing an emulsifying agent. The resulting O/W emulsion is finally stirred for several hours under ambient conditions in order to allow the solvent evaporation [42]. A modified solvent evaporation method has also been widely described [10,31,33]. In this technique the lipids are also first dissolved in an organic solvent. By mixing, the drug is then incorporated into the organic phase either as a solid (S/O/W) which has been first grinded in a mortar in the presence of liquid nitrogen, or dissolved in an aqueous solution (W/O/W). The obtained preparation is then emulsified into an aqueous surfactant solution. The emulsion is poured into an ice-cooled aqueous phase and stirred. Obtained

microparticles are filtered, rinsed with water and dried in a dessicator.

2.2.2 O/W melt dispersion technique (for lipophilic drugs) [10,27,29,31,34,37,38,42]

This is also called hot melt microencapsulation technique (which can be carried out by normal or phase inversion technique). The drug is dissolved in the melted lipid (the melting temperature depending on the lipid used). The hot mixture is emulsified into an aqueous surfactant solution that is heated above the lipid melting point. The O/W emulsion can then be poured into a larger volume of ice-cooled aqueous phase [10,29,31]. The emulsion, which is obtained by mixing with a high shear device (e.g., Ultra-Turrax® [IKA], or Silverson mixer), is finally allowed to cool either at room temperature or in an ice bath (**Figure 1**).

Hardened microparticles are filtered, rinsed with water and dried in a vacuum dessicator.

2.2.3 W/O melt dispersion technique (for hydrophilic drugs) [21]

This method is a variant of the O/W melt dispersion technique, but it is used for water-soluble drugs. This process does not use water in order to avoid excessive drug solubility into the external aqueous phase and thereby low drug loading in microparticles. First, the drug is dispersed into the melted lipid together with the surfactant. A hot nonaqueous continuous phase (e.g., silicone oil) is poured into the molten lipid phase. The obtained dispersion is then rapidly cooled through cold oil addition and immersion in an ice bath. Solidified microparticles are separated from oil by centrifugation and are finally washed and dried.

2.2.4 W/O/W multiple emulsion technique for water-soluble drugs

A heated aqueous drug solution is emulsified into the melted lipid. The obtained primary W/O emulsion is put into an external aqueous phase and stirred so as to get a W/O/W emulsion. The latter is then cooled either in an ice bath [30] or at room temperature under stirring [42] (**Figure 1**). Hardened microparticles are filtered, rinsed with water and finally dried in a vacuum dessicator.

2.2.5 High-pressure homogenisation

The homogeniser can reduce particle size to the micro- or even the nanometre range of size depending on composition and process parameters.

2.2.5.1 Hot homogenisation

A pre-emulsion is obtained by mixing a hot aqueous surfactant solution with the drug-loaded lipid melt, using a high shear device. The high-pressure homogeniser is preheated at a temperature above the lipid melting point [25,26,43]. The pre-emulsion is put through the homogeniser once or several times. Formulations are then allowed to cool at room temperature.

2.2.5.2 Cold homogenisation [26,43]

The drug is dissolved into the melted lipid. After solidification, the mixture is milled in liquid nitrogen or dry ice with the help of a mortar mill. Grinded particles are then dispersed into an aqueous surfactant solution heated at 5 – 10°C below the lipid melting point. Particles can be disrupted by putting them through the homogeniser once or several times.

2.2.6 Microchannel emulsification technique [44,45]

This technique is considered to be a novel method used to prepare monodisperse O/W and W/O emulsions without high mechanical stress and at lower energy input compared with conventional emulsification processes.

A silicon microchannel (MC) plate, which is fabricated by micromachining technology, is used, and droplets are produced by forcing the dispersed phase into the continuous phase through the MCs. The droplet size is precisely regulated by the structure of the MCs. This manufacturing technique yields monodispersed droplets. A SLM suspension is obtained after cooling the emulsion at room teperature.

2.2.7 Cryogenic micronisation

Lipid matrices, obtained either by melt dispersion (the drug is added to the molten lipid under magnetic stirring, the melting temperature depending on the lipid used) or solvent stripping (the drug and lipid are dissolved into a solvent mixture under stirring, e.g., benzyl alcohol/ethanol [24]), are stored at -80°C and then micronised in a customised apparatus supplying liquid nitrogen during the process. Obtained powders are finally sieved in an automatic sieving apparatus. This technique can be used for particles of ± 5 – 5000 µm in diameter according to the chosen sieves.

2.2.8 Spray congealing (also called spray chilling) [20-23,28,36,41,46].

Lipophilic material is heated to a temperature above its melting point. The drug is then dissolved into the melt. The hot mixture is atomised with a pneumatic nozzle into a vessel that is stored in a carbon dioxide ice bath. Obtained particles are finally vacuum dried at room temperature for several hours [20,28,41].

In the first variant of this technique, the melted mixture is atomised by ultrasound energy into small droplets that fall freely and solidify by cooling at room temperature [21,23,36].

Another variant of the spray chilling method, using a rotating disc, has also been described [22]. With this method the melted mixture is dropped onto a high-speed rotating disc. The rotation causes the molten mixture to spread and spray from the disc periphery onto a chilled surface from which microparticles are collected (**Figure 2**).

2.2.9 Spray drying [28,39,46]

Lipids and the lipophilic drug are dissolved simultaneously into an organic solvent. The mixture is then spray dried in order to get solid lipid particles (**Figure 3**).

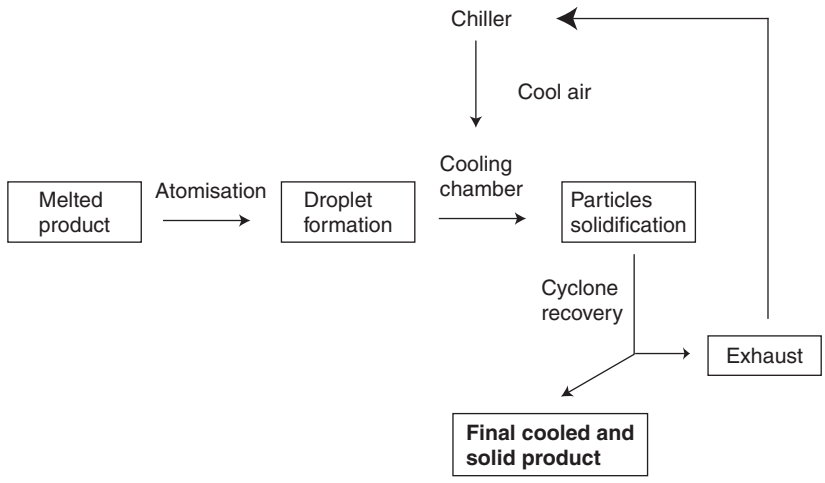

Figure 2. Schematic representation of the spray congealing process.

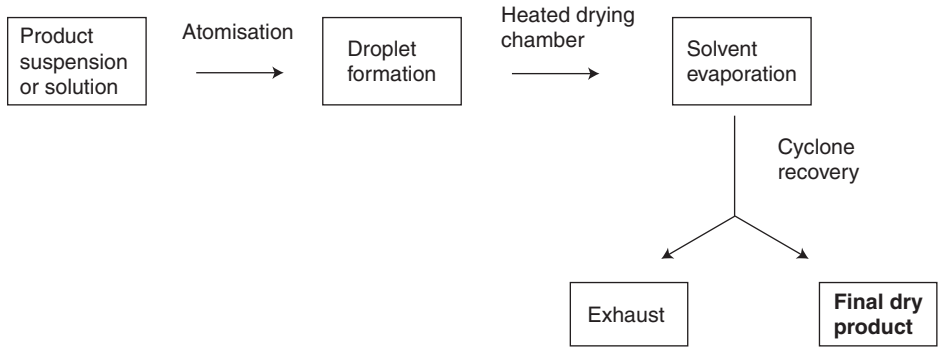

Figure 3. Schematic representation of the spray drying process.

3. Solid lipid microparticles characterisation

3.1 Determination of particle size distribution

3.1.1 Laser diffractometry

Laser diffractometry (LD) size analysis is based on the principle that particles of a given size diffract light through a given angle, which increases with decreasing particle size. Two different diffraction theories can be used (Mye and Fraunhofer) to determine the size distribution from the light intensity reaching the detectors. However, it is important to notice that the LD technique does not measure particle size in the strict sense, but rather calculates size from light scattering effects.

The laser diffraction technique has the advantage of covering a broad size range (from the nanometre to the lower millimetre range [15,47]) while being usable with wet as well as dry samples. This way, LD seems to be one of the most convenient techniques for SLM size determination: submicronic particles as well as aggregates can be identified in microparticles populations.

The results can be expressed in terms of standard percentiles D(v, 0.9), D(v, 0.5) (= mass median diameter) and D (v, 0.1), which correspond to size values below which 90, 50 or 10% of sample particles lie. The span value is the measurement of size distribution width and is calculated as follows [24,48]:

$$Span = [D(v, 0.9) - D(v, 0.1)] / D(v, 0.5)$$

3.1.2 Electrical zone sensing method

The electrical zone sensing method, also called electroresistance particle counting method (with 'Coulter counter multisizer' or 'Elzones' instruments), is based on the principle that when a particle suspended in a conducting liquid gets through a small orifice, on either side of which are electrodes, a change in electric resistance occurs. A known suspension volume is actually drawn through an orifice, which is the only conducting path between two electrodes. The resistance between those electrodes is monitored. When a particle gets through the orifice, a pulse increase in resistance appears. The increase in resistance is proportional to the particle volume. As a result, the distribution of pulse magnitudes can be used as a measurement of particle volume distribution [48-50].

3.1.3 Scanning electron microscopy and optical microscopy [48,50]

Both techniques are used to determine particle size, particle shape and surface characteristics simultaneously. The main disadvantage of such techniques is that they can only examine

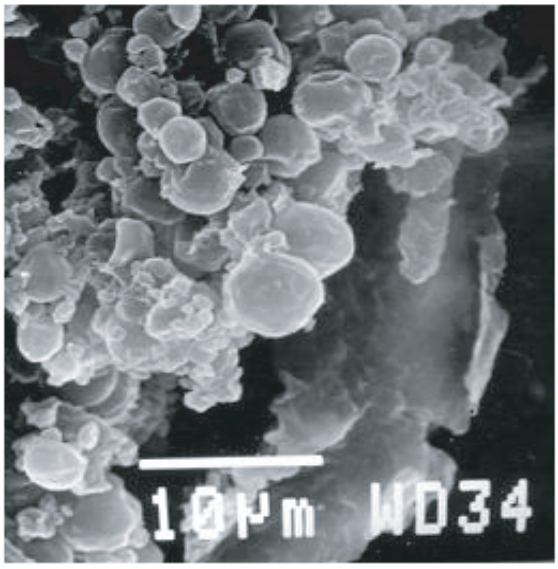

Figure 4. Scanning electron microscopy photomicrographs of solid lipid microparticles. Taken from SANNA V, KIRSCHVINK N, GUSTIN P *et al.*: Preparation and *in vivo* toxicity study of solid lipid microparticles as carrier for pulmonary administration. *AAPS PharmSciTech* (2004) **5**(2): Article 27 [27], with permission.

a rather small number of particles. Indeed, the number of particles that need to be counted (300 – 500) to obtain a good distribution estimate causes the method to be slow and tedious. In addition, the diameter is obtained from only two particle dimensions (i.e., length and breadth). No estimation of particle thickness is available.

We generally tend to consider that optical microscopy makes it possible to measure particles of 1 – 100 µm in size. Electron microscopy can measure particles of 0.01 – 1 µm.

Optical microscopy seems to be sufficient to determine SLM size if distribution is monodispersed. However, SLM populations often contain some submicronic particles that can only be detected by using scanning electron microscopy (**Figure** 4).

3.1.4 Sieving analysis

This method uses a series of standard sieves in a range of standard diameters. A given powder mass is placed on the first sieve (with the broadest mesh) in a mechanical shaker. The powder is shaken for a given period of time and the material that gets through one sieve and is retained on the next, finer sieve, is collected and weighed [50,51]. Sieving is a straightforward technique able to produce a separated size fraction for possible further studies. This technique should be avoided for particles < 40 µm, fragile particles, irregular particles such as elongated needles, particles sticking to the sieves or forming clumps, and particles electrostatically charged [48].

3.1.5 Image analysis system

The image analysis system is a new technology developed to determine and analyse particle size (0.7 – 2000 µm) and shape. This technology can be seen as a kind of automated microscope: combining the precision and sensitivity of an ordinary microscope with the statistical significance of the number of analysed particles – this being carried out either in real time [101] or within a few minutes [52]. Its ability to analyse particle shape provides users with high-quality, helpful information to characterise materials completely (emulsions, suspensions or powders) [52]. As a result, the image analysis system can be used in order to better understand material behaviour (e.g., powder flowability). Morphological parameters determined by the software include sieving diameter [101], mean diameter, convexity, roundness and elongation, among others.

This technology is bound to become increasingly popular, although the apparatus still remains rather expensive [48].

3.1.6 Determination of aerodynamic size distribution [48,53,54]

Aerodynamic size analysis only concerns the inhalation field. The aerodynamic diameter of particles or droplets is actually the most important parameter influencing aerosol deposition. This parameter is defined as the diameter of a unit-density sphere with the same settling velocity, generally in air, as the particle. This includes particle shape, density and physical size, all of which influence the particle aerodynamic diameter [53]. The determination of aerodynamic size distribution is useful to determine the respirable fraction [54]. Such determination is generally carried out with a cascade impactor. The principle on which these impactors operate is based on the aerodynamic behaviour of aerosol particles. They use the principle of inertial separation to size particulate samples from the gas stream. The impactor usually has several stages for particle size determination, each of which gives a cut off point based on the particle aerodynamic diameter.

3.2 Determination of solid lipid microparticle morphology

The general morphology of SLMs is most often determined by microscopy (scanning electron microscopy or optical microscopy, see Section 3.1.3), but can also be studied by using new image analysis technology (see Section 3.1.5). The shape of SLMs can be significantly different from a spherical shape.

The surface characteristics of SLMs (smooth or rough, regular or not) can be visualised by microscopy. Their surface morphology has proved to vary depending on the excipients used [41].

X-ray photoelectron spectroscopy (XPS), also known as electron spectroscopy for chemical analysis (ESCA), is a high-resolution technique for the elemental analysis of solid materials surfaces. Consequently, XPS can determine the atomic composition of the particles surface. XPS is based on the emission of electrons from materials in response to photon

irradiation, with sufficient energy to cause the core level electron ionisation. These electrons are emitted at energies characterising the atoms from which they are emitted. In view of the fact that photons have a low penetration energy, only electrons belonging to surface atoms or just underneath surface atoms (up to 100 Å) escape and are counted.

This technology is used to gather information on drug distribution in microparticles; in particular, to know if the drug is present on the surface of particles or really entrapped within them. Surprisingly, XPS is still rarely used in the microencapsulation field [21,31,55,56]. This technique can be used when the compound to be localised contains atoms that can emit electrons after photon irradiation and are not present in carrier materials (e.g., Cl, N).

3.3 Solid-state analysis of solid lipid microparticles

This characterisation step is necessary in order to detect possible modifications in the physicochemical properties of the drug incorporated into SLMs and of the lipophilic excipients. It has been proved that although particles were produced from crystalline raw materials, the presence of emulsifiers, the preparation method and the high-shear dispersion may account for changes in the crystallinity of matrix constituents compared with bulk materials. This may lead to liquid, amorphous or only partially crystallised metastable systems [57,58].

It has also been proved that with lipid drug delivery systems polymorphic transformations may occur during dosage form preparation and subsequent storage. Indeed, during the melt solidification, triglycerides and fatty acids in particular can crystallise into different polymorphic forms (i.e., the thermodynamically instable α-form, the β′-form, the stable β-form) depending on lipid composition and cooling rates. Polymorphic transformations may cause changes in active and auxiliary substances solubilities and melting points. In particular, the conversion of one polymorph into another may change the physical properties of the substance [10,28,59].

Indeed, considering that the degree of lipid crystallinity and the possible modifications in the lipid's solid state are correlated with drug incorporation and release rates, and considering that the drug's solid-state form (amorphous or crystalline) in solid dispersions influences dissolution rates, it is important to pay special attention to these parameters [1,41].

The solid states of bulk materials, as well as solid states obtained from solid dosage forms (SLMs), are generally analysed by means of the following different techniques:

3.3.1 *Differential scanning calorimetry*

Differential scanning calorimetry (DSC) is one of the most widely used techniques to study solid state, and especially to determine compound purity, stability and polymorphism. This technique relies on the principle that solid-state modifications are characterised by different melting points and melting enthalpies [1]. DSC indeed measures transition temperatures (solidification and melting temperatures, glass transition temperature, thermal degradation temperature) as well as transition enthalpies [60].

3.3.2 *X-ray diffraction*

X-ray diffraction is based on the principle that X-rays are diffracted by crystals, considering that their wavelengths have about the same magnitude as the distance between crystal atoms or molecules. This technique makes it possible to investigate a crystal structure [61] to assess the compound possible amorphisation, elucidate some polymorphic transformations and study interactions between active substances and microparticle excipients [36].

3.3.3 *Hot stage microscopy*

Hot stage microscopy (HSM) is an analytical technique that combines the best properties of microscopy and thermal analysis in order to carry out characterisation of the physical properties of the material as a function of temperature. Combined with high-resolution cameras and image manipulation software, this technique is often used to confirm the transitions observed with other techniques [62]. The solid states of bulk drugs (lipophilic excipients and active substances) as well as the solid state of obtained SLMs can be characterised by this technique. The main advantages of HSM are the possibility to identify which particles (characterised by their shapes and sizes) are first concerned by state transition, and the possibility to distinguish between the excipient's behaviour and the drug.

3.3.4 *Fourier transform raman spectroscopy and infrared spectroscopy*

Fourier transform raman spectroscopy and infrared spectroscopy are useful tools for investigating the structural properties of lipids [1]. These techniques have proved to be highly sensitive to structural differences in a molecule's functional groups that can take place during crystallisation or polymorphic transformations [63]. As a result, they can be used in the field of SLMs to study the solid-states of bulk materials or solid dosage forms, and in particular to detect interactions between active substances and lipophilic excipients in molten samples [20,41].

3.4 Drug loading determination

A few drugs or peptides with various lipophilicity degrees have been incorporated into SLMs; for example, carbamazepine [36], theophylline [22,23,29], fenbufen [23], hydrocortisone [29], ibuprofen [29], ketoprofen [29], indomethacin [29], pseudoephedrine HCl [30], fluorouracil [35,38], ftorafur [38], insulin [31,33], thymocartine [31], gonadotropin release hormone [24], DNA [32], piribedil [26,64], medroxyprogesteron acetate [28], estradiol 17-β cypionate [28], somatostatin [10], verapamil HCl [21] and felodipine [20,41].

The determination of drug loading (or drug incorporation) is an important tool to evaluate a potential drug carrier system. It is obviously desirable to produce microparticles with high drug content in order to decrease the amount of

microparticles to be administered, whatever the administration route.

Drug incorporation into microparticles can be expressed in terms of theoretical drug loading, real drug loading or entrapment efficacy:

- Theoretical drug loading is expressed in a percentage related to the lipidic phase (lipidic matrix + drug). Theoretical drug loading (%) = (drug weight x 100)/(drug weight + excipient weight).
- (Real) drug loading or drug content is expressed as a percentage related to the lipidic phase (lipidic matrix + drug). Drug loading (%) = (real amount of drug, assayed in SLM sample x 100)/total weight of SLM sample.
- Encapsulation efficiency (or entrapment efficacy or loading efficiency) (EE) is calculated as a percentage related to the total amount of drug initially used. EE (%) = (amount of drug incorporated x 100)/amount of drug initially used.

The drug loading and EE can be influenced by a large number of factors. The most often quoted parameters are the following:

- The drug solubility in melted lipids should be high enough to obtain a sufficient drug loading [29] and thereby a relatively higher EE.
- The chemical and physical state of the solid lipid matrix and of the drug to be incorporated have an influence on EE.
- The choice of the preparation method can also influence the EE of the drug into SLMs. For example, the melt dispersion technique generally gives higher encapsulation efficiency than does the solvent evaporation technique [10,31], whereas the cold homogenisation technique generally gives higher drug loading than hot homogenisation [26].
- The way the drug is initially dispersed into the lipid at the initial stage of the preparation (i.e., in the solid state or as a solution) can also be considered as a relevant factor influencing EE [10].
- Increasing SLM's particle size generally leads to a higher drug loading. This parameter has been studied by determining and comparing drug loadings of SLM in different size fractions of the sample [23,36]. It has also been noticed that some of the smallest particles are formed by pure excipients only (empty spheres) [23].
- The theoretical initial drug loading influences encapsulation efficiency, which generally decreases when the theoretical loading increases [10,31,33]. In this case, it is important to use relatively high theoretical drug loading in order to get sufficient drug content, but the theoretical drug loading must also be limited to avoid a decrease in encapsulation efficiency and a resulting waste of drug. Some research has reached contrasting conclusions which could be accounted for by poor water solubility of the drug, and therefore by a smaller relative drug loss with increasing theoretical loading [29].
- In some special cases, the external aqueous phase pH can influence SLM's drug loading; for example, if the drug is hydrophilic (e.g., when the drug is a peptide, such as insulin [31,33]), the external aqueous phase pH can be adjusted in order to decrease drug aqueous solubility and thereby enhance drug-loading efficiency.

Because of all these parameters can influence encapsulation efficiency, various formulations and processes have to be studied in order to maximise drug content in microparticles. Drug content optimisation can be achieved with the help of experimental design methodology, which makes it possible to study several parameters simultaneously on one or many chosen responses; for example, drug content [65,66]. It is also important to notice that drug loading might lead to some changes in SLM size distribution [35].

Drug loading and encapsulation efficiency are generally determined as follows. SLMs are first isolated from the aqueous phase. The aqueous SLM suspension is either filtered or centrifuged, or even ultrafiltered (for the smallest microparticles), to separate SLMs from the aqueous phase. Particles are then rinsed with water in order to eliminate the drug crystals that are not incorporated into SLMs. Finally, obtained particles are dried. SLMs are then either dissolved into an appropriate solvent or heated with a suitable aqueous solvent in which the drug is soluble and shaken in order to extract the drug in the solvent. The drug assay is carried out on the obtained solution: generally by means of a spectrophotometrical technique.

The preparation of SLMs by spray congealing or spray drying does not use water, which makes it possible to avoid the separation step between SLMs and the aqueous phase.

As described in Section 3.2 of this review, XPS analysis can give further information about the encapsulation of drugs into SLMs. This technique is used to localise the drug inside the particle, and thereby to know whether the drug is present on the surface of the particles or really entrapped within them.

4. *In vitro* drug release study

As described in Section 1, SLMs are mainly used to ensure the incorporated drug release is controlled. Therefore, a drug release study has to be carried out on obtained SLMs.

Drug release profiles are determined by an *in vitro* dissolution test. This test is generally carried out according to the Pharmacopeia (USP or European Pharmacopeia) guidelines; for example, by using a basket or paddle stirring apparatus. The dissolution medium is chosen depending on the intended administration route. The sample can be put either into a cell with two chambers (one chamber contains the sample, the other chamber is the acceptor compartment) separated by a stainless steel sieve plate (with pores of a chosen diameter) [31,33], or into a dialysis tubing device [34]. In order to improve the wettability of microparticles, a surfactant is generally

added to the dissolution medium [22,38]. Drug release is finally assayed spectrophotometrically. In the special case of topical administration, the drug release study was completed with the help of the Franz diffusion cell technique [37].

It has also been noticed that a 24-h time interval is considered sufficient to study the sustained-release potential of drug carrier systems (i.e., SLMs) [24]. Release profiles can be further studied by determination of two statistical moments: i) the *in vitro* mean dissolution time ($MDT_{in\ vitro}$); and ii) the variance in dissolution time ($VR_{in\ vitro}$); and an associated statistical parameter, the concentration–time profile relative dispersion (RD). The way to calculate these parameters has been described by Passerini *et al.* [21]. The statistical moment approach has the advantage of allowing the dissolution curve to be separated into stages and, therefore, to check for modifications in the release mechanism during the dissolution test [21].

Drug release is expected to be affected by several parameters. First, the dissolution medium [26,41,67] and the dissolution method [68] must be both correctly chosen in order to get a correct prediction of the *in vivo* drug release from microparticles [24]. The choice of the dissolution medium and method can actually affect the *in vitro* drug release study results and, therefore, their correlation with the *in vivo* drug release study. The nature (hydrophobicity) of the excipient is considered to be the most important parameter influencing drug release. More hydrophobic materials are expected to reduce the drug release rate [22-24,41]. The choice of matrix materials influences the release process rate. Another way to change the matrix hydrophobicity is by adding a hydrophobic or hydrophilic excipient [20,22,41].

The drug's physicochemical characteristics (its water solubility) also play a part [23]. The release rate and the amount of drug released from SLMs increase with drug hydrophilicity. The preparation method of the SLMs can affect the drug's release rate by influencing the matrix wettability properties [24].

The particle size is also considered as a relevant parameter influencing drug release. Drug release from smaller particles is higher than release from larger ones because of the larger specific surface area of smaller microparticles [22,41].

A faster release is obtained with higher drug and/or adjuvant content (e.g., lactose) in SLMs because matrix diffusion is easier due to an increase in the number of pores created during the release process [22,30,35,41].

The drug release increases when the medium agitation rate in the dissolution apparatus increases [35].

Storage can induce polymorphic changes in SLMs and thereby modify the drug release rate [41].

Consequently, a suitable choice of SLM formulation (in terms of excipient nature, drug nature and drug loading) can bring about the intended *in vitro* release profiles (e.g., sustained release [24], enhanced release [36]).

If obtained SLMs are not rinsed after separation from the aqueous phase, the dissolution profile shows a rapid release from the external drug fraction towards the dissolution medium, followed by a phase of decrease in the release rate [26,31,33].

At the end of the release study, some of the drug may remain enclosed in the particles [33], in particular if the drug is adsorbed into the lipid matrix material [31].

5. Administration routes, *in vivo* drug release and biocompatibility studies

Despite their high potential as promising drug carrier systems, SLMs have been rather unexploited. So far, only a few complete studies on SLMs have been published. Consequently, little data are currently available on SLM *in vivo* administration, drug release and biocompatibility.

Nevertheless, as assumed for SLNs [1], it could be anticipated that SLMs are well tolerated in living systems because they are made of physiological compounds. On the other hand, the toxicity of the surfactants has to be considered, although not more than other drug carrier systems. No problems should occur, provided an approved surfactant is chosen.

This section presents an overview of tested SLM administration routes and corresponding *in vivo* drug release and biocompatibility studies carried out so far.

5.1 Peroral administration

The peroral route is the most often cited SLM administration route in the literature [20-23,26,38,41]. It includes aqueous SLM dispersion, SLM tablets, pellets or capsules. However, data on *in vivo* drug release and biocompatibility studies are most often missing. Demirel [26] has nevertheless perorally administered SLM suspensions to rabbits; such suspensions were composed of Compritol® 888 ATO (Gattefosse) and Labrasol® (Gattefosse) as a lipidic matrix, Tween® 80 (ICI America) as a surfactant and piribedil as the active substance. The bioavailibility of piribedil-SLMs was found to be higher than with pure piribedil.

Considering that SLM lipidic matrices are composed of physiological lipids and that most surfactants have already been used perorally, the authors cast no doubt on the biocompatibility SLMs after oral administration.

5.2 Parenteral administration

SLMs could also be parenterally administered apart from the intravenous route, owing to particle micronic size (in opposition to SLNs, which are often used for the intravenous administration). Some studies have been carried out on the *in vivo* drug release and biocompatibility of SLMs.

Reithmeier [10,31] has tested the biocompatibility of SLMs composed of a glyceryl tripalmitate (Dynasan® 116, Hüls AG) lipidic matrix and polyvinyl alcohol as a surfactant by implanting SLMs subcutaneously in mice. Polymeric microparticles composed of poly(D,L-lactide-co-glycolide), a well known approved polymer often used for parenteral applications, were also implanted and used as a reference. The study showed only a slight inflammation reaction in the implantation area, but

that was the case for both SLMs and polymeric microparticles. It has been concluded that studied SLMs showed comparable biocompatibility to polymeric microparticles that have been approved and used for parenteral administration.

Del Curto [24] has produced SLMs composed of glyceryl monobehenate (Compritol® E ATO, Gattefossé) and containing gonadotropin release hormone (antide) by co-melting process. After subcutaneous injection in rats, studied SLMs proved to give the incorporated active substance a sustained-release profile. Therefore, Antide-SLMs are potentially useful as a depot formulation when prolonged action is required.

5.3 Topical administration

SLM topical applications have been seldom used. However, Yener [37] has studied SLMs prepared with beeswax as matrix material, polysorbate 80 (Tween® 80) as a surfactant and containing a UV absorber (octyl methoxy cinnamate, OMC). Obtained SLMs were put into topical vehicles (oleaginous cream, carbopol gel and o/w emulsion). OMC release from SLMs and the OMC penetration rate and amount were tested through application on excised rat skin. Obtained results were those expected: a decrease in OMC release rate and amount (and therefore sustained action compared with free OMC action), and a decrease in the penetration rate and amount.

5.4 Pulmonary administration

SLMs can be considered as a promising drug carrier system for pulmonary administration even if they have been rather unexploited so far [65,66]. However, a preliminary *in vivo* tolerance study has been carried out with rats with SLMs composed of glyceryl behenate (Compritol 888 ATO) as a lipidic matrix and poloxamer 188 (Lutrol® F68, BASF) as a surfactant. SLM dispersions in phosphate buffer saline were administered intratracheally. Bronchoalveolar lavages were performed on the anaesthetised rats. Total and differential cell counts (i.e., inflammatory cells) were then done with the collected bronchoalveolar liquids. Results did not show significant differences between placebo groups and SLM-treated rats. It has been concluded that the studied SLMs seem to be well tolerated by the lower airways, but tolerance must still be assessed after repeated administrations [27].

6. *In vivo* fate

The *in vivo* fate of SLMs has not been studied thoroughly so far. However, in view of their similar composition, SLMs are expected to behave in the same way as SLNs *in vivo*. Consequently, the *in vivo* fate of SLMs should depend on administration routes and especially on enzymatic processes. Because SLM lipidic matrices are composed of physiological lipids, they should be exposed to metabolisation *in vivo*. Lipases should then be the most involved enzymes in the degradation of SLMs. This kind of enzyme, which is present in various organs and tissues (notably in the gastrointestinal tract, at the subcutaneous or intramuscular injection sites), works by splitting the ester linkage and thereby forming partial glycerides or glycerol and fatty acids. Olbrich notably proved that SLNs composed of glyceryltrimyristate glyceryltripalmitate, glyceryltristearate and cetylpalmitate, are decomposed by enzymes such as lipases, and that such degradation is influenced by several parameters (i.e., surfactant composition and storage time) [10,69,70]. These conclusions could reasonably be extrapolated to SLMs, but should be confirmed by experimentation.

7. Expert opinion and conclusion

SLMs can reasonably be considered as a promising drug carrier system even if they have not been widely investigated. Actually, SLMs present several advantages: a physiological composition and thereby a supposed limited toxicity; a possibility of producing SLMs on a fairly large industrial scale; and the relative low cost of their raw materials and production processes. Some drawbacks must, however, be taken into account. For instance, the drug to be incorporated into SLMs must preferably be lipophilic enough in order to achieve a high entrapment efficiency. The latter is also affected by several other parameters such as the preparation method, the chemical and physical state of the drug and excipients, and the size of particles obtained. In practice it appears that one of the main difficulties in using SLMs is to optimise formulation parameters (excipients and drug nature, initial theoretical drug loading etc.) and production techniques in order to obtain SLMs that have simultaneously a high entrapment efficiency, a high drug loading, the intended size according to the desired administration route and presenting the desired drug release profile.

Most research articles present a complete characterisation of SLMs, including size and shape determinations, solid-state analysis and drug loading capacity studies, although such a characterisation requires several analytical methods and is generally slow and tedious.

The *in vitro* drug release studies that have been up to now carried out tend to prove the ability of SLMs to provide a controlled release of the incorporated substances. Nevertheless it must be taken into account that the dissolution medium and the dissolution method are both critical parameters, which must be suitably chosen in order to get a good correlation between the *in vitro* and *in vivo* drug release studies. The main difficulty in studying the rate of drug release from a carrier lies in mimicking as close as possible the expected *in vivo* conditions. Especially in the case of SLMs, the presence of enzymes such as lipases exerts an important influence on drug release, but this parameter is difficult to mimic in the *in vitro* dissolution tests. It is also interesting to notice that owing to the lipidic nature of SLMs the drug release studies require the use of surfactant in the dissolution medium in order to improve the microparticle's wettability. However, it is regrettable that the eventual influence of the addition of a surfactant on the *in vitro* drug release rates has up to now never been

studied. This is why the promising drug release results obtained by *in vitro* experimentation must be confirmed by *in vivo* studies.

Concerning their application fields and administration routes, SLMs have until now been most often studied for peroral administration. So far, only a few applications of subcutaneous, topical and pulmonary administrations have been considered and should thereby be further investigated.

To sum up, given their numerous advantages and interesting *in vitro* drug release results, SLMs can be considered as a promising drug carrier system, which could be used by different administration routes (i.e., peroral, parenteral, topical and pulmonary routes). However, drug release results obtained by *in vitro* experimentation and suggesting an ability for SLMs to provide a controlled release to the incorporated substances must be confirmed by *in vivo* studies.

In the same way, the biocompatibility and *in vivo* fate of SLMs are in most research works disregarded, and should be considered and assessed even if the physiologically used materials tend to suggest that SLMs are biocompatible.

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Affiliation

Dr Séverine Jaspart†, Dr Géraldine Piel, Dr Luc Delattre & Dr Brigitte Evrard
†Author for correspondence
University of Liège, Laboratory of Pharmaceutical Technology, Tour 4, étage 2,
Avenue de l'hôpital 1, 4000 Liège, Belgium
Tel: +32 4 3664306; Fax: +32 4 3664302;
E-mail: sjaspart@ulg.ac.be