Solid lipid microparticles as an approach to drug delivery

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Abstract

Introduction: Solid lipid particles have been introduced since the early 1990s as an alternative drug carrier system to emulsions, liposomes and polymeric microparticles. While lipid nanoparticles have been the object of a substantial number of reviews, fewer are available on lipid microparticles despite their distinct advantages, including biocompatibility, ease of production and characterization, extended release properties and high loading.

Area covered : This review presents an overview of the advantages and drawbacks of lipid microparticles (LMs), i.e. lipid-based particles with dimensions in the micrometer range. Specific focus is on the role of the main excipients used for LMs formulations, lipids and surfactants, and their effect on LM properties. Furthermore, an update on preparation techniques and characterization methods are also presented, with particular emphasis on more recent technologies. The interaction of LMs with biological systems and in particular will cells is reviewed. Moreover, the various LM administration routes are examined, with special attention to most recent applications (i.e., pulmonary and nasal delivery).

Expert opinion: LMs represents an attractive and versatile carrier system, however their pharmaceutical applicability has been rather limited. Investigation on the use of LMs for less established administration routes, such as pulmonary delivery, may provide further interest within the area of LM-based systems, both in industry and in the clinic.

Keywords: Lipid microparticles, preparation methods, characterization, interaction with biological systems, administration routes

1. Introduction

Particulate delivery systems have gained great interest for the modulation and targeting of active ingredient release in the pharmaceutical, cosmetic and food fields [1,2]. The *in vivo* efficacy of therapeutic agents is influenced not only by the drug activity itself, but also by the excipient system used for its formulation which, ideally, should ensure a modulation in drug release according to therapeutic requirements. The controlled release of a drug has several advantages including enhanced therapeutic efficiency and extended duration of action, reducing dosing frequency and improving patient compliance by facilitating therapy

management. In addition, a lower amount of drug is required and its accumulation in non-target tissue is minimized thus decreasing the adverse side-effects.

One of the major drug delivery particle systems is based on lipid particles [3-5]. These lipid-based carriers consist of a hydrophobic core, solid at ambient and body temperature, stabilized by a layer of surfactant embedded in their surface [1,3,6]. The active compound is dissolved or dispersed in the solid lipid matrix. Lipid particles are derived from conventional oil-in-water (o/w) emulsions by replacing the liquid oil by a solid (at room temperature) lipid component [1,4]. They are based mainly on physiologically compatible and biodegradable constituents, providing good *in vivo* tolerability and optimal biodegradability [1,3,7]. Most of the excipients used are GRAS (generally recognized as safe) [7-9]. However, it must be emphasize that substances in the micrometer range may exhibit biological properties that are different from their larger counterparts, although the major concerns about particle toxicity are associated to nanoscale materials [2] Additional advantages of lipid particles include: increase bioavailability of sparingly water soluble substances [4,7], modified release of incorporated drug, the possibility of avoiding the use of organic solvents for their preparation, relatively low production cost, feasibility of large scale production and adequate physico-chemical stability [1,3,10,11]. Moreover, their solid matrix protects encapsulated labile substances against degradation [8,12,13]. Lipid particles exhibit high loading capacity for lipophilic substances, their main limitation being the inefficient incorporation of hydrophilic substances [3, 13,14].

Because of the above characteristics, lipid particles overcome most of the drawbacks associated with polymeric particles, such as inefficient biodegradation, polymer accumulation effects, toxicity and the general requirement of organic solvents for their production [1,3,15-17], and therefore represent an alternative carrier system to traditional polymeric micro- and nano-particles.

A distinction is made between micro- and nano-lipid particles, which generally refer to particles with dimensions measured in the micrometer- and nanometer-size range, respectively [3]. However, currently there is no world-wide accepted definition of nanoparticles. This has a profound effect on the interpretation of data that can be found in literature [18]. For the purpose of this review and according to the metric system, the terms microparticles and nanoparticles will refer to particles with dimensions in the micrometer and submicrometer or nanometer range, respectively [19-21].

While lipid nanoparticles (LNs) are currently attracting a great deal of attention in the research community due to their relevant size dependent properties, lipid microparticles (LMs) have been less studied. Consequently, while a substantial number of reviews have been devoted to LNs [1,4,6-8,10,22-24], fewer are available on the use of LMs [3, 20].

Lipid microparticles have received less attention because their applicability is more limited than the submicron particles. However, LMs do exhibit some interesting advantages compared to LNs, including simpler production and characterization methods, superior extended release properties due to their increased size [3,6,25] and a higher loading capability, which reduces the amount of microparticles to be administered [3, 26]. Moreover, for specific administration routes (e.g., nasal and pulmonary) or applications (e.g., skin delivery), their size is the most appropriate. In addition, due to their micron dimensions, major toxicological concerns related to nanoparticles and their ability to cross biological membranes, are not a concern [2]. Due to the lower specific surface area compared to LNs, a reduced amount of potentially toxic surfactants is required for LMs stabilization. For the same reason, chemical and physical stability is higher for LMs than LNs. For instance, water can be removed from LMs by freeze-drying without altering the particle characteristics, whereas cryo-protectors are necessary to decrease LNs aggregation during lyophilization [1,24].

Several aspects of LMs have been discussed in detail in an excellent review by Jaspart et al. [3]. This review article gives an update on several aspects of LMs including preparation techniques, physicochemical characterisation and *in vitro* evaluation methods. Additionally, it covers the strategy for the optimization of the different parameters influencing the formulation of the LMs. Moreover, the dynamic of the interaction of the LMs with cellular structures were explored, this aspect being often overlooked [27].

2. LM components

The essential components of LM systems are lipids and surfactants. The lipids forming the hydrophobic core of the LMs are solid at room and body temperature (melting point > ~ 45 °C) and includes different compound classes, namely triacylglyceride (e.g., tristearin, tripalmitin), partial glycerides and their mixtures (e.g., mono-, di- and tri-esters of glycerol and behenic acid), fatty acids (e.g., stearic acid), fatty alcohol (e.g., cetyl alcohol), steroids (e.g., cholesterol), non-glyceride esters of saturated fatty acids with saturated fatty alcohols (e.g., cetylpalmitate) and waxes (complex mixtures containing mainly esters of fatty acids and hydroxy acids with monohydric fatty alcohols, free fatty acids, and hydrocarbons) (see Table 1) [1,3].

Different types of surfactants (mainly anionic, non-ionic and amphoteric surfactants but also cationic surfactants) have been used for the preparation of LMs (Table 2), however the physiologically compatible substances, such as phosphatidylcholine and bile salts (sodium cholate, sodium taurocholate, sodium glycocholate), are generally preferred as stabilizers [1,3,7,14].

3. Scientific approach for the design of lipid microparticles

A wide variety of formulation components can be used to prepare LMs. Since the choice of the excipients will affects the properties of the delivery system, the selection of suitable lipid carrier material and stabilizer for a specific active ingredient is of paramount importance. Several studies have been carried out for the optimization of the LM in vitro performance [14,21,26,28]. Generally, these investigations are based on trial-and-error experiments but this approach is time-consuming, since the LM performance is difficult to predict and control.

One of the key factors affecting drug loading capacity in the lipid particles and its release kinetics is its solubility and miscibility in the melted lipid. Consequently, the lipid with the maximum solubilizing potential for the active ingredient is initially selected in order to achieve maximum drug loading [3,7,29]. Solubility parameters for lipid excipients and drugs can be calculated experimentally and used to select the most suitable LM matrix [29]. Alternatively, the visual observation of the amount of drug that can be dissolved in the melted lipid phase was performed by some researchers [21,30]. The selection of the lipid excipient has also been performed by assessing the partition behaviour of the active ingredient in the melted lipid and hot water. On the basis of the measured partition coefficients, the best lipid for the preparation of the LM system is selected [31,32]. The entrapment of hydrophilic drugs in LMs has been shown to be enhanced by using polar lipids, such as cetyl alcohol, mono- and di-glycerides [33-35]. Conversely, higher entrapment efficiency and release modulation have been obtained for lipophilic drugs by tristearin as compared to the polar lipids stearic acid, stearyl alcohol and di-glycerides [28,36].

In order to overcome the drawback of the inefficient incorporation of polar drugs into lipid particles, the synthesis of lipophilic derivatives of the target drug has been proposed [37,38]. Alternatively, Dalpiaz et al. [13] have exploited acid-base interactions between the

drug and the lipid excipient to enhance the entrapment efficiency of a hydrophilic drugs in LMs. For substances with limited solubility in both water and lipid, Malzert-Fréon et al. have shown that the addition of solubility enhancers (Labrasol®) to the lipid matrix improves the loading rates of lipid particles [39].

Another critical parameter for drug incorporation is the lipid crystal structure and its possible modifications during the preparation of the particles [3]. More ordered crystal structures reduce the drug incorporation rates. Conversely, thermodynamically less stable crystal configurations have lower packing density which consequently allow more 'space' to accommodate the active ingredient, giving rise to increased loading capacity [1,8,29]. Therefore, less-ordered crystals, which are more easily formed with lipid mixtures, the amorphous state or multiple systems based on oil-in-solid lipid-in-water dispersion should enhance drug incorporation into lipid particles [8,29]. In addition, polymorphic modifications from unstable to more stable and ordered forms (e.g., higher energy α and β' polymorph conversion to the stable β form) lead to drug expulsion [8], which is minimized for lipids with low polymorphic transition rate (e.g., carnauba wax).

The selection of the type and concentration of surfactant for LM formulations is important for the physical stability of the particle system and it is generally dictated by safety consideration (surfactants of natural origin are preferred) and maximum stability. Higher surfactant/lipid ratios result in a reduction of the particle size, since adequate coverage of the particle surface is ensured, but also increase the risk of toxic side effects [1, 34,40]. All types of surfactants have been studied in the preparation of LMs (see Table 2), however, non-ionic (e.g., Poloxamer, Polysorbate) and amphoteric (phosphatidylcholine) surfactants are the most commonly used. It has been reported that lipid particles prepared with surfactant mixtures exhibit higher stability than formulations with a single surfactant [1,21,41], which is probably due to a synergic effect of surfactants on the interface tension.

In order to select the optimum surfactant concentration, surface tension measurements have been performed by Bose et al. [21]. However, the selection of the surfactant or surfactant mixture for the formulation of LMs has been mainly based on empirical approaches [42,43], since it is difficult to predict the packing density of the surfactant distribution on the particle surface. In addition the kinetic of the redistribution process of surfactant molecules between particle surfaces, monomers and micelles also affects the stability of the LMs systems [1]. However, according to Zhang et al. [29], polymeric surfactants with longer polymer chains, high molecular weight (e.g., Poloxamer 407) and better solubility in the dispersion medium are more suitable for lipid particle stabilization.

Some authors [29,37,44] have described the optimization of the LM properties by computer simulation, based on experimental design methodology. The advantage of this approach is the reduction of the number of trial-and-error experiments, however computational time and expense must be taken into consideration. A starting base formulation is selected in order to identify the key manufacturing variables (e.g., drug loading, surfactant concentration, mixing time), and then the optimal production parameters are calculated, speeding up LM development.

4. LM preparation methods

The production processes of lipid nano- and micro-particles have been described in detail in the literature [1,3,20,45]. This review will focus on the methods specifically used for the preparation of the micron-sized lipid particles, with particular emphasis on more recent technologies. To note, high-pressure homogenization methods will not be reviewed since they are more effective for the production of submicron size dispersion of solid lipid [1].

The main advantages of the production of LMs are the ease of scale-up and the fact that they are generally based on technologies already available to the pharmaceutical industries [3,45]. Limitations include: reproducibility, homogeneity in particle size and shape, optimal drug loading and release.

4.1. Melt emulsification method

For the melt emulsification technique, the solid lipid is melted and the drug (e.g., aceclofenac, progesterone, quercetin, rifampicin, budesonide) dissolved or dispersed into it. This melted phase is subsequently mixed into a pre-heated polar phase (mainly water) containing the surfactant. Generally, the hot aqueous phase is poured into the molten lipid to avoid loss of excipient and drug during the preparation. The mixture is then vigorously homogenised with a high-shear apparatus at a temperature above the lipid melting point and the obtained emulsion cooled at room temperature to form a microparticle suspension (Fig.1). The water-free solid LMs are then recovered by filtration, centrifugation and/or lyophilisation. Solvent removal enhances the stability of the LM preparation and facilitates characterization studies and formulation of the particles into a dosage form. Melt emulsification represents the most commonly used method for the preparation of LMs [26,28,36,42,46,47], the main advantage being the lack of the use organic solvent, although reproducibility can be lower compared to other techniques.

4.2. Solvent emulsification-evaporation method

This technique differentiates from the melt emulsification method since the lipid matrix instead of being melted, is dissolved with the drug (e.g., somatostatin, avobenzone, clozapine) in a water-immiscible organic solvent (e.g., dichloromethane, cyclohexane), which is then emulsified in an aqueous phase containing the surfactant by high-speed

homogenization. Thereafter, the organic solvent is evaporated from the emulsion by mechanical stirring at room temperature or under reduced pressure (rotary evaporator), resulting in the precipitation of the lipid as LMs. The main advantage of this procedure is the limited exposure of thermolabile substances to high temperature [1,3,28,34,45]. However, the use of organic solvents represents a disadvantage for pharmaceutical applications.

4.3. Solvent emulsification-diffusion method

In the solvent emulsification-diffusion technique the organic solvent used is partially water-miscible (butyl lactate, benzyl alcohol, ethyl acetate). The lipid and active ingredient (e.g., insulin) are dissolved in the organic solvent (saturated with water) and emulsified with the external aqueous phase (saturated with the organic solvent), containing the surfactant under high-speed mixing. Water is then added to the obtained emulsion in order to favour the organic solvent diffusion from the droplets to the external phase, producing the solidification of the dissolved lipophilic material [48,49]. The LMs are isolated by centrifugation or filtration. This technique has the advantages of satisfactory reproducibility and reduced exposure to high temperature. The major drawback is low drug entrapment due to its diffusion into the aqueous phase [49].

4.4. Double emulsion (w/o/w) method

This technique is used for polar drugs (e.g., serratiopeptidase, hepatitis B surface antigen, salbutamol) in order to overcome their poor entrapment in the hydrophobic matrix of the LMs. A hot aqueous solution of the hydrophilic drug is added to the molten lipid containing a lipophilic surfactant under mixing. This primary w/o emulsion is then dispersed in a secondary aqueous phase maintained at a temperature above the lipid melting point and

containing a hydrophilic surfactant. The mixture is stirred and the resulting double emulsion cooled at room temperature. The obtained LMs are isolated by centrifugation or freeze-drying [34,42,43,45]. Particles formed from such technique are, by nature, poorly controlled in both size and structure, thus limiting their use [49].

4.5. Sonication method

The dispersion of the emulsions obtained according to one of the methods reported in the previous sections, can be performed by sonication alone or in combination with stirring. Sonication of the sample using an ultrasonic probe leads to a reduction of the particle size to few microns (Fig. 2), suitable for specific administration routes such as pulmonary delivery [11,14,43].

4.6. Spray congealing method

Spray congealing represents a relatively novel and attractive technique for the preparation of LMs, which is not based on emulsification methods. With this method a fluid mixture consisting of the drug (e.g., felodipine, tetracycline, lidocaine, verapamil, theophylline) dissolved or dispersed in the molten lipid carrier (with a relatively low melting temperature, < 90 °C) is atomized through an atomization device. The atomization leads to the formation of molten droplets, which then solidify into a vessel maintained at ambient temperature or in dry ice. The obtained LMs are then collected and in some cases dried in a desiccator [3,33,42,50,51]. The performance of the spray congealing process depends primarily on the atomization efficiency of the molten mixture. The different types of atomization devices available include: (A) centrifugal atomizers (the molten mixture is dropped onto a high speed rotating disk and the rotation spread and spray the molten fluid) [3]; (B) airless atomizers, seldom employed because of the high pressure required (70-80

bar) to atomize viscous fluids such as melted lipids and (C) the air- or pneumatic- nozzle (air must be heated to avoid rapid solidification) which is the most widely applied [33,42,43]. The latter can be in the internal- (the molten fluid and the atomization air come in contact in the mixing chamber) or external- (the molten fluid and the atomization air come in contact outside the nozzle) mixing configuration (Fig. 3). The inner diameter of the pneumatic nozzle, the air temperature and pressure represent the main operating parameters influencing the performance of the obtained LMs. Rodriguez et al. [52] have described the use of ultrasound-assisted atomizer for the production of LMs (mean particle size, 375 μm) by spray congealing. The spray congealing method has the advantage of being completely solvent free [33,42,53] and hence it does not require removal of water from the particle formulation, thus improving the stability of the LM preparation and producing a free flowing dry powder ready to be used. Moreover, a study by Albertini et al. [53] demonstrated that, particularly at high drug loading levels, enhanced microparticle entrapment efficiency is achieved by spray congealing, compared to conventional melt emulsification. Spray congealing has also been shown to be more efficient than melt emulsification for the incorporation of polar drugs into the lipid matrix of the LMs [42,51]. The main disadvantage of spray congealing is that rather larger particles are produced (mean diameter > 30-40 μm; see Fig. 4) [33, 42,51], which is suitable for oral or topical delivery, but limits their applicability for other administration routes (e.g., unsuitable for inhalation drug delivery).

4.7. Supercritical fluid-based method

Techniques based on supercritical fluids represent an alternative and innovative approach for the preparation of LMs. As for spray congealing, this method does not involve emulsification, being based on generation of particles via aerosol formation. Supercritical

fluid technology uses fluids whose temperature and pressure are both above their critical point values. These fluids exhibit properties (e.g., solvating characteristics, solute diffusivity, viscosity) intermediate between a liquid and a gas. Moreover, the solvation power of a supercritical fluid can easily be tuned simply by changing the applied pressure and temperature, thus enhancing the selectivity and flexibility of the process [54]. Additional advantages are produced when CO_2 is used as supercritical fluid, due to its relatively low critical temperature (31.1 °C) and hence the possibility to work under mild conditions and minimising the contact with air [55]. The supercritical technique is environmentally friendly since it circumvents the use of organic solvent. Moreover, CO_2 is fairly non-toxic, non-flammable easily removed from the sample by decompression and it is readily available from the atmosphere (fermentation processes). The main techniques developed for the formation of particulate systems include the rapid expansion of supercritical solutions (RESS) and the supercritical anti-solvent technique (SAS).

RESS involves the rapid depressurization of the supercritical fluid phase in which the solute of interest is dissolved (Fig. 5A). Small particles are obtained owing to the large supersaturation associated with the rapid loss of density and solvent strength of the compressible fluid phase. In SAS (Fig. 5B) the solute is dissolved in a liquid organic phase (which is contradictory with the main goal of supercritical fluids, the reduction of the use of organic solvents) and the supercritical fluid is used as anti-solvent. Particle formation is achieved by bringing the solution into contact with a supercritical fluid, having low affinity for the solute but miscible with the organic phase. Both processes produce dry particles under mild operating conditions and in a single step, which is an advantage compared to the multi-steps required by the traditional methods [49,53]. The supercritical fluid technology is particularly suited for the preparation of lipid microparticles because of the satisfactory solubility of the lipid excipients in the most common supercritical fluid, CO_2. For the

preparation of LMs by supercritical fluids a modification of the RESS method, called particles from gas saturated solution (PGSS), has been used [49]. Instead of solubilizing the solute in CO_2, the melted lipid excipient containing the active to be encapsulated is saturated with supercritical CO_2. Particles are then formed by decompression of the supercritical fluid through a nozzle. Different types of lipids alone or in combination with polymers (polyethylene glycol, polymethacrylates) have been used for the preparation of LMs loaded with different drugs (e.g., bovine serum albumin, caffeine, ketoprofen) by the supercritical fluid technology [56-60].

4.8. Spray drying method

Using the spray drying technique, the lipid excipient and the active (e.g., glibenclamide, etoricoxib, estradiol) are dissolved or dispersed in an organic solvent. The obtained mixture is then atomized into a current of warm filtered air, which removes the solvent from the droplets yielding solid microparticles. Due to the heat supplied by the air for solvent evaporation, the use of lipids with high melting points (e.g., > 70 °C) is required, to avoid the melting of the LMs during the spray process, this aspect being probably the reason of the limited application of the method to LM preparation [3,40,61].

5. Lipid microparticle characterization

5.1. Drug Loading

Drug loading indicates the amount of drug incorporated into the lipid matrix. The drug loading is determined as the percentage ratio between the amount of active ingredient assayed in the LMs and the total weight of the particle sample according to this equation:

$$\text{Drug loading (\%)} = \frac{\text{mass of drug in microparticles}}{\text{mass of microparticles recovered}} \cdot 100$$

The encapsulation efficiency is calculated as the percentage ratio between the quantity of active entrapped in the microparticles and added to the melted lipid phase during particle production, according to the following equation:

$$\text{Entrapment efficiency (\%)} = \frac{\text{mass of drug in microparticles}}{\text{starting mass of drug}} \cdot 100$$

LMs with high loading levels have the advantage that a reduced amount of particles is required to attain the effective drug concentration. By increasing the amount of active principle incorporated during the LM preparation, the size and loading increase, but the efficiency of encapsulation decreases, due to the reduced mass of lipid available for the drug to be entrapped [21,34,62]. Drug loading and encapsulation efficiency are determined on the LMs after removal of the aqueous phase by filtration or centrifugation. Particles can be rinsed with water in order to eliminate drug adsorbed on their surface and lyophilized to obtain water-free microparticles. The extraction of the incorporated drug from the LMs is performed by heating the sample above the melting point of the lipid used, followed by stirring or sonication. The obtained sample, after dilution to volume and filtration, is assayed by spectrophotometric or chromatographic techniques.

5.2. Particle size

The particle size depends on various parameters such as composition of LMs (matrix material, surfactant type and concentration, incorporated drug) production method and conditions (e.g., higher stirring speed produce a reduction of the LM mean diameter) [1,14,42,63]. The particle size distribution can be expressed in terms of average particle diameter ± S.D. The results are also reported as volume or mass median diameter (D_{50}), namely the particle size below which 50% of the sample (in terms of volume or mass) lies. The D_{10} and D_{90} (particle size below which 10 and 90% of the sample lies in terms of volume or mass) are also used. The polydispersity index (P.I.), useful to measure the size distribution width, is calculated according to the following equation:

$$P.I. = D_{90} - D_{10} / D_{50}$$

Small P.I. values (ca. < 0.3) indicate narrow particle size distribution [21].

5.2.1. Optical microscopy and scanning electron microscopy

Optical- and scanning electron-microscopy are both used to measure particle size and evaluate particle shape. The latter method can also determine the surface morphology characteristics (Fig. 4). The optical microscopy technique is less accurate in the dimensional range of few microns. The main disadvantage of the microscopic techniques is the rather small number of particles examined (300-500) which limits the statistical significance of the results [64]. Moreover the procedure is lengthy and tedious [3,34,51,65]. However, both are extremely useful as they allow the direct evaluation of the particle

morphology and the presence of agglomerates.

5.2.2.Coulter counter

This method is based on the principle of measurement of change in the electric resistance across the sensing zone, when a suspension of electrolyte containing particles is drawn through a small orifice on either side of which two electrodes are immersed [24,51,65]. The main advantage of the method is that the instrument is capable of measuring a high number of particles in a short time [65].

5.2.3. Laser diffraction

This technique is based on the correlation between the angle of the laser light scattered by particles and their size (the angle increases with decreasing particle size). The dimensions of the particles are calculated from the light scattering angle through complex algorithms based on Fraunhofer and/or Mye calculations. Laser diffraction covers a broad size range from about 0.1 μm to 1000 μm and can be used with dry powder or particle suspension. It is a simple and fast sizing method [64] and represents the most commonly used technique for the analysis of particle size distribution [3].

5.2.4. Dynamic light scattering

Dynamic light scattering measures the rate of fluctuations in laser light intensity scattered by particles diffusing through a fluid in the sample cell. The smaller the particles, the faster they diffuse, the faster the intensity will change. Its measurement size range is limited to few nanometers to about 3 microns only [1]. Therefore the method is rarely used to measure the size of LMs.

5.2.5. Atomic Force Microscopy

This technique utilizes the force acting between the particle surface and a probing tip for imaging. One of the advantages of atomic force microscopy is the simplicity of sample preparation, as no vacuum is applied during operation [1]. Although atomic force microscopy is mainly used to examine the three-dimensional topography of the particles, it has also been used to measure the size of micro- or submicro- lipid particles [66,67].

5.2.6. Dynamic image analysis

Dynamic image processing measurements is based on the shadow projection principle. The particles move through the measuring field between a light source, which illuminate them and digital cameras which take their pictures from the other side. The software evaluate the projections of the particles to determine the size distribution of the sample in the range 1-1000 μm.

5.3. Zeta potential

The zeta potential (the electrical potential between the tightly bound surface liquid layer of a dispersed particle and the bulk phase of the suspension) affects the electrostatic interaction between dispersed particles. The greater the zeta potential, less likely particle aggregation will occur (due to electric repulsion) and hence its magnitude gives an indication of the potential stability of the LM dispersion. In addition the zeta potential can influence the interaction of the lipid particle systems with biological membranes [1,7]. Zeta potential can be measured by a traditional technique called electrophoretic light scattering, a technique which measures the velocity of the particles suspended in a fluid medium using a cell with electrodes at either side, through which an electric field is applied. The velocity of the particles is determined by detecting the scattered light emitted from the

particles. A minimum zeta potential value of ± 25-30 mV is generally considered necessary to ensure the stability of a dispersion stabilized only by electrostatic repulsion [1,7].

5.4. Crystallinity and Polymorphism

The characterization of degree of lipid crystallinity and modification of the lipid crystal lattice during particle preparation and storage are very important parameters as they influence drug incorporation and release rate. In fact, thermodynamically less stable crystal configurations have a lower density which give rise to increase mobility of the incorporated substance and hence differences in entrapment efficiency and release behaviour [1,3]. Moreover, drug solid-state modifications (crystalline or amorphous) in the LMs, can provide information on its dispersion/miscibility in the lipid matrix and/or interactions with the excipients [37,53]. Differential scanning calorimetry (DSC) and powder X-ray diffraction are the most commonly used techniques for LM solid-state studies.

Differential scanning calorimetry is a thermal method which measures the thermal response of a sample to a temperature gradient, thus enable the measurement of transition enthalpies and the related transition temperatures (solidification and melting points, glass transition temperatures) [65]. DSC analysis is based on the fact that different polymorphic forms exhibit different melting temperatures and enthalpies. For instance, tristearin can crystallize in LM producing polymorphs: the stable β-form (melting point, about 65 °C) and the unstable α (melting point, about 48 °C) and β' (melting point, about 62 °C) forms [62,68]. A drawback of DSC is related to possible modification of lipid carrier or incorporated substance solid state, upon heating or cooling of the sample.

Powder X-ray diffraction analysis exploits the diffraction of X-rays by crystals, due to their wavelengths being comparable to the distance between atoms and molecules of crystals. A powder can be also investigated by X-ray diffraction by comparing the position

and intensity of the diffraction peaks with those of a known substance or polymorphic form. This method has the advantage that the sample is examined as presented. Polymorphic modifications can be detected by the presence of diffraction signals at different positions and with varying intensities [53]. Moreover, since amorphous forms produce broad and diffuse maxima in diffraction patterns [69], it is generally recognized that the disappearance of the crystalline peaks of a substance indicates its amorphisation.

Fourier transform infrared and Raman spectroscopy have also been utilized for solid-state analysis of LMs [7, 40]. Although infrared spectroscopy is used for the identification of polymorphs [70], its applicability is limited due to the complexity of the spectra with strongly overlapped bands. Raman spectroscopy is a particularly useful technique, as it involves no sample preparation [24]. It has been shown [71] that Raman spectra of lipids are sensitive to conformational, packing and dynamical changes involving hydrocarbon chains.

5.5. Release

Since the general aim of microparticle systems is protection and controlled release of the incorporated drug, *in vitro* release studies have to be carried out on the obtained particles to ascertain their release profiles [1,3]. These studies are performed according to the Pharmacopoeia dissolution tests such as apparatus I, II and IV [3,42], although non-official apparatus are also often used [14,48,62,70]. The method measures the diffusion of the incorporated substance through the particle matrix into a suitable dissolution fluid (generally phosphate buffer, pH 7.4). In order to ensure sink conditions, surfactants, cyclodextrins, water miscible organic solvents (e.g, propylene glycol, methanol) or proteins have been added to the release medium [43,62,72]. The release fluid should ensure adequate solubility for the incorporated drug, while it should not affect the integrity of the

LMs. Consequently, the selection of a solvent system can be challenging, because encapsulated substances generally exhibit physico-chemical properties similar to the LM matrix. For actives which are not stable in the release medium, the amount of drug remaining in the LMs, rather than the amount of drug released has to be determined [62]. For specific administration routes, such as pulmonary or topical delivery, evaluation of the release profile in a large volume of fluid is not representative of real conditions and hence might give results that do not reflect the actual *in vivo* behaviour. Accordingly for topical and pulmonary applications, Franz cells have been employed [14,46].

Rapid release indicate a short diffusion path of the active compound and hence its presence on the surface region of the LMs. This has been associated with a drug-enriched shell incorporation model. For stabilization effects, fast release will reduce the fraction of the active ingredient protected by the lipid particle matrix. Slow release suggests an effective incorporation of the substance inside the particle matrix, which has been described by the drug-enriched core incorporation model [1,8,10]. Several authors have described biphasic release profiles [21,42,51,53] exhibiting an initial burst release followed by a continuous slow release. This type of release could be utilized to deliver the drug at different rates, producing both rapid (e.g., to deliver an initial dose when required) and prolonged therapeutic effects.

In general the release of the active substance incorporated in the microparticles is lower than its dissolution, however release rates higher than dissolution have been also reported for the LMs [73,74]. This can be traced to improve wetting and solubilisation of the encapsulated drug due to the presence of the surfactant used as stabilising agent for the preparation of the particles. Moreover, the reduced size of the LMs as compared to the raw drug substance, would contribute to the higher release rate, because of the increase in specific surface area [75].

Release from the LMs is influenced not only by the nature and concentration of the incorporated drug, the type of lipid matrix and surfactant, but also by the method of LM preparation and their particle size [3,14,37,42,53]. Another critical parameter influencing the drug release rates is the lipid crystal structure, since the thermodynamically less stable configuration has a lower density which give rise to increase mobility of the incorporated substance and hence differences in the release behaviour. The advantage of higher incorporation rates in unstable polymorphic form is counterbalanced by an increased mobility of the drug [1].

5.6. Drug distribution within the particles

Drug localization within the LMs is difficult to ascertain, because of the lack of suitable techniques. However, information on drug distribution on the particle surface can be achieved by X-ray photoelectron spectroscopy (XPS) technique, if the encapsulated substance contains atoms that are not present in the carrier material (e.g., Cl, N). XPS is based on the emission of electrons from materials at specific energy values, following irradiation by photons with sufficient energy to cause ionisation of core level electrons. Since photons have a low penetration energy, only electrons belonging to atoms present in the surface or just below (up to 10 nm) can be detected [3,50].

6. Interaction with biological systems

6.1.Biocompatibility

Since LMs are composed mainly of biocompatible and biodegradable lipids, they are well tolerated [1,3]. However the toxicity of the surfactant used for their preparation has to be

considered, although physiologically compatible emulsifiers (e.g., phosphatidylcholine, bile acids) have also been used for the preparation of LMs [43,76]. The tolerability of LMs has been investigated *in vitro* by cell viability studies. This approach involves the incubation of cell cultures with a suspension of the unloaded microparticles in a suitable medium. Following exposure to the particles, the cytotoxicity is assessed by evaluation of cell viability using different kind of commercially available assays [43,77,78], such as the release of lactate dehydrogenase. For this *in vitro* approach murine macrophage J774, human type lung alveolar (A549) and epithelial (Calu-3) cell lines have been used and no significant cytotoxic effects were observed in a wide range of particle concentrations [43,78,79], confirming the satisfactory tolerability of LMs. Erni et al. [80] reported no cytotoxic effects for neutral LMs in different cell lines (293 embryonic kidney cells, murine macrophage cells), while cationic LMs displayed concentration-dependent cytotoxicity comparable to that of the cationic lipid. Moreover, a number of *in vitro* studies have demonstrated the low cytotoxicity of lipid nanoparticles on different cell lines [1,81] and these observations could be reasonably extrapolated to LMs.

In vivo biocompatibility of LMs has been evaluated by Reithmeier et al. [82] by subcutaneous implantation in mice. Only slight inflammation was observed in the implantation area. Sanna et al. [83] performed *in vivo* acute pulmonary toxicity studies of LMs in rat lung tissue. No significant inflammatory airway response were induced by the LMs after a single administration. The good pulmonary tolerability of lipid particles was confirmed *in vivo* for nanoparticles after repeated inhalations [77].

6.2. Biodegradation

The LM matrix is composed of naturally occurring lipids and hence biological mechanisms for their metabolisation are present *in vivo*. The main enzymes responsible for the

degradation of LMs are lipases and esterases [1,3]. As lipases are presents in various body regions, including intestine, subcutaneous and muscle tissues and serum fluids [84], biodegradation of lipid particles can occur independently of the administration route. Following *in vitro* incubation in physiological media containing lipases, LMs have been shown to undergo degradation (80% mass loss after 48 days) [84]. However, further studies should be performed in order to confirm whether these data could be transferred to the more complex *in vivo* situation. It should be stressed that the enzymatic degradation of the LM matrix will affect, together with diffusion, the in vivo drug release [1,38]. Lipid nanoparticles have been intensively investigated with relation to the influence of their composition on the kinetic of degradation by lipolytic enzymes [1,3]. It is reasonable to assume that the obtained data could be relevant for the LMs too.

6.3. Interaction with cells

Although the preparation and characterization of LMs have been thoroughly investigated [3,7,49], their effect on cells has been mostly overlooked. In general, microparticles are unlikely to cross most biological barriers, whereas nanoparticles due to their smaller size can cross such barrier, depending on their size and type of membrane [27,85]. Microparticles can enter the cells that can perform phagocytosis (e.g., macrophages, neutrophils), a mean of internalize material up to 10 μm in size. In comparison, pinocytosis uptake mechanism for sub-micron material can be performed by almost all type of cells and hence in general nanoparticles can be intracellularly delivered [86].

Efficient internalization of neutral and positively charged LMs by human primary macrophages was demonstrated *in vitro* by Erni et al. [80]. Phagocytosis was followed by a rapid (24 h) intracellular particle degradation, making LMs a suitable carrier for the immediate delivery of therapeutics to these cells. The research group of Maretti et al. [79]

studied the internalization ability of rifampicin loaded LMs on murine macrophage J774 cell lines. The interaction of LMs containing the flavonoid quercetin with A549 lung alveolar and Calu-3 bronchial epithelial cells has been examined by Scalia et al. [43,78]. For both cell lines, the intracellular delivery of quercetin was significantly increased when the flavonoid was encapsulated in the LMs, as compared to its free form (Fig.6). The enhanced cellular uptake achieved by the LMs was due to reduced quercetin degradation after encapsulation and a more effective interaction between the encapsulated quercetin and the cell surface [43,78]. Further studies are required to understand the dynamic of the interaction of the LMs with cellular structures *in vivo* and *in vitro*.

7. Administration routes

LMs have been used for the delivery of various types of drugs by various routes of administration, which will be examined in the following sections.

7.1. Oral administration

By oral delivery, the LM may provide sustained release and protection of labile drugs (e.g. proteins and peptides) against chemical and enzymatic degradation inside the gastrointestinal tract [7]. In particular, lipid matrices are more biocompatible and provide a less detrimental environment for peptides and proteins than synthetic polymers. Lipid-based microparticulate systems have also been shown to improve the oral bioavailability of poorly-water soluble drugs [7,87]. Since the absorption of intact microparticles through the gastrointestinal tract is extremely low (2-3%) [88], this effect can be traced to stimulation of endogenous solubilization processes and increased solubility due to the presence of

surfactants [7]. An additional advantage of LMs is the lack of toxicity after oral administration [3]. However, the stability of the lipid carriers in the gastric and intestinal fluids should be verified [89]. LMs were found to be effective as oral delivery system for the peptide drug serratiopeptidase (a proteolytic enzyme), being able to entrap high level of peptide and ensure sustained release [90]. Controlled-release tablets of the poorly-soluble drug, felodipine, were prepared by the research group of Savolainen et al. [33] by incorporation of the drug in LMs, which were then compressed. After oral administration in diabetic rats of phospholipid-modified LMs loaded with glibenclamide, Nnamani et al. [91] reported a blood-glucose lowering effect higher than that produced by a commercial non-encapsulated drug sample. Momoh et al. [92] prepared LMs containing a hydrophilic oral antihyperglycemic agent using a solidified reverse micelle approach. *In vivo* studies on induced diabetic rats indicated that administration of LMs produced a greater glucose level reduction compared to the free drug. Using the solidified reverse micelle system, LMs loaded with diclofenac potassium were developed and were found to achieve *in vitro* drug sustained release and *in vivo* good anti-inflammatory and gastro-protective properties in rats [93]. LMs containing mesalazine were embedded in a lipid micro-reservoir to produce a lipid microcapsule delayed release dosage form to be dispersed in water to facilitate oral administration in patients unable to swallow [94]. Moreover, LMs have also been investigated as a taste-masking approach using loratadine, as a model drug [95].

7.2. Topical administration

Whole lipid microparticles offer little value as carrier for transdermal delivery because they cannot penetrate healthy skin owing to the micrometer size [5, 96]. However, for skin with impaired barrier function appropriate information is lacking. In fact, only for very small nanoparticles (< 10 nm) permeation through the stratum corneum into the underlying viable

epidermis and dermis has been reported [2,5,97]. In general, nanoparticles greater than 20 nm has been shown to penetrate deeply into the hair follicles and into the stratum corneum, but not into the viable epidermis [2,5]. Larger microparticles (> 10 μm) neither penetrate into the follicular orifices, nor the horny layer [95,98]. Several researchers have demonstrated that microparticles with diameter < 3-5 μm can diffuse through hair follicles and were also observed in the superficial layers of the stratum corneum [97,99]. Maximum penetration depth into the follicular duct was observed for the smallest particles [99]. It should be considered that movement of the hairshaft would enhance microparticles permeation into the hair follicle canal [100].

Because of the preferential penetration within the hair follicle, smaller LMs can deliver substances into the hair follicles, thus enabling high concentrations of active ingredients within the reservoir of the follicular infundibulum. Accumulation of the particles in the follicular ducts could be followed by release of the incorporated drug and its diffusion to the perifollicular epidermis [2]. Moreover, because of their micron size, LMs increase the contact surface of the encapsulated active substance with the stratum corneum, which could enhance the cutaneous delivery and achieve sustained release of the drug [28,49]. On the other hand, since microparticles do not penetrate the stratum corneum, the LM formulation forms a film on the cutaneous surface fixing the active ingredient on the superficial portion of the skin. This represents an advantage for active substances, such as sunscreen agents, whose site of action is localized in the outermost cutaneous layers.

Nasr et al. [28] prepared LMs loaded with aceclofenac for the topical delivery of this anti-inflammatory agent. *In vivo* studies on rats demonstrated that the LMs after incorporation in topical lotion and paste formulations, achieved superior anti-inflammatory activity compared to the marketed product. Passerini et al. [101] using porcine skin, compared *in vitro* LMs (18-44 μm size range) with lipid nanoparticles (140-154 nm size

range) for the topical delivery of econazole nitrate. No significant difference in drug permeation was observed between lipid micro- and nano-particles, suggesting no size dependent effect in the examined dimensional range. LMs (mean particle size, 4.2 μm) containing a model retinoid drug were developed by Lauterbach and Mueller-Goymann [102] for the delivery of the active ingredient to the hair follicle. *In vitro* studies with porcine ear skin confirmed the follicular penetration and dissolution of the particles in sebum. Work from this group and from other researchers has shown that the incorporation of several commonly used sunscreen agents in LMs decreases their penetration into animal and human skin, both *in vitro* and *in vivo* [26,46,47,103]. This effect not only ensures the efficacy of the UV filters by retaining them on the skin surface where they act, but also limits potential toxic reactions. Moreover, encapsulation of sunscreen agents in the LM matrix has been shown to reduce the light-induced degradation of photolabile sunscreen agents, thereby maintaining their photoprotective capacity [36,53,68].

7.3. Pulmonary administration

Drug inhalation represents the most popular route for the treatment of lung diseases and shows promise for systemic action [11]. The delivery of particulate agents to the respiratory tract by dry powder inhaler (DPI) has become a popular method since it achieves greater drug chemical stability, the dosing option are broader and the passive nature of the dry powder devise ensures better patient compliance [11]. In order to achieve efficient respiratory deposition, the drug particles should have an aerodynamic diameter < 5 μm, together with suitable density and morphology [11,14,76]. LMs exhibit several advantages for pulmonary delivery, including physico-chemical stability, avoidance of organic solvents

for their preparation and the ability to control release after deposition. Moreover, since they are based on physiological compounds, they should be well tolerated in the pulmonary tract. As reported in section 6.1., *in vitro* and *in vivo* published studies have demonstrated that lipid particles do not induce inflammatory responses in the lung tissue, even after repeated inhalation exposure [77, 83].

Sebti and Amigi [76] studied the performance of budesonide-loaded LMs for lung delivery, based on cholesterol and phospholipids, and reported improved aerosolization efficiency, when compared to commercially available DPIs. The production of LMs for inhalation based on glycerol behenate and Pluronic F-68 and containing budesonide has also been reported by Mezzena et al. [11]. Advantages of the latter system include *in vitro* controlled drug release and the lack of organic solvent for their preparation. The development of LMs for pulmonary administration and containing the polar bronchodilator agent, salbutamol has been described by Jaspart et al. [37] and Scalia et al. [14]. In order to overcome the drawback of the inefficient incorporation of this hydrophilic drug into the lipid matrix, different approaches based on the use of a hydrophobic derivative of salbutamol [37] or the unionised salbutamol free base [14] were described. LMs loaded with the antioxidant flavonoid, quercetin were developed by Scalia et al. [43,78] and were found to exhibit *in vitro* satisfactory aerosolisation, sustained release properties and enhance quercetin uptake by A549 and Calu-3 pulmonary cells (Fig.6). Respirable LMs loaded with rifampicin were found to be taken up by murine macrophages which should enhance the effectiveness of conventional tuberculosis therapy [79]. However, further studies should be conducted to evaluate whether the above results obtained *in vitro* can be confirmed in *in vivo* experiments.

Pandey and Khuller [104] have reported the encapsulation of various antitubercular drugs (rifampicin, isoniazid and pyrazinamide) into LMs (size range 1.1–2.1 μm) for

pulmonary administration. Delivery of the particles to guinea pig lungs [104] by nebulization improved drug bioavailability and reduced dosing frequency. Sebti et al. [105] compared *in vivo* budesonide-loaded LMs with a commercial drug product following a single inhalation in healthy volunteers. Pulmonary administration of LMs produced a significant increase in budesonide lung deposition as determined by scintigraphic evaluation of the labelled drug.

7.4. Nasal administration

The delivery of drugs to the nasal mucosa is used not only for the treatment of local diseases, but also for systemic delivery (across the mucosa of the respiratory region) and represents an interesting option for brain uptake of active agents through the olfactory region. Powder preparations offer several advantages for nasal administration including higher stability, simpler formulations and improved residence time. In addition, the micrometric size is preferred in order to induce the deposition of particles on nasal mucosa [75,106], and differently from nanoparticle systems, hamper their inhalation [107] and absorption in the blood stream, avoiding the induction of unwanted effects.

Few studies have appeared in the literature on the application of LMs to the nasal route. Hepatitis B antigen was loaded in LMs for mucosal administration via the nasal route [108]. *In vivo* studies in rats demonstrated that LMs induced a marked mucosal immune response, superior to that achieved by intranasal and intramuscular administration of plain antigen. Dalpiaz et al. [75] produced LMs containing a prodrug of the antiretroviral agent, zidovudine. Following nasal administration, LMs were shown to induce *in vivo* the uptake of the prodrug by rat cerebrospinal fluid. Under the same conditions, no prodrug was

detected in the cerebrospinal fluid following its administration in the non-encapsulated form.

7.5. Parenteral administration

LMs are not suitable for intravenous administration, because of their size [1]. The diameter of the fine capillaries is about 7-9 μm and therefore due to the possibility of capillary blockage which could lead to a fatal embolism, for safety reasons it is recommended that the size of the particles should be in the submicron range [1,7] . LMs are suitable for intramuscular (i.m.) or subcutaneous (s.c.) administration, provided that their size is below 150 μm to ensure their injectability [62]. Injected microparticle remain at the site of injection, creating a depot for local delivery and prolonged effect [86] and therefore biodegradation is desirable. Due to their physiological compatibility, biodegradability and release properties, LMs are suitable for parenteral administration and represent a valuable alternative to polymeric carriers [84]. A systematic *in vitro* study on lipase induced degradation of triglyceride-based implants or microparticles, indicated that the latter exhibit a more rapid degradation/erosion, and therefore are more suitable for short-term drug delivery [62,84]. Oxytetracycline-loaded LMs were developed by Domb [109] and after a single intramuscular injection in turkey, were shown to achieve extended drug release (3-5 days) compared to 1 day for the commercial drug. The biodegradability of the formulation was also demonstrated. The peptide hormone, somatostatin which exhibits a very short half-life, was incorporated in LMs with a suitable size distribution for i.m. or s.c. injection. The peptide was release almost continuously for over 10 day [62]. Reithmeier et al. [82] produced LMs suitable for i.m. and s.c. injections and containing insulin and thymocartin (immunomodulating peptide). Biocompatibility of the LMs in mice was similar to

polymeric particles already approved for parenteral application. The *in vitro* insulin release was slower (incomplete after 13 days) than thymocartin (5 days). Entrapment of gonadotropin release hormone antagonist in LMs has been reported by Del Curto et al. [110] . Following subcutaneous administration in rats, drug plasma levels were detectable for 30 days.

8. Commercial products

Despite LM potential clinical applications, as reviewed in the previous section, and favourable characteristics (e.g., avoidance of organic solvent during preparation, ease of large-scale production, physical stability), their commercialization is very limited. From the perusal of the literature only two examples were found of marketed formulations based on LMs, namely an ultrasound contrasting agent (perflutren lipid microsphere; Definity®) and a topical product containing an insect repellent incorporated in LMs (Skedaddle ™).

9. Conclusions

During the past few years, the potential of utilizing LMs as efficient drug carriers for different administration routes with modulated drug release properties has been recognised by several researchers, and has been extensively studied both *in vitro* and *in vivo*. LMs constitute an attractive drug carrier system. However, despite the advantages linked to the presence of the lipid systems over traditional systems, and particle engineering efforts that many research groups have worked on, currently there are only few commercially available systems. Until the pharmaceutical industry does not embrace fully this very flexible formulation technology it will be challenging to advance research in this area.

Expert Opinion

Lipid microparticles (LM) are a versatile drug delivery system, primarily suited for the controlled delivery of hydrophobic active ingredients. In comparison to lipid nanoparticles, LMs have the advantage of higher drug loading with reduced issues surrounding potential toxicity of their nanoparticle counterparts. Unlike nanoparticles, LMs have poor cellular-uptake in non-phagocytising cells, while providing a platform for macrophage targeting applications if required. LMs utilise GRAS and endogenous excipients, so new formulations should be relatively easy to transfer into the clinic. Furthermore, the manufacture of LMs is relatively easy and scalable from the bench to production site, using equipment and techniques that are widely used throughout the pharmaceutical and food sectors, with minimal modification (i.e. emulsification and sonication). The current methodologies for the productions of LMs represent an important step toward more delivery options. Recent developments have demonstrated a revival in the research interest for these systems and advances in single-step manufacturing methods, such as spray congealing, may further popularise this drug delivery technology. Furthermore, considering the majority of active ingredients in pre-clinical study are hydrophobic, LMs are likely to provide a platform for the development of a number of products and applications over the coming years that require a compatible carrier, stabilizer and/or release modifying component. One current drawback is in the empirical nature of formulation of LMs. There is still no standardised formula for choice of excipient and stabilising components with respect to active ingredient. However, our understanding of LM structure and physico-chemical properties are likely to become more mature as work is continued in the field. Thus, the development of robust formulation and processing methodologies are likely to be brought in line with conventional solid dosage and emulsion based systems in future where

quality-by-design approaches can be utilised in the development and implementation of new LM based dosage forms. Clearly, there is a renewed interest in LMs for drug delivery and some delivery routes are more established than others. The application of LMs to oral drug delivery appears to be the most mature, however, applications for delivery of topical formulations containing sunscreens may provide alternatives to nano-based formulations. Of particular interest is the use of LMs to modulated therapies for lung treatment; the 'holy grail' of the lung delivery arena. Application of LMs for lung delivery would allow for controlled and sustained release of a number of molecules in the lung for treatment of inflammation, infection and for targeting respiratory macrophages in diseases such as tuberculosis. There is already some evidence to suggest pulmonary administration may be a key aspect in optimizing drug release in the lung. However, there are still few studies performed *in vitro* and on animals. Furthermore studies on humans are inexistent. Ultimately, the key goals within the area of LM R&D should be to (I) expand knowledge in the area of understanding physico-chemical properties and active ingredient function; (II) develop robust production technologies with predictable drug loading and release; (III) establish a coordinated study of microparticle excipient and LM toxicity via preclinical trials before conducting Phase I studies. Such studies will provide the fundamental proof that this system can be used clinically and provide new therapies worldwide.

Article highlights box

- The essential components of lipid microparticles (LMs) systems are lipids, surfactants and active ingredient.
- A wide variety of formulation components can be used to prepare LMs, since the choice of the excipients will affects the properties of the delivery system.
- The LMs can be prepared by several methods, each with their own advantages and disadvantages.

- Several analytical methods can be employed to characterise the physico-chemical properties of the LMs, using direct and indirect methods.
- LMs can be delivered by several routes: oral, topical, nasal, pulmonary and parenteral.
- LMs are biocompatible, bio-degradable and have demonstrated bio-affinity in vitro and in vivo

Declaration of interest

The authors report no conflicts of interest

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Table 1.

Major lipids used for the preparation of LMs

Lipid class	Structure	Melting point
Triglycerides	tristearin	73.0 °C
	tripalmitin	65.5 °C
	glyceryl trimyristate	
Partial glycerides	glyceryl dibehenate	65-77 °C
	glyceryl palmitostearate	52-56 °C
	glyceryl monostearate	58-59 °C
Non-glyceride esters	cetyl palmitate	54 °C

Fatty alcohols	stearyl alcohol	60-61 °C
	behenic alcohol	71 °C
Fatty acids	palmitic acid	62.9 °C
	stearic acid	69.0 °C
	behenic acid	81.0 °C
Waxes	carnauba wax	82-86 °C
	beeswax	62-65 °C
Others	cholesterol	148-149 °C
	phosphatidylcholine	225-231°C

Table 2.

Major surfactants used for the preparation of LMs

Surfactant class	Chemical name
Anionic	Sodium cholate
	Sodium taurocholate
	Sodium glycocholate
	Sodium lauryl sulphate
	Dioctyl sodium sulfosuccinate
Amphoteric	Phosphatidylcholine
Non-ionic	Polysorbate 80

	Polysorbate 60
	Sorbitan palmitate
	Sorbitan stearate
	Poloxamer 188
	Poloxamer 407
	Alkylpolyglucosides
	Sucrose esters
Cationic	Stearyl amine
	Alkyltrimethylammonium bromide

Figure legends

Figure 1. Schematic representation of the melt emulsification method.

Figure 2. SEM micrographs of salbutamol-loaded LMs produced by (A) high-speed stirring (17500) or (B) high-speed stirring (17500) followed by sonication (1 min; power output, 40W). From ref. [14] with permission.

Figure 3. Diagrams of (A) an external mixing nozzle (the molten fluid and atomization air come in contact outside the nozzle) and (B) an internal mixing nozzle (the molten fluid and the atomization air come in contact in the mixing chamber inside the nozzle).
From ref. [70] with permission.

Figure 4. SEM micrographs at different magnifications of avobenzone-loaded LMs produced by (A) melt emulsification or (B) spray-congealing. From ref. [53] with permission.

Figure 5. Schematic diagrams of the (A) rapid expansion of supercritical solution, and (B) supercritical anti-solvent techniques. From ref. [55] with permission.

Fig. 6. In vitro uptake of free and LM-loaded quercetin by lung cells. Values are means±SD (n=6). From ref. [43] and [78] with permission.