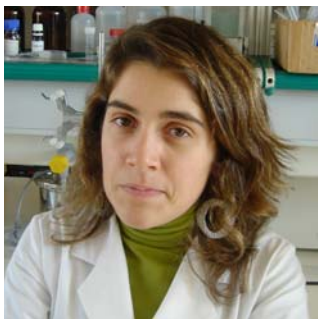
Oral delivery of drugs by means of lipid nanoparticles



Silva, A.C.[1,2], Santos, D.[1], Ferreira, D.C.[1], Souto, E.B.[2]
[1] Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Porto, Porto, Portugal; [2] Department of Pharmaceutical Technology, School of Health Sciences, University of Fernando Pessoa, Porto, Portugal; Corresponding author: anete@netcabo.pt

Introduction

Lipid-based carrier systems have several distinct advantages for oral administration of drugs, e.g. they are composed of biodegradable and well-tolerated lipids, and can also be produced on large scale. Examples of such systems are oral o/w emulsions and aqueous dispersions of lipid nanoparticles. The oral o/w emulsions include the "traditional" simple emulsions, but also the novel SolEmuls technology [1-3]. This technology allows the localisation in the interfacial lecithin layer of drugs which are poorly soluble in the water phase and simultaneously in the oil phase of emulsions (e. g. Amphotericin B). Alternative systems are the nanoparticulate dispersions made from solid lipids. There are two types, solid lipid nanoparticles (SLN) and the second generation nanostructured lipid carriers (NLC).
The features of SLN and NLC for oral delivery of drugs are related to their adhesive properties. Nanoparticles are known as adhesive systems thus, once adhered to the gut wall these particles are able to release the drug exactly where it should be absorbed increasing its bioavailability. Furthermore, the lipids are known to have absorption promoting effects for lipophilic, hydrophilic and poorly soluble drugs [4, 5], depending on the structure of the lipids [6]. For the production of SLN and NLC for oral administration all the lipids and surfactants used in traditional oral dosage forms such as tablets, pellets and capsules can be used.
Aqueous SLN and NLC dispersions cannot be directly used for oral administration of drugs due to the high ionic strength that enhance the risk of particle aggregation. Moreover, the performance of such carriers is also dependent on the presence/absence of food. Advantages of SLN and NLC is the fact that they can be converted into solid dosage forms, such as tablets, capsules, pellets or powders in sachets, and they can also be transferred to a powder (by spray-drying or lyophilization) and added to the tabletting powder mixture. The obtained powders can be used to produce classic solid dosage forms, can be redispersed in water or juice prior to administration, and can also be used for the filling of hard gelatine capsules. Alternatively, the SLN can be produced directly in liquid PEG 600 and filled into soft gelatine capsules. These lipid nanoparticles might be a promising approach to protect drugs from hydrolysis, and also to increase the bioavailability of drugs and control their release [7].

Materials and methods

Materials

Cetyl palmitate (Gattefossé GmbH, Weil am Rhein, Germany) was used for preparation of SLN. The surfactant polyglycerol-methylglucose distearate (Tego Care®450) (Goldschmidt, Deisenhofen, Germany) was the selected emulsifying agent for stabilization of the aqueous SLN dispersion. The water used for all experiments was purified water obtained from a MilliQ Plus, Millipore.

Methods

Production of SLN

SLN were prepared having 20% (m/m) of lipid phase. Cetyl palmitate was melted at 5-10ºC above its melting point (56ºC [8]). Simultaneously, an aqueous surfactant solution was prepared and heated at the same temperature. A pre-emulsion was further obtained by dispersing the hot lipid phase in the hot surfactant solution at 8000 rpm for 1 min, using an Ultra-Turrax T25 (Janke & Kunkel GmbH, Staufen, Germany). The pre-emulsion was homogenized at 75ºC, applying 500 bar and 3 cycles by means of an APV Micron Lab 40 (Homogenizer Systems, Germany). A thermostated water bath adjusted to 25ºC was used as cooling system to control the rate of cooling of the obtained dispersions.

Lyophilization of SLN

Aqueous SLN dispersions were lyophilized using a Gamma 2-20 apparatus (Christ, Osterode a.H., Germany). The vials containing the samples were closed under vacuum in the freeze-drier. The freeze-drying procedure consisted of freezing right after SLN production at -70ºC in a deep freeze, thermal treatment at -22ºC for 2 hr, cooling down to -40ºC for more 2hr, primary drying at 1.03 mbar and secondary drying for 3 hr at 0.001 mbar. Reconstitution of the lyophilized products was performed by manual shaking followed by vortex.

Physicochemical characterization

The particle size analysis was performed by photon correlation spectroscopy (PCS) and by laser diffractometry (LD). The PCS yielded the mean diameter of the main population and polydispersity index (PI) as a measure for the width of the particle size distribution. For PCS measurements, all the samples were diluted with bidistilled water to suitable concentration and measured with a Malvern Zetasizer 4 (Malvern Instruments, UK). For LD analysis the diameters 50, 90 and 99% were used. LD99% means that 99% (volume distribution) of the measured particle are below the given value. LD was performed using a Coulter®LS 230 (Coulter Electronics, Germany).
Crystallinity studies were assessed by means of x-ray analysis and by differential scanning calorimetry (DSC). X-ray analysis was performed by wide-angle x-ray scattering (WAXS, 2 Theta = 4-40º) on a Philips PW 1830 x-ray generator (Amedo, The Netherlands) with a copper anode. DSC analysis was performed using a Mettler DSC 821e (Mettler Toledo, Gießen, Germany). DSC scans were recorded at a heating and cooling rate of 5 K/min. The samples were heated up to 85°C, and cooled down to 25°C. To compare the crystallinity between NLC dispersions, the re-crystallization index (RI), which is defined as the percentage of the lipid matrix that has re-crystallized during storage time, was calculated according to the following equation [9]:

$$RI\ (\%) = \frac{\Delta H_{\ aqueous\ \ SLN\ dispersion}}{\Delta H_{\ bulk\ material}\ \ x\ \ Concentration_{\ lipid\ phase}}\ x\ 100$$

where ΔH is the molar melting enthalpy given by J/g and the concentration is given by the percentage of lipid phase.
SLN morphology was observed using scanning electron microscopy (SEM). Aliquots of aqueous dispersions before and after lyophilization were mounted on metal stubs using adhesive tape, gold coated under vacuum and examined on a JEOL JSM-840 SEM (10kV, Japan).
Bulk and tapped densities of SLN powders obtained after lyophilization were measured using a tap density tester (Stampfvolumeter, STAV 2003, Jel, Germany). The apparent volume occupied by a mass of powder of about 1 g, placed into a 10 ml graduated cylinder, was determined before and

after packing, tapped more than 1250 strokes in order to obtain the closest packed densities. The determination of the Carr's index was performed using the following equation:

$$Carr's\ index\ (\%) = \frac{Density_{1250\ Strokes} - Density_{0\ Strokes}}{Density_{1250\ Strokes}} \times 100$$

Results and discussion

Previously to lyophilization, the samples were diluted with a solution of cryoprotectant in order to preserve the physicochemical properties of SLN after their reconstitution with double distilled water. Table 1 shows the particle size parameters obtained before and after freeze-drying procedure.

Table 1: Particle size analysis of SLN before and after freeze-drying.

Particle size parameters	Before freeze-drying	After freeze-drying
PCS (nm)	233.10	1055.42
PI	0.121	0.472
LD50% (µm)	0.215	11.10
LD90% (µm)	0.467	25.43
LD99% (µm)	0.536	48.19

Spherical-shaped particles were oproduced by high pressure homogenization (Figure 1, left). This shape was maintained after freeze-drying, however some particle aggregation has been detected (Figure 1, right). These results confirm those depicted in Table 1.

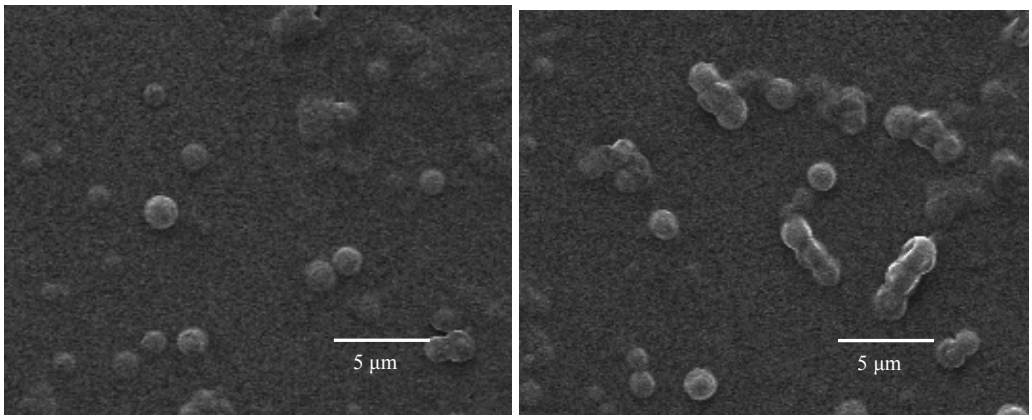


Figure 1: SEM analysis of SLN before (left) and after (right) freeze-drying.

To assess the crystallinity of lipid matrix, x-ray diffraction patterns were compared before and after freeze-drying in the presence of cryoprotectant. Only a slight increase of the peaks intensity was recorded after freeze-drying which impair that during lyophilization lipid molecules reorganize in a way that is quite similar to the x-ray pattern of the original SLN dispersions (data not shown). This means that these carriers are sufficiently stable and remain hydrated. These observations can also be pointed out after analysis of DSC results. DSC results also support the increase in lipid crystallinity after freezing the samples (Table 2). RI means recrystallinity index and it defined as the percentage of lipid phase that has recrystallized. The molar enthalpy (ΔH) is given by J/g and the concentration is given by the percentage of lipid phase in the formulation.

Table 2: DSC parameters of SLN recorded before and after freeze-drying.

DSC parameters	Before freeze-drying	After freeze-drying
Melting point (ºC)	49.13	51.33
Integral (mJ)	367.25	422.11
Enthalpy (J/g)	12.61	14.09
Onset (ºC)	51.12	58.44
RI (%)	58.79	63.71

Once having solid and individualized particles after freeze-drying the analysis of the Carr's index is useful to evaluate the flow properties of solids. Values lower than 25 are usually taken as an indication of good flow characteristics. Cetyl palmitate-based SLN revealed a Carr's index of 13, which is interpreted that SLN powders have suitable rheological properties e.g. to fill capsules, and matrices for tablet production.

Conclusions

These systems offer the possibility to develop well tolerated oral delivery systems, revealing optimal particle sizes and flow properties, in addition to the fact that remain crystalline after suffering lyophilization.

References

1. Akkar, A., Müller, R.H., *Formulation of intravenous Carbamazepine emulsions by SolEmuls technology.* Eur. J. Pharm. Biopharm., 2003. **55**: p. 305-312.
2. Akkar, A., Müller, R.H., *Intravenous itraconazole emulsions produced by SolEmuls technology.* Eur. J. Pharm. Biopharm., 2003. **56**: p. 29-36.
3. Akkar, A., *Poorly soluble drugs: formulation with nanocrystal and SolEmuls® technology*, in *PhD Thesis*. 2004, Freie Universität Berlin: Berlin.
4. Charman, W.N., *Lipids, lipophilic drugs, and oral drug delivery - Some emerging concepts.* J. Pharm. Sci., 2000. **89**: p. 967-978.
5. Porter, C.J., Charman, W.N., *In vitro assessment of oral lipid based formulations.* Adv. Drug Deliv. Rev., 2001. **50**: p. S127-S147.
6. Sek, L., Porter, C.J., Kaukonen, A.M., Charman, W.N., *Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products.* J. Pharm. Pharmacol., 2002. **54**: p. 29-41.
7. Müller, R.H., Runge, S.H., Ravelli, V., Mehnert, W., Souto, E.B., *Oral bioavailability of cyclosporine: Solid lipid nanoparticles (SLN®) versus drug nanocrystals.* Int. J. Pharm., 2006. **in press**.
8. Lippacher, A., *Pharmazeutisch-technologische Characterisierung von flüssigen und halbfesten SLN Dispersionen für die topische Applikation*, in *PhD Thesis*. 2000, Freie Universität Berlin: Berlin.
9. Freitas, C., Müller, R.H., *Correlation between long-term stability of solid lipid nanoparticles (SLN™) and crystallinity of the lipid phase.* Eur. J. Pharm. Biopharm., 1999. **47**: p. 125-132.