Drug Delivery and Translational Research (2018) 8:1807–1814
https://doi.org/10.1007/s13346-018-0521-9

ORIGINAL ARTICLE

Preparation of a novel lipid-core micelle using a low-energy emulsification method

Hans F. Fritz[1,2] · Andrea C. Ortiz[1,2] · Sitaram P. Velaga[3] · Javier O. Morales[1,2,3]

Published online: 16 April 2018

Abstract

High-energy methods for the manufacturing of nanomedicines are widely used; however, interest in low-energy methods is increasing due to their simplicity, better control over the process, and energy-saving characteristics during upscaling. Here, we developed a novel lipid-core micelle (LCM) as a nanocarrier to encapsulate a poorly water-soluble drug, nifedipine (NFD), by hot-melt emulsification, a low-energy method. LCMs are self-assembling colloidal particles composed of a hydrophobic core and a hydrophilic shell. Hybrid materials, such as Gelucire 44/14, are thus excellent candidates for their preparation. We characterized the obtained nanocarriers for their colloidal properties, drug loading and encapsulation efficiency, liquid state, stability, and drug release. The low-energy method hot-melt emulsification was successfully adapted for the manufacturing of small and narrowly dispersed LCMs. The obtained LCMs had a small average size of ~ 11 nm and a narrow polydispersity index (PDI) of 0.228. These nanocarriers were able to increase the amount of NFD dispersible in water more than 700-fold. Due to their sustained drug release profile and the PEGylation of Gelucire 44/14, these nanocarriers represent an excellent starting point for the development of drug delivery systems designed for long circulation times and passive targeting.

Keywords Lipid-core micelles · Low-energy method · Poorly water soluble drugs · Hot-melt emulsification · Nanocarriers

Introduction

The delivery of poorly water-soluble drugs has challenged pharmaceutical scientists for decades. Several strategies have been explored to tackle this issue, including the use of solid dispersions [1], cyclodextrin inclusion complexes [2], emulsion and microemulsion development [3], and nanosuspensions [4, 5]. Over the past years, increasing attention has been paid to the use of nanocarriers as a means to deliver drugs to target sites. As part of current efforts in nanocarrier development, key questions to be addressed revolve around the type of drug formulation to be used and possible fabrication methods. In this paper, we focus on the latter issue.

Lipid carriers are well-suited for drug delivery and transport of poorly water-soluble drugs to different parts in the body, e.g., the brain [6] or accumulation in the liver [7]. A number of lipid carriers are currently available, including solid lipid nanoparticles (SLN) [8], self-(micro)emulsifying drug delivery systems (SMEDDS) [9], nanostructured lipid carriers (NLC) [10], nanoemulsions (NE) [11], and hybrids combining lipidic and polymeric materials, such as lipid nanocapsules (LNC) [12] and lipid-core micelles (LCM) [13]. Lipid carriers are made up of at least one type of lipid, which constitutes the matrix or core, and a surfactant, which stabilizes the matrix in aqueous media. The advantages of these nanosystems include their high affinity for poorly water-soluble drugs, high drug loading capacity, their biocompatibility, and their ability to cross membranes, making them excellent candidates for the delivery of poorly water-soluble drugs [14]. Nevertheless, these systems also have a number of limitations, such as temperature-related stability problems and the fact that lipid

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13346-018-0521-9) contains supplementary material, which is available to authorized users.

✉ Javier O. Morales
jomorales@ciq.uchile.cl

[1] Department of Pharmaceutical Science and Technology, School of Chemical and Pharmaceutical Sciences, University of Chile, Santos Dumont 964, 4to piso, Of. 09, Independencia, 8380494 Santiago, Chile

[2] Advanced Center for Chronic Diseases (ACCDiS), 8380494 Santiago, Chile

[3] Pharmaceutical and Biomaterial Research Group, Department of Health Sciences, Luleå University of Technology, 97187 Luleå, Sweden

chains may reorganize over time, thus altering release profiles [14]. As a consequence, the efficient delivery of poorly water-soluble drugs depends not only on the choice of nanosystem, but also on the materials constituting the system.

Current advances in material technology are opening up new avenues for nanocarrier development. LCMs are self-assembling colloidal particles measuring less than 100 nm in diameter and composed of a hydrophobic core and a hydrophilic shell [15]. Their production therefore requires hybrid materials, such as Gelucire 44/14, to address the poor water solubility of a drug. Gelucire 44/14 is composed of polyethylene glycol (PEG) glycerides, a mixture of monoglycerides, diglycerides, and triglycerides and monoesters and diesters of PEG. Due to its amphiphilic character and surface-active properties, it has been used extensively in different drug delivery strategies, including self-emulsifying drug delivery systems [16] and SLN [17], in addition to other uses as a co-surfactant. Gelucire 44/14 is thus an excellent candidate for the development of a new formulation for LCMs.

Micelle formation depends on critical micelle concentration (CMC), a surfactant-specific value which is affected by temperature, pressure, and other factors. At room temperature, the CMC of Gelucire 44/14 is 72 mg/L. Micelle fabrication can be a complex and energy-intensive process, as described by Friedrich and Müller-Goymann [18], who developed an inverse micelle nanosuspension using Ultra-Turrax (conventional high-energy method) followed by a high-pressure homogenization technique, in order to increase the efficiency of the process.

Gelucire 44/14 is solid at room temperature, with a melting point of 44 °C, thus requiring heat for the quick and efficient dissolution of a poorly water-soluble drug. The characteristics of Gelucire 44/14 make it a promising candidate for the development of LCMs. LCMs have been used for the solubilization of various poorly water-soluble drugs, including paclitaxel [19], camptothecin [20], and vitamin K3 [21]. While LCMs are self-assembling, assembly can be improved through the use of hot-melt emulsification, a method that is well-suited to the characteristics of Gelucire 44/14. It is worth noting that while hot-melt emulsification has ample use in the production of nanoemulsion/microemulsion [22] and SLNs [23], no previous investigations have described its use in the production of small LCMs. Furthermore, when used for SLNs, hot-melt emulsification usually requires an additional step of high-energy homogenization, such as high-pressure homogenization [24]. Other high-energy methods include high-shear stirring and ultrasonication. In contrast with high-energy methods, low-energy methods rely on the materials' chemical potential [25], examples include phase inversion temperature, self-emulsification, and solvent diffusion methods [26]. While high-energy methods are more common, the interest in low-energy methods has steadily been growing. Their advantages over high-energy methods include their non-destructive nature and greater energy efficiency; they are thus of particular interest for large-scale production [27]. Here, we used a low-energy method, hot-melt emulsification, for the first time in the fabrication of small size LCMs for the delivery of a model poorly water-soluble drug, such as nifedipine (NFD).

Materials and methods

Materials

Gelucire® 44/14 was kindly donated by Gattefosse (Lyon, France). Tween 20 (Sigma-Aldrich, Germany), Span 80 (Sigma-Aldrich, Germany), nifedipine HPLC grade 97% pure (AK Scientific, CA, USA), methanol (Panreac Química SLU, Spain), and dichloromethane (Merck, Germany) were purchased and used as received. A Milli Q water Ultrapure Simplicity® (Merck, Germany) was used to produce ultrapure water.

Fabrication of micelles

Micelles were produced by hot-melt emulsification with the two selected compositions depicted in Table 1. First, water was heated to 70 °C, and a lipid phase composed of Gelucire® 44/14, Tween 20, and Span 80 was melted by stirring in an oil bath at 70 °C. When both phases reached 70 °C, they were mixed under rapid stirring, and cold water was immediately added (Table 1). The mixture was taken out of the oil bath and stirred for an additional 5 min. For nifedipine-loaded micelles, 30, 45, or 60 mg of NFD were added to the lipid phase prior to heating to 70 °C. Finally, samples were centrifuged for 30 min at 5000 rpm to eliminate excess NFD and stored for further characterization.

Micelle size, polydispersity index, and zeta potential

The physicochemical properties of the obtained micelles were determined in a Malvern® Zetasizer Nano ZS. Particle size and polydispersity index (PDI) were determined by dynamic light scattering. The zeta potential was measured by Doppler laser velocimetry. Number distributions were used to express size results. Each sample was measured in triplicate and each measurement corresponds to the average of 10–15 determinations by the instrument.

Table 1 Composition of formulations F30 and F20

Materials	F30	F20
Gelucire 44/14 (mg)	1200	800
Tween 20 (mg)	188	125
Span 80 (mg)	52	35
Hot water (mg)	2560	3040
Cold water (mL)	60	60

Characterization of micellar state

The liquid state was determined by differential scanning calorimetry (DSC) in a DSC131 Evo (Calisto Inc., USA). DSC measures the difference in heat needed to increase the temperature of a sample and that of a reference. The temperature gradient was 25 to 65 °C at 5 °C/min.

Characterization of micelle morphology

The morphology of the obtained nanoparticles was analyzed using the transmission electron microscopy (TEM) mode of an Inspect F50 scanning transmission electron microscope (FEI, Hillsboro, OR, USA) after loading the samples onto a copper grid. Samples were stained with 0.5% phosphotungstic acid for 2 min, washed with water, dried at room temperature and visualized under the microscope.

Encapsulation efficiency and drug loading

Encapsulation efficiency (EE) and drug loading (DL) were determined by directly measuring the NFD in micelles by dissolving nifedipine-loaded micelles in methanol:dichloromethane 80:20 (*%v/v*) in a 1:6 (*%v/v*) ratio of sample:solvent (see equations below). The NFD content was determined in a Dionex UltiMate 3000 High Pressure Liquid Chromatography system (Thermo Scientific, USA) after separation of excess nifedipine by centrifugation. The column used was Inertsil ODS-4 (5 μm 4.6 × 150 mm) and kept at 40 ± 1 °C. The mobile phase consisted of methanol:water 60:40 (*% v/v*) at a flow rate of 1 mL/min. The volume injected was 20 μL, and detection was performed at 237 nm. For NFD, the detection limit was 0.002 mg/mL, the quantification limit was 0.25 mg/mL, and the calibration curve had an $R^2 = 0.9993$.

$$\%\text{Encapsulation Efficiency } (EE) = \frac{\text{Amount of NFD measured (mg)}}{\text{Amount of NFD wheighed (mg)}} \times 100$$

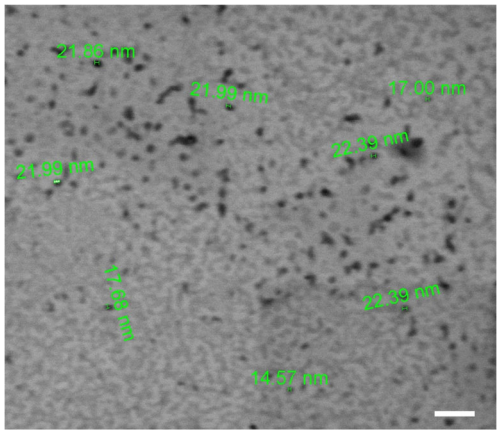

Fig. 2 Representative TEM micrograph of F30 LCMs. The bar represents 100 nm

$$\%\text{Drug Loading } (DL) = \frac{\text{Amount of NFD measured (mg)}}{\text{Amount of solids in formulations (mg)}} \times 100$$

Release study

A release study by dialysis was conducted in phosphate buffer (10 mM, pH 7.2) at 37 °C in an incubator (Labtech, Chile) at 75 rpm. Dialysis bags (SnakeSkin® Dialysis Tubing, Thermo Scientific, USA) with a molecular weight cut-off of 3500 Da were cut to a length of 7.5 cm, sealed at the bottom, loaded with 4-mL sample, sealed at top, and kept in 26 mL of phosphate buffer to maintain sink conditions. Samples were taken at 0, 3, 6, 24, 48, 96, and 120 h, and NFD released was quantified using the HPLC method described above. Each formulation was studied in three independent batches.

Colloidal stability test

The stability of the formulation was assessed over a period of 1 month, both at room temperature and at 4 °C. In both cases,

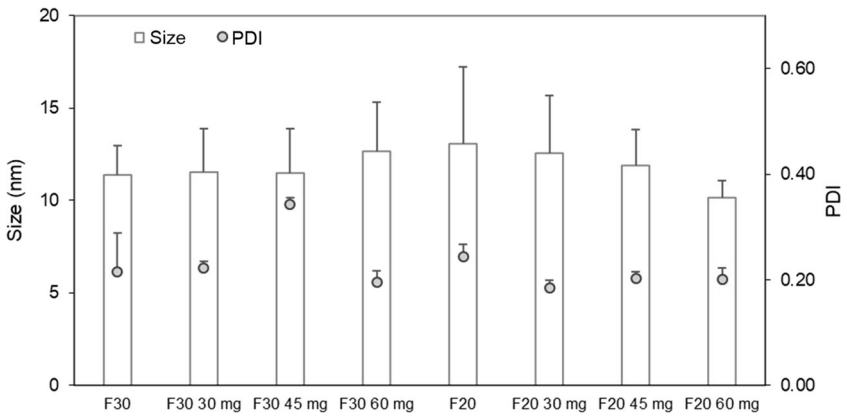

Fig. 1 Size (nm) and PDI of both formulations in triplicate, with and without NFD load

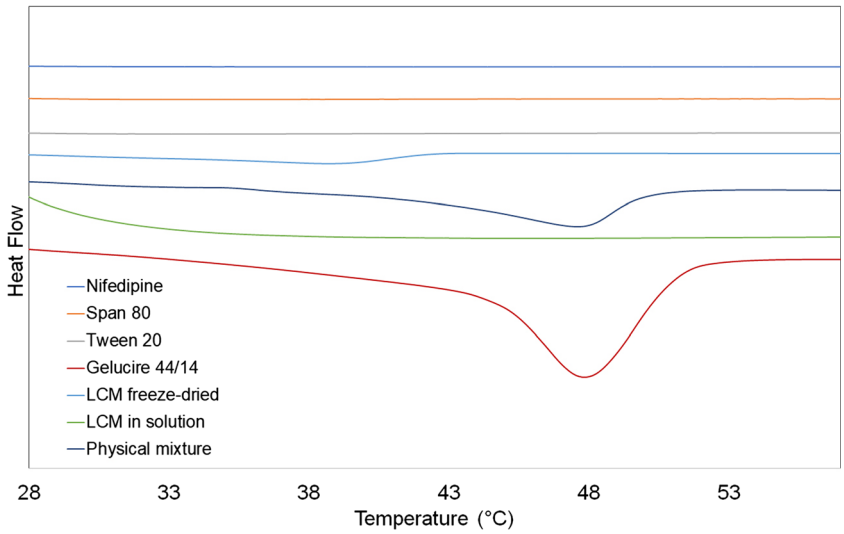

Fig. 3 Comparison of DSC thermograms obtained from LCMs in solution, LCMs freeze-dried, and Gelucire 44/14

the formulation was protected from light for the duration of the test period. Two formulations with three different amounts of NFD each, for a total of six, were tested. For each formulation, a control without the drug was also tested. For each sample, size, PDI, and ZP were determined at 0, 1, 2, 3, and 4 weeks.

Results and discussion

Lipid core micelle fabrication, size distribution, and zeta potential characterization

The final formulations of micelles were achieved after testing various micelle-forming materials, including Gellucire 44/14, Gellucire 50/13, sodium stearate, and sodium taurocholate. Preliminary formulation development included studies of additional surfactants such as Tween 20 and Span 80. Most studied formulations were not able to elicit monodisperse nanoscale particle size distributions and conversely presented large particle sizes (over 100 nm) and polydispersity indices (over 0.4). Only Gelucire 44/14 with the inclusion of surfactants exhibited narrowly dispersed nanoscale particle size. Thus, this work presents the findings of further testing of the selected Gelucire 44/14 lipid core micelles. These micelles were prepared by hot-emulsification, a procedure frequently used for solid lipid nanoparticles but without prior reports on lipid core micelles. As shown in Fig. 1, the average size of the obtained micelles was 11.5 ± 2.0 nm, with a high reproducibility given the small data dispersion of three independent batch productions in different dates. Particle size was less variable in formulation F30 than in F20, and the incorporation of NFD did not produce any statistically relevant changes. The relatively low mean PDI of 0.228 ± 0.024 might be related to the width of the particle size population. PDI values were similar across formulations. Only for F30–45 mg NFD, the PDI was above average; nevertheless, at 0.343 ± 0.01, it still is a low value. Compared to similar LCMs prepared by both

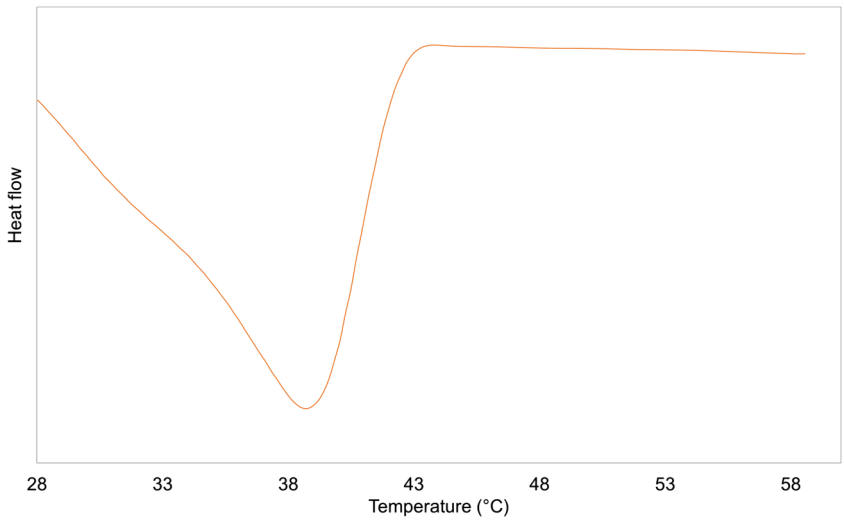

Fig. 4 Auto scaled DSC thermogram of freeze-dried LCMs

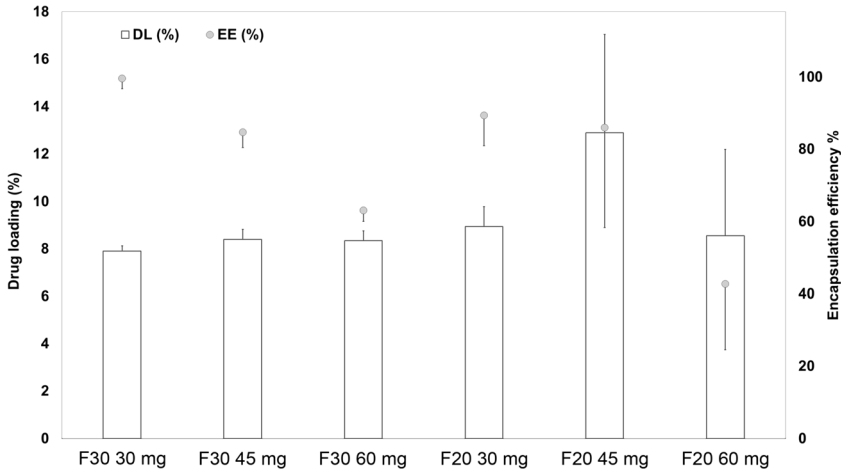

Fig. 5 Encapsulation efficiency (EE) and drug loading (DL) for F20 and F30 formulations with different amounts of NFD

high- and low-energy methods, the LCMs obtained here were of a small size and had a low PDI. LCMs prepared using high-energy methods such as extensive vortexing of a diacyllipid-PEG film [28] and filtration [19] have been found to fall into size ranges of 7–20 nm or 10–35 nm, respectively, to give a few examples. When using low-energy methods, SMEDDS systems have a similar size range to LCMs. A SMEDDS system prepared with Gelucire 44/14 using a water titration method resulted in particle sizes between 31.7 ± 7.8 nm and 73.1 ± 22.7 nm, with PDI values of 0.720 and 1.105, respectively [29]. Other studies using melt emulsification to render nanosuspensions have included the use of micelle-forming and stabilizing materials such as poloxamer 188, Tween 80, polyvinyl alcohol, polyvinyl polyvinylpyrrolidone K25, and their mixtures [22] without reaching the small particle size of our LCMs nor the narrow particle size dispersion. The mean ZP for the LCMs produced here is − 7.4 ± 5.1 mV. The large standard deviation is due to the small value of zeta potential because of the absence of ionizable groups in the hydrophilic PEG corona of LCMs. The developed LCMs were still stable due to the PEG shell provided by Gelucire and resulting in steric hindrance stabilization [30]. Here, the addition of NFD did not significantly affect any of the measured parameters. The method described here is reproducible and has the advantage of being a low-energy method, eliminating the need for ultrasonication or high-pressure homogenization to achieve small sizes and low polydispersity. The homogeneity and narrow size distribution of the micelles can be observed in the TEM micrograph in Fig. 2.

Thermal analysis by differential scanning calorimetry

Since Gelucire 44/14 has a melting point of 44 °C and is thus solid at room temperature, the physical state of the particles obtained was investigated in two DSC experiments. First, we analyzed a concentrated solution of micelles in a hermetic pan; and second, a freeze-dried sample of micelles in a conventional DSC pan. Figure 3 shows the comparison of these two samples with unprocessed Gelucire 44/14. Only the freeze-dried sample showed an endothermic peak; for micelles in solution, no peak was obtained. Therefore, micelles are solid when freeze-dried, but not in solution. This experiment

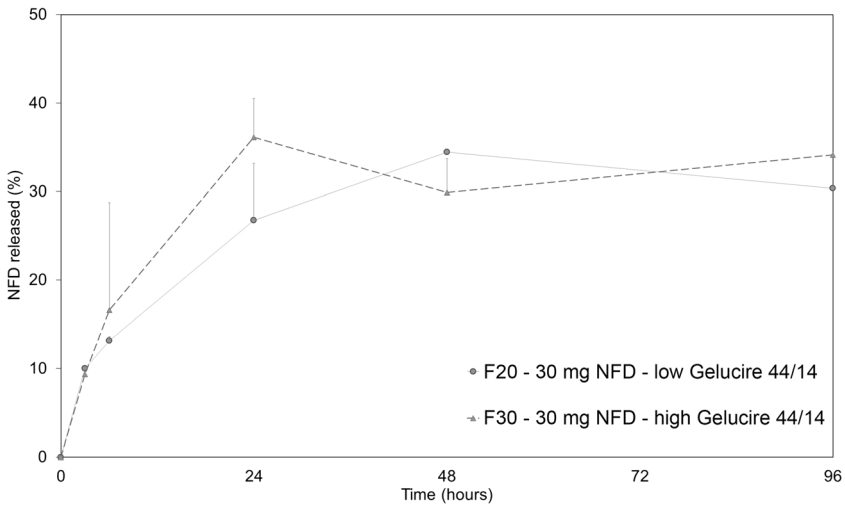

Fig. 6 NFD release (30 mg load in each formulation) of F20 (low Gelucire 44/14 amount) and F30 (high Gelucire 44/14 amount) in phosphate buffer (pH 7.2)

Table 2 Stability test of blank formulations F30 and F20 and FND loaded at 30, 45, and 60 mg NFD in weeks 0 and 1. Storage was conducted at 4 and 25 °C. Full details of stability results are in Table S1 in Electronic Supplementary Materials

Formulation	Time (weeks)	Temperature (°C)	Size (nm)	PDI	Zeta potential (mV)
F30	0	25	13.1 (1.4)	0.184 (0.136)	− 11.2 (1.7)
F30	1	25	26.4 (7.4)	0.491 (0.029)	− 1.5 (0.3)
F30	1	4	27.5 (1.9)	0.465 (0.036)	− 2.5 (0.1)
F30 NFD 30	0	25	12.6 (0.8)	0.350 (0.037)	− 7.8 (4.6)
F30 NFD 30	1	25	34.4 (21.0)	0.366 (0.053)	− 3.7 (0.2)
F30 NFD 30	1	4	29.0 (14.2)	0.460 (0.044)	− 3.2 (0.2)
F30 NFD 45	0	25	9.60 (1.4)	0.278 (0.010)	− 9.8 (0.5)
F30 NFD 45	1	25	21.4 (1.8)	0.398 (0.034)	− 2.1 (0.1)
F30 NFD 45	1	4	22.6 (3.0)	0.361 (0.075)	− 2.9 (0.3)
F30 NFD 60	0	25	13.6 (0.4)	0.306 (0.043)	− 4.7 (1.8)
F30 NFD 60	1	25	18.7 (3.0)	0.426 (0.126)	− 3.4 (0.4)
F30 NFD 60	1	4	27.1 (3.7)	0.477 (0.174)	− 3.3 (0.6)
F20	0	25	12.0 (5.6)	0.266 (0.021)	− 6.2 (3.0)
F20	1	25	22.4 (1.8)	0.351 (0.035)	− 2.2 (0.1)
F20	1	4	24.3 (1.2)	0.424 (0.061)	− 3.0 (0.1)
F20 NFD 30	0	25	13.8 (0.3)	0.361 (0.019)	− 5.0 (3.7)
F20 NFD 30	1	25	22.4 (2.9)	0.438 (0.052)	− 3.3 (0.3)
F20 NFD 30	1	4	26.6 (3.1)	0.461 (0.043)	− 3.4 (0.1)
F20 NFD 45	0	25	12.7 (0.9)	0.147 (0.010)	− 19.2 (1.3)
F20 NFD 45	1	25	22.2 (0.7)	0.421 (0.030)	− 1.3 (0.5)
F20 NFD 45	1	4	28.5 (5.2)	0.358 (0.077)	− 2.5 (0.5)
F20 NFD 60	0	25	10.9 (0.2)	0.322 (0.035)	− 7.7 (1.2)
F20 NFD 60	1	25	35.5 (16.4)	0.565 (0.280)	− 5.3 (1.1)
F20 NFD 60	1	4	70.9 (46.5)	0.914 (0.115)	− 5.4 (0.7)

is commonly used to characterize the melting of SLNs, and we conducted this to clarify the state of the PEGylated lipids of LCMs that are solids at room temperature [31]. In Fig. 4, the graph representing the freeze-dried micelle from Fig. 3 is re-plotted at an enlarged scale, at which an endothermic peak can clearly be seen. Figure 3 shows an interesting decrease in the melting point of Gelucire, a result of the added surfactants, such as Tween 20 and Span 80. It has previously been described that the mixture of different surfactants can have a plasticizing effect, thereby affecting physical properties, such as the melting point [32]. This effect is further corroborated by analyzing the physical mixture where the Gelucire 44/14 melting point appears at a similar range as that of the pure material. Results of the other pure materials in the temperature range indicate no significant changes after LCM production.

Encapsulation efficiency and drug loading

We found the micelles to have a better encapsulation efficiency (EE) profile in F30 formulations, probably due to the increased proportion of Gelucire in these formulations, allowing the encapsulation of greater amounts of micelles and NFD. As can be seen in Fig. 5, the formulation with the best EE was F30 with 30 mg of NFD. EE decreased with increasing amounts of NFD, an effect related to the formulations' maximum loading capacity. Excess drug that has not been loaded into micelles by the second week usually precipitates and crystalizes. Nevertheless, for F30 30 mg NFD, this was not seen within the whole month. Of note, the solubility of nifedipine in water is 0.0059 mg/mL [33], while our F30 30 mg NFD formulation allowed for the dispersion of around 0.47 mg/mL, a more than 700-fold increase. Variations in drug loading (DL) were not statistically significant. The average DL across formulations was 8.2, with the highest standard deviations observed for F20–45 mg NFD and F20–60 mg NFD. These two formulations showed the least stability over the duration of experiments.

Nifedipine release study

Release studies were conducted for the formulations with the highest EE; the results are shown in Fig. 6. The main difference between both formulations is that the standard deviation for F30 shows a greater variability across triplicates. However, both formulation released more than 25% over 4 days. There are no statistically significant differences between release profiles, and after 24 h, both release profiles seem to reach a plateau around 30%. The low amount of

drug released and the similarity of release values between 24 and 96 h characterize the release profile as one of long-term releases. The low release rate might represent an advantage for intracellular delivery, which has been described for nanoparticles [34–37]. Furthermore, the use of PEG has been described to result in stealth nanosystems, thus extending the time that nanoparticles can remain in circulation. In addition, the nanosystem developed here is a good candidate for targeted release [38]; as has been described, the addition of a ligand to the PEG corona enables nanosystems to target specific cells or tissues. The use of biotin and folate as ligands in polymeric micelles is an example of targeting cancer with PEG-coated nanoparticles [39–41].

Stability test results

Another important aspect to consider is micelle stability. Loaded micelles were more stable, although starting in the first or second week, excess NFD began to crystallize at the bottom of the reaction containers. Micelles were most stable at room temperature, but even then, stability was not maintained after 2 weeks, after which there was a marked increase in size over 50 nm in almost all cases (see Table 1S in electronic supplementary materials). The zeta potential generally does not fall below − 10 mV; and over time, it approaches 0. The PDI shows a noticeable increase from the second week onwards, which could reflect an increase in the micelle size populations, as well as indicating that at room temperature, the growth and aggregation of particles to larger sizes occurs between the first and second week. In contrast, for refrigerated micelles, the value was relatively stable over time, which could mean that the growth of the particles is insignificant (Table 2 and electronic supplementary materials).

Conclusions

Hot-melt emulsification, a low-energy method previously described for SLNs, should be considered for the preparation of LCMs. The energy savings resulting from the lack of mechanical homogenization, such as high-pressure homogenization, is of importance for the industry, and studies using low-energy methods are becoming more frequent. Beyond energy efficiency, hot-melt emulsification shows real promise for the preparation of these novel Gelucire 44/14-based LCMs, including the resulting small particle sizes and low PDI values shown here, indicating just one population of LCMs. Further advantages lie in the capacity for stealth nanocarriers resulting from the PEG corona, and the high increase of dispersible NFD—used here as a model of a poorly water-soluble drug—in an aqueous medium. Together, with a long-term release profile, these characteristics could be exploited to develop nanocarriers for intravenous administration and passive and active targeting. Further optimization of these LCMs should address the issues of nanocarrier stability.

Funding information The authors acknowledge the funding support from FONDECYT 1181689, FONDAP 15130011, and STINT IB2015-6087.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kawakami K. Supersaturation and crystallization: non-equilibrium dynamics of amorphous solid dispersions for oral drug delivery. Expert Opin Drug Deliv. 2017;14:735–43. https://doi.org/10.1080/17425247.2017.1230099.
2. Semalty A. Cyclodextrin and phospholipid complexation in solubility and dissolution enhancement: a critical and meta-analysis. Expert Opin Drug Deliv. 2014;11:1255–72. https://doi.org/10.1517/17425247.2014.916271.
3. Rehman FU, Shah KU, Shah SU, Khan IU, Khan GM, Khan A. From nanoemulsions to self-nanoemulsions, with recent advances in self-nanoemulsifying drug delivery systems (SNEDDS). Expert Opin Drug Deliv. 2017;14:1325–40. https://doi.org/10.1080/17425247.2016.1218462.
4. Al-Kassas R, Bansal M, Shaw J. Nanosizing techniques for improving bioavailability of drugs. J Control Release. 2017;260:202–12. https://doi.org/10.1016/j.jconrel.2017.06.003.
5. Fahr A, Liu X. Drug delivery strategies for poorly water-soluble drugs. Expert Opin Drug Deliv. 2007;4:403–16. https://doi.org/10.1517/17425247.4.4.403.
6. Alam MI, Beg S, Samad A, Baboota S, Kohli K, Ali J, et al. Strategy for effective brain drug delivery. Eur J Pharm Sci. 2010;40:385–403. https://doi.org/10.1016/j.ejps.2010.05.003.
7. Esposito E, Boschi A, Ravani L, Cortesi R, Drechsler M, Mariani P, et al. Biodistribution of nanostructured lipid carriers: a tomographic study. Eur J Pharm Biopharm. 2015;89:145–56. https://doi.org/10.1016/j.ejpb.2014.12.006.
8. Natarajan J, Baskaran M, Humtsoe LC, Vadivelan R, Justin A. Enhanced brain targeting efficacy of olanzapine through solid lipid nanoparticles. Artif Cells Nanomedicine Biotechnol. 2017;45:364–71. https://doi.org/10.3109/21691401.2016.1160402.
9. Prajapat MD, Patel NJ, Bariya A, Patel SS, Butani SB. Formulation and evaluation of self-emulsifying drug delivery system for nimodipine, a BCS class II drug. J Drug Deliv Sci Technol. 2017;39:59–68. https://doi.org/10.1016/j.jddst.2017.02.002.
10. Albekery MA, Alharbi KT, Alarifi S, Ahmad D, Omer ME, Massadeh S, et al. Optimization of a nanostructured lipid carriers system for enhancing the biopharmaceutical properties of valsartan. Dig J Nanomater BIOSTRUCTURES. 2017;12:381–9.
11. Patel RHP and Nanoemulsions RJ For intranasal delivery of riluzole to improve brain bioavailability: formulation development and pharmacokinetic studies. Curr Drug Deliv 2016. http://www.eurekaselect.com/137454/article (accessed October 9, 2017).
12. Jakubiak P, Thwala LN, Cadete A, Preat V, Jose Alonso M, Beloqui A, et al. Solvent-free protamine nanocapsules as carriers for mucosal delivery of therapeutics. Eur Polym J. 2017;93:695–705. https://doi.org/10.1016/j.eurpolymj.2017.03.049.
13. Kumari P, Muddineti OS, Rompicharla SVK, Ghanta P, Karthik ABBN, Ghosh B, et al. Cholesterol-conjugated poly(D, L-lactide)-based micelles as a nanocarrier system for effective

delivery of curcumin in cancer therapy. Drug Deliv. 2017;24:209–23. https://doi.org/10.1080/10717544.2016.1245365.
14. Martins S, Sarmento B, Ferreira DC, Souto EB. Lipid-based colloidal carriers for peptide and protein delivery-liposomes versus lipid nanoparticles. Int J Nanomedicine. 2007;2:595.
15. Torchilin VP. Lipid-core micelles for targeted drug delivery. Curr Drug Deliv. 2005;2:319–27.
16. Čerpnjak K, Zvonar A, Vrečer F, Gašperlin M. Development of a solid self-microemulsifying drug delivery system (SMEDDS) for solubility enhancement of naproxen. Drug Dev Ind Pharm. 2015;41:1548–57. https://doi.org/10.3109/03639045.2014.971031.
17. Hosny KM, Aljaeid BM. Sildenafil citrate as oral solid lipid nanoparticles: a novel formula with higher bioavailability and sustained action for treatment of erectile dysfunction. Expert Opin Drug Deliv. 2014;11:1015–22. https://doi.org/10.1517/17425247.2014.912212.
18. Friedrich I, Müller-Goymann C. Characterization of solidified reverse micellar solutions (SRMS) and production development of SRMS-based nanosuspensions. Eur J Pharm Biopharm. 2003;56:111–9. https://doi.org/10.1016/S0939-6411(03)00043-2.
19. Gao Z, Lukyanov AN, Singhal A, Torchilin VP. Diacyllipid-polymer micelles as nanocarriers for poorly soluble anticancer drugs. Nano Lett. 2002;2:979–82. https://doi.org/10.1021/nl025604a.
20. Mu L, Chrastina A, Levchenko T, Torchilin VP. Micelles from poly(ethylene glycol)–phosphatidyl ethanolamine conjugates (peg-Pe) as pharmaceutical nanocarriers for poorly soluble drug camptothecin. J Biomed Nanotechnol. 2005;1:190–5. https://doi.org/10.1166/jbn.2005.030.
21. Wang J, Mongayt DA, Lukyanov AN, Levchenko TS, Torchilin VP. Preparation and in vitro synergistic anticancer effect of vitamin K3 and 1,8-diazabicyclo[5,4,0]undec-7-ene in poly(ethylene glycol)-diacyllipid micelles. Int J Pharm. 2004;272:129–35. https://doi.org/10.1016/j.ijpharm.2003.12.011.
22. Kocbek P, Baumgartner S, Kristl J. Preparation and evaluation of nanosuspensions for enhancing the dissolution of poorly soluble drugs. Int J Pharm. 2006;312:179–86. https://doi.org/10.1016/j.ijpharm.2006.01.008.
23. Vivek K, Reddy H, Murthy RS. Investigations of the effect of the lipid matrix on drug entrapment, in vitro release, and physical stability of olanzapine-loaded solid lipid nanoparticles. AAPS PharmSciTech. 2007;8:16–24.
24. Harivardhan Reddy L, Vivek K, Bakshi N, Murthy RSR. Tamoxifen citrate loaded solid lipid nanoparticles (SLN™): preparation, characterization, in vitro drug release, and pharmacokinetic evaluation. Pharm Dev Technol. 2006;11:167–77. https://doi.org/10.1080/10837450600561265.
25. Solans C, Izquierdo P, Nolla J, Azemar N, Garciacelma M. Nano-emulsions. Curr Opin Colloid Interface Sci. 2005;10:102–10. https://doi.org/10.1016/j.cocis.2005.06.004.
26. Yukuyama MN, Ghisleni DDM, Pinto TJA, Bou-Chacra NA. Nanoemulsion: process selection and application in cosmetics—a review. Int J Cosmet Sci. 2016;38:13–24. https://doi.org/10.1111/ics.12260.
27. Koroleva MY, Yurtov EV. Nanoemulsions: the properties, methods of preparation and promising applications. Russ Chem Rev. 2012;81:21–43. https://doi.org/10.1070/RC2012v081n01ABEH004219.
28. Lukyanov AN, Gao Z, Mazzola L, Torchilin VP. Polyethylene glycol-diacyllipid micelles demonstrate increased accumulation in subcutaneous tumors in mice. Pharm Res. 2002;19:1424–9. https://doi.org/10.1023/A:1020488012264.
29. Mandawgade SD, Sharma S, Pathak S, Patravale VB. Development of SMEDDS using natural lipophile: application to β-artemether delivery. Int J Pharm. 2008;362:179–83. https://doi.org/10.1016/j.ijpharm.2008.06.021.
30. Sawant RR, Torchilin VP. Multifunctionality of lipid-core micelles for drug delivery and tumour targeting. Mol Membr Biol. 2010;27:232–46. https://doi.org/10.3109/09687688.2010.516276.
31. Behbahani ES, Ghaedi M, Abbaspour M, Rostamizadeh K. Optimization and characterization of ultrasound assisted preparation of curcumin-loaded solid lipid nanoparticles: application of central composite design, thermal analysis and X-ray diffraction techniques. Ultrason Sonochem. 2017;38:271–80. https://doi.org/10.1016/j.ultsonch.2017.03.013.
32. Gumede TP, Luyt AS, Pérez-Camargo RA, Iturrospe A, Arbe A, Zubitur M, et al. Plasticization and cocrystallization in LLDPE/wax blends. J Polym Sci Part B Polym Phys. 2016;54:1469–82. https://doi.org/10.1002/polb.24039.
33. Ran Y, He Y, Yang G, Johnson JL, Yalkowsky SH. Estimation of aqueous solubility of organic compounds by using the general solubility equation. Chemosphere. 2002;48:487–509.
34. Weissleder R, Cheng H-C, Bogdanova A, Bogdanov A. Magnetically labeled cells can be detected by MR imaging. J Magn Reson Imaging. 1997;7:258–63.
35. Moore A, Basilion JP, Chiocca EA, Weissleder R. Measuring transferrin receptor gene expression by NMR imaging. Biochim Biophys Acta BBA-Mol Cell Res. 1998;1402:239–49.
36. Schoepf U, Marecos EM, Melder RJ, Jain RK, Weissleder R. Intracellular magnetic labeling of lymphocytes for in vivo trafficking studies. BioTechniques. 1998;24:642–51.
37. Chou LYT, Ming K, Chan WCW. Strategies for the intracellular delivery of nanoparticles. Chem Soc Rev. 2011;40:233–45. https://doi.org/10.1039/C0CS00003E.
38. Otsuka H, Nagasaki Y, Kataoka K. PEGylated nanoparticles for biological and pharmaceutical applications. Adv Drug Deliv Rev. 2003;55:403–19. https://doi.org/10.1016/S0169-409X(02)00226-0.
39. Yoo HS, Park TG. Folate receptor targeted biodegradable polymeric doxorubicin micelles. J Control Release. 2004;96:273–83. https://doi.org/10.1016/j.jconrel.2004.02.003.
40. Patil YB, Toti US, Khdair A, Ma L, Panyam J. Single-step surface functionalization of polymeric nanoparticles for targeted drug delivery. Biomaterials. 2009;30:859–66. https://doi.org/10.1016/j.biomaterials.2008.09.056.
41. Yuan H, Miao J, Du Y, You J, Hu F, Zeng S. Cellular uptake of solid lipid nanoparticles and cytotoxicity of encapsulated paclitaxel in A549 cancer cells. Int J Pharm. 2008;348:137–45. https://doi.org/10.1016/j.ijpharm.2007.07.012.