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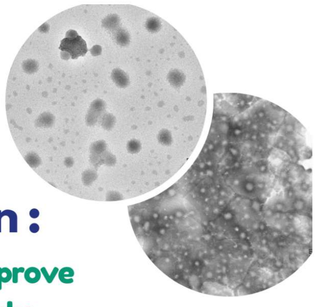

Microfluidization : an eco-friendly process to improve oral bioavailability of poorly soluble API

Skyepharma LAGEPP Université Claude Bernard Lyon 1

INSIGHT

Microfluidization : an eco-friendly process to improve oral bioavailability of poorly soluble API

Skyepharma reinforces its strong collaboration with Lyon University to develop an eco-friendly process to take up the challenge of improving bioavailability of poorly soluble APIs. Very promising results are obtained with different Solid Lipid Nanoparticles and Nanostructured Lipid Carriers.

INTRODUCTION

Oral drug administration is the preferred route because it ensures better patient compliance to their therapies. However, many factors limit the effectiveness of oral treatments, including the poor bioavailability of some Active Pharmaceutical Ingredients (API). It is estimated that 60% of new available API are poorly water-soluble and belong to the Biopharmaceutics Classification System (BCS) classes II and IV. Most of them also have a low oral bioavailability due to significant hepatic metabolism or efflux by P-glycoprotein. Considering these issues, encapsulation of BCS class II or IV API in lipid-based nanoparticles has become an innovative strategy to improve their solubility but also to control its release and avoid hepatic first pass effects (1-2). The main objective of the present work is to develop Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC) in order to improve the oral efficacy of therapeutic molecules with poor water solubility and permeability. Both SLN and NLC have the advantage of being biocompatibles and biodegradables. They are set by an ecological-friendly manufacturing process without any solvents and using an advanced technology, the microfluidization (high pressure homogenization process), presenting also the advantage to be easily scaled-up (3-4). Thus, the final aim of this project is to transpose the encapsulation process to an industrial scale at Skyepharma and propose new innovative and efficient oral drug delivery systems for BCS II or IV API.

MATERIALS AND METHODS

Materials

Spironolactone (SPI) was the selected model of BCS class II API for the proof of concept. Concerning the lipidic excipients, Precirol® ATO5 (PATO5; melting point 51-58°C), Capryol® 90 (C90) and Maisine® CC (MAI) were a generous gift of Gattefossé S.A.S. (Saint Priest, France). The surfactant; poloxamer 188, Kolliphor® P188 (KOL188) was a gift from BASF (Levallois-Perret , France).

Preparation of SLN and NLC nanosuspensions

Formulation of SLN and NLC were developed according to a previous work of Dumont et al.(5). SLN nanosuspensions was composed of two phases: the lipidic with spironolactone dissolved in PATO5 and the aqueous with a surfactant KOL188 dissolved in deionized water. The two phases were heated separately to 70°C and then homogenized by high shear agitation (Ultra-turrax®, IKA) at 11000 rpm during 3 min. This pre-emulsion was inserted in the Microfluidizer® LM20 (Microfluidics) tank and went through the system until the Z-type chamber G10Z at 750 bars. Finally, this nanoemulsion was roughly cooled down at 4°C during 7 min, allowing PATO5 to solidify and become lipid nanoparticles. For NLC formulation, SPI was previously dissolved in a liquid lipid (C90 or MAI) and added to melt PATO5 to form the lipid phase of the pre-emulsion.

Particle size characterization

Nanoparticle mean diameters (D50) and their polydispersity index (PDI) were quantified by Diffraction Light Scattering with a Zetasizer Nano ZS (Malvern).

Transmission Electron Microscopy

Morphology of nanoparticles was characterized by Transmission Electron Microscopy (TEM) with a JEM-1400 Flash Electron Microscope (Jeol). The samples were diluted 10-fold in deionized water to allow a clearer identification.

Entrapment efficiency quantification

The amount of loaded SPI was expressed with the encapsulation efficiency (EE), which represents the amount of API effectively encapsulated compared to the total amount of API used.

Sample preparation

The samples were prepared using ultra-filtration and centrifugation. 500µL of nanosuspension were placed in Eppendorf tubes containing Amicon® Ultra-0.5, with a 30kDa cut-off. (Millipore) and were centrifuged at 10.000 x g for 10 min at 4°C. 200 µL of the aqueous supernatant were then collected, diluted in methanol, filtered through membrane filters 0.22 µm and analyzed by UHPLC. The total amount of SPI in nanosuspensions was determined bydissolving a sample of SLN or NLC in acetonitrile and UHPLC quantification after sample dilution.

Ultra-HPLC determination

SPI was quantified with an Acquity Arc UHPLC (Waters) equipped with a Cortecs C18 2.7µm, 4.6×50 nm column (Waters). The mobile phase was composed of 60% methanol and 40% water with 0.1% acetic acid, at the temperature of 50°C. The flow rate was 0.4 mL/min, the injection volume was 10µL during a 5 min run time and the UV detection wavelength was at 238 nm. The calibration curve was comprised between 0.1 to 50 µg/mL.

RESULTS AND DISCUSSION

Blank nanoparticles

Particle size characteristics of blank NLC were satisfactory with mean diameter less than 200 nm and PDI below 0.2 (Table 1). The SLN formulation provided higher results even if they are still acceptable.

	SLNs	NLC-C90	NLC-MAI
D50 (nm)	192.84 ± 13.92	168.22 ± 32.94	143.37 ± 0.49
PDI	0.174 ± 0.026	0.180 ± 0.042	0.184 ± 0.018

Table 1. Particle size characteristics of blank SLNs and NLCs (n=3), mean ± SEM

These data were confirmed by TEM with respect to the particle size distribution. The nanosuspension observation also demonstrated that SLN and NLC have a spherical shape, in line with our expectations (Figure 1).

Figure 1. TEM images of blank SLN (left) and NLC (right)

SPI-loaded nanoparticles

Compared to the blank nanoparticles, the SPI encapsulation in both SLN and NLC improved their size results with smaller nanoparticles and PDI (Table 2).

	SLNs	NLC-C90	NLC-MAI
D50 (nm)	183.74 ± 33.39	184.97 ± 59.02	176.50 ± 37.53
PDI	0.167 ± 0.055	0.213 ± 0.070	0.217 ± 0.053
EE (%)	60.7 ± 5.3	50.2 ± 18.2	72.8 ± 1.7

Table 2. Particle sizes of SPI-loaded nanoparticles (n=9) and encapsulation efficiency (n=3), mean ± SEM

Concerning NLC formulation, MAI allowed the formation of slightly smaller nanoparticles with a narrow distribution, similarly to C90. Concerning the EE, NLC-CMAI allowed the highest rate of EE since 72.8 % of SPI was encapsulated whereas only 60.7 and 50.2% of SPI was retrieved in SLN and NLC-C90 respectively.

CONCLUSION

As expected, microfluidization allowed the formation of SLN and NLC with mean diameters lower than 200 nm. The addition of a liquid lipid did not have a significant impact on the size or polydispersity index of the nanoparticles but significantly improved the entrapment efficiency of spironolactone, especially for Maisine®CC NLC. In addition, both SLN and NLC had a spherical shape. However, the release profile of SPI from these nanoparticles has to be investigate. The microfluidization method thus presents a real interest in formulation but also an ability of industrial transposition, which will be the main prospect of this project. Moreover, a drying process of these lipid nanoparticles will be study in order to develop solid dosage forms for oral administration of these BCS II or IV API encapsulated in lipid nanoparticles.

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Oksana Lemasson1; Sandrine Bourgeois2; Vanessa Bourgeaux3; Stéphanie Briançon4
1 Université Claude Bernard Lyon 1, LAGEPP UMR CNRS 5007, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France , oksana.lemasson@univ-lyon1.fr
2 Université Claude Bernard Lyon 1, ISPB-School of Pharmacy, LAGEPP UMR CNRS 5007, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France , sandrine.bourgeois@univ-lyon1.fr
3 Skyepharma Production S.A.S, 55 rue du Montmurier, F-38070 Saint-Quentin-Fallavier, France, v.bourgeaux@skyepharma.fr
4 Université Claude Bernard Lyon 1, ISPB-School of Pharmacy, LAGEPP UMR CNRS 5007, 43 boulevard du 11 novembre 1918, F-69100 Villeurbanne, France, stephanie.briancon@univ-lyon1.fr