International Journal of Pharmaceutics 379 (2009) 260–265

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

The gastrointestinal stability of lipid nanocapsules

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ARTICLE INFO

Article history:
Received 31 January 2009
Received in revised form 27 March 2009
Accepted 18 May 2009
Available online 12 June 2009

Keywords:
Lipid nanocapsules
Paclitaxel
Stability
Gastrointestinal tract
Simulated media

ABSTRACT

The *in vitro* gastrointestinal stability of lipid nanocapsules (LNCs) was studied in different media. The size of LNCs was determined in simulated gastric and intestinal media. In updated fasted state simulated intestinal fluid (FaSSIF-V2) and updated fed state simulated intestinal fluid (FeSSIF-V2) media, the encapsulation ratio of paclitaxel-loaded LNCs was also measured. The size of LNCs was not modified after 3 h in simulated gastric fluid and simulated intestinal fluid described by the United States Pharmacopeia, in FaSSIF, FaSSIF-V2, and in FeSSIF. Moreover, in the presence of pancreatin in FeSSIF-V2, a decreased above 30% of the loading of paclitaxel was observed. This was attributed to the presence of lipase in pancreatin that could interact with Lipoid® (a mixture of phosphatidylcholine and phosphatidylethanolamine), present on the shell of LNC. As a conclusion, LNCs were stable on gastric medium and fasted state intestinal medium.

1. Introduction

Lipid nanocapsules (LNCs) form a new generation of nanovectors that can encapsulate some anticancer agents (Allard et al., 2008; Garcion et al., 2006; Lamprecht and Benoit, 2006; Peltier et al., 2006) and can be administered by different routes. One of the recent applications of these colloidal carriers is the oral administration of drugs that allows an improved quality of life for the patient. For some lipophilic drugs, such as paclitaxel for example, oral administration is problematic because of paclitaxel's low water solubility and its affinity for Cytochrome P-450 3A4, or 2C8 enzymes and the multidrug efflux pump (Schellens et al., 2000; Sparreboom et al., 1997). Thus, its encapsulation in a lipid formulation could provide a solution to improve the oral bioavailability of these compounds. Some publications have already shown the interest of using these novel drug delivery systems orally (Bromberg, 2008; Gao et al., 2003; Zhang and Feng, 2006). An *in vivo* study of paclitaxel-loaded LNCs has already shown an increase in oral bioavailability by a factor of 3 (Peltier et al., 2006). Nevertheless, the reason for this improvement is not yet understood. The first barrier to cross after oral administration is constituted by the physicochemical environment of the gastrointestinal tract. The drug must then be transported across the intestinal epithelium. Some polymeric nanoparticles have already shown degradation in the gastrointestinal tract due to the variation of the pH level, the presence of enzymes or bile salts (Kuentz, 2008). On the other hand, lipid-based delivery systems like self-emulsifying or self-microemulsifying drug delivery system (SEDDS or SMEDDS, respectively) form emulsions and thus "dissolve" the drug in the gastrointestinal tract (Constantinides, 1995; Porter et al., 2007). Moreover, it is well known that lipids are rapidly digested by gastric and intestinal enzymes and absorbed in the form of micellar structures by enterocytes (Pouton, 2000; Subramanian and Ghosal, 2004). Consequently, the gastrointestinal tract is a barrier that can limit LNCs absorption by disruption. Over the last few years, some research teams have tried to establish simulated gastrointestinal fluids with *in vitro–in vivo* correlations (Dressman and Lennernas, 2000; Horter and Dressman, 2001; Lipka and Amidon, 1999), but to date, no standard model has been recognised. The aim of this study was to investigate LNCs stability in different gastrointestinal fluids.

2. Materials and methods

2.1. Materials

Captex® 8000 (tricaprylin) was a gift from Abitec Corp. (Colombus, Ohio, USA) via Unipex (Rueil-Malmaison, France). Lipoid® S75-3 (soya bean lecithin at 70% phosphatidylcholine and 10% phosphatidylethanolamine) and Solutol® HS15 (a mixture of free polyethylene glycol 660 and polyethylene glycol 660 hydroxystearate) were gifts from Lipoid Gmbh (Ludwigshafen, Germany) and BASF (Ludwigshafen, Germany), respectively. NaCl was purchased

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doi:10.1016/j.ijpharm.2009.05.069

from Prolabo VWR International (Fontenay-sous-Bois, France). Paclitaxel powder used for LNCs formulation was from Bioxel Pharma (Quebec, Canada). Purified water was obtained from a MilliQ185 System (Waters, Saint-Quentin-en-Yveline, France). Acetonitrile, acetone, methanol and tetrahydrofurane HPLC grade were from Sigma–Aldrich (Saint-Quentin-Fallavier, France) and Carlo Erba Reactifs (Val-de-Reuil, France).

Sodium monobasic phosphate anhydrous, maleic acid, and pancreatin (8× USP specification) were purchased from Sigma–Aldrich. Pepsin (European Pharmacopeia), sodium taurocholate, lecithin, sodium hydroxide and 1-oleoyl-*rac*-glycerol were obtained from Fluka (Buchs, Switzerland). Calcium chloride and acetic acid were purchased from Prolabo. Sodium oleate (purity >82%) was obtained from Riedel-de Haen (Selze, Germany).

2.2. Methods

2.2.1. Preparation and characterisation of blank LNC or paclitaxel-loaded LNC

LNCs were prepared according to the original process described by Heurtault et al. (2002) including several changes. Briefly, Captex® 8000 (29%, w/w) and Lipoid® S75-3 (1.6%, w/w) were mixed and heated at 85 °C. Solutol® HS15 (24.15%, w/w), NaCl (1.77%, w/w) and water (43.48%, w/w) were added and homogenised under magnetic stirring. Three cycles of progressive heating and cooling between 70 and 90 °C were then carried out and followed by an irreversible shock induced by dilution with 2 °C purified water (73%, v/v) added to the mixture at 78 °C. Slow magnetic stirring was then applied to the suspension of LNCs for 5 min at room temperature.

To prepare paclitaxel-loaded LNCs, 29.3 mg of paclitaxel was dissolved in Captex® 8000 in the presence of ethanol and the solvent was evaporated at 80 °C before use. LNCs were then prepared as described previously.

The size of the nanoparticles was measured by dynamic light scattering (DLS) on a Zetasizer Nano series DTS 1060 (Malvern Instruments S.A., Worcestershire, UK).

To determine the paclitaxel encapsulation ratio, LNCs were filtered using a Minisart® 0.2 μm filter (Vivascience AG, Hanovre, Germany) after formulation in order to eliminate paclitaxel crystals (paclitaxel not encapsulated). Three samples of filtrate were prepared by dissolution of an exact quantity of LNCs dispersion in a 96/4 (v/v) methanol/tetrahydrofurane solution and then filtrated on a Minisart® 0.2 μm filter in order to eliminate the residual components of the LNCs. A 15 μL aliquot of each filtrate was injected in triplicate onto the high-performance liquid chromatograph (HPLC) column. Chromatography was performed using a Waters 717 plus autosampler, Waters 600 controller and Waters 2487 Dual Absorbance Spectrometer (Waters S.A., Saint-Quentin-en-Yvelynes, France) with an XTerra® C_{18}-ODB 150 mm × 4.60 mm column (Waters, Milford, Ireland) and a UV detector set at 227 nm. The flow rate was set to 1 mL/min. The gradient was obtained by mixing proportion of phase A (water) and phase B (acetonitrile). Initially, the mobile-phase composition was 50% B; a linear gradient was applied to reach a composition of 85% B after 7 min, maintained for 2 min and then returned to the initial conditions. Quantification was achieved by comparing observed peak area ratios of paclitaxel in the samples to a calibration curve obtained under the same experimental conditions. Linearity was observed in the range from 39.0 to 390.2 mg/L with a correlation coefficient above 0.99. The detection limit was 10.0 mg/L and the quantification limit was 20.0 mg/L. The mean drug payload (mg of paclitaxel/g of LNCs dispersion) of each batch of LNCs dispersion and the standard deviation were calculated from three samples. The encapsulation efficiency (%) was determined by dividing the measured-drug payload by the drug payload calculated with the exact quantity of each component of the formulation.

Table 1
Composition of the medium to simulate the fasted state, upper small intestine: FaSSIF and FaSSIF-V2.

Composition/medium	FaSSIF	FaSSIF-V2
Sodium taurocholate	3 mM	3 mM
Lecithin	0.75 mM	0.2 mM
Sodium monobasic phosphate anhydrous	28.65 mM	–
Maleic acid	–	19.12 mM
Sodium hydroxide	8.71 mM	34.8 mM
Sodium chloride	105.85 mM	68.62 mM
pH	6.5	6.5

2.2.2. Composition of various media

Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared according to USP XXIV (US.Pharmacopeia.XXIV, 2006). The SGF medium contained 0.32% (w/v) of pepsin; the pH was 1.2. The SIF medium was composed of 1% (w/v) pancreatin with a pH of 7.5.

The FaSSIF/FeSSIF fluids represent a simplification of the proximal small intestine composition in the fasted state (FaSSIF) or in the fed state (FeSSIF). These media were made up according to the literature (Galia et al., 1998; Lobenberg et al., 2000; Nicolaides et al., 2001). The composition is summarised in Tables 1 and 2.

The FaSSIF-V2 and FeSSIF-V2 fluids are updated (V2) versions of the standard FaSSIF and FeSSIF media. Revision of FaSSIF and FeSSIF media was performed by Jantratid et al. (2008b) in order to better mimic *in vivo* conditions (Porter et al., 2007). FaSSIF-V2 contained one modification: a decrease in the amount of lecithin, whereas in FeSSIF-V2, the pH, the buffer, and the bile component concentration (contains bile salt and lipolysis products) were modified (Tables 1 and 2) (Jantratid et al., 2008b).

2.2.3. Stability study

The LNCs were diluted at a final concentration of 10% (v/v) and incubated at 37 °C in different media. Samples were collected at times 0, 1, 2 and 3 h. The FeSSIF-V2, samples were centrifuged for 5 min at 13,600 × *g* to eliminate aggregates of pancreatin in medium. The size distribution of the particles in suspension was measured by dynamic light scattering on a Zetasizer Nano series DTS 1060 (Malvern Instruments S.A., Worcestershire, UK). Experiments in FaSSIF-V2 and FeSSIF-V2 were also performed with paclitaxel-loaded LNCs for 6 h and the encapsulation efficiencies were determined by LC–MS/MS after filtration using a Minisart® 0.2 μm filter of samples to eliminate free paclitaxel.

Chromatography was performed using a Waters Alliance® 2695 system (Waters S.A.) with an Uptisphere® C_{18}-ODB 150 mm × 2.0 mm, 5 μm column (Interchrom, Montluçon, France). The mobile phase consisted of phase A (0.1% formic acid in water) and phase B (0.1% formic acid in methanol). Under initial conditions, the mobile-phase composition was 30% B; a linear gradient was applied to reach a composition of 98% B after 5 min, maintained

Table 2
Composition of the medium to simulate the fed state, upper small intestine: FeSSIF and FeSSIF-V2.

Composition/medium	FeSSIF	FeSSIF-V2
Sodium taurocholate	15 mM	10 mM
Lecithin	3.75 mM	2 mM
Acetic acid	144.04 mM	–
Maleic acid	–	55.02 mM
Glyceryl monooleate	–	5 mM
Sodium oleate	–	0.8 mM
Sodium hydroxide	101.02 mM	81.65 mM
Sodium chloride	203.18 mM	125.5 mM
Pancreatin	–	100 unit/mL
Calcium chloride	–	5 mM
pH	5	5.8

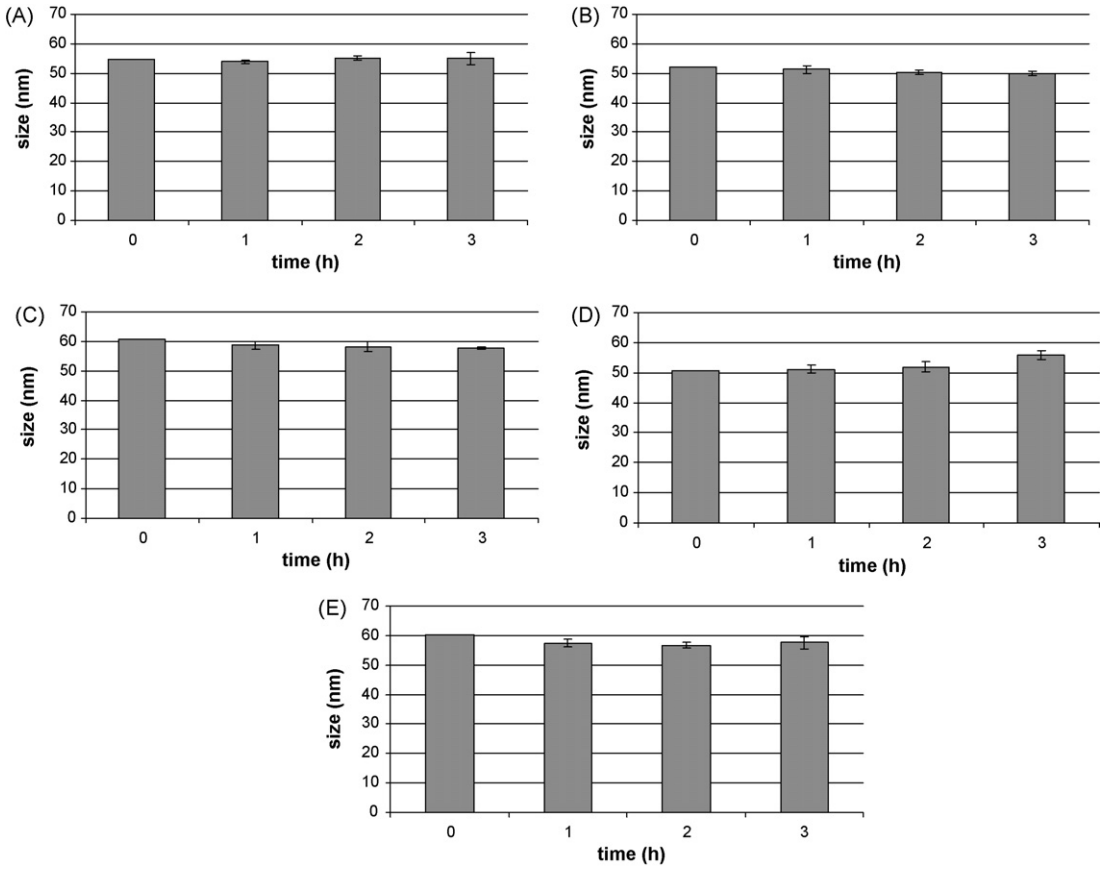

Fig. 1. LNCs particle size following incubation in simulated gastric fluid (SGF) (A), in simulated intestinal fluid (SIF) (B), in simulated intestinal fluid in a fasted state (FaSSIF) (C), in simulated intestinal fluid in a fed state (FeSSIF) (D) and in updated simulated intestinal fluid in a fasted state (FaSSIF-V2) (E) ($n = 3$; data are shown as mean ± S.D.).

for 30 s and then returned to the initial conditions. The flow rate was 0.3 mL/min and the column temperature was set at 40 °C. The total HPLC effluent was directed into a Quattro Micro® triple quadruple mass spectrometer (Waters S.A.). Ionisation was achieved using a turbo ion spray in positive ion mode. The mass spectrometer operated in the multiple reaction monitoring (MRM) mode. The $(M-H)^+$ m/z transitions for each compound were 854.6 → 286.1. A typical retention time of paclitaxel was 6.46 min. Quantification was achieved with QuantLynx® (Waters S.A.) comparing the observed peak area ratios of paclitaxel samples to a calibration curve made under the same conditions. The range of linear response was 0.015–7.2 μg/mL. The lower limit of detection was 0.015 μg/mL and the lower limit of quantification was 0.75 μg/mL.

3. Results

3.1. Characterisation of lipid nanoparticles

Blank LNCs were obtained with a mean size of 53 ± 1 nm with a polydispersity index of 0.058 ± 0.005. In the case of paclitaxel-loaded LNCs, the mean diameter of the particles was 51 ± 2 nm and the polydispersity index was 0.051 ± 0.008. For both formulations the polydispersity index was inferior to 0.1 which demonstrates the monodispersity of preparation. The encapsulation of the paclitaxel was very efficient (93 ± 6%) and the drug payload was 1.84 ± 0.15 mg/g of LNCs dispersion.

3.2. Stability study in simulated gastric medium

The size stability of LNCs in SGF was estimated by DLS. As shown in Fig. 1A, the size of the LNCs was not affected by the gastric environment after 2 h of incubation. Nevertheless, the encapsulation ratio of paclitaxel in LNCs was measured during 2 h of incubation in the gastric medium and a release of 12% of the initial amount of encapsulated paclitaxel was measured (Fig. 2A).

3.3. Stability in simulated intestinal media

In the literature, different media of simulated intestinal fluid have been described and used to evaluate the stability of colloidal carriers. In this paper, different media were used and compared. In the presence of only pancreatin (SIF medium), the size of the LNCs was conserved after 3 h of incubation (Fig. 1B). Using the same experimental procedure, in the presence of bile salts at fed or fasted concentrations, no change of size was observed (Fig. 1C–E). However, in the modified fed state simulated intestinal fluid, which contained bile salts and pancreatin, the size measurement of blank media, i.e. without nanoparticles (Fig. 3B), showed a size above 9 nm, corresponding to micelles. Unfortunately, during the stability experiment on the LNCs, in this media, the same size was measured (Fig. 3A), and no size above 50 nm was observed.

Moreover, in order to investigate the stability of paclitaxel-loaded LNCs in simulated intestinal media, the encapsulation ratio of paclitaxel in LNCs was measured during 6 h of incubation in the medium. As shown in Fig. 2B, the release of paclitaxel after incubation in the FaSSIF-V2 medium is less than 6.5%. However, in the FeSSIF-V2 medium, approximately 30% of the initial amount of encapsulated paclitaxel was released (Fig. 2C).

4. Discussion

In order to evaluate the stability of LNCs in the gastrointestinal tract, the first difficulty was to find the *in vitro* media which have a

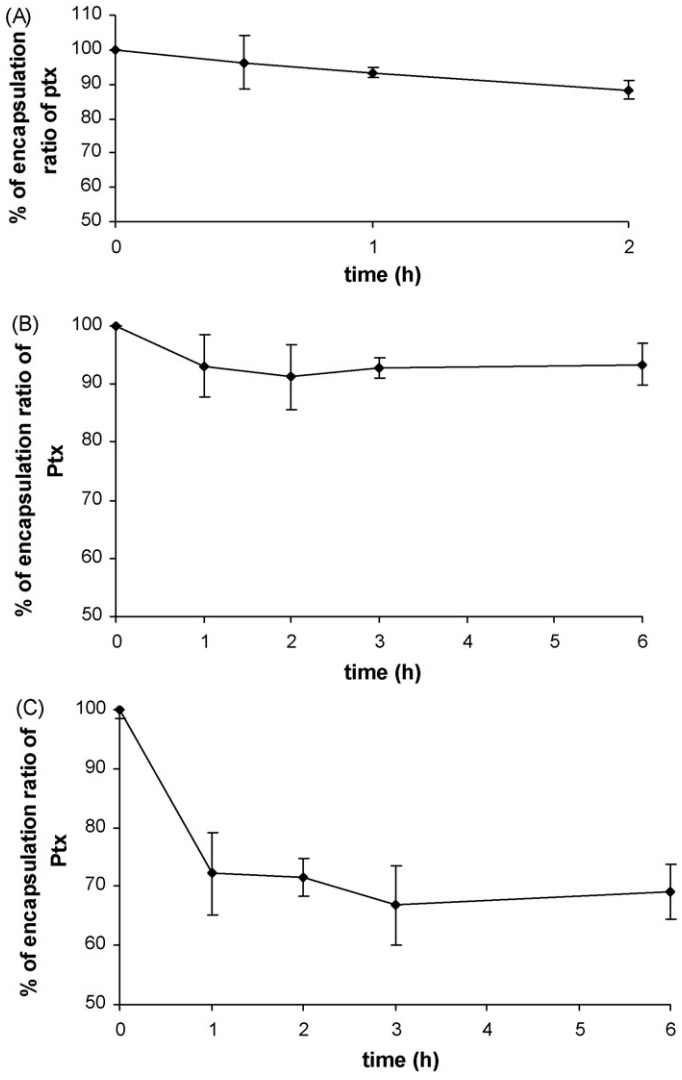

Fig. 2. Percentage of encapsulation rate of paclitaxel following incubation in an updated simulated gastric fluid (SIG) (A), in an updated simulated intestinal fluid in the fasted state (FaSSIF-V2) (B) and in updated simulated intestinal fluid in a fed state (FeSSIF-V2) (C). 100% corresponding to the initial ratio of encapsulation ($n = 3$; data are shown as mean ± S.D.).

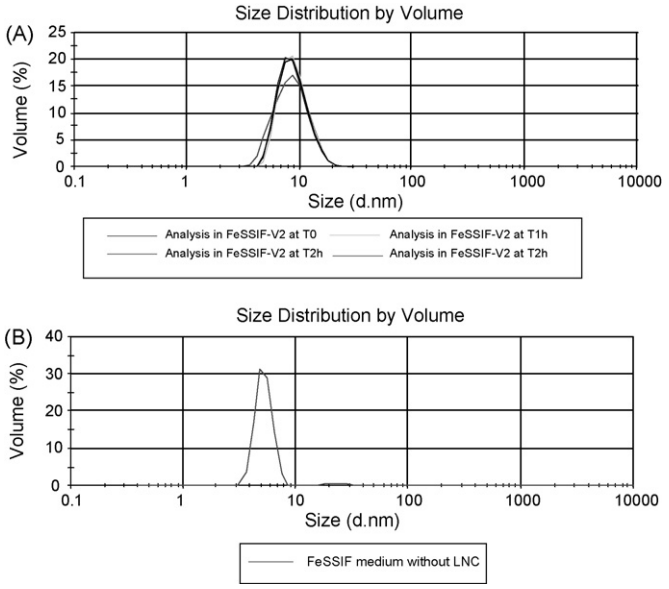

Fig. 3. Example of LNCs size distribution (A) following incubation in updated simulated intestinal fluid in a fed state (FeSSIF-V2) and size distribution of the medium (B) ($n = 3$; data are shown as mean ± S.D.).

chance to be predictive of the *in vivo* fate of LNCs. One difficulty is the complexity of gastrointestinal fluid and by consequence the difficulty of creating a representative model. Indeed, the composition, the volume, the dynamics, the motility, and the gastrointestinal transit vary amongst people. Because of these different characteristics of gastrointestinal media, there are many of simulated fluids developed and used to study drug solubility. Nevertheless, no media are used to study the stability of nanovectors. An *in vitro* lipolysis model was described to simulate dynamic digestion of the lipid formulations (Porter et al., 2008) but in our case lipid was inside nanocapsules. The goal of this study was to investigate the stability of particles. But, in future work, it could be interesting to study the lipolysis of LNCs. Moreover, external influences such as food, disease or drug-taking can modify the transit conditions (Lindahl et al., 1997; McConnell et al., 2008). Because of these different characteristics of gastrointestinal media, there are many of simulated fluids developed and used to study drug solubility. Nevertheless, no media are used to study the stability of nanovectors. Accordingly, the stability of LNCs was analysed in different media. Firstly, simulated fluids, as described by the United States Pharmacopeia, were studied. These media are an essential reference in the evaluation of relating data on drug formulations (US.Pharmacopeia.XXIV, 2006). The simulated gastric fluid has a pH of 1.2 and contains pepsin, a digestive protease that simulates fasting conditions in the stomach. As shown in Fig. 1A, LNCs were stable in gastric media for 3 h and their size was conserved, and approximately 12% of initial amount of encapsulated paclitaxel was released. In the literature, some studies have demonstrated the influence of the particle surface on gastrointestinal stability. PLA nanoparticles presented degradation and an aggregation in gastric medium (Landry et al., 1996; Tobío et al., 2000). In the presence of a polyoxyethylene (PEG) coating, (des Rieux et al., 2007; García-Fuentes et al., 2003; Sahu et al., 2008) the stability of the suspension was improved. The authors explained this difference by the effect of the acidity of the gastric medium on PLA. Indeed, at low pH levels, PLA was rapidly degraded (Makino et al., 1986) whereas PEG surfactants remained stable. A similar result was found with PEG stearate lipid nanoparticles (García-Fuentes et al., 2003). Consequently, PEG managed to consolidate nanoparticles and to protect PLA from degradation. In our case, LNCs contained Solutol® HS15 (a mixture of free PEG 660 and PEG 660 hydroxystearate) on their surface; this could protect Lipoid® and Captex® from acidic degradation but some Paclitaxel could diffuse across the shell of LNCs. Other alternative simulated gastric media are described in the literature (Dressman et al., 1998; Galia et al., 1998; Klein et al., 2004; Vertzoni et al., 2004). Modifications are principally based on the addition of surfactants but while conserving the pH level. As it was demonstrated that it was the pH that affected nanoparticle stability, the lipid nanoparticles were not tested with other media.

After demonstrating the stability of LNCs in the stomach, intestinal stability was studied. Firstly, United States Pharmacopeia media were used, as previously. The size of LNCs particles was conserved (Fig. 1B) after contact with USP SIF. The same stability was observed with PLA or PA nanoparticles coated with PEG (Sahu et al., 2008; Tobío et al., 2000). However, PLA nanoparticles uncoated or coated with albumin (Landry et al., 1996) as well as chitosan nanoparticles (Trapani et al., 2008), have shown rapid degradation that was directly related to the enzymatic activity of pancreatin. This degradation phenomenon was related to the action of the lipase on PLA or was dependent upon an alkaline pH. In the latter case a

reaction with the protons of chitosan was observed. Consequently, the PEG being at the surface of the LNCs protected the particles against pancreatin enzyme activity. Moreover, it was important to notice that the pancreatin concentration given by the USP does not reflect *in vivo* conditions (McConnell et al., 2008). Stability studies were then performed in the FaSSIF and FeSSIF media in order to study the potential action of the bile salts and lecithin (Dressman et al., 1998; Galia et al., 1998). In these media, the mean size of LNCs particles remained unchanged. Recently some modifications of these media have been proposed. Firstly, phosphate buffer was substituted by maleate buffer (Vertzoni et al., 2004). Then, in both, FaSSIF-V2 or FeSSIF-V2, the amount of lecithin was decreased in accordance to *in vivo* data. Moreover, in the fed state simulated intestinal fluid medium two lipid digestion products (glyceryl monooleate and sodium oleate) as well as pancreatin were added (Jantratid et al., 2008b). The amount of pancreatin added was based on the lipase activity because it was the mean enzyme responsible for lipid digestion. Indeed, lipase requires bile salts in order to become functional as well as having a pH above 6 (Borgström, 1954). In both media, based on physiological parameters which are required for the prediction of drug dissolution, the size stability of LNCs was measured and the release of paclitaxel was studied. In FaSSIF-V2, the size of LNCs particles was not modified and paclitaxel remained encapsulated. In FeSSIF-V2, the size analysis was not appropriate because micelles of medium were more abundant than the nanocapsules themselves. Indeed, as in the upper small intestine, there was a strong emulsification process which was due to the presence of bile salts, glyceryl monooleate and sodium oleate (Embleton and Pouton, 1997). On the other hand, paclitaxel-loaded LNCs stability was studied in FeSSIF-V2. After 6 h, a decreased encapsulation ratio was observed (30%). In view of this result, it could be preferable to administrate LNCs in pre-prandial state. These updated media were recently described (Jantratid et al., 2008b). For the moment, only the behaviour of one formulation in these media has been published (Jantratid et al., 2008a). A difference of drug release rates was observed between the FeSSIF and FeSSIF-V2 (called $FeSSIF_a$ in the original paper); the release was increased in the presence of pancreatin and the author suggested that pancreatin facilitated the 'digestion' of lipid formulations. This explanation could apply in the case of LNCs. Furthermore, the concentration of bile salts was at the upper limit of the *in vivo* secretion, being between 5.2 and 11.2 mM in the fed state in humans (Kalantzi et al., 2006). It may therefore be interesting to study the effect of bile salts on paclitaxel release via LNCs. It would be also interesting to study the activity of lipase on lipid nanocapsules. In the literature, an enzyme degradation assay was described in the presence of lipase and co-lipase, and, more especially, this experiment was performed on solid lipid nanoparticles (SLNs) that were composed of a lipid matrix and a surfactant (Müller et al., 1996; Olbrich et al., 2002; Olbrich and Müller, 1999). Contrary to LNCs, the SLN contained cholic acid sodium salt in their formulation and more especially on their surface. Bile salts have the capacity to accelerate the adsorption of lipase on particle surfaces and consequently, the complex lipase/co-lipase can degrade the particles. A similar experiment was performed with LNCs (no different sizes were observed (data not shown)) but this result could be explained by the absence of bile salts in the LNCs formulation. Therefore, SLN stability was different to that of LNCs in the gastrointestinal tract.

5. Conclusion

As no standard relevant *in vitro* media are validated to study the stability of lipid colloidal carriers, a stability study of LNCs was performed on various media in order to check how different stress conditions could affect the integrity of lipid nanocarriers. According to our results LNCs present gastrointestinal stability because of their surface coated with PEG. The intestinal stability experiments showed a better stability in fasted state media as LNCs are more stable in absence of pancreatin. The relevance of the *in vitro* media in predicting *in vivo* stability of LNCs has to be assessed with *in vivo* experiments. This is important to progress in validating standard *in vitro* media reliable for stability studies of colloidal carriers such as LNCs. After this milestone passed it will be possible to benchmark different colloidal carriers that exist.

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