Lauroyl polyoxylglycerides, functionalized coconut oil, enhancing the bioavailability of poorly soluble active substances

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Abstract: *Gelucire® 44/14, a lauroyl polyoxylglycerides obtained by polyglycolysis of hydrogenated coconut oil with PEG-32, is used to increase the oral bioavailability of poorly-water soluble drugs. It is a solid dispersion composed of a PEG ester fraction under a lamellar phase of 120 Å with a helical conformation and an acylglycerol fraction under a hexagonal packing. This excipient spontaneously evolves to its most stable phase of 120 Å after storage at 25 °C for 21 hours leading to physically stable formulations. Gelucire® 44/14 is a hydrophilic system that hydrates and swells in contact with water and forms cubic mesophases before complete erosion/emulsification. It is also lipolyzed by various enzymes such as gastric lipase or carboxyl ester hydrolase. After an in vitro gastrointestinal lipolysis simulation, the main components remaining are mono and diesters of PEG-32. These amphiphilic metabolites can explain the beneficial role of Gelucire® 44/14 on the solubility of poorly-water soluble drugs such as cinnarizine even after partial lipolysis of the lipid-based system. Finally that excipient can also increase the bioavailability of active substances by interacting with enterocyte-based proteins like P-glycoprotein or cytochromes P450.*

Key words: *coconut oil, crystalline structure, emulsification, lauroyl polyoxylglycerides, lipolysis, poorly-water soluble drug*

Introduction

Most of the new chemical entities developed by the pharmaceutical industry are practically insoluble in water and consequently possess a low oral bioavailability [1]. These poorly-water soluble molecules are classified in the class 2 and 4 of the Biopharmaceutics Classification System [2]. In order to efficiently formulate these active substances for the oral route, formulators should either increase the dissolution of the drug in the gastro-intestinal tract or predissolve the drug into the formulation and avoid its precipitation when in contact with the biological fluids. Many formulation techniques can increase the dissolution of poorly-water soluble drugs such as micronization, inclusion in cyclodextrins, addition of surfactants or lipid-based excipients.

The use of lipids and lipid-based excipients in self-emulsifying systems is more and more described in the literature as well as used in marketed products. Recently a classification of these lipid-based systems was introduced and characterized [3] and in addition, many formulation techniques have been developed to produce solid or semi-solid systems [4].

Among these self-emulsifying systems Gelucire® 44/14, a PEG-32 lauroyl polyoxylglycerides (Gattefossé SAS, Saint-Priest, France), is obtained by polyglycolysis of hydrogenated coconut oil (medium and long chain triacylglycerols) and PEG-32. It is composed of a defined admixture of C8-C18 mono-, di- and triacylglycerols (20% w/w), PEG-32 mono- and diesters and free PEG-32 (80% w/w). The main fatty acid present is lauric acid which accounts for 45% on average of the total fatty acids [5-7]. Gelucire® 44/14 has been widely used and characterized during the last five years in order to increase the solubility and bioavailability of many drugs: carbamazepine [8], glibenclamide [9], antiviral agent PG301026 [10], piroxicam [11, 12], propranolol [13, 14], flurbiprofen [15], aceclofenac [16], carvedilol [17], griseofulvin [18], spironolactone [19], and cinnarizine [11].

The aim of this paper is to present the physical and biopharmaceutical characterizations needed to develop a successful formulation with Gelucire® 44/14.

Physical characterization

As Gelucire® 44/14 is a semi-solid crystalline excipient, formulators should characterize the structure of the mixture containing this lipid-based vehicle and the drug substance to ascertain that their formulation is in its most stable form and retains its self-emulsifying properties. In this chapter, the main physical characterization tests needed are presented firstly on the raw material and secondly with two model drug substances.

Thermal analysis

Figure 1 shows the thermogram of the first melting of a Gelucire® 44/14 sample recorded with a Differential Scanning Calorimeter (Pyris Diamond, Perkin-Elmer, USA) calibrated with benzoic acid (T_m = 122.4 °C) and indium (T_m = 156.6 °C, ΔH_m = 26.6 J/g). The thermal analysis was carried out between – 20 and 120 °C at a heating rate of 3 °C/min on a 10 mg sample. Gelucire® 44/14 presents a broad endotherm ranging from 10 to 45 °C with an onset melting temperature of 38.2 °C and a peak melting temperature of 43.2 °C. This thermal behaviour can be explained by the composition of the excipient, a mixture of acylglycerols and PEG esters. The Differential Scanning Calorimetry (DSC) analyses of these two fractions separately *(figure 1)* show that the acylglycerol fraction melts first (representing the first two endothermic events), and then the PEG ester and free PEG fraction melts last (representing the last and main endothermic event).

Crystalline structure

The combined use of X-Ray Diffraction (XRD) and DSC allows the detection of all polymorphs formed after various thermal treatments ranging from quenching into liquid nitrogen to slow crystallization. XRD allows the study of

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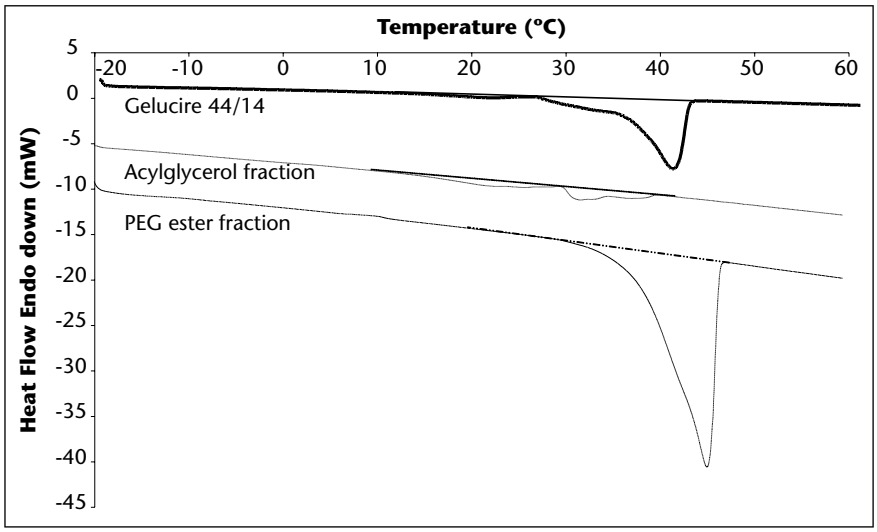

Figure 1. *Thermograms of the first melting of Gelucire® 44/14 (solid line), the acylglycerol fraction of Gelucire® 44/14 (dotted line), and the PEG ester fraction of Gelucire® 44/14 (dashed dot dot line) at a heating rate of 3 °C/min.*

the structure and the polymorphism of lipid-based compound. Wide-Angle X-ray Scattering (WAXS) region corresponds to short reticular distances between hydrocarbon chains while Small-Angle X-ray Scattering (SAXS) domain corresponds to long spacing. DSC, by temperature and enthalpy of phase-transition measurement, shows energy transfers that occur during the heating or cooling of the sample. By combining these two techniques, one can link structural changes to phase transitions.

Gelucire® 44/14 is crystallized in lamellar phases with the PEG chains under a helical conformation. This crystalline structure has already been reported for another polyoxylglycerides containing the same ethylene oxide unit: Gelucire® 50/13 [20]. SAXS analysis of the untreated sample shows a lamellar phase of 120 Å *(figure 2)*. No signal of the acylglycerol fraction was detected. Various polymorphs with shorter lamellar phases such as 90, 94, 99 or 105 Å were detected when the product is melted and crystallized with different crystallization rates. Shorter structures were due to the more or less important tilt of the PEG chains. However during the heating of these freshly crystallized samples, we observed a progressive phase transition from the tilted lamellar phases to the most stable phase of 120 Å. *Figure 3* shows that the sample evolves by itself to its most stable form after storage of 21 hours at 25 °C. This phenomenon was confirmed with WAXS analysis of samples crystallized slowly at 0.1 °C/min or by quenching into liquid nitrogen *(figure 4)*. WAXS measurement also allowed detecting acylglycerols under a hexagonal lattice.

This study demonstrates that Gelucire® 44/14 evolves to the most stable form (lamellar phase of 120 Å) whatever the crystallization rate applied during the formulation process if left 21 hours at 25 °C.

Hydration and emulsification performance

Naproxen and sodium naproxen were chosen as model drugs to evaluate the impact of drug polarity on the wettability and emulsification performance of Gelucire® 44/14. Model drugs were added at 10% w/w to the molten excipient under stirring. The mixture obtained with Gelucire® 44/14 was either a solid solution in the case of naproxen or a solid dispersion with sodium naproxen as detected by DSC and XRD [21]. Gelucire® 44/14 and these two mixtures were used to form films with an Automatic Film Applicator (Sheen-1137, height = 2 mm, spreading rate = 0.05 ms^{-1}). Gelucire® 44/14 film shows an irregular surface, with slopes and localized folds [21]. The inclusion of naproxen results in a smoother surface. The inclusion of sodium naproxen gives a surface which is more broken; numerous edges and deep cavities of 50 µm of diameter can be observed. This film crystallizes quicker than the other ones, giving a more porous structure. In this case the drug acts as a nucleation enhancer where newly-formed crystals diffuse toward existing drug crystals creating cavities in the film. The surface of the film containing sodium naproxen is also affected by the morphology of the drug crystals as this substance is not soluble in Gelucire® 44/14 [22].

Wettability of these films by water was characterized by goniometry (G1 Krüss goniometer, Krüss GmbH, Germany). Initial contact angles are similar whatever the drug used and are identical to those obtained with Gelucire® (53.1 ± 4.9°, 57.9 ± 5.6°, and 64.8 ± 7.8° for Gelucire® 44/14, the mixture containing naproxen, and the mixture with sodium naproxen, respectively). This shows that Gelucire® 44/14 hydrophilicity dominates the polarity differences of the two drug models as all initial contact angles were below 65°, implying a hydrophilic surface which is required for rapid emulsification in a lipid-based self emulsifying system. However the contact angle at the equilibrium was statistically higher for the mixture containing naproxen due to the hydrophobicity of the drug. On the other hand, the rate of spreading of the water drop (*i.e.* wettability) is similar for Gelucire® 44/14 alone and the mixture with naproxen (– 1.68 ± 0.5°/s, and – 1.86 ± 0.5°/s, respectively), but dramatically decreased for the mixture with sodium naproxen due to the broken surface of the sample (– 2.89 ± 0.5°/s) [21].

Figure 5 presents the variation of viscosity and refraction index of Gelucire® 44/14 during the discrete addition of water at 45 °C under stirring (100 rpm). The addition of up to 13% w/w of water into the molten Gelucire® 44/14 leads to a liquid solution with the same viscosity as the excipient itself (below 100 mPa.s). This amount of water is needed to completely hydrate the ethylene oxide units in the PEG ester and free PEG fraction [23]. The system slowly becomes a transparent gel with the addition of 13 to 43% w/w of water, the viscosity steadily increasing from 0.1 to 1 Pa.s. Then a high viscosity gel is formed between 43 and 61% w/w of water. The increase of viscosity measured up to 7 Pa.s is due to the formation of cubic mesophases. This gel slowly erodes and emulsifies with the addition of increasing amounts of water to become a turbid gel with low viscosity (61 to 75% w/w of water), then a turbid liquid system (75 to 90% w/w of water) and finally a translucent liquid system for high aqueous dilutions. This last system is characterized as a microemulsion with a particle size distribution of 80 nm (measured with a photon correlation spectrophotometer, PSS Nicomp, USA).

These studies demonstrate that Gelucire® 44/14 hydrophilicity dominates the polarity of the model drugs and favour the hydration of the lipid-based systems leading to a cubic phase system that erodes/emulsifies with the hydrodynamic of the aqueous environment.

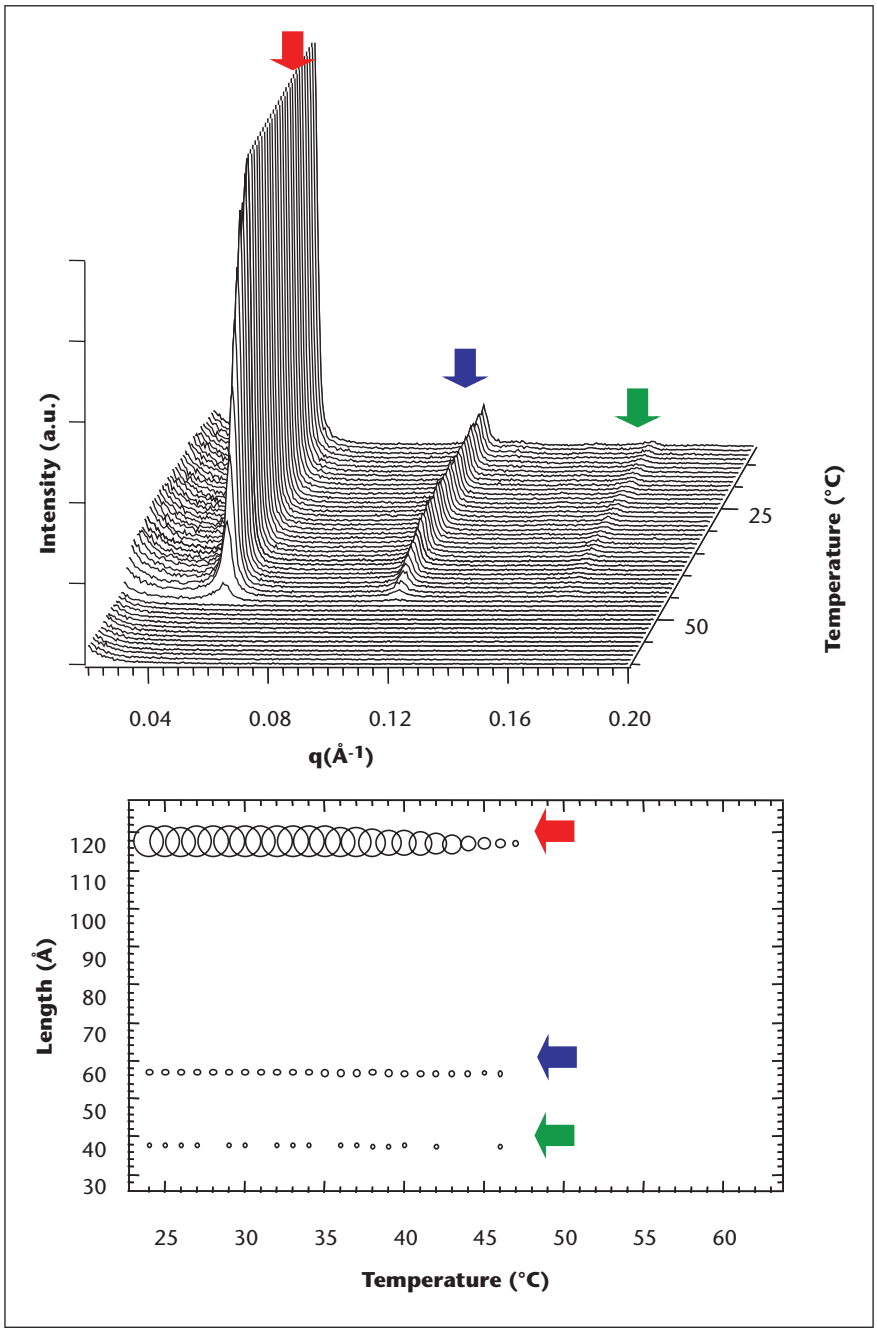

Figure 2. *3D evolution of SAXS patterns versus scattering vector q ($Å^{-1}$) upon the first heating of a Gelucire® 44/14 sample (top). The lower part of the figure shows the evolution of the X-Ray Diffraction peak positions d (Å) as a function of temperature, where d(Å) = (2*pi)/q($Å^{-1}$).*

Biopharmaceutical characterization

In the previous chapter the ability of Gelucire® 44/14 to evolve into a stable crystalline form and to self-emulsify in contact with water in vitro was presented. However, this excipient is a lipid-based system, containing both acylglycerols and PEG esters that can be hydrolyzed by lipases. In this chapter enzymes able to lipolyze Gelucire® 44/14 and the effects of this hydrolysis on drug performance are presented. In addition, the influence of Gelucire® 44/14 on enterocyte-based proteins and the absorption of drugs into the enterocyte are briefly discussed.

Lipolysis

The lipolysis of Gelucire® 44/14 and its fractions was evaluated by measuring the release of free fatty acids (FFAs) with a pH-stat apparatus (718 STAT Titrino, Metrohm, Switzerland) adjusted to a constant end-point value [24]. An emulsion of 500 mg Gelucire® 44/14 in 15.0 mL of an assay solution (NaCl 150 mM; NaTDC 4 mM; $CaCl_2$ 1.4 mM; Tris-HCl 1 mM) was mechanically stirred (450 rpm) in a temperature-controlled reaction vessel at 37 °C. Before adding enzymatic solution, we waited for 5 min until the gel phase disappeared and a translucent medium is obtained. The pH was kept constant using an automated burette to titrate FFAs with a 0.1 M NaOH solution. Activities were expressed in international units: 1 U corresponds to 1 µmol of FFAs released per minute. Specific activities were expressed as U per mg of pure enzyme.

Table 1 presents the specific activities of four lipases on Gelucire® 44/14 and its components: acylglycerol fraction and PEG fraction [7]. Human Pancreatic Lipase (HPL), the main lipase involved in the digestion of dietary triacylglycerols, does not show any significant activity on Gelucire® 44/14 (2 ± 2 U/mg) nor on either of its fractions. Other pancreatic lipases such as Human Pancreatic Lipase-Related Protein 2 (HPLRP2) show low activity on Gelucire® 44/14 (12 ± 1 U/mg) although the highest activity of HPLRP2 is that observed on the acylglycerol fraction (333 ± 0 U/mg). In addition, this enzyme shows low activity on the PEG ester fraction. Carboxyl Ester Hydrolase (CEH) shows high activity on Gelucire® 44/14 (96 ± 2 U/mg), and the highest activity of CEH is that recorded on the PEG ester fraction (50 ± 12 U/mg). The highest activity of all the enzymes tested is that of Dog Gastric Lipase (DGL) on Gelucire® 44/14 (108 ± 10 U/mg), although DGL shows low activity on the PEG ester fraction.

Gastric lipase probably plays an essential role in the in vivo digestion of Gelucire® 44/14, although it is less abundant than pancreatic lipase in the human digestive system. In addition, the main pancreatic enzyme involved in the intestinal digestion step of Gelucire® 44/14 is probably CEH.

Impact of lipolysis on the solubilising performance of Gelucire® 44/14

The in vitro gastrointestinal lipolysis of Gelucire® 44/14 was then investigated to understand which compounds are, after digestion, responsible for keeping poorly water-soluble drugs in solution [11]. Experimental conditions were adapted from in vivo data recorded at 50% gastric emptying of test meals, both in the stomach and in the duodenum, and

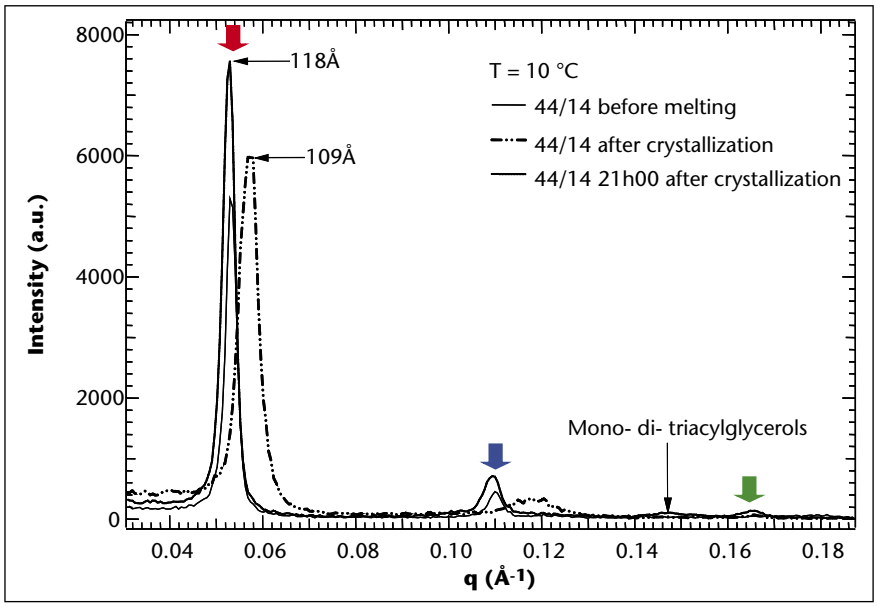

Figure 3. *Comparison of SAXS patterns versus scattering vector q (Å^{-1}) of Gelucire® 44/14 samples measured at 10 °C before melting (solid line), after crystallization (dashed dot dot line), and after storage at 25 °C for 21 hours (dotted line).*

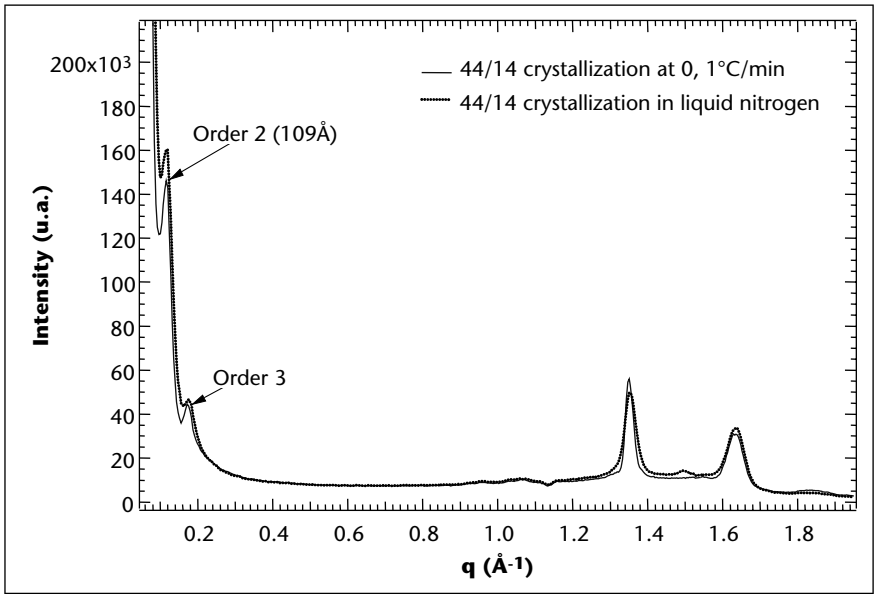

Figure 4. *Comparison of WAXS patterns versus scattering vector q (Å^{-1}) of two Gelucire® 44/14 samples crystallized at either 0.1 °C/min (solid line) or by quenching in liquid nitrogen (dotted line) and stored thereafter at 25 °C for 21 hours.*

enzymatic solutions were prepared according to *in vivo* secretions of lipases during a meal [25]. Experiments were performed over a period of 90 minutes to simulate the gastrointestinal digestion of lipids. An emulsion of Gelucire® 44/14 with either piroxicam or cinnarizine in the assay solution was mechanically stirred in a temperature-controlled reaction vessel at 37 °C. Then a freshly prepared gastric enzymatic solution (rDGL) was added to the reaction vessel and the pH was kept constant at 5.5 during 30 min (gastric step of lipolysis), via an automated titration of FFAs with 0.1 M NaOH using a pH-stat device. After the gastric step, a freshly prepared pancreatic enzymatic solution was added to the mixture (dilution by 1.7-fold) and the pH was shifted to 6.25 and kept constant for 60 min. At different time points, samples were taken to assay each component of Gelucire® 44/14 and also the percentage of drug dissolved in the aqueous phase.

During the gastrointestinal lipolysis of Gelucire® 44/14, monoacylglycerols and PEG esters are the largest compounds present at the end of gastric phase, and PEG mono and diesters are the largest compounds after the duodenal phase.

Solutions of Gelucire® 44/14 with either piroxicam or cinnarizine were formulated to evaluate the precipitation of these active substances during the gastrointestinal lipolysis of the excipient [11]. The precipitation of piroxicam is mainly due to the gastric lipolysis of Gelucire® 44/14 nevertheless the aqueous solubility of this drug is increased 4-fold due to the metabolites of the lipid-based excipient. With respect to the formulation of cinnarizine with Gelucire® 44/14, drug precipitation is only associated with the dilution of the gastric medium by the pancreatic juice until it reaches the composition of the duodenal medium. However, at the end of simulation of the gastrointestinal lipolysis, the aqueous solubility of cinnarizine formulated with Gelucire® 44/14 is increased 132-fold when compared with its aqueous solubility without excipient. This study highlights the importance of gastrointestinal lipolysis and the associated phenomena such as the dilution of chyme by biliary and pancreatic secretions *in vivo*, on the solubilisation of poorly water-soluble drugs formulated with Gelucire® 44/14 [11].

Interaction with enterocyte-based proteins

The absorption and bioavailability of active substances can be limited by enterocyte-based proteins such as P-glycoprotein (P-gp), an efflux protein that transports the drug out of the cell, or by cytochrome P450 enzyme that transform active substances into metabolites. In both cases the access of the drug to systemic circulation is limited and its bioavailability reduced.

Gelucire® 44/14 demonstrated its inhibitory effect on efflux proteins such as P-gp both in vitro diffusion chambers [26] and with Caco-2 cells monolayer [27, 28]. The inhibition of the efflux of Rhodamine 123 in diffusion chambers is observed for concentrations of Gelucire® 44/14 ranging from 1.0 to 10.0% v/v. These concentrations are higher than the critical micellar concentration of the excipient (0.01% v/v) suggesting that the drug should be included into micelles in order to increase its absorption [26]. Recently it has been proposed that Gelucire® 44/14 specifically inhibits P-gp and not Breast Cancer Resistance Protein (BCRP) another efflux protein [29].

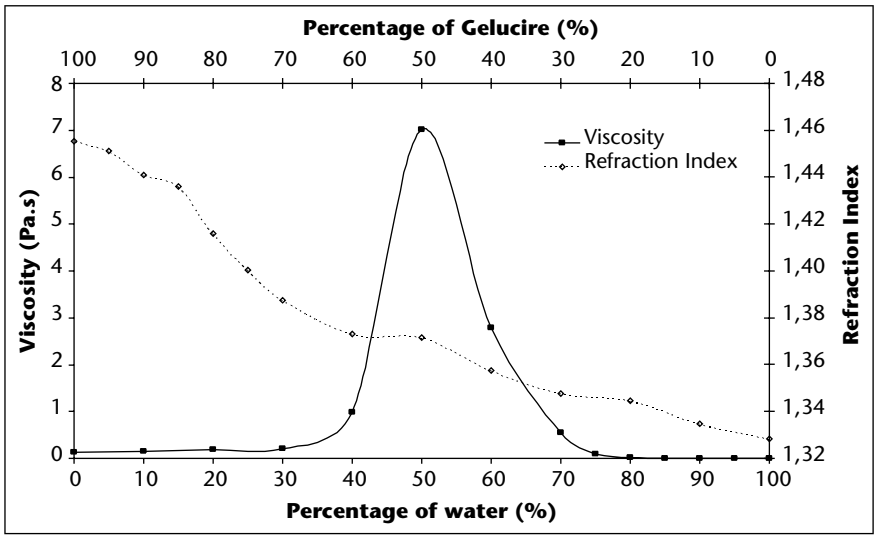

Figure 5. *Evolution of the refraction index and viscosity during the addition of water to Gelucire® 44/14 at 45 °C.*

Table 1. *Specific activities of Human Pancreatic Lipase (HPL), Human Pancreatic Lipase Related Protein 2 (HPLRP2), Carboxyl Ester Hydrolase (CEH), and Dog Gastric Lipase (DGL) on Gelucire® 44/14 and its two fractions: acylglycerols and PEG esters. Specific activities are expressed in U/mg as mean ± standard deviation (n = 2).*

Enzymes	Specific activity (U/mg)		
	Gelucire® 44/14	Acylglycerols fraction	PEG esters fraction
rHPL	2 ± 2	20 ± 0	6 ± 0
rHPLRP2	12 ± 1	333 ± 0	5 ± 2
CEH	96 ± 2	163 ± 7	50 ± 12
DGL	108 ± 10	106 ± 3	21 ± 6

Gelucire® 44/14 has also shown its ability to inhibit the metabolism of active substances by cytochrome P450 on human liver microsomes [28, 30].

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