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Applications of Lipid Nanoparticles (SLN and NLC) in Food Industry

[1]Souto E.B. and [2]R.H. Müller
[1]Department of Pharmaceutics, Biopharmaceutics and Biotechnology, Free University of Berlin, Kelchstr.31
[2]Pharma Sol GmbH, Blohmstr. 66A, D-12307 Berlin, Germany

Abstract: The aim of the present study is the description of the special features that the well known Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Nanoparticles (NLC) have for the oral delivery of food nutrients and active ingredients. Here, the main lipid materials used for the production of these carriers, as well as their production methods are given. Furthermore, the morphological differences between SLN and NLC are pointed out and the advantages of these carriers for food industry are referred. The question of how to formulate oral forms based on lipid nanoparticles is also discussed and several useful examples are given.

Key words: Solid Lipid Nanoparticles, SLN, Nanostructured Lipid Carriers, NLC, food nutrients, food industry

INTRODUCTION

The Gastrointestinal Tract (GIT) is recognized to be an organ that it is always in a state of continuous motility. This dynamic behaviour is of digestive and of inter-digestive modes that are involved in the digestion of food. When administering systems other than food or drinks, the GIT behaves itself in the same way. Therefore, the transit time of a delivery system intended for oral administration of active ingredients (nutrients or drugs) along the GIT, is the most limiting physiological factor in the development of such formulations.

In addition the motility patterns, the GIT transit depend on whether the person is in a fasted or in a fed state. Furthermore, the physical state of the delivery system, either a solid or a liquid, also influences the transit through the GIT. To successfully modulate the GI transit time of a transport system administered orally, one needs to have a good fundamental understanding of the anatomic and physiologic characteristics of the human GI tract (Table 1) and develop a system that is physiologically accepted concerning its excipients status.

To overcome the physiological difficulties encountered in the administration of active ingredients via oral route one can use the special features of lipid nanoparticle systems, i.e. Solid Lipid Nanoparticles (SLN) and Nanostructured Lipid Carriers (NLC). These are novel delivery systems with absorption increasing effects, such as occlusion, absorption enhancement and controlled release of food nutrients and active ingredients. SLN® is the first generation of lipid nanoparticles consisting of a solid matrix, which were developed at the beginning of the nineties in parallel by the research group of R. H. Müller in Germany[1] and M. R. Gasco in Italy[2]. NLC® is the second generation, which has been developed at the turn of the millennium by Müller *et al.*[3] and consists of a blend of solid and liquid lipids that is solid at both room and body temperatures (melting temperatures higher than 40°C).

The use of lipid materials is of major importance, particularly if they are of physiological and biodegradable nature, in addition to the fact that they enhance the oral absorption in the same way as the lipid components of the normal food. Under physiological conditions, the gastric absorption of most active ingredients is insignificant as a result of its limited surface area (0.1-0.2 m^2) covered by a thin layer of mucus coating, the lack of villi on the mucosal surface and the short residence time of most actives in the stomach. If those actives are suitable to be entrapped into the matrix of the above mentioned lipid nanoparticles, these systems may enhance the absorption of the active ingredients and other nutrients in the stomach but also in the small intestine, where most of the food is absorbed.

FEATURES OF LIPID NANOPARTICLES (SLN and NLC)

To understand the special features of lipid nanoparticles a brief description of their morphology and structure, as well as the main production methods will be give in this section.

Morphology and structure: The concept of lipid nanoparticle systems was realized when the liquid lipid (oil) from oil-in-water emulsions was replaced by a lipid being solid both at room and body temperature. Depending on the purpose of application, the melting

Corresponding Author: Müller R.H. Department of Pharmaceutics, Biopharmaceutics and Biotechnology, Free University of Berlin Kelchstr. 31, D-12169 Berlin, Germany

Table 1: Physiologic characteristics of gastrointestinal tract (GIT) modified by Weber[4]

GIT section	pH	Constituents	Relative osmolarity	Dilution	Sensitivity to absorption promoter
		HCl			
Stomach		Mucus			
Fasted	1-3	Pepsin			
Fed	3-5	Rennin	Non-		
		Cathepsin	-issosmotic	Large	Low
		Lipase	(Variable)		
		Intrinsic factor			
Small intestine	4.6-6.0	Amylase			
Duodenum	8	Bile acids	Isoosmotic		
Jejunum		Mucus	(330 mOsmol)	Large	Medium
		Glucohydrolase			
		Galactohydrolase			
		Lipase			
		Trypsin			
		Chymotrypsin			
Large intestine	7.5-8.0	Mucus	Non-	Small	High
			-isoosmotic		
Colon	5-7	Flora	Non-	Small	High
			-isoosmotic		

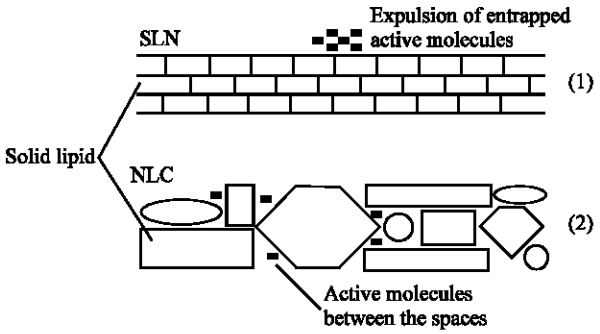

Fig. 1: Schematic representation of the different morphology of SLN (system 1) and NLC (system 2)

point can be changed between 50°C and 100°C selecting the composition of the lipid matrix.

To produce SLN the lipid can be a highly pure lipid e.g. tristearin[4] or tripalmitin[5], or a less defined mixture of acylglycerols, such as Compritol[6] or Imwitor[7]; while to produce NLC one needs to admix a solid lipid with a liquid lipid (oil). This mixture is chosen in order to obtain a particle matrix which is in a solid state at the anticipated melting point. Relatively high amounts of oil can be incorporated when choosing a mixture with a high melting lipid, e.g. blending an oil such as Miglyol (medium chain triacylglycerols) with Carnuba wax (melting point between 80-90°C). Up to above 2/3 of oil can be admixed but still remaining the melting point above 50°C[8]. A schematic representation of the different morphology of both systems is shown in Fig.1.

When producing a lipid particle from a solid lipid only (SLN), especially in case of highly pure lipids such as tristearin (C_{18}), tripalmitin (C_{16}) or cetyl palmitate (wax of cetyl acetate and palmitic acid), the particles can form relatively perfect lipid crystals (Fig. 1, system 1), then recrystallizing after being prepared by a hot homogenization process (c.f. section 2.2), or during precipitation when using the microemulsion technique. This means that the loading capacity of SLN can be limited, especially when high loadings of active molecules are required. However, when mixing especially very different molecules, such as long chain acylglycerols of the solid lipid with short chain acylglycerols of the liquid lipid, crystals with many imperfections are formed (Fig. 1, system 2). Apart from localizing the active ingredient molecules in between fatty acid chains or lipid lamellae, these imperfections provide a space for additional loading of active molecules. These latter can be incorporated in the particle matrix in a molecular dispersed form, or be arranged in amorphous clusters. High resolution X-ray analysis can be used to determine the type of drug incorporation[9]. The type of the selected lipids used for preparation of nanoparticles also seems to be responsible for their physical shape. In case of highly pure lipids the nanoparticles have a more cubic shape[10]. On the contrary, in case of using rather polydispersed mixtures the lipid nanoparticles obtain a nice spherical shape. When using identical lipid molecules the cubic shape occurs because they can build up a crystal like a dense brick wall. In case of larger and smaller and simultaneously differently shape stones (crystallizing molecules), the creation of a spherical structure is possible[11].

Production methods and scale up: In order to prepare SLN or NLC, the most important technologies described in the scientific literature are the preparation by the high pressure homogenization technique[12-14] and by the hot microemulsion technique[2]. Other methodologies used by

single research groups are precipitation of lipid particles using water-immiscible organic solvents (cyclohexane, chloroform or methylene chloride)[15-18]., or semi-polar water-miscible solvents (ethanol, acetone or methanol) [19-22], or applying a solvent diffusion method using a partially water soluble solvent (benzyl alcohol or tetrahydrofuran)[23, 24]. These latter three methodologies are comparable to solvent-based preparation methods for the production of polymeric nano- and microparticles. Apart from the high pressure homogenization procedure, all these methods have the disadvantage of requiring the use of organic solvents. This is less desired regarding the need to remove the solvent after the production of nanoparticles and potential solvent residues in the obtained product. Therefore, the high pressure homogenization seems to be the most suitable technology for production of lipid nanoparticles, which can be performed via hot or cold homogenization.

When applying the hot homogenization, the lipid is melted and the active ingredient is dissolved in the melted lipid. A typical temperature is 5°C above the melting temperature of the lipid. In the next step, the active-containing lipid melt is dispersed in a surfactant-stabilizer solution at identical temperature using a rotor-stator high speed stirrer. A pre-emulsion is obtained having a mean droplet size between 2 and 5 μm. The pre-emulsion is passed through a high pressure homogenizer using between 250 and 500 bar applying 1 to 3 homogenization cycles. In the cold homogenization process, the active-containing lipid melt is solidified, grounded to microparticles which are then suspended in a cold surfactant-stabilizer solution. In this case a pre-suspension is homogenized, i.e. the lipid microparticles will be reduced to lipid nanoparticles in a solid state.

For the homogenization process a piston-gap homogenizer or a jet-stream homogenizer (microfluidizer type) can be used for both lab and scale-up production. Due to technical reasons, such as temperature control, cost of large scale equipment and availability in industry, the piston-gap homogenizers are typically preferred, e.g. equipment from APV Gaulin or from Avestin. For lab-scale production a Micron LAB40 (APV, batch size 40 mL or for even smaller volumes) or an Avestin B3 (minimum batch size of approximately 3.5 mL) can be used. For larger volumes the continuous version of the LAB40 can be applied being equipped with product containers having a capacity of up to 500 mL (minimum volume of approximately 200 mL due to the dead volume of 50 mL). For large-scale production the LAB60 has a homogenization capacity of 60 l/h and can be used for first technical batches. The Gaulin 5.5 has also been successfully employed for large scale production, having a homogenization capacity of 150 l/h. Larger machines are the Rannie 118 with up to 2 tons/h (APV systems) or the Avestin 1000 with the capacity of 1 ton/h (Avestin).

SLN AND NLC FOR DELIVERY OF ACTIVES BY ORAL AND PERORAL ROUTES

The features of SLN for oral and peroral delivery are related to their adhesive properties. Once adhered to the GIT wall these particles are able to release the active ingredients exactly where they should be absorbed. In addition, the lipids are known to have absorption promoting properties not only for lipophilic drugs[25-27]. There are even differences in the lipid absorption enhancement depending on the structure of the lipids. For example, Medium Chain Triacylglycerols (MCT) lipids are more effective than Long Chain Triacylglycerols (LCT) [26]. The body will take up the lipid and the solubilized actives at the same time. Fig. 2 shows a schematic representation of oral administration of a SLN/NLC formulation.

For these routes of administration all the lipids used in traditional dosage forms such as tablets, pellets and capsules can be used. Some examples of the most used lipids are summarized in Table 2.

In addition, all compounds of Generally Recognized As Safe (GRAS) status or accepted GRAS status can be employed, as well as from the food industry[13]. Even such unpleasant surfactants with regard to parenteral administration such as Sodium Dodecyl Sulphate (SDS) can be applied to stabilize aqueous dispersions of SLN or NLC. These kind of surfactants possess excellent dispersing properties and are accepted up to a certain concentration in oral products[28].

The release of active ingredients from lipid nanoparticles in the GIT is also dependent on the lipase/co-lipase activity for the GIT digestion of the lipid matrix (Table 1). The lipase/co-lipase complex leads to a degradation of food lipids as pre-step of the absorption. To get basic information about the degradation velocity of lipid nanoparticles as a function of lipid and surfactant used, an *in vitro* degradation assay based on pancreas lipase/co-lipase complex was developed[29,30]. It has been found that degradation velocity and consequently drug release could be adjusted in a controlled way by the selection of the surfactant mixture. Degradation could be accelerated by using sodium cholate in the surfactant mixture. Sodium cholate acts as an activator for the lipase complex by promoting the anchoring of the enzymes on the particle surface. On the contrary, the use of poloxamers (polyoxyethylene-polyoxypropylene block co-polymers) as steric stabilizers delays the degradation velocity.

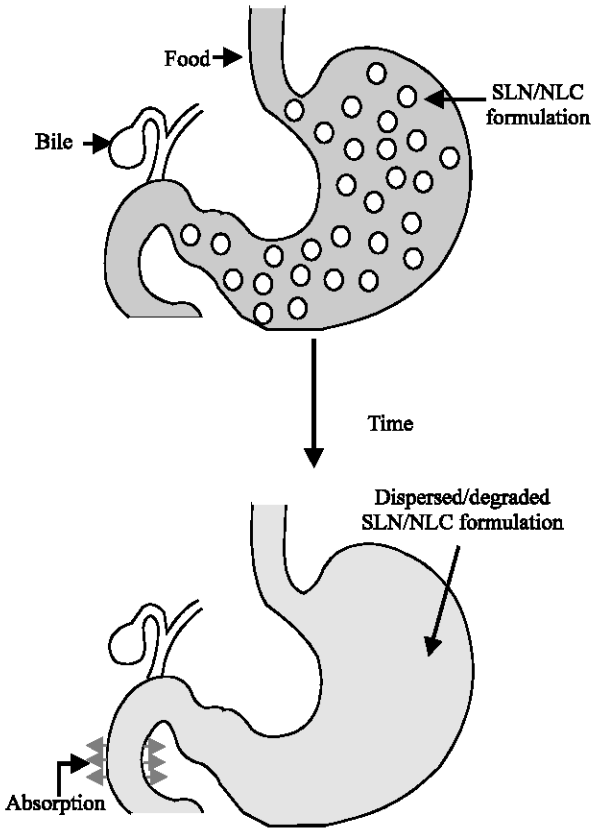

Fig. 2: Schematic representation of oral administration of a SLN/NLC formulation. The absorption of active ingredients delivered by the SLN/NLC formulation occurs at the same time and in the same way as the food

The anchoring of the enzyme complex is minimized or even prevented by the steric hindrance of the poloxamer adsorption layer.

Once the stomach acidic environment and high ionic strength (Table 1) favour the particle aggregation, aqueous dispersions of lipid nanoparticles might not be suitable to be administered as dosage form. In addition, the food will also have a high impact on their performance. Therefore, they can be transformed into solid dosage forms, such as tablets, capsules, pellets or powders in sachets. Figure 3 shows an example of how lipid nanoparticles can be formulated in a tablet.

Other advantages are the fact that packing lipid nanoparticles in a sachet for re-dispersion in water or juice prior to administration will allow an individual dosing by volume of the reconstituted lipid nanoparticles. For the production of tablets the aqueous lipid particle dispersion can be used instead of a granulation fluid in the granulation process. Alternatively, lipid nanoparticles can be transferred to a powder (by spray-drying or by freeze-drying) and added to the tabletting powder mixture. In both cases it is beneficial to have a higher solid content to avoid the necessity of having to remove too much water. For cost reasons spray-drying might be the preferred method for transforming lipid nanoparticle dispersions into powders, with previous addition of a protectant[31]. For the production of pellets the particle dispersion can be used as a wetting agent in the extrusion process[32]. The powders of lipid nanoparticles can be used for the filling of hard gelatine capsules, or even be added directly in liquid PEG 600 and filled into soft gelatine capsules.

Table 2: Examples of lipid molecules used for production of SLN and NLC

Lipids	References
Triacylglycerols	
Trimyristin	[5, 29-51]
Tripalmitin	[5, 17-19, 29, 31, 35, 36, 38, 39, 43, 44, 47, 51-71]
Tristearin	[5, 11, 29, 31, 35, 39, 46, 50-52, 59, 72-76]
Acylglycerol mixtures	
Witepsol®bases	[20, 35, 48, 52, 54, 56]
Glyceryl monostearate	[22-24, 39, 42, 44, 46, 60, 77-81]
Glyceryl behenate	[30, 32, 33, 35, 38-40, 42, 44, 46, 48, 49, 60, 61, 72, 73, 79, 80, 82-107]
	[30, 40, 44, 48, 73, 80]
Glyceryl palmitostearate	
Waxes	[11, 20, 32, 38, 42, 43, 46, 59-61, 73, 74, 86, 87, 90, 93, 95, 98, 99, 108-119]
Cetyl palmitate	
Beeswax	[39, 44, 60]
Hard fats	
Capric acid	[120]
Palmitic acid	[120, 121]
Stearic acid	[44, 46, 63, 69, 76, 77, 122-150]
Behenic acid	[129, 130]

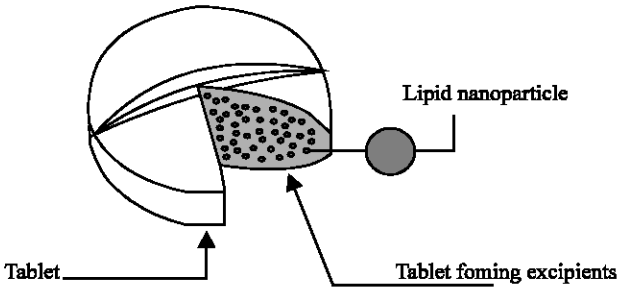

Fig. 3: Schematic representation of lipid nanoparticles formulated as tablets

Advantages of the use of SLN for oral and peroral administration are the possibility of drug protection from hydrolysis[33], as well as the increase in drug bioavailability and prolonged plasma levels[34]. When products are to be administered orally in dry forms, capsules and tablets are the most frequently used. They are effective and provide the costumer with convenience of handling, identification and administration. From a technological point of view, solid forms are generally more stable than are their liquid counterparts and thus are preferred for actives of poor chemical stability. Dry powders are taken orally (usually after mixing with water or juices) to a much lesser extent than are capsules and tablets, but are preferred by some patients who are unable to swallow the solid forms. Other advantages to be pointed out are physicochemical stabilization of various labile compounds, weaken or minimization of undesired colours of special actives, flavour enhancement, carrier for lipophilic compounds intended for addition to soft drinks or energy drinks and also for taste masking by incorporation of compounds inside the particle matrix.

CONCLUSIONS

The GIT transit time varies from one person to another. It also depends upon the physical properties of the object ingested and the physiological conditions of the alimentary canal. When using lipid nanoparticles as carriers for food nutrients or active ingredients the differences in the absorption patterns between people can be minimized, because of the lipid nature of these carriers. In addition, due to their small particle size, they can be degraded and absorbed in the same way as food, as well as during its digestion. The results will be an increase of the actives bioavailability if entrapped into SLN or NLC. These carriers show promising features not only for pharmaceutical and cosmetic fields, but also for food industry.

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