Expert Opinion

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Nanostructured lipid carrier-based hydrogel formulations for drug delivery: A comprehensive review

Slavomira Doktorovova & Eliana B Souto†
†*Faculty of Health Sciences, Fernando Pessoa University, Rua Carlos da Maia, 296, 4200-150 Porto, Portugal*

The scientific literature today provides several systems that can deliver active pharmaceutical ingredients (APIs) across the skin. These include reservoir matrices, matrix diffusion-controlled devices, multiple polymer devices and multilayer matrix assemblies. Among these, nanostructured lipid carriers (NLC) have emerged as novel systems composed of physiological lipid materials suitable for topical, dermal and transdermal administration. This review focuses on the design characteristics, production and composition of semi-solid formulations containing NLC as API carriers. One of the useful semi-solid systems are hydrogels, which can be used as vehicles to provide appropriate consistency for NLC formulations to be applied onto the skin. In the present review recent developments in the field are highlighted, including examples of APIs successfully entrapped within NLC now amenable for delivery via the skin. Further innovations in NLC composition and formulation, as well as in semi-solid hydrogel assemblies, are likely to expand the number of APIs available for topical, dermal and transdermal delivery.

Keywords: carbopol, chitosan, hydrogels, lubricity, nanostructured lipid carriers, semi-solids, spreadability, xanthan gum

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1. Introduction

The development of formulations for poorly water soluble or water insoluble active pharmaceutical ingredients (APIs) has been one of the most challenging tasks in pharmaceutical technology over the past decades. The interest in lipid-based formulations has increased and novel drug delivery systems have been developed. Other trends are related to the development and improvement of existing colloidal carriers able to overcome the low bioavailability (the fraction of the administered drug that reaches the systemic circulation) and to provide a better controlled release of many APIs.

At the beginning of the 1990s, the concept of solid lipid nanoparticles (SLN) was introduced [1]. SLN are colloidal carriers consisting of lipids that are solid at room temperature, with a structure that is very similar to physiological lipids, which provides the advantage of low toxicity and good *in vivo* tolerance [2-4]. Moreover, it has been claimed that carriers can protect sensitive APIs against degradation and thus enhance their stability. Due to their small mean particle size, SLN could also enhance the amount of the API delivered to the site of action. Nanostructured lipid carriers (NLC) were introduced at the turn of the millennium, being described as the second generation of lipid-based nanoparticles [3,4]. The difference between NLC and SLN is the inner structure. Besides solid lipid, the NLC structure also contains a certain amount, usually up to 30%, of an oil, that is a lipid that is liquid at room temperature. It is considered a 'solid lipid', the lipid raw material having a melting point above

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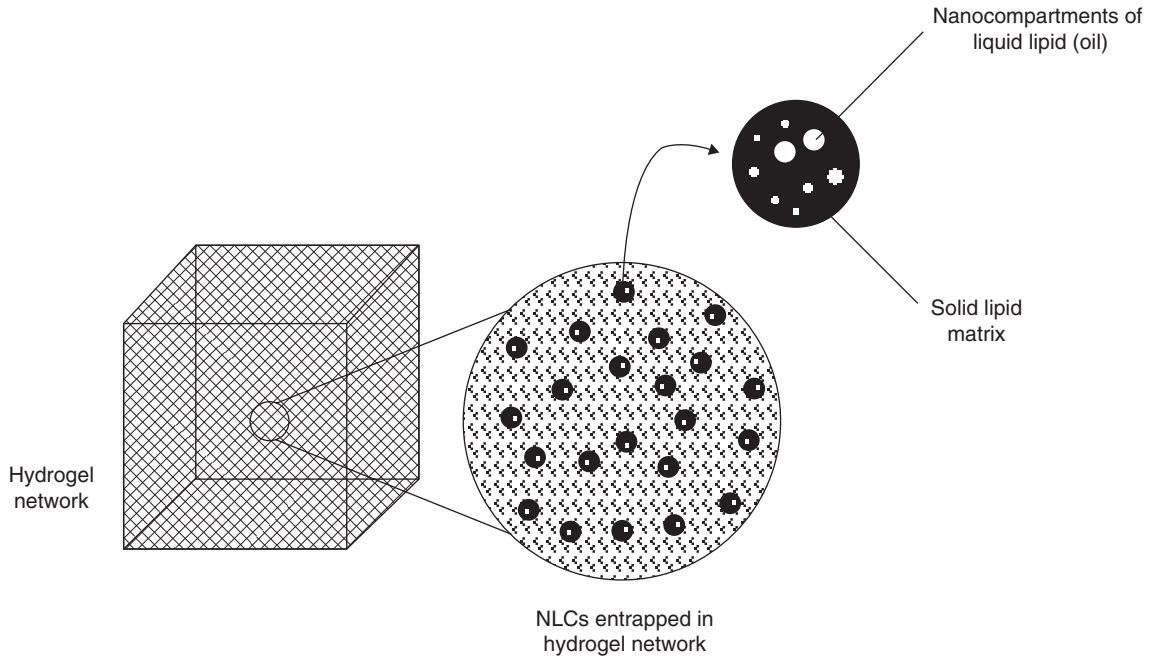

Figure 1. Schematic representation of NLC dispersed in a hydrogel network highlighting the inner structure of the lipid particles.

room and body temperatures, whereas a 'liquid lipid' shows its melting event much below room and body temperatures (usually below 0°C). **Figure 1** provides a schematic representation of the inner structure of NLC. The advantages of including small amounts of liquid lipid into a solid lipid matrix are related to the possibility of entrapping APIs which are better solubilized in liquid lipids, followed by higher loading capacity achieved in NLC in comparison to SLN.

To develop suitable formulations composed of lipid nanoparticles for topical, dermal and transdermal administration, usually a semi-solid vehicle is required to disperse the colloidal carrier formulations. Recently, hydrogel formulations composed of NLC have been described as one of the possible semi-solid systems for topical, dermal and transdermal administration of APIs [5,6]. These systems comprise a hydrogel-based vehicle where API-loaded NLC are dispersed and entrapped in the network (**Figure 1**).

By adjusting the hydrogel viscosity and the NLC concentration within its network, the semi-solid system can be suited for a wide-area application or for application in a particular region. The aim of this review paper is to provide a comprehensive overview of the use of NLC-based hydrogels as a promising delivery system for topical, dermal and transdermal administration of APIs.

2. Nanostructured lipid carriers as drug delivery systems

NLC are colloidal carriers characterized by a solid lipid core consisting of a mixture of solid and liquid lipids, and having a mean particle size in the nanometer range [7]. The liquid lipid is entrapped within the solid lipid matrix, so that the particles are solid at room and body temperatures. There are three types of inner composition of NLC, describing the distribution of the liquid lipid within the solid lipid matrix [8]. The lipids suitable for NLC production are similar to physiological molecules, being therefore well tolerated, biodegradable and non-toxic.

The first marketed NLC products were cosmetics, employing these particles for coenzyme Q10 delivery to the skin for anti-aging purposes [3,9]. Topical application of aqueous NLC dispersions is known to create a mono-layered lipid film onto the skin, which avoids water evaporation, and thus increases the skin's moisture and hydration [10]. Furthermore, as colloidal carriers, NLC can be used to modify the bioavailability of several APIs (in particular, those of lipophilic character) administered topically, and when compared to other colloidal carriers, can even provide a controlled API release. **Table 1** gives examples of APIs successfully incorporated in NLC and their lipid core composition.

2.1 Structure, morphology and production

As mentioned previously, the literature describes three types of NLC, which differ in their core morphology depending on the lipid composition of the matrix [2-4]. The imperfect crystal NLC type is composed of a blend of solid and liquid lipids. The mixing of two lipids with spacially very different molecules may prevent perfect crystal formation, leading to the creation of imperfections within the lipid core. These imperfections provide additional space for the accommodation

Table 1. Examples of APIs incorporated in NLC and the lipid core composition of the particles.

Active pharmaceutical ingredients	Ref.	Lipid core composition
Ascorbyl palmitate	[11-13]	Glyceryl monostearate/cetyl alcohol/PEG-8 beeswax and linoleoyl macrogolglycerides
Beta-carotene	[14]	Propylene glycol monostearate
Celecoxib	[6]	Glyceryl dilaurate and glyceryl mono-dicaprylate
Clobetasol propionate	[15]	Monostearin and caprylic/capric triglycerides
Clotrimazole	[16-18]	Glyceryl tripalmitate and caprylic/capric triglycerides
Coenzyme Q10	[19,20]	Cetyl palmitate and caprylic/capric triglycerides
Flurbiprofen	[21]	Glyceryl trimyristate and caprylic/capric triglycerides
Ibuprofen	[22]	Glyceryl behenate and caprylic/capric triglycerides
Indomethacine	[23,24]	Glyceryl behenate and caprylic/capric triglycerides
Ketoconazole	[18]	Glyceryl behenate and alpha-tocopherol
Nitrendipine	[25,26]	Glyceryl trimyristate and caprylic/capric triglycerides
Progesterone	[27]	Monostearin/stearic acid and oleic acid
Psoralen	[28]	Glyceryl palmito-stearate and squalene
Valdecoxib	[5]	Glyceryl dilaurate and propylene glycol monocaprylate

of APIs. Pure lipids will recrystallize in very perfect assemblies, such as triclinic parallel β polymorphic forms. These, in comparison to the less perfect polymorphic forms such as β′(orthorhombic perpendicular) or α (amorphous), will depict a lower number of voids and vacancies able to accommodate the API molecules. Immediately after producing the particles, if the pure lipid crystallizes in a less perfect form (β′, α), during shelf life it will be organized in its most stable form β. As a result, the API molecules previously entrapped in the former system will be expelled during the transformation from $\alpha \rightarrow \beta' \rightarrow \beta$ [29-31]. As a result, a higher loading capacity can be achieved compared to lipid nanoparticles produced with very pure lipid molecules which create perfect crystals resembling brick wall structures. The amorphous NLC type describes the situation when the lipid mixture forms a solid matrix at room temperature, but does not fully recrystallize during the production process, creating an amorphous blend. This occurs when employing special lipids, such as hydroxyoctacosanylhydroxystearate and isopropyl myristate. The advantage is the limited recrystallization, which minimizes the expulsion of APIs immediately after production and during shelf-life. The multiple NLC type consists of separate liquid lipid compartments entrapped within a solid lipid matrix. This occurs in formulations with such a high concentration of liquid lipid that is above its solubility in the solid lipid at room temperature. During production, the miscibility gap is reached and phase separation occurs during the cooling down step. As the solid lipid solidifies, liquid droplets are subsequently created and entrapped in the particle core. This type of NLC is particularly useful for the incorporation of APIs with higher solubility in oils than in solid lipids. To produce NLC, several oils are usually screened regarding their solubility for a particular API. Thus, in the majority of the cases the multiple NLC type is obtained (**Figure 1**).

As previously reported, NLC shape and structure is influenced by the choice of the solid lipid [32]. NLC composed of cetyl palmitate as a solid lipid and caprylic and capric triglycerides as a liquid lipid (being 10% of NLC weight) revealed spherical, but not perfectly round particles [32]. In contrast, Jores *et al.* [33] observed platelet-shaped particles with the liquid lipid separated from the solid lipid, but stuck onto the particle surface.

As the role of NLC is to deliver APIs, the ability of NLC to accommodate active molecules is an important property. It can be expressed by the entrapment efficiency (*Ee*) and loading capacity (*Lc*). The *Ee* is determined as [15]:

$$Ee = \frac{Wa - Ws}{Wa}.100\%$$

where *Wa* is the weight of API added to the formulation and *Ws* is the amount of API determined in supernatant after separation of the lipid and aqueous phase. *Ee* defines the ratio between the weight of entrapped API and the total weight of API added to the dispersion. *Lc* expresses the ratio between the entrapped API and the total weight of the lipids. It is determined as follows:

$$Lc = \frac{Wa - Ws}{Wa - Ws + Wl}.100\%$$

where *Wl* is the weight of lipid added in the formulation [15,34]. Both *Ee* and *Lc* are dependent on several

parameters, such as the lipophilic properties of the API, the screening of the most appropriate lipid composition/ratio (solid/liquid lipids) and surfactant combination, as well as the production procedure used.

To produce NLC several methods are possible, that is by microemulsion, high pressure homogenization (HPH), solvent diffusion or by melt–emulsification processes. The selection of the method is dependent on the physicochemical properties of the API and on the production scale [35,36]. Production parameters such as temperature, pressure and number of homogenization cycles in the case of HPH may influence the size of the particles produced [37].

The microemulsion technique is based on the production of a warm microemulsion at a temperature above the melting point of the solid lipid, which is then dispersed in a cold aqueous surfactant solution, leading to the breaking of the inner oil droplets into even smaller droplets, which – after solidifying – form solid nanoparticles. This technique was first described to produce lipid nanoparticles without a liquid lipid [38] (i.e., SLN), but it has been recently adapted for the production of NLC [5,6,39]. The boundaries of the microemulsion domains are usually determined by means of pseudoternary phase diagrams with the percentage of lipid phase, aqueous phase and surfactant as the three diagram components [6]. The lipid phase is previously heated to melt the solid lipid. The required quantities of surfactant phase and the lipid phase are heated to the same temperature and gently mixed to form a monophasic mixture that is slowly titrated with aliquots of distilled water and stirred at, for example, > 60°C for a sufficiently long time to reach the equilibrium. After equilibrium has been reached, the mixture should be visually checked for transparency and through crossed polarisers for optical isotropy. Only those systems which appear black when visualized through the crossed polarisers are considered to be within the microemulsion region, thus confirming the absence of other phases. Advantages of the microemulsion technique are the absence of specialized equipment [40] and energy required to create the particles [41]. For API-loaded NLC, the molten lipid is previously mixed with oil and API molecules, and then emulsified with the aqueous phase, comprised of surfactant, solubilizer and water. Both phases are maintained at a temperature above the melting point of the lipid (usually > 60°C). At this temperature, the two phases are mixed using a cyclomixer to form a microemulsion. This warm microemulsion is then diluted in cold water (2 – 3°C) under a mechanical stirring overhead stirrer for several minutes to form the NLC dispersion with the required concentration of API.

The HPH was first described by Müller and Lucks [1] and it has been successfully used for NLC preparation for s everal years [2]. The method requires heating the mixture of liquid lipid and solid lipid to a temperature 5 – 10°C above the melting point of the solid lipid. It is followed by admixing the lipid phase into the hot aqueous phase containing the surfactant to obtain a hot pre-emulsion, applying several homogenization cycles/minutes at, for example, 500 bar. A hot nanoemulsion is produced which is then cooled down to room temperature. The solidification of the solid lipid occurs while cooling down, forming the aqueous NLC dispersion. This technique is the most suitable for medium and large scale production, requiring special equipment such as the piston-gap high pressure homogenizer or the microfluidizer [2-4]. For the incorporation of APIs, these need to be added to the melted lipid phase prior to pre-emulsification.

The solvent diffusion and melt emulsification methods have also been recently described to produce NLC [1-3]. Employing the solvent diffusion method, the solid lipid and liquid lipid are mixed together, dissolved in organic solvent miscible with water (e.g., acetone, ethanol) at a temperature of, for example, 70°C, the organic solution is added into excess of distilled water under mechanical stirring [42,43,44,46]. The resulting pre-emulsion (melted lipid droplets) is cooled down to obtain nanoparticles [45]. The principle of the melt emulsification method is preparation of a lipid phase consisting of liquid lipid and molten solid lipid, and dispersing the melt into aqueous surfactant solution and further processing using ultrasonication. Then the emulsion is cooled down to obtain solid nanoparticles [1].

2.2 Physicochemical properties

The stability of a formulation is a measure of its ability to maintain the set-up characteristics for a required period of time. The characteristics of an NLC formulation include, first the size of the particle and size distribution within the batch. Furthermore, zeta potential analysis may also help in estimating the stability of the aqueous NLC dispersion. The assessment of the presence of liquid lipid domains and API loading can be performed using DSC and x-ray scattering methods.

The basic characteristic of the NLC is the size, which needs to be verified to comply with the definition of nanoparticles. The size of NLC can be determined by photon correlation spectroscopy (PCS) and by laser diffractometry (LD). The result of PCS analysis is the mean diameter of the nanoparticle population. Information about particle size distribution is given by the polydispersity index (PI) and by the data obtained by LD. PI reflects the variation of particle size around the mean diameter. Low PI means that all particles are sized in a narrow range, and therefore the dispersion may be considered relatively homogenous in size. If the PI is lower than 0.1 and related to particles with a size range of 100 nm, this indicates a dispersion which can be considered monodisperse [2,33]. High PI reflects great differences between particle sizes within the formulation and indicates potential problems with the stability of the formulation, as the greater probability of coupling of the particles has to be considered. This is a phenomenon also known from suspensions: mixtures of very different sizes

tend to collide and to form aggregates. This also applies to nanoparticles, in which aggregation is obviously undesirable. PI may be obtained by PCS, from one assay along with particle size, using an equation proprietary to Malvern. Prior to measurement, the NLC dispersion needs to be diluted in distilled water to weak opalescence. This applies to both liquid and semi-solid dispersions of nanoparticles.

The information about size distribution may also be given by LD analysis, the outcome is the percentage of volume of a defined size. LD reflects the volume distribution below a given value, for example, D99% indicates that 99% of assessed particles are of size below the given value.

The zeta potential is defined as the surface charge of the particles. As it is known from suspensions, solid particles dispersed in aqueous medium are electrically charged onto the surface. The value of the surface charge also indicates the stability of the dispersion. Zeta potential below |30mV| indicates the risk of particle collision and subsequent coupling. To be considered stable, a zeta potential higher than |30mV| is desirable. Zeta potential can be calculated from electrophoretic mobility of the particles according to the Helmholtz–Smoluchowsky equation.

DSC is a method used to observe the changes of crystallinity and melting/solidification behaviour within the sample as a function of temperature changes. With regard to NLC the method helps to verify the incorporation of the liquid lipid domains into the particles, as well as the presence of API molecules entrapped within the lipid matrix. Comparison of DSC patterns of bulk materials and those from NLC formulations provides information about the behaviour of the solid and liquid lipid as a mixture. The blend of two lipids usually exhibits a lower melting temperature and lower enthalpy of the melting event, due to the interrupted lattice of solid lipid. In comparison to particles composed solely of solid lipids of similar composition, the NLC should exhibit lower enthalpies for phase transitions [39]. Likewise, the presence of API molecules decreases the melting enthalpies of the lipid blend. An example of DSC patterns of blends of Compritol 888 ATO (glycerol behenate)/sunscreen mixture with increasing amounts of lipid is given in **Figure 2** [47].

In **Figure 2** the upper curve is the bulk material of Compritol as reference. The amount of API was lowest in the second top curve, and increased sequentially in the curves below. A clear reduction of the melting point and melting enthalpy could be detected by the reduction of the area under the curves. Useful information provided by the DSC analysis is also the occurrence of polymorphic changes of the material. Changes from a less stable crystal modification (α form) to a more stable polymorphic form (β) during storage may lead to API expulsion from the nanoparticles [48]. An example can be the polymorphic behaviour of Compritol 888 ATO [49]. During the heating run, the DSC pattern shows two peaks, one at 71.7°C and another very small peak at 51.2°C. After heating the lipid for one hour at 90°C, the smaller peak, belonging to unstable α form, is not present in the second DSC run, and the peak at 71.7°C broadens indicating a greater amount of material in this most stable β modification. As such, the acyl chains are packed more tightly and therefore provide less space for API accommodation [49].

Wide angle x-ray scattering (WAXS) is a method used to determine the state of acyl chains within the lipid layers [29-31]. Incorporation of the liquid lipid into the solid lipid can be accurately verified. Both lipids as a bulk material have their distinct x-ray pattern, which can be partially recognized in the pattern of NLC. Liquid lipid is believed to disturb the crystallinity of the solid lipid; subsequently, the peaks of solid lipid become weaker. No changes in peak positions occur; the changes in peak intensity are observed due to changes in the order of the acyl chains. WAXS is even more suitable for the identification of polymorphs than DSC, as each modification has its unique pattern in WAXS diffractogram.

Information about particle size, zeta potential and crystallinity may facilitate the estimation of the stability of the NLC formulation. Changes in crystallinity during storage lead to changes (increasing) of the surface area, and subsequently particles tend to form aggregates. Changes in the particle dispersity can be observed by monitoring the formulation viscosity. In fact, when viscosity increases, the dispersion becomes more semi-solid due to particle coalescence and subsequently transforms into an even more solid system, which is undesirable. This instability is described as gel formation. Gelling phenomena is the transformation of a system of low viscosity into a highly viscous gel [50]. Suitable surfactants may prevent this instability, preventing the coalescence of the particles. Kaur *et al*. showed that a combination of ionic and non-ionic surfactants could enhance the stability of nanoparticle formulations for at least one year [51]. Another approach to prevent gel formation is incorporating the NLC dispersion into semi-solid formulations such as creams and hydrogel [13,52,53]. Particle growth generally precedes the gelling step. Furthermore, such phenomena is related to some inductors similar to crystallization phenomena (high temperatures, light) and the degree of gelling can be correlated to the degree of crystallization of the lipid phase. Crystal interfaces with low concentrations of adsorbed emulsifier molecules represent the preferred sites of particle aggregation over which gel formation can proceed. Gel formation is usually an irreversible process, which involves the loss of the colloidal particle structure.

3. Hydrogel-based semi-solid formulations

Important parameters for multiple-type topical formulations are their emolliency, which is affected by lubricity and spreading properties. Lubricity and spreadibility are related to the formulation's ability to cover a surface and reduce

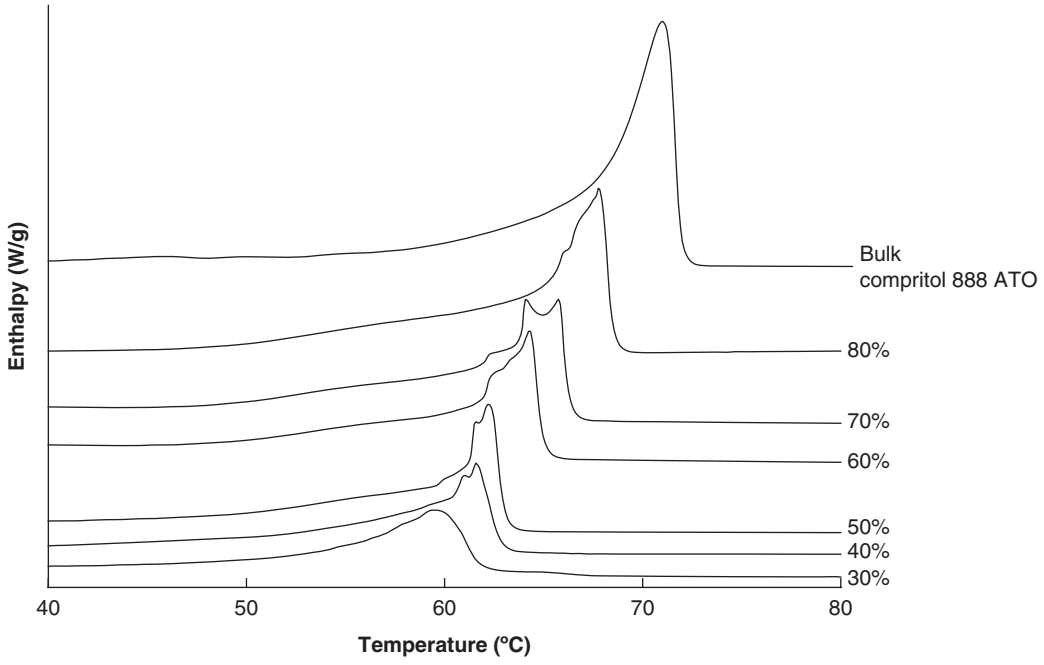

Figure 2. DSC thermograms of blends of Compritol 888 ATO/sunscreen mixture with increasing amounts of lipid (30, 40, 50, 60, 70, 80).
Modified from Xia *et al.* [47].

the attrition, and these properties can be determined quantitatively. Although being independent on the properties of the applied surface, lubricity is expressed as a friction factor described by Stocke's law of friction [54]:

$$F = 6\pi\eta rv$$

where η stands for viscosity, r is the film thickness and v is the speed of moving film. In contrast, spreadibility is highly dependent on the applied surface, being described by the contact angle [54]:

$$S = R/r$$

where R is the radius of the wetted area and r the radius of the initial droplet. Young's law describes the force balance at interfaces between solid substrate, liquid and gas phase. The contact angle plays an important role since it describes the curvature of the droplet on the surface. Spreadibility is an important property of topical formulation from a patient compliance point of view [5]. Application of the formulation to sensitive skin is more comfortable if the vehicle (i.e., semi-solid formulation) spreads easily, exhibiting maximum 'slip' and 'drag'. It has been reported that semi-solids composed of larger diameter particles have better spreadibility. In contrast, those formulations having higher consistency are more difficult to spread. The formulation's components and its consistency also govern the API release profile. Consistency index is a measure of consistency, being related to the apparent viscosity at a shear rate of 1/sec. The flow index n is a measure of the deviation of a system from Newtonian behaviour ($n = 1$). A value of $n < 1$ indicates pseudoplastic flow or shear thinning, whereas a value of $n > 1$ indicates dilatant or shear thickening flow. Flow index confers an idea of the flowability of the formulation from the container. Usually, the thicker the base, the lower the flow index. NLC-based hydrogel formulations usually reveal a pseudoplastic-like behaviour typical of well-known commercial topical formulations [9,52].

It has been reported that aqueous NLC dispersions themselves exhibit viscoelastic behaviour [55], which is dependent on the concentration of lipid phase [52]. The storage modulus (G′), loss modulus (G″) and complex viscosity (Eta) of NLC were evaluated applying a frequency sweep test, recorded at a constant stress amplitude of 5 Pa (**Figure 3**). During this test the dispersion suffers sinusoidal stress, providing information on its inter-molecular and inter-particle forces [56], thus viscous and elastic components can be distinguished [57].

From **Figure 3** it is clear that the elastic modulus (G′) is higher than the viscous modulus (G″) in the investigated frequency range. For pure elastic materials, as soon as the force is lowered or released, the deformation recovers, whereas pure viscous materials have a phase shift of 90°, because when the applied force reaches its maximum, the material is pulled apart with its highest speed [58,59]. In practice, these results show that NLCs are sufficiently viscous (or fluid) to be easily applied, and sufficiently elastic to adhere

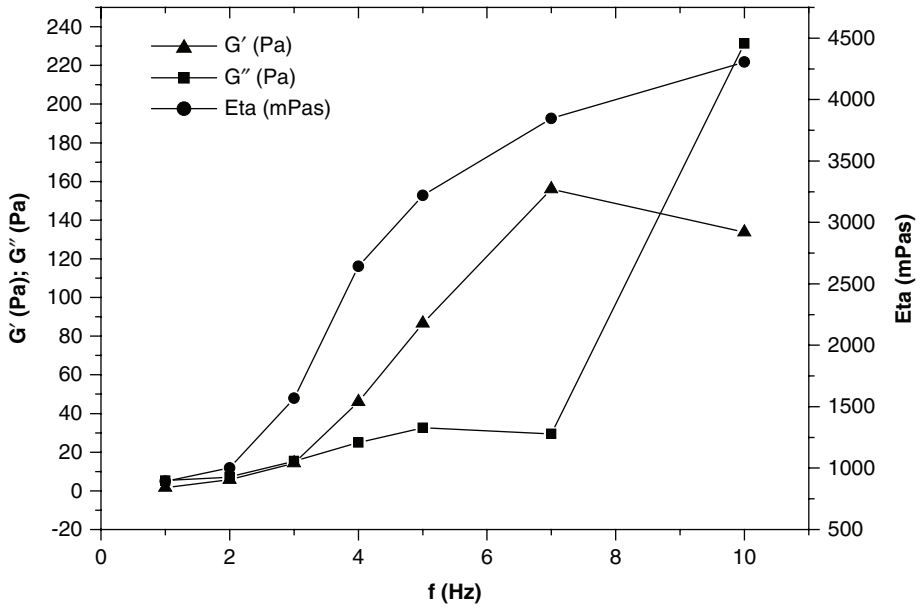

Figure 3. Storage modulus (*G′*), loss modulus (*G″*) and complex viscosity (η) of NLC as a function of the frequency at a constant stress amplitude of 5 Pa.
Modified from Souto *et al.* [52].

and self-immobilize onto the skin. If the NLC formulation is aimed to be applied between adjacent skin areas or into areas that might rub against clothing, it should have a lubricant effect. Having a spherical shaped NLC impairs excellent lubricating action. Nevertheless, to have appropriate consistency these particles have usually been dispersed in semi-solid, *in situ* gelling hydrogels [5,6,13,24,34,53,60-63]. This approach usually creates semi-solid formulations with a pseudoplastic behaviour (i.e., decrease of viscosity with increase in frequency). In general, hydrophilic semi-solid gels have a further number of advantages, such as low toxicity, availability, unique physical properties, biocompatibility and muco-adhesiveness, and these can be natural, semi-synthetic or synthetic polymers.

Examples of natural polymeric hydrogels used to disperse lipid nanoparticles include chitosan, dextran and xanthan gum. Chitosan is a natural based polymer obtained from chitin. This material is biocompatible, biodegradable and non-toxic. Chitosan hydrogels have been used in various biomedical applications [64] and, since the molecule itself provides the possibility for further adjustment by copolymerisation, hydrogels with improved characteristics for novel drug delivery systems can be developed. The molecule contains a polar group with positive charge, which needs to be considered in case of nanoparticle incorporation, as this can cause changes in particles' zeta potential [52]. Chitosan-based systems have been described to improve the retention and biodistribution of APIs applied topically onto the eye [65]. Besides its low toxicity and good ocular tolerance, chitosan exhibits favourable biological behaviour, for example bioadhesiveness and permeability enhancing properties.

Dextran gels and their derivatives may also be used as semi-solid systems for nanoparticle dispersions. Dextran hydrogels are also suitable for macromolecules delivery, they are non-toxic, do not cause tissue irritation and show a low tendency of incorporated particles to adhere to the gel matrix [34,66]. Xanthan gum hydrogels are also easily prepared and widely used in pharmaceutical applications; however, the gels can exhibit too low viscosity and tackiness [6,67].

Semi-synthetic derivates of cellulose are widely used in pharmaceutical applications [68]. Several derivates have the advantages of time-proven safety in topical use, simple preparation which does not require neutralization and low price. Examples of synthetic polymers are carbopols. These are polyacrylates cross-linked with other polymers, depending on the concrete type of carbopol, providing a wide portfolio of hydrogels with favourable characteristics. For hydrogel preparation, a low concentration of carbopol powder is required, usually below 1%. The gels are easily and quickly prepared, are well tolerated and provide biological intactness. To start the gelation process, the dispersion needs to be neutralized. Typically, electrolytes such as kalium hydroxide or sodium hydroxide in small amounts are used to start carbopol gelation, but these are not appropriate for lipid nanoparticle-based gels due to possible changes in the zeta potential of the particles. Instead, triethanolamine may be used. Carbopol hydrogels have showed their suitability for nanoparticle incorporation [62].

The mechanical barrier and lubricating effect of lipid nanoparticles protect and support the skin, which is particularly

useful in case of skin irritation and allergic reactions. When dispersing NLC formulations in semi-solid vehicles, the rheograms revealed by shear flow investigations usually depict the characteristic shape of a classical pseudoplastic flow (**Figure 4**).

Figure 4 depicts the rheological behaviour of NLC dispersed in polyfluorocarbon (PFC) hydrogels [69]. As shown, besides the pseudoplastic flow, thixotropy was also observed since the up and down curves did not overlap. The rheological behaviour of thixotropic fluids is usually a time-dependent pattern, that is the flow and viscosity of the systems are dependent on the formulation time history. Pseudoplastic semi-solids reveal a rheological behaviour between Newtonian and plastic flows. While Newtonian materials flow at low shear rates, liquid materials shows plastic flow at higher shear rates. PFC hydrogels' viscosity decreases as the shear rate is increased, which is dependent on the shear. Since the viscosity decreases with increasing shear rate, thixotropy is similar to pseudoplastic flow, but the time scale of the experiment also controls the viscosity, that is if the experiment stops, the viscosity will fall.

Several gels composed of different polymers (polyacrylates, poloxamers and xanthan gum) have been produced and evaluated for *in vitro* skin penetration studies [67]. The results showed almost two times higher API concentration in the skin with lipid nanoparticle-enriched gel as compared to conventional gel, thus indicating better localization of the API in the skin. *In vivo* skin hydration studies in albino rats revealed an increase in the thickness of the stratum corneum with improved skin hydration. The developed formulations were shown to be non-irritant to the skin with no erythema or edema and had a primary irritation index of 0.00.

For topical application, semi-solid formulations are the most common, whether for skin penetration or superficial activity. A wide range of APIs can be incorporated, showing little tendency for phase separation. By adjusting the formulation viscosity, it can be suited for wide-area application or for application on a particular region. Other advantages are easy manipulation and acceptable appearance. To obtain NLC dispersions in a semi-solid form, various approaches can be followed [4].

NLC dispersions may be used as they are, provided the dispersion viscosity allows using it without further processing. Highly concentrated nanoparticle dispersions may have a cream-like appearance. When using NLC, creamy formulations are obtained if they are composed of up to 50 – 60% of lipid content [9]. The viscosity of nanoparticle dispersions increases with lipid content; above 50 – 60% paste-like formulations are obtained and further increase of lipid content may even lead to solid formulations. The advantage of using a highly concentrated nanoparticle dispersion is its one-step preparation in case the HPH technique is applied [70]. However, NLC dispersions are usually produced at a lower lipid content, leading to low viscosity and a milk-like appearance, for example below 40% lipid content, viscosity of 100 mPas has been reported [71]. Secondly, the NLC can be incorporated into an already-established semi-solid formulation, that is into a cream or hydrogel formulation established specially for the nanoparticles' incorporation. This requires replacing a part of the aqueous phase of the cream with nanoparticle dispersion before the production of the cream, or the production of a cream formulation with decreased water content, which would subsequently be replaced by aqueous nanoparticle dispersion after the production of a cream. Another alternative would be the incorporation of nanoparticles into a semi-solid hydrogel formulation. The incorporation of NLC into hydrogels is obtained by mixing the gel components and adding a concentrated nanoparticles dispersion before starting the gelation process. As neutralizing agents, as mentioned previously, electrolytes (e.g., sodium or kalium hydroxides) should be avoided as they cause a rapid decrease of zeta potential, thus leading to particle aggregation. Instead, Tristan® (tromethamine [BASF, Germany]) or Neutrol®TE (N,N,N,Ntetra (2-hydroxypropyl)[BASF, Germany]) ethylenediamine can be used (BASF, Germany) [5,72].

The stability of NLC incorporated into Carbopol 934 (polyacrylate), xanthan gum, hydroxyethylcellulose 4000 and chitosan hydrogels has been tested [25,52]. The average diameter and zeta potential of blank NLC prepared from tripalmitin and caprylic and capric triglycerides has been determined prior to incorporation into hydrogel and subsequently after incorporation. Particles remained in their colloidal size range, and no nanoparticle aggregation was observed during 90 days of storage at room temperature. Only a small decrease of zeta potential was observed in Carbopol, xanthan gum and hydroxyethylcellulose hydrogels. Polar groups of chitosan and the need to use acetic acid caused an increase of originally negative zeta potential [52]. Nitrendipine-loaded NLC prepared from trimyristin and caprylic and capric acid triglycerides were also evaluated before and after incorporation into polyacrylate, xanthan gum, hydroxypropylcellulose and chitosan hydrogels [25]. After 90 days of storage, all prepared hydrogels remained transparent, no particle aggregation was observed and the particle size remained below 250 nm. Higher *Ee* of nitrendipine was achieved when employing NLC compared to SLN consisting of trimyristin only. All hydrogels performed sustained release of the API during 24 h; those using NLC exhibited faster drug release compared to SLN-based hydrogels. As the *in vivo* testing showed a longer half-life of API and more sustained release compared to oral dosage forms, these hydrogels proved a useful tool to overcome low bioavailability and high first-pass effect of certain APIs.

In another study, contact mode atomic force microscopy imaging showed that *p*-dodecanoyl-calix[4]arene-based lipid nanoparticles dispersed in several hydrogel formulations were characterized by a mean diameter of 150 nm with little or no aggregation [73]. The simultaneous use of lateral force, topographic and force modulation mode imaging allows a clear interpretation of the observed images, showing

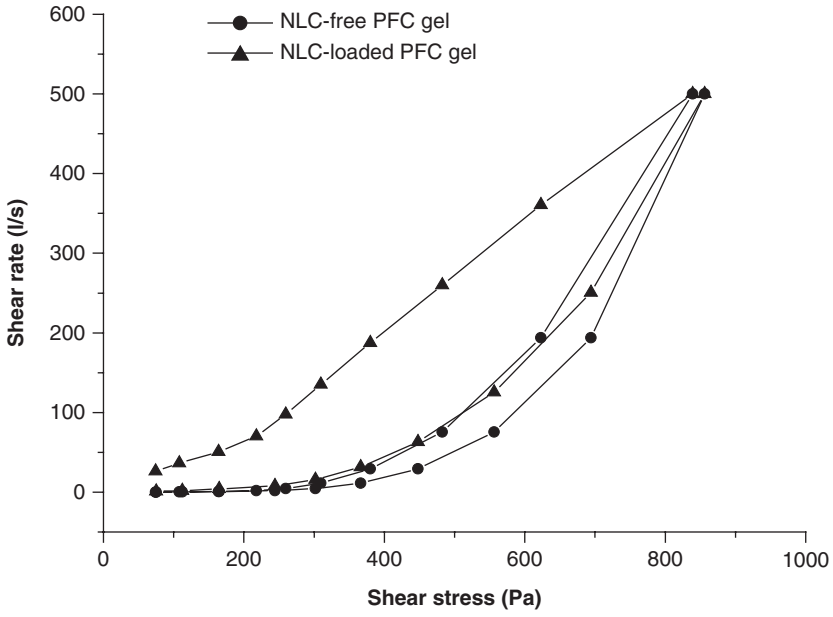

Figure 4. Shear flow investigations of NLC-free perfluorocarbon (PFC)-based hydrogels and of NLC-loaded PFC-based hydrogels.
Modified from Souto *et al.* [69].

the presence of nanoparticles in the sub-surface region and that those affect the local mechanical properties of the gels. A NLC-based Carbopol gel containing valdecoxib dissolved in a mixture of solid lipid and liquid oil revealed a faster onset but a prolonged action, as was evident from *in vitro* release and *in vivo* studies [5]. The gel showed a flow index of 0.386, indicating pseudoplastic flow behaviour. The gel was also found to be safe and did not cause drying or irritation of the skin, as revealed by the Draize patch test. Similarly, an NLC-based gel containing celecoxib also showed an initial burst–release phase followed by steady state release up to 24 h. Compared to micellar gel or a marketed hydrogel formulation, NLC-based celecoxib gel showed higher anti-inflammatory efficacy, as proved by *in vivo* testing [6]. In contrast, no burst release of indomethacin from NLC-based xanthan gum hydrogel was observed [24].

4. Conclusion

Several colloidal carriers have been studied as delivery systems for APIs across the skin. A relatively new type of system composed of NLC-loaded semi-solid has been presented in this review. If the NLC are physicochemically stabilized in the hydrogel network, this system may combine the properties of both classes of materials and may find a variety of biomedical applications. Biocompatibility and stability, the ability to deliver a broad range of APIs, and the individual specificity of both phases (gel versus NLC) turn this into a versatile delivery system relevant for topical, dermal and transdermal administration, since the skin penetration degree of API can be critically controlled. New findings on reversible and irreversible aggregation of particles can lead to novel combined drug delivery systems regarding NLC as multipurpose carriers.

5. Expert opinion

Colloidal carriers allow the properties of the API being carried to be hidden and then to control and target its release. They allow API protection against chemical and biological degradation related to the administration route. The physicochemical characteristics of the carriers themselves govern the types of application. Among the numerous nanoparticle systems, lipid structures were developed for various administration routes. Liposomes, micelles, nanoemulsions, microemulsions and lipid nanoparticles are very common due to their low toxicity, their ability to carry hydrophilic or lipophilic drugs, their ability to control and localize the release of the API and their small size. Generally, the lipid systems present the advantage of low toxicity due to their physiological lipid composition compared to polymeric particles. However, a chemical transformation of these lipids might change the structure of the system, the load and/or release capacity, the interfacial properties, and its *in vivo* fate. Physical modifications can also occur. The consequences of size control and nanoparticle growth are important considerations in preparing dispersions, and particular attention has to be focused on their evolution. For API release, the profile depends on the homogeneity of the initial product. If precipitation occurs during storage, the quantity of API delivered for each administration is unknown. Furthermore, API itself may

sometimes destabilize the initial system. The physicochemical stability of the lipid carriers may show shelf-life variations due to their complex composition and structure.

The topical route provides many advantages compared to other routes of administration. The administered API avoids systemic load as well as systemic side effects. Another advantage is the possibility to avoid passage through the liver, as well as fluctuations of plasma levels.

The delivery of APIs through the skin using conventional semi-solid formulations may not be sufficient as the molecules may not permeate through the stratum corneum in sufficient amounts. Several techniques have been developed to increase skin penetration of APIs, including the temporary weakening of the horny layer of the skin by means of iontophoresis or microporation, using penetration enhancers or pro-drug forms. One of the most promising approaches over conventional semi-solid formulations would be the use of colloidal systems carrying the APIs. The submicro-nmeter size allows close contact to the horny layer of the skin, providing occlusion and decrease of moisture loss (through evaporation), increasing its hydration and thus permeation/penetration. NLCs have the additional advantage of their lipid composition being therefore biocompatible, biodegradable and safe, achieving a controlled and/or prolonged release profile of the entrapped API in their solid matrix. Developing NLC-based semi-solid hydrogels may hold further advantages, in particular if the API is localized both within the lipid NLC matrix and in the semi-solid hydrogel. A supersaturation effect may occur, contributing to the enhanced permeability of the skin from the API. In addition, NLC-based semi-solid hydrogels increase skin hydration due to occlusiveness of the NLC, which also allows increasing the skin health and permeation. During recent years scientists have reported that lipid nanoparticles can act as efficient promoters of API delivery through the skin.

Declaration of interest

The authors state no conflict of interest and have received no payment in the preparation of this manuscript.

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Affiliation

Slavomira Doktorovova[1] & Eliana B Souto[†1,2]
[†]Author for correspondence
[1]Faculty of Health Sciences, Fernando Pessoa University, Rua Carlos da Maia, 296, 4200-15o Porto, Portugal
E-mail: eliana@ufp.edu.pt
[2]Institute of Biotechnology and Bioengineering, Centre of Genetics and Biotechnology, (IBB/CGB-UTAD)