Iranian Journal of Pharmaceutical Sciences
Summer 2011: 7(3): 139-150
ijps.sums.ac.ir

Review Article

Pharmaceutical Nanoemulsions and Their Potential Topical and Transdermal Applications

Samira Sadat Abolmaali[a], Ali Mohammad Tamaddon[a,*], Fakhr Sadat Farvadi[a], Saeed Daneshamuz[a], Hamidreza Moghimi[b]

[a]*Department of Pharmaceutics, Faculty of Pharmacy, and Pharmaceutical Sciences Research Center, Shiraz University of Medical Sciences, Shiraz, Iran*
[b]*Department of Pharmaceutics, Faculty of Pharmacy, Shaheed Beheshti University of Medical Sciences, Tehran, Iran*

Abstract

Topical and transdermal drug delivery systems are noninvasive and can be self-administered with the minimization of side-effects, have received increased attention during the past few years. Nanoemulsions, emulsions sized between 20-200 nm with narrow distributions, offer several advantages for topical and transdermal delivery of pharmaceutical agents including controlled droplet size, the ability to efficiently dissolve lipophilic drugs, enhanced skin permeation and extended release of lipophilic and hydrophilic drugs. Moreover, they exert good sensorial and physical properties such as complete dispersion on skin and skin hydration in cosmetic products. The review deals with nanoemulsion applications in topical and transdermal drug and gene delivery.

Keywords: Drug Delivery; Nanoemulsion; Nanotechnology; Skin, Topical; Transdermal.
Received: December 22, 2010; *Accepted:* March 18, 2011.

1. Introduction

Nanoemulsions: definition, composition, preparation and characterization

An emulsion is generally described as a heterogonous system composed of two immiscible liquids. Emulsions like other disperse systems are thermodynamically unstable as a result of excess free energy associated with the surface of the internal phase. Nanoemulsion is a type of emulsion sized between 20-200 nm with narrow distributions. They are transparent or translucent with a bluish coloration. So, the definition is different from that of sub-micron emulsions. It is worth saying that, while the distinction between a nanoemulsion and an emulsion, in terms of their size, is rather arbitrary, nanoemulsions because of their small droplet size, cause a large reduction in gravity force; therefore, Brownian motion may be sufficient to possess a higher stability against sedimentation or creaming than an emulsion [1].

The distinction between a microemulsion and a nanoemulsion is blurred because the

*Corresponding author: Dr. Ali Mohammad Tamaddon, Pharmaceutical Nanotechnology and Cellular Delivery Lab, Department of Pharmaceutics, Shiraz University of Medical Sciences, Shiraz, 71345, Iran.
Tel. (+98)711-2426070; Fax: (+98)711-2424126

description of a nanoemulsion is very similar to that of a microemulsion, though the physical appearance of a nanoemulsion resembles that of a microemulsion, in that both systems are transparent (or translucent) and of low viscosity, there is an essential difference between the two systems; namely that a nanoemulsion (i.e. an emulsion) is, at best, kinetically stable or metastable, while a microemulsion is thermodynamically stable [2]. Besides, nanoemulsions are two-phase systems where the dispersed phase droplet size has been made in the nanometer size range, the microemulsions, and micellar systems are single-phase systems. As a consequence, many of the nanoemulsions reported in the literature do not possess long-term stability. They may experience Ostwald ripening or coalescence instabilities that could be controlled by modifying oily phase solubility, surfactant quantity and molecular weight [1, 3]. Some nanoemulsions have, however, exhibited sufficiently high levels of stability to be proposed as vehicles for drug delivery.

One supposed advantage of a nanoemulsion over a microemulsion is that it requires a lower surfactant concentration for its formation. When comparing this surfactant concentration with the 20% surfactant typically needed to prepare a microemulsion containing a comparable amount of oil, one should realize that the droplet size of a microemulsion thus produced would typically be ~10 nm. Consequently, in order to produce nanoemulsion droplets of the comparable size, the amount of surfactant required would increase (the surface area of the droplet varies with the square of the droplet radius) to a comparable value. Moreover unlike microemulsions, they can be diluted with water without changing the droplet size distribution [4]. There are several reports in literature which seems wrongly addressed nanoemulsions prepared by titration method based on pseudoternary phase diagram at relatively high levels of surfactant and co-surfactant [5-16].

Nanoemulsions, as a consequence of their relatively high kinetic stability, low viscosity, and transparency/translucency, are very attractive for a range of industrial applications, including the pharmaceutical field where they have been explored as drug delivery systems. They offer several advantages for the delivery of drugs, biological, or diagnostic agents. Traditionally, nanoemulsions have been used in clinics for more than four decades as total parenteral nutrition fluids. Several other products for drug delivery applications such as Diprivan, Liple, and Ropion have been marketed [17] (Table1).

Although nanoemulsions are chiefly seen as vehicles for administering insoluble drugs, they have more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, genes, photosensitizers, or diagnostic agents. Research with perflurochemical nanoemulsions has shown promising results for enhanced sonography imaging and the treatment of cancer in conjugation with other treatment modalities or by targeted delivery to the neovasculature [17].

Nanoemulsions contain oil phase, surfactants or emulsifiers, active pharmaceutical ingredients (drugs or diagnostic agents), and additives. The oil phases are mainly natural or synthetic lipids, fatty acids, oils such as medium or long chain triglycerides, or perflurochemicals. The most commonly used emulsifiers and co-emulsifiers include either natural or modified lecithins, poly (ethylene oxide) (PEO)-containing block copolymers, PEG-conjugated castor oil derivatives (Cremophore EL), glycerides, and positively charged lipids. Other pharmaceutical additives such as pH adjustment agents, antioxidants, flavors, and preservatives may also be included in the final formulation, if requested [17].

Nanoemulsions are thermodynamically

unstable and require considerable mechanical energy for their preparation. The mechanical energy can be supplied in the form of high pressure homogenizer, Microfluidizer, or an ultrasonic generator.

High energy methods cannot be used for some cases, especially for labile molecules and if there is a limited access to their respective expensive equipments. In these cases, low energy emulsification methods, such as spontaneous emulsification or phase inversion are employed. Bouchemal *et al*. prepared nanoemulsions by injecting oil phase solution in a water miscible organic solvent, e.g. alcohols, into aqueous phase under magnetic stirring [18]. Diffusion of organic solvent into the external aqueous phase leads to the formation of nanodroplets. Fernandez *et al*. proposed the method of phase inversion temperature for polyoxyethylene type non-ionic surfactants [4]. Increasing emulsion temperature over phase inversion point causes oil swollen droplets (o/w emulsions) transform into water swollen droplets (w/o emulsions). The system crosses a point of zero curvature and minimal interfacial tension promoting the formation of finely dispersed nanoemulsions.

Nanoemulsion systems are routinely characterized for their particle size and surface properties (surface electrostatic charge and morphology). Size of nanoemulsion droplets determines their behavior both *in vitro* and *in vivo*. This can be measured using an ensemble (e.g., spectroscopic methods such as light scattering), counting (e.g., microscopy such as freeze fracture electron microscopy) or separation method (e.g., analytical ultracentrifugation). Similar to particle size, surface charge of the nanoemulsion droplets has marked effect on the stability of the emulsion system and the droplets' *in vivo* disposition and clearance. Conventionally, the surface charge on the emulsion droplets has been expressed in terms of zeta potential, which is routinely measured using a Zetasizer or the ZetaPlus instrument. As the emulsion droplets are a result of interfacial phenomenon brought out by surface active agents, their zeta potential is dependent on the extent of ionization of these surface active agents and counter-ion concentration. According to DLVO electrostatic theory, the stability of the colloid is a balance between the attractive van der Waals' forces and the electrical repulsion because of the net surface charge. If the zeta potential falls below a certain level, the emulsion droplets will aggregate as a result of the attractive forces. Conversely, a high zeta potential (either positive or negative), typically more than 30 mV, maintains a stable system [17].

2. Rationales for topical and transdermal applications of nanoemulsions

Nanoemulsions have been formulated for a broad variety of topical and transdermal applications in fields of cosmetic, drug and gene delivery. They are generally advantageous because of low skin irritation, high drug loading capacity and potential for skin hydration and drug permeation enhancement. The previously reported publications considering topical and transdermal applications of nanoemulsions are categorized into cosmetic, drug and gene delivery sections.

2.1. Cosmetic applications

Nanoemulsions have attracted considerable attention for application in personal hair products. They were found useful for an optimized dispersion on skin and controlled delivery of cosmetics. They are easily valued in skin care because of their good sensorial properties and their biophysical properties especially hydrating power [19].

Yilmaz and Borchert have shown the effect of lipid content and charge of nanoemulsions on skin hydration, elasticity and erythema [20, 21]. Positively charged nanoemulsion with *stratum corneum* lipids (PNSC),

positively charged nanoemulsion without *stratum corneum* lipids (PN) and negatively charged nanoemulsion with *stratum corneum* lipids (NNSC) were compared in 14 healthy female subjects. The formulations were prepared by high-pressure homogenization followed by addition of Carbopol 940 as thickener to improve low viscosity of the nanoemulsions. The formulations were stable, indicated by no significant change of the mean droplet size and the viscosity due to the presence of the co-surfactants phytosphingosine (PS) and myristic acid, providing zeta potential values of +35±4 mV for PNSC creams, +38±5 mV for PN creams and −43±5 mV for NNSC creams. These high zeta-potentials lead to strong repulsion of the nanodroplets and prevent them from aggregation, flocculation and coalescence. PNSC cream was compared to Physiogel® cream with similar compositions regarding the content of ceramide. For both formulations, the levels of skin humidity and elasticity were determined similar using Corneometer® 825 and Cutometer® SEM 575. If PNSC cream was compared to PN, all values of PNSC creams were significantly higher than those of PN creams, indicating the requirement of SC lipids in order to prolong the effect on skin properties and to improve the barrier function of skin by leading to an increase of skin humidity and thus an increase in skin elasticity. All values of PNSC creams were significantly higher than the ones of NNSC creams, indicating that PS, inducing the positive charge, was crucial for the enhanced efficacy on skin humidity and elasticity. The combined results suggested that, PNSC was significantly more effective in increasing skin hydration and elasticity than PN and NNSC indicating that phytosphingosine inducing the positive charge, SC lipids and ceramide 3B are crucial for the enhanced effect on skin hydration and viscoelasticity. In another study, Zhou and coworkers developed lecithin nanoemulsion with droplet sizes below 100 nm and improved skin hydration capacity if incorporated into o/w cream by 2.5 times of general emulsion [22]. Besides, a significant improvement in dry hair product is obtained with a prolonged effect after a cationic nanoemulsion use. Hair becomes more fluid and shiny, less brittle and non glassy [23].

2.2. Drug and gene delivery

In the field of topical and transdermal drug and gene delivery, there are several studies based on the enhancement of skin permeation and extended release for hydrophilic and lipophilic drugs, application of nanoemulsions for topical gene delivery, and photodynamic therapy.

2.2.1. Enhancement of skin permeation

Wu *et al*. [24] described topical transport of hydrophilic compounds using w/o nanoemulsion containing sorbitan monooleate (Span®80), polyoxyethylene 20 sorbitan monooleate (Tween®80), olive oil and water. Nanoemulsions were tested for their ability to facilitate transport of a model hydrophilic solute, inulin, across hairless and hairy mouse skin and hairy rat skin following topical in vitro application. The similarity of permeation profiles of inulin incorporated in water-in-oil nanoemulsions through hairy rat and hairless and hairy mouse skin strongly implies that transport of inulin from nanoemulsions has been independent of animal skin characteristics such as *stratum corneum* thickness and follicle-type. Besides, they have found that the rate and the extent of inulin transport across hairy mouse skin were highly dependent on the hydrophile-lipophile balance (HLB) of the surfactant mixture in the nanoemulsion. Nanoemulsions prepared using mixtures with lower HLB exhibited significantly higher rate and extent of transport. The authors concluded that water-in-oil nanoemulsions prepared with a lipid

phase whose HLB is compatible with normal sebum would efficiently facilitate skin transport of large hydrophilic molecules dissolved in the aqueous core and such transport is expected via a transfollicular pathway.

Mou *et al*. [25] have developed a hydrogel-thickened nanoemulsion system for topical delivery of lipophilic drugs such as camphor, menthol and methyl salicylate. The nanoemulsions had been prepared using high pressure homogenization followed by dispersion into carbomer 940-based gel matrix which had no significant influence on droplet size. The formulation, containing 5% drug, soy lecithin, Tween 80, Poloxamer 407 and propylene glycol, had spherical shape, small diameters (50-60 nm) and high permeation rate. The high permeation rates of the formulations had been attributed to several factors. The high concentration (5%) of drugs resulted in high concentration gradient, which might be the main permeation mechanism of drugs into the skin and could act as drug reservoirs where drug is released from the inner phase to the outer phase and then further onto the skin. In addition, due to the small droplet diameters, the oily droplets might embed into the *stratum corneum* and the drug molecules could directly be delivered from oily droplets into the *stratum corneum* without a transfer via hydrophilic phase of nanoemulsions. Then drug molecules permeate more easily into *stratum corneum*.

There are several classes of drugs considered for nanoemulsion formulations with enhanced topical and transdermal delivery such as steroids, non-steroidal anti-inflammatory and cytotoxic drugs.

A. Non-steroidal anti-inflammatory agents

Kuo *et al*. [26] investigated bioavailability and anti-inflammatory effect of Microfluidizer based nanoemulsions containing alpha, delta or gamma tocopherol compared to their respective nanosuspensions. The antioxidant nanoemulsion formulations, made of phosphatidyl choline and soybean oil in Tween 80 and water, had mean sizes in range of 42-56 nm. The formulations exhibited a significant anti-inflammatory effect in croton oil induced inflammation in CD-1 mouse that were associated with decreased auricular thickness and production of IL-1α and TNF-α. Nanoemulsions raised blood concentrations of delta and gamma tocopherol 2.2-2.4 fold of nanosuspension formulations, while the effect was not significant for alpha tocopherol. A similar nanoemulsion formulation was developed for aspirin [27]. The nanoemulsions, having mean particle size of 90 nm, increased twofold anti-inflammatory property of aspirin in a CD-1 mouse model of induced inflammation. This was associated with similar changes in the accumulation of auricular levels of pro-inflammatory cytokines.

Application of nanoemulsions for topical administration of ketoprofen was reported in some publications. Sakeena *et al*. have evaluated nanoemulsions of palm oil esters prepared by spontaneous emulsification method for delivery of ketoprofen in carrageenan-induced rat hind paw edema. The nanoemulsions demonstrated a significant drug release through methyl acetate membrane *in-vitro* and a comparable efficacy as compared with Fastun® gel in-vivo [28-30]. In another study, Kim *et al*. prepared nanoemulsions of ketoprofen with an acceptable stability and a high skin permeation rate [31].

Baboota *et al*. investigated the potential of nanoemulsions for transdermal delivery of celecoxib. It was revealed that the steady state flux and permeability coefficient increased significantly compared to the gel formulations. In addition, the anti-inflammatory effects were higher on carrageenan-induced paw edema in rats [32]. Wang *et al*. [33] developed different o/w

nanoemulsions of 1% curcumin, a natural polyphenolic phytochemical. They aimed to compare two methods of nanoemulsion preparation, high-speed (24000 rpm) and high-pressure homogenization (1500 bar) o/w using medium chain triacylglycerols as oil and Tween 20 as emulsifier. Mean droplet sizes of 618.6 nm for high speed homogenization and 79.5 nm for high pressure homogenization have been achieved. Enhancing effect of o/w nanoemulsions for anti-inflammation activity of curcumin was studied. Approximately, twofold more inhibitory effect of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced edema of mouse ear was resulted for high pressure homogenizer compared to high speed homogenizer. Such anti-inflammation activity was attributed to drug permeation enhancement when the emulsion droplet sizes were reduced to below 100 nm.

B. Steroids

Holler *et al*. [34] have described a comparative study that shows the influence of negative (sucrose laureate), non-ionic (polysorbate 80) and the cationic surfactant (phytosphingosine) on physicochemical behavior of o/w nanoemulsion and skin permeation of the model drugs (fludrocortisone acetate and flumethasone pivalate). The nanoemulsions were prepared by mixing the separately prepared aqueous and oily phases. The aqueous phase containing sucrose laureate or polysorbate 80 and distilled water, heated up to 50 °C under slight mixing. But, phytosphingosine was dissolved in the oil phase, containing PCL-liquid, Lipoid S75, tocopherol and the model drug (1%). The two phases were merged and pre-homogenized at 2500 rpm. Then, raw formulation was high pressure homogenized for 12 cycles at 600 bars until mean size of 100-200 nm was obtained. The phytosphingosine-free nanoemulsions with sucrose laureate and polysorbate 80 showed a uniform particle size over the whole pH-range, whereas the phytosphingosine nanoemulsions exhibited that particle sizes increase dramatically up to 1200 nm at pH 8.0. The positively charged nanoemulsions containing phytosphingosine were able to carry more efficiently fludrocortisone acetate and flumethasone pivalate into the skin than the negatively charged ones and subsequently promote the penetration of the drugs through skin. It was hypothesized by the author that the degree of skin binding is probably more important with the positively charged particles than with the negatively one as it is known that the skin is negatively charged at neutral pH. In other studies, Khang *et al*. [35, 36] were demonstrated an enhanced stability and skin permeation of lecithin and sucrose stearate based nanoemulsions of progesterone by cyclodextrins. This enhancement was more pronounced for gamma cyclodextrin and lecithin based nanoemulsions. Stecova and coworkers developed topical nanoparticulate systems (NLC, SLN and nanoemulsion) to improve skin permeation of cyproterone acetate. The highest penetration enhancement was observed for SLN formulation [37].

C. Cytotoxic agents

Tagne *et al*. [38] studied nanoemulsions of dacarbazine, which is a highly lipid soluble cytotoxic drug, and the topical application in xenograft nude mice model of a human melanoma cell line. The nanoemulsion, having mean particle size of 131 nm, demonstrated decreased negative charges which associated with better skin bilayer permeability. The formulation caused up to tenfold greater reduction in tumor size as compared with the drug suspension. In addition, during drug cessation period (12 weeks), the nanoemulsion showed fivefold greater efficacy in preventing tumor growth. Similarly, Kakumenu *et al*. [39] demonstrated in-vivo efficacy of dacarbazine nanoemulsion in an epidermoid carcinoma xenograft mouse model in comparison with dacarbazine suspension after topical administration. This

Table 1. Commercial formulations of therapeutic nanoemulsions in market.

Drug	Brand name	Manufacturer	Therapeutic indication
Propofol	Diprivan	Astrazeneca	Anesthetic
Propofol	Troypofol	Troikaa	Anesthetic
Dexamethasone	Limethason	Mitsubishi Pharmaceutical	Steroid
Alprostadil palmitate	Liple	Mitsubishi Pharmaceutica l	Vasodilator platelet inhibitor
Flurbiprofen axetil	Ropion	Kaken Pharmaceuticals	Nonsteroidal analgesic
Vitamin A, D, E, K	Vitalipid	Fresenius Kabi	Parenteral nutrition

could be attributed to the reduced particle size (111 nm versus 6000 nm), the reduced zeta potential (-3.2 versus -89.1 mV), and the greater drug dispersibility and stability.

3. Extended drug release

Alves *et al*. [40, 41] reported human skin penetration and distribution of a lipophilic drug model, nimesulide, from hydrophilic gels containing nanocarriers. They compared nanocapsules (GNM-NC), nanospheres (GNM-NS) and nanoemulsion (GNM-NE) formulations in modulating the skin penetration of nimesulide. Nanoprecipitation, interfacial deposition and spontaneous emulsification methods had been used to prepare the nanostructured dispersions. The average hydrodynamic diameters were determined 252 nm for the nanoemulsion, 277 nm for the nanocapsules and 202 nm for the nanospheres containing nimesulide. The encapsulation efficiencies were about 99%. The nanoemulsion demonstrated shear-thining rheological characteristics with no thixotropic phenomenon. The different nanocarrier systems were incorporated into the hydrophilic gels and their ability for delivery of the drug into the human skin was investigated using stripping technique and Franz-type diffusion cells. The drug was detected in the *stratum corneum* for the gel containing nimesulide-loaded nanocapsules and nanospheres, while no drug was detected for that of nanoemulsion (GNM-NE). The hypothesized mechanism by the authors is that, the formulations prepared with poly caprolactone (GNM-NC and GNM-NS) had presented a higher affinity for the horny layer. So, the result is in agreement with other studies, which reported that particulated drug carriers (microparticles and nanoparticles) improve the drug residence in the skin without increasing transdermal transport. Additionally, the high specific area of the carrier facilitates the contact of the encapsulated molecules with the *stratum corneum* [25]. The function of these particles is to deliver an ingredient to the upper layer of the skin and to prolong the time of their delivery.

Several publications concerned about application of nanoemulsions for extended release topical administrations. Fontana *et al*. prepared hydrogels containing lipid core nanocapsules and nanoemulsions of clobetasol propionate. They were compared according to their controlled release behavior and activity using *in-vitro* and in-vivo model of contact dermatitis. The authors concluded that nanoencapsulation of clobetasol in the nanocapsules led to a better control of the drug release and a better dermatological efficacy [42, 43]. Wang and coworkers prepared w/o nanoemulsion formulations with droplet sizes in range of 50-200 nm for a sustained release of morphine and an extended analgesic activity [44]. Silva *et al*. [45] described incorporation of genistein into topical nanoemulsions composed of egg lecithin, medium chain triglycerides or octyldodecanol by spontaneous emulsification. Nanoemulsions had droplet sizes in range of 230-280 nm. The loaded amount was close to 100%. The nanoemulsions exhibited a sustained permeation through pig ear skin in Franz cells.

4. Topical gene delivery

Wu *et al*. [46] described the preparation of water-in-oil nanoemulsions containing expression plasmid DNA that appear to facilitate follicular transfection following topical application *in vivo*. The nanoemulsions without DNA had mean particle sizes of 14.6-42.3 nm; while the nanoemulsion with DNA showed sizes of 20.2-32.1 nm. Expression plasmids encoding chloramphenicol acetyl-transferase (CAT) or human interferon-2 cDNA had been formulated in water-in-oil nanoemulsions and applied to murine skin. It had been shown that the deposition site of plasmid DNA was primarily in follicular keratinocytes. The transgene expression was optimal at 24 h following topical application of a single dose of water-in-oil nanoemulsion containing plasmid DNA as quantified by RT-PCR and ELISA. The efficiency of nanoemulsion mediated transfection was most effective in the context of normal versus atrophic hair follicles. The results of this study have suggested that the efficiency of transfection and the dynamics of transgene expression appear to be augmented by the use of a nanoemulsion vehicle. This may be the result of simple physical protection of plasmid DNA from endogenous deoxyribonucleases present in the skin. It is also possible that this effect is a consequence of alterations in cell membrane fluidity or membrane integrity that results from the presence of non-ionic detergents in the oil phase of the nanoemulsion. Other possibilities include undefined components of the organic plant oil that may facilitate transfection.

5. Photodynamic therapy

Photodynamic therapy was ranked as first line therapy for actinic keratosis and is acceptable for treatment of neoplastic skin diseases. The application of topical nanoemulsions for photodynamic therapy of skin conditions was reported for 5-aminolevulonic acid (ALA) and temoporfin (Foscan®). Zhang *et al*. [47] prepared o/w and w/o nanoemulsions of ALA and the methyl ester by high pressure homogenization and probe ultrasonication. The nanoemulsions were negatively charged and sized below 256 nm. It demonstrated higher loading of the methyl ester to about 68% in soybean oil o/w nanoemulsions. A higher skin permeation rate was observed for o/w nanoemulsions as compared with w/o nanoemulsions. The o/w nanoemulsion was advantageous over aqueous solution for successful topical application of ALA which required an extended release for about 24-48 h. Besides, the o/w nanoemulsions were able to exert the highest *in-vitro* flux without compromising skin barrier function. Dirschka *et al*. demonstrated that nanoemulsion based gel formulation of ALA (BF-200 ALA) had a better stability and skin permeation than ALA solution. It was shown clinically that the nanoemulsion was superior over the methyl ester cream in ex-vivo skin model [48]. Similarly, Primo *et al*. evaluated the application of o/w nanoemulsions for delivery of Foscan *in-vitro* and demonstrated confirming results [49].

6. Protection of natural antioxidants

There are reports of using nanoemulsion for stabilization of antioxidants. Junyapresert *et al*. prepared nanoemulsions of medium chain triacylglycerols (MCT) and nanostructured lipid carriers (NLC) of cetyl palmiate and MCT to load coenzyme Q10. The particles remained in the nanosize range and preserved above 90% of their content for 12 months after storage at 4, 25 and 40 °C. Due to skin occlusiveness effect, the particles promoted deep penetration of Q10 into skin *in-vitro* [50]. Mitri *et al*. have reported the application of lipid nanocarriers including solid lipid nanoparticle (SLN), NLC and nanoemulsion for ultraviolet protection of lutein, a lipid soluble antioxidant. The negatively charged nanocarriers prepared by

high pressure homogenization had sizes in range of 150-350 nm. A better skin permeation (60% in 24 h), but a lower protection (14% after 10 minimal erythema dose) was demonstrated for nanoemulsions [51].

7. Antimicrobial activity

Nanoemulsions have an enhanced antibacterial activity due to discrete droplets of oil that fuse with bacterial cell walls or viral envelopes, destabilizing the organism's lipid envelope and initiate disruption of pathogens.

Hamouda *et al*. [52, 53] have presented a novel nanoemulsion formulation with a unique topical antimicrobial activity against bacteria, enveloped viruses and fungi. The nanoemulsion prepared by mixing of heated oil phase (8 volumes tributyl phosphate, 64 volumes soybean oil) and 8 volumes Triton X-100 at 82 °C for one h with 20 volumes of deionized water with a reciprocating syringe pump. The final particle size of the nanoemulsion was reported in the range of 400-800 nm using a laser light scattering technique. The nanoemulsion has the potential to be used as a topical antimicrobial agent against several pathogens. It has bactericidal activity against vegetative forms of most Gram-positive bacteria and few Gram-negative bacteria, including *H. influenzae, N. gonorrhoeae* and *V. cholera*. *P.aeruginosa*. Enteric Gram-negative bacilli were resistant which may be attributable to their cell wall lipopolysaccharide (LPS) and negative surface charge. It was reported the nanoemulsion had a negative charge that might result in repulsive forces preventing the attachment to the bacterial cell wall.

All the tested enveloped viruses (*Herpes* simplex, influenza A and vaccinia) were sensitive to the nanoemulsion treatment to different extents. Moreover, it has been reported that the nanoemulsion is virucidal to the HIV virus. This consistent inactivation of enveloped viruses suggests that the nanoemulsion may affect the viral lipid membrane. This is further supported by the fact that nonenveloped viruses, such as adenovirus, are resistant to the treatment. The resistance of yeast cells, e.g. *Candida albicans*, to the cidal effects of the nanoemulsions is most likely due to the rigid cell wall structure in yeast. The demonstrated fungistatic action may be due to disruption of the budding process by lysing newly formed yeast buds.

In other studies, Pannu *et al*. developed novel o/w nanoemulsions with broad antifungal activity against dermatophytes and activity against *propionibacterium acne* for treatment of skin, hair and nail infections [54, 55].

8. Conclusion and future perspectives

Nanoemulsion formulations offer several advantages for topical delivery of cosmetic and pharmaceutical agents including controlled droplet size, lower concentration of surfactant and the ability to efficiently dissolve and stabilize lipophilic drugs. The trapped drug molecules in the inner phase of nanoemulsion may result in an extended drug release and a prolonged activity.

There are several mechanisms suggested for enhancement of skin permeation from nanoemulsion formulations following topical administration. Surface charge-modified nanoemulsion droplets had significant influence on the binding affinity of droplets to the skins; nanoemulsion could act as drug reservoirs and the high concentration of drugs in nanoemulsion resulted in high concentration gradient; due to the small droplet diameters of nanoemulsion, the oily droplets might embed into the *stratum corneum* and the drug molecules could directly be delivered from oily droplets into the *stratum corneum* without a transfer via hydrophilic phase of nanoemulsions. It has been shown that the positively charged lipid such as phytosphingosine may play an important role for droplet attachment to

stratum corneum for successful drug delivery. Besides, nanoemulsions are easily valued in cosmetics because of their good sensorial and physical properties such as complete dispersion on skin and hydration action in skin, hair and nail care products.

Further basic researches are required to be carried out for better understanding of how such system modifies the diffusion of actives in to the skins, how they interact with *stratum corneum* and how they affect penetration of active materials. Definitely, more human studies need to be carried out to have a "real life" data.

References

[1] Sing AF, Graciaa A, Lachaise J, Brochette P, Salager JL. Interaction and coalescence of nanodrops in transluscent o/w emulsions. *Colloid Surface A* 1999; 152: 31-9.

[2] Sarker DK. Engineering of nanoemulsions for drug delivery. *Curr Drug Deliv* 2005; 2: 297-310.

[3] Chiesta M, Garg J, Kang YT, Chen G. Thermal conductivity and viscosity of water-in-oil nanoemulsions. *Colloid Surface A* 2008; 326: 67-72.

[4] Fernandez P, Andre V, Reiger J, Kuhnle A. Nano-emulsion formation by emulsion phase inversion. *Colloid Surface A* 2004; 251: 53-8.

[5] Dixit N, Kohli K, Baboota S. Nanoemulsion system for the transdermal delivery of a poorly soluble cardiovascular drug. *PDA J Pharm Sci Technol* 2008; 62: 46-55.

[6] Shakeel F. Criterion for excipients screening in the development of nanoemulsion formulation of three anti-inflammatory drugs. *Pharm Dev Technol* 2009; 15: 131-8.

[7] Shakeel F, Baboota S, Ahuja A, Ali J, Aqil M, Shafiq S. Nanoemulsions as vehicles for transdermal delivery of aceclofenac. *AAPS Pharm Sci Tech* 2007; 8: E104.

[8] Shakeel F, Baboota S, Ahuja A, Ali J, Shafiq S. Celecoxib nanoemulsion: skin permeation mechanism and bioavailability assessment. *J Drug Target* 2008; 16: 733-40.

[9] Shakeel F, Baboota S, Ahuja A, Ali J, Shafiq S. Skin permeation mechanism and bioavailability enhancement of celecoxib from transdermally applied nanoemulsion. *J Nanobiotechnology* 2008; 6: 8.

[10] Shakeel F, Baboota S, Ahuja A, Ali J, Shafiq S. Enhanced anti-inflammatory effects of celecoxib from a transdermally applied nanoemulsion. *Pharmazie* 2009; 64: 258-9.

[11] Shakeel F, Baboota S, Ahuja A, All J, Shafiq S. Skin permeation mechanism of aceclofenac using novel nanoemulsion formulation. *Pharmazie* 2008; 63: 580-4.

[12] Shakeel F, Faisal MS. Nanoemulsion: a promising tool for solubility and dissolution enhancement of celecoxib. *Pharm Dev Technol* 2009; 15: 53-6.

[13] Shakeel F, Ramadan W. Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. *Colloids Surf B Biointerfaces* 2009; 75: 356-62.

[14] Shakeel F, Ramadan W, Ahmed MA. Investigation of true nanoemulsions for transdermal potential of indomethacin: characterization, rheological characteristics, and ex vivo skin permeation studies. *J Drug Target* 2009; 17: 435-41.

[15] Shakeel F, Ramadan W, Gargum HM, Singh R. Preparation and *in vivo* evaluation of indomethacin loaded true nanoemulsions. *Sci Pharm* 2009; 78: 47-56.

[16] Zheng WW, Zhao L, Wei YM, Ye Y, Xiao SH. Preparation and the in vitro evaluation of nanoemulsion system for the transdermal delivery of granisetron hydrochloride. *Chem Pharm Bull (Tokyo)* 2010; 58: 1015-9.

[17] Tiwari SB, Amiji MM. Nanoemulsion formulations for tumor targeted delivery In: Amiji M.M., Nanothechnology for cancer therapy, 2007: New York: CRC press, pp. 723-40.

[18] Bouchemal K, Briancon S, Couenne F, Fessi H, Tayakout M. Stability studies on colloidal suspensions of polyurethane nanocapsules. *J Nanosci Nanotechnol* 2006; 6: 3187-92.

[19] Sonneville-Aubrun O, Simonnet JT, L'Alloret F. Nanoemulsions: a new vehicle for skincare products. Adv Colloid Interface Sci 2004; 108-109: 145-9.

[20] Yilmaz E, Borchert HH. Design of a phytosphingosine-containing, positively-charged nanoemulsion as a colloidal carrier system for dermal application of ceramides. *Eur J Pharm Biopharm* 2005; 60: 91-8.

[21] Yilmaz E, Borchert HH. Effect of lipid-containing, positively charged nanoemulsions on skin hydration, elasticity and erythema--an *in vivo* study. *Int J Pharm* 2006; 307: 232-8.

[22] Zhou H, Yue Y, Liu G, Li Y, Zhang J, Gong Q, Yan Z, Duan M. Preparation and characterization of a lecithin nanoemulsion as a topical delivery system. *Nanoscale Res Lett* 2009; 5: 224-30.

[23] Patravale VB, Mandawgade SD. Novel cosmetic delivery systems: an application update. *Int J Cosmet Sci* 2008; 30: 19-33.

[24] Wu H, Ramachandran C, Weiner ND, Roessler BJ. Topical transport of hydrophilic compounds using water-in-oil nanoemulsions. Int J Pharm 2001; 220: 63-75.
[25] Mou D, Chen H, Du D, Mao CH, Wan J, Xu H, Yang X. Hydrogel-thickened nanoemulsion sytem for topical delivery of lipophilic drugs. *Int J Pharm* 2008; 353: 270-6.
[26] Kuo F, Subramanian B, Kotyla T, Wilson TA, Yoganathan S, Nicolosi RJ. Nanoemulsions of an antioxidant synergy formulation containing tocopherol have enhanced bioavailability and anti-inflammatory properties. *Int J Pharm* 2008; 363: 206-13.
[27] Subramanian B, Kuo F, Ada E. Enhancement of anti-inflammatory property of aspirin in mice by a nano-emulsion preparation. *Int Immunopharmacol* 2008; 8: 1533-9.
[28] Sakeena MH, Elrashid SM, Muthanna FA, Ghassan ZA, Kanakal MM, Laila L, Munavvar AS, Azmin MN. Effect of limonene on permeation enhancement of ketoprofen in palm oil esters nanoemulsion. *J Oleo Sci* 2010; 59: 395-400.
[29] Sakeena MH, Muthanna FA, Ghassan ZA, Kanakal MM, Elrashid SM, Munavvar AS, Azmin MN. Formulation and *in vitro* evaluation of ketoprofen in palm oil esters nanoemulsion for topical delivery. *J Oleo Sci* 2010; 59: 223-8.
[30] Sakeena MH, Yam MF, Elrashid SM, Munavvar AS, Azmin MN. Anti-inflammatory and analgesic effects of ketoprofen in palm oil esters nanoemulsion. *J Oleo Sci* 2010; 59: 667-71.
[31] Kim BS, Won M, Lee KM, Kim CS. In vitro permeation studies of nanoemulsions containing ketoprofen as a model drug. *Drug Deliv* 2008; 15: 465-9.
[32] Baboota S, Shakeel F, Ahuja A, Ali J, Shafiq S. Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. *Acta Pharm* 2007; 57: 315-32.
[33] Wang X., Jiang Y, Wang Y, Huang M, Ho C, Huang Q. Enhancing anti-inflammation activity of curcumin through o/w nanoemulsions. *Food Chem* 2008; 108: 419-24.
[34] Hoeller S, Sperger A, Valenta C. Lecithin based nanoemulsions: a comparative study of the influence of non-ionic surfactants and the cationic phytosphingosine on physicochemical behaviour and skin permeation. *Int J Pharm* 2009; 370: 181-6.
[35] Klang V, Matsko N, Raupach K, El-Hagin N, Valenta C. Development of sucrose stearate-based nanoemulsions and optimisation through gamma-cyclodextrin. *Eur J Pharm Biopharm* 2011; 79: 58-67.
[36] Klang V, Matsko N, Zimmermann AM, Vojnikovic E, Valenta C. Enhancement of stability and skin permeation by sucrose stearate and cyclodextrins in progesterone nanoemulsions. *Int J Pharm* 2010; 393: 152-60.
[37] Stecova J, Mehnert W, Blaschke T, Kleuser B, Sivaramakrishnan R, Zouboulis CC, Seltmann H, Korting HC, Kramer KD, Schafer-Korting M. Cyproterone acetate loading to lipid nanoparticles for topical acne treatment: particle characterisation and skin uptake. *Pharm Res* 2007; 24: 991-1000.
[38] Tagne JB, Kakumanu S, Nicolosi RJ. Nanoemulsion preparations of the anticancer drug dacarbazine significantly increase its efficacy in a xenograft mouse melanoma model. *Mol Pharm* 2008; 5: 1055-63.
[39] Kakumanu S, Tagne JB, Wilson TA, Nicolosi RJ. A nanoemulsion formulation of dacarbazine reduces tumor size in a xenograft mouse epidermoid carcinoma model compared to dacarbazine suspension. *Nanomedicine* 2011; 7: 277-83.
[40] Alves MP, Scarrone AL, Santos M, Pohlmann AR, Guterres SS. Human skin penetration and distribution of nimesulide from hydrophilic gels containing nanocarriers. *Int J Pharm* 2007; 341: 215-20.
[41] Alves PM, Pohlmann AR, Guterres SS. Semisolid topical formulations containing nimesulide-loaded nanocapsules, nanospheres or nanoemulsion: development and rheological characterization. *Pharmazie* 2005; 60: 900-4.
[42] Fontana MC, Coradini K, Guterres SS, Pohlmann AR, Beck RC. Nanoencapsulation as a way to control the release and to increase the photostability of clobetasol propionate: influence of the nanostructured system. *J Biomed Nanotechnol* 2009; 5: 254-63.
[43] Fontana MC, Rezer JF, Coradini K, Leal DB, Beck RC. Improved efficacy in the treatment of contact dermatitis in rats by a dermatological nanomedicine containing clobetasol propionate. *Eur J Pharm Biopharm* 2011; 79: 241-9.
[44] Wang JJ, Hung CF, Yeh CH, Fang JY. The release and analgesic activities of morphine and its ester prodrug, morphine propionate, formulated by water-in-oil nanoemulsions. *J Drug Target* 2008; 16: 294-301.
[45] Silva AP, Nunes BR, De Oliveira MC, Koester LS, Mayorga P, Bassani VL, Teixeira HF. Development of topical nanoemulsions containing the isoflavone genistein. *Pharmazie* 2009; 64: 32-5.

[46] Wu H, Ramachandran C, Bielinska AU, Kingzett K, Sun R, Weiner ND, Roessler BJ. Topical transfection using plasmid DNA in a water-in-oil nanoemulsion. *Int J Pharm* 2001; 221: 23-34.

[47] Zhang LW, Al-Suwayeh SA, Hung CF, Chen CC, Fang JY. Oil components modulate the skin delivery of 5-aminolevulinic acid and its ester prodrug from oil-in-water and water-in-oil nanoemulsions. *Int J Nanomedicine* 2011; 6: 693-704.

[48] Dirschka T, Radny P, Dominicus R, Mensing H, Bruning H, Jenne L, Karl L, Sebastian M, Oster-Schmidt C, Klovekorn W, Reinhold U, Tanner M, Grone D, Deichmann M, Simon M, Hubinger F, Hofbauer G, Krahn-Senftleben G, Borrosch F, Reich K, Berking C, Wolf P, Lehmann P, Moers-Carpi M, Honigsmann H, Wernicke-Panten K, Helwig C, Foguet M, Schmitz B, Lubbert H, Szeimies RM. Photodynamic therapy with BF-200 ALA for the treatment of actinic keratosis: results of a multicentre, randomized, observer-blind phase III study in comparison to a registered methyl-aminolaevulinic acid cream and placebo. *Br J Dermatol* 2012;166:137-46.

[49] Primo FL, Bentley MV, Tedesco AC. Photophysical studies and in vitro skin permeation/retention of foscan/nanoemulsion (NE) applicable to photodynamic therapy skin cancer treatment. *J Nanosci Nanotechnol* 2008; 8: 340-7.

[50] Junyaprasert VB, Teeranachaideekul V, Souto EB, Boonme P, Muller RH. Q10-loaded NLC versus nanoemulsions: stability, rheology and in vitro skin permeation. *Int J Pharm* 2009; 377: 207-14.

[51] Mitri K, Shegokar R, Gohla S, Anselmi C, Muller RH. Lipid nanocarriers for dermal delivery of lutein: preparation, characterization, stability and performance. *Int J Pharm* 2011; 414: 267-75.

[52] Hamouda T, Hayes MM, Cao Z, Tonda R, Johnson K, Wright DC, Brisker J, Baker JR Jr. A novel surfactant nanoemulsion with broad-spectrum sporicidal activity against bacillus species. *J Infect Dis* 1999; 180: 1939-49.

[53] Hamouda T, Myc A, Donovan B, Shih AY, Reuter JD, Baker JR Jr. A novel surfactant nanoemulsion with a unique non-irritant topical antimicrobial activity against bacteria, enveloped viruses and fungi. *Microbiol Res* 2001; 156: 1-7.

[54] Pannu J, McCarthy A, Martin A, Hamouda T, Ciott Si, Fothergill A, Sutcliffe J. NB-002, a novel nanoemulsion with broad antifungal activity against dermatophytes, other filamentous fungi, and Candida albicans. *Antimicrob Agents Chemother* 2009; 53: 3273-9.

[55] Pannu J, McCarthy A, Martin A, Hamouda T, Ciotti S, Ma L, Sutcliffe J, Baker JR Jr. In vitro antibacterial activity of NB-003 against Propionibacterium acnes. *Antimicrob Agents Chemother* 2011; 55: 4211-7.