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NANOEMULSION FOR SKIN DRUG DELIVERY

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ABSTRACT

The use of nanoemulsion in augmenting dermal and transdermal effectiveness of drugs has now well established. The development of nanoemulsion based semisolid dosage forms is an active area of present research. However, thickening or liquid-to-semisolid conversion of the nanoemulsions provides opportunities to the formulation scientist to explore novel means of solving instability issues during transformation. Extending knowledge about the explicit role of nature/magnitude of zeta potential, types of emulsifiers and selection of appropriate semisolid bases could place these versatile carriers from laboratory to industrial scale. Nanoemulsions are termed as biphasic (O/W or W/O) or multiple nanoemulsions (W/O/W). Oil phase components include fatty acids (e.g., oleic acid), esters of fatty acids and alcohols (e.g., isopropyl myristate, isopropyl palmitate, ethyl oleate), medium chain triglycerides, triacetin, terpenes (e.g., limonene, menthol, cineole) and other penetration enhancers. Till date several approaches have been employed to develop nanoemulsions (size range 20–200 nm) which are High-energy emulsification and Low-energy emulsification. NanoemulsionS are found effective to treat psoriasis, dermatitis and cancer.

Key words: Nanoemulsion, Composition, development, drug release, characterization

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INTRODUCTION

Despite large number of therapeutics have been discovered, very few drugs have achieved clinical success. The lesser success rate is most often attributed to the lower 'bioavailability' and poor site specificity. These metrics mainly depend on the route of drug administration, organ physiology and metabolism **[1]**. Systemic route portrays rapid fluctuation in drug plasma level i.e. below or above, requires frequent dosing and causes pain at the injection site. As a consequence, sometimes delivery of drug in systemic route becomes uncomfortable **[2]**.

Oral route is the most common and preferred route of administration, however it may be associated with possible drug and food interactions, susceptibility to the degradation in gastric environment, first pass metabolism and many more **[3]**. In parallel, the versatile nature of the skin makes it the most useful site for drug administration. It has been used preferably for the management of several skin diseases such as inflammation, microbial infections, psoriasis, dermatitis and many more. Transdermal route of drug administration has been employed

constantly in the management of various systemic disorders such as hypertension, arthritis, diabetes, cancer etc. It helps overcome drawbacks associated with oral and intravenous routes **[4]**. Dermal and transdermal routes offer larger surface area available for drug absorption, ease in accessibility and termination of therapy whenever required **[4, 5]**. Drug delivery through skin helps in the management of both topical as well as systemic disorders **[5]**. It is pain free method of administration, facilitate self-medication in the patients, preferred in long term management of the ailments like chronic pain **[6]** and avoids hepatic first pass metabolism **[7]**. These routes are cost effective on a monthly cost basis, as these are designed to deliver drugs from days to months. Acceptability of these routes of drug administration is high as evident from increasing market. The global market of topical dosage forms alone was worth $9.44 billion in 2013 and going to reach at worth $11.21 billion by 2018 with the growth rate of 3.50% **[8]**. The delivery of drugs through skin has become an attractive area for researchers **[6]**. Application of most of the existing drugs are limited that require modification in their physicochemical characteristics before administration. Drugs those belong to the class II and IV of Biopharmaceutical Classification System (BCS) and have unintended physicochemical properties like rapid degradation in the gastric environment **[9]**, high first pass metabolism **[10]** could be the suitable candidates for dermal and transdermal administration. Till date many efforts have been made to exploit the different formulation strategies to deliver the drug through skin. However, dermal and transdermal administration of semisolid preparations face several limitations due to the larger globule size, slow penetration, rapid volatilization of highly volatile compounds, degradation by the environmental effect, photo-instability and many more. Exhaustive investigations have been carried out towards the exploration of nanotechnologies so as to deliver even less soluble, highly irritant, highly volatile, charged surfaces and photosensitive molecules **[11]**. As evident from the literature, nanoemulsion technology is anticipated as the best technology because of its fluidic nature, prominent interaction with the skin cells, small droplet size, efficient permeation ability, protection ability to deliver even irritant, volatile and high molecular weight molecules. Nanoemulsions interact with the skin cells rapidly due to its fluidic nature and surfactant/emulsifier interface. Considering the prospects and industrial significances of nanoemulsion, an effort was made to assemble strategies to formulate nanoemulsions, challenging issues like long term stability and fate of nanoemulsion in the treatment of major disorders following dermal and transdermal route of administration.

NANOEMULSION

Nanoemulsions are oil-in-water (O/W), water-in-oil (W/O) dispersion of two immiscible liquids stabilized using an appropriate surfactant **[11]**. The mean droplet diameter attained is usually <500 nm **[12]**. Small droplet size gives them a clear or hazy appearance which differs from milky white color associated with coarse emulsion (whose micronsized droplets partake in multiple light scattering) **[13]**. The word nanoemulsion is sometimes used interchangeably with submicron emulsion or mini emulsion; however it should not be confused with microemulsion. Nanoemulsions despite having the same droplet size range as microemulsions, differ tremendously in structural aspects and long term thermodynamic stability **[14]**. Nanoemulsions can be rendered into several dosage forms, like liquids **[15]**, creams **[16,17]**, sprays **[18]**, gels **[19,20]**, aerosols **[21, 22]**, foams **[23]**; and can be administered by equally varying routes like topical **[24]**, oral **[25]**, intravenous **[26]**, intranasal **[27]**, pulmonary and ocular **[28]**. They possess higher solubilization capacity than

simple micellar dispersions, greater kinetic stability than coarse emulsions and have found use in cosmetic **[29]** and pesticide industry **[30]** as aqueous base for organic deliverables. Their long-term physical stability is a direct consequence of small droplet size, which impairs conventional destabilization phenomena like creaming, sedimentation and coalescence. Often brownian motion is strong enough to offset gravity or viscosity induced kinetic instability.

TYPES OF NANOEMULSIONS

Depending on constituents and relative distribution of the internal dispersed phase/phases and the more ubiquitous continuous phase, nanoemulsions are termed as biphasic (O/W or W/O) or multiple nanoemulsions (W/O/W). Phase volume ratio (Φ) measures comparative volumes of internal and external phase comprising a nanoemulsion and determines its droplet number and overall stability. Normally, phase present in greater volume becomes the external phase. To predict type of nanoemulsion formed under given conditions, interaction of various components making up the nanoemulsion must be estimated. If chief surfactant is water soluble it favors O/W emulsification, and conversely if surfactant is oil soluble it favors W/O emulsification. Polar portion of an emulsifier is generally better barrier to coalescence than hydrocarbon region. It is therefore possible to make O/W emulsion with relatively high internal phase volumes. On the other hand W/O emulsions invert easily if water content is raised and therefore continuous dilution of a nanoemulsion either with water or oil can reveal its type. Usually increasing Φ beyond 40% can lead to phase inversion of a W/O emulsion. Some oils fluoresce when excited with UV light and if incorporated in an O/W nanoemulsion, produce a dotted pattern of fluorescence upon illumination. Conversely, in case of a W/O nanoemulsion the entire field lights up. Conductivity tests can also be used to differentiate between O/W and W/O subtype of nanoemulsions **[31]**. In multiple nanoemulsions inner water phase is dispersed in an oil phase which in turn is distributed within a bulk aqueous phase within a single system.

COMPOSITION

The choice of emulsion components and ratios of these components is critical in generating stable emulsion systems with appropriate particle sizes. Oil phase components include fatty acids (e.g., oleic acid), esters of fatty acids and alcohols (e.g., isopropyl myristate, isopropyl palmitate, ethyl oleate), medium chain triglycerides, triacetin, terpenes (e.g., limonene, menthol, cineole) and other penetration enhancers. These may be used alone or in combination to form the oil phase. The aqueous phase may include sodium chloride and buffer salts, preservatives and penetration enhancers. Viscosity enhancing agents (e.g., Carbopol®, Aerosil®, gelatin) are incorporated to reduce the fluidity and generate the desired final consistency of the product .A wide range of materials has been used as surfactants and cosurfactants. Consideration must be given to combinations that effectively reduce interfacial tension and produce stable emulsions with appropriate particle size, but which also ensure minimal skin irritancy; thus, the preference for non-ionic surfactants. Commonly used surfactants include Tween® (polysorbates), Cremophor® (mixture of macrogol glycerol hydroxystearate, PEG-40 castor oil, polyoxyl 40 hydrogenated castor oil), Transcutol® P (diethylene glycol monoethyl ether), Plurol Oleique® (polyglyceryl-3-oleate), Plurol Isostearique® (isostearic acid ester of poly-glycerols and higher oligomers) and Labrasol® (mixture of mono-, di- and tri-glycerides of C8 and C10 fatty acids, and mono- and di-esters of PEG) **[32]**. Lecithin, an amphiphilic compound, has been widely investigated as the “ideal” surfactant because it is a natural compound with a low skin irritancy profile. Organogels are w/o ME based on lecithin and an a polar organic

solvent, that form gel-like reverse micellar systems with high viscosity, solubilisation capacity and thermodynamic stability, and are transparent and biocompatible **[33]**. Cosurfactants are generally short and medium chain alcohols and polyglycerylderivatives, including ethanol, isopropanol, isopropyl myristate and propylene glycol (PG). Nonionic surfactants have also been used to provide low irritancy cosurfactants **[34, 35]**.

METHODS OF PREPARATION

Selection of ingredients, their appropriate concentration, order of addition, appropriate method of preparation, optimum stirring speed/shear stress are the key points which are considered before the development of the quality product. Using excess amount of emulsifiers generates new surface area during emulsification process and hence inhibits shear induced coalescence of the nanoemulsion droplets. The extra molecules of emulsifier remain in the system in the form of micelles, get dissociate into monomers whenever required and rapidly adsorb over the surfaces of newly form droplets. The nanoscale droplet formation should be ensured either by applying extreme shear or phase inversion emulsification approach **[35]**. Till date several approaches have been employed to develop nanoemulsions (size range 20–200 nm) which are High-energy emulsification and Low-energy emulsification.

High Energy Emulsification Methods

High energy methods depend on mechanical devices to create powerful disruptive forces for size reduction. Disruptive forces are achieved via ultrasonicators, microfluidizer and high pressure homogenizers which are industrially scalable. Their versatility lies in the fact that almost any oil can be subjected to nanoemulsification. Major disadvantages include instrumental cost and generation of high operational temperatures which sometimes rules out thermolabile drugs.

Microfluidizer

A microfluidizer (Microfluidics TM Inc., U.S.A.) concomitantly useshydraulic shear, impact, attrition, impingement, intense turbulence and cavitation, to effect size reduction. It forces feed material through an interaction chamber consisting of microchannels under influence of a high pressure displacement pump (500–50,000 psi), resulting in very fine droplets. Usually a coarse emulsion is passed repeatedly (sometimes up to100 cycles) through a microfluidizer until desired size and dispersity is obtained. Impaction energy generated by collision of droplets dissipates in form of heat and requires cooling. Weber number is a dimensionless number in fluid mechanics which analyses pattern of fluid flow and correlates homogenization efficiency with viscosity ratio of dispersed and continuous phase and can be a good starting point to gauge overall efficiency of high pressure homogenization **[36]**. Biggest advantage of this highly scalable process is zero contamination of feed material as reduction is effected by source material itself.

Piston Gap Homogenizer

Piston gap homogenizers work on principle of colloid mills. A coarse emulsion is made to pass through a narrow gap (of dimension b 10 μm) between a fixed stator and a rapidly moving rotor. Size reduction is caused by high shear, stress and grinding forces generated between rotor and stator **[37]**. The upper ceiling of droplet size can be ascertained by fixing dissipation gap to required size, which implies that a yield will not be obtained unless and until emulsion is ground down to a size which is equal or lower to that of the gap between rotor and stator.

Ultrasonication

Ultrasonication methods depend on high-frequency sound waves (20 kHz and up). They can be used to form a nanoemulsion in situ or reduce size of a pre-formed emulsion. Bench-top sonicators consist of a piezoelectric probe which

generates intense disruptive force at its tip **[38]**. When dipped in a sample, ultrasonic waves produce cavitation bubbles which continue to grow until they implode .This implosion setsup shock waves, which in turn create a jet stream of surrounding liquid, pressurizing dispersed droplets and effecting their size reduction **[39]**. Investigation into operational parameters has revealed that droplet size decreases with increasing sonication time and input power **[40]**. Probes in an ultrasonicator are available in variety of dimensions which affect their functionality. Usually narrower probes are preferred
for working on small volume batches. Relative placement of probe in the sample, i.e. depth to which it is dipped alters pattern of wave reflection and pressure distribution and consequently it should not touch any solid surface. Procedurally, a coarse emulsion is prepared by addition of
a homogenous oil phase to aqueous phase under mechanical stirring. The emulsion is then subjected to ultrasonication at different amplitudes for short time cycles until desired properties are obtained for nanoemulsion. Compared to other high energy methods, ultrasonication requires least energy expenditure. One serious downside of this technique is contamination induced by probe. For scale up applicability, commercial homogenizers based on sonication have been developed, in which nanoemulsion is made to flow through a special column capable of producing ultra-sonic waves.

Low Energy Emulsification Method

Nanoemulsions prepared by low-energy emulsification methods were developed after studying cumulative behavior of oil, surfactants, co-surfactants, drug, aqueous component, hydrophilic lipophilic balance of utilized oil surfactant blend, and operative temperature **[41]**.Low-energy methods include spontaneous emulsification **[42]**, phase inversion and the less utilized catastrophic phase inversion method. A key character of these methods is utilization of energy stored in the system to produce ultra-fine droplets. Low energy methods are sometimes limited by oil type and emulsifiers that can be used.

Spontaneous Emulsification

Spontaneous emulsification is akin to nanoprecipitation method utilized in manufacturing polymeric nanoparticles. However, instead of polymer, oil is used. The procedure involves preparation of two phases, one a hydrophilic surfactant containing aqueous phase and other an organic or oil phase such as mygliol containing a drug, an oil soluble surfactant such as Span and a partially water miscible organic solvent such as acetone or ethyl acetate. Organic phase is added drop wise to aqueous stirred phase (although the reverse i.e. adding water to oil is equally feasible in case of W/O emulsions) to form small nanoscale emuslions.

Why does spontaneous emulsification occur?

For a process to be spontaneous, overall change in ΔG should be negative. Since nanoemulsions traditionally have a positive finite interfacial tension it has to be offset by increased entropy generated by diffusion of oil droplets. Energy of emulsification which is expended in development of new surface area is thus provided by accompanying turbulence. In some cases small amount of external energy may still be required (supplied by a magnetic stirrer), yet the actual process of emulsification occurs spontaneously. To further elaborate; suppose oil is dissolved in a water miscible organic solvent like acetone which is to be emulsified into aqueous surfactant solution. Upon introduction, acetone in organic phase and water move towards each other. This leaves behind tiny droplets of oil, which are immediately covered up by surfactant molecules. Mild magnetic stirring is helpful in setting up tiny convection currents which consistently distribute oil droplets in bulk so that any new surface

generated by solvent diffusion is immediately covered by surrounding surfactant molecules. To prepare very small droplets, it is usually necessary to use a high ratio of water miscible component-to-oil in the organic phase prior to mixing.

Phase Inversion Method

Phase inversion temperature (PIT) methods form nanoemulsions by exploiting changes in aqueous/oil solubility of surfactants in response to temperature fluctuation. It involves ordered conversion of a W/O to O/W emulsion or vice versa via an intermediary bicontinuous phase. Usually an oil, water, and surfactant blend is heated past a predetermined temperature, termed as PIT (specific for the utilized formulation blend), and then cooled rapidly. Temperature change from low to high leads to opening and reversal of interfacial structure causing phase inversion. When this is followed by rapid quenching, interfacial structure closes again trapping oil or water. This is a bottom up process and nascent droplets remain stable over a considerable period of time due to rich surfactant coverage. Since input of heat is necessary, PIT methods may rule out utilization of thermosensitive drugs. Also good mutual solubility of water, oil, surfactant and drug is a prerequisite to facilitate smooth phase transition. Any destabilization is governed by Ostwald ripening only.

IN-VITRO CHARACTERIZATION OF NANOEMULSION

Adherence to a strict droplet size is a perquisite whilst fabricating nanoemulsions, and size estimation is mandatorily performed following formulation. Droplet size influences many properties. Larger, more spherical drops will typically flow easier than smaller or distorted droplets which tend to stick together. Uniformity of droplet size distribution is measured by polydispersity index; nanoemulsions are generally referred to as ‘monodisperse’ if polydispersity index is <0.2. Particle size analyzers measure droplet radius using photon correlation spectroscopy (PCS) or laser diffraction. PCS has limitations though, in terms of overall derivable information. It sometimes misses out on smaller populations, which differ substantially from average population. It is also impossible to differentiate blank droplets (which do not possess any drug molecule), surfactant aggregates, liposomes, micelles, nanoparticles or one colloidal form from other. Additionally, shape of oil dropletsis taken as a perfect sphere which is not always the case. Furthermore, dilution of a sample is often required, which alters its native state. Therefore, for exact visualization (globule size, volume fraction, shape,) electron microscopy (SEM, TEM, cryo-TEM, freeze-fracture), neutron and X-ray scattering are applied to substantiate data obtained via PCS. SEM produces considerably deep two dimensional images and is beneficial in identifying topography, contours and morphology of a droplet. Freeze-fracturing can add value to SEM data, wherein rapidlyfrozen nanoemulsion droplets are ruptured open, to observe their interior which allows exact identification, localization and capturing of relative symmetry of constitutive lipids, surfactants, and co-surfactants **[43]**. To study real time droplet dynamics, nanoemulsions are fixed in glassy solids undergoing phase transition and then visualized underan electron microscope or directly analyzed using small-angle X-ray scattering. TEM is also employed for observing nanoemulsion characteristics. It gives a wholesome picture, since it can capture coexisting structures (such as coated droplets, blank vesicles, any deviation from spherical structure, presence of aggregates, etc.), and microstructural details of the nanoemulsion itself. Grapentin et al. have comparatively utilized PCS and cryo-TEM to evaluate stability of perfluorocarbon nanoemulsions for up to one year. Results suggested that PCS alone produced

misleading results with respect to stability however its combination with cryo-TEM gave greater insight into evolution of droplet dynamics, with both techniques assisting one another **[44]**. Optical characterization tools further include refractive index measurements, static light scattering, diffusing-wave spectroscopy etc. Refractive index of a developed nanoemulsion (measured by a refractometer) attains special significance when it is intended for ophthalmologic administration. Ophthalmic formulations should be as transparent as possible, to preclude any discrepancy between normal and post administrational patient vision. Diffusing-wave spectroscopy is used to analyze thick concentrated samples as it is not constrained by multiple light scattering. Zeta Potential is used for gauging charge on nanoemulsion surface, which provides clues towards its long term stability and in some cases interaction with target matrix. It is determined indirectly using principle of electrophoretic mobility. As a rule of thumb zeta potential values N +30 mV or b-30 mV are considered as good indicators of long term stability. Nanoemulsions with lower zeta potential may eventually aggregate and even phase separate. Manipulating zeta potential is therefore a method of enhancing emulsion stability. Using a combination of zeta potential measurements, absorption spectroscopy, and fluorescence quenching it is possible to delineate conformational changes occurring in the molecules making up a nanoemulsion with respect to alterations in drug loading pH, temperature and other processing variables **[45]**.

STABILITY IN NANOEMULSIONS

Droplet Aggregation

Instability affecting nanoemulsion is usually mediated by droplet aggregation which causes growth of droplet size, implying that all special characteristics imparted due to nano scale are lost. Greater aggregation may ultimately result in phase separation which causes irreversible damage.

Whilst designing a nanoemulsion, interaction (IE) between droplets should be reduced to a minimum by calibrating all the factors stated above and plotting a potential energy curve against inter-droplet distance. The minimum in this potential energy curve coincides with distances to which any two droplets could approach each other without interacting and can give a crude idea of the overall stability of nanoemulsion. As a rough guide, higher zeta potential is a guaranteed indicator of stability.

Ostwald Ripening

Ostwald ripening (OR) is a diffusive phenomenon, which leads to growth in droplet size; coarsening of emulsion and ultimately phase separation. Ostwald ripening is loosely interchangeable with flocculation and is especially evident in nanoemulsions. In Ostwald ripening, larger droplets grow at the expense of smaller ones. Ostwald ripening is driven by magnitude of Laplace pressure, which is pressure difference across a curved interface. Ostwald ripening of nanoemulsions can therefore be very rapid in comparison to regular emulsions/dispersions as differences in Laplace pressures are much greater **[46]**.

Prevention of Ostwald Ripening

Theoretically, employing an oil phase which has very low aqueous solubility can prevent Ostwald ripening forever. This however is not always feasible, and in practice, lipidic blends of MCT and LCT are employed to increase complexity of formulation in order to stall Ostwald ripening. One such approach is the trapped species method, wherein, a susceptible dispersed phase is trapped inside a normally Ostwald ripening insensitive phase. Internal osmotic pressure created by the trapped species counters Laplace pressure and reduces coarsening of nanoemulsion **[47]**. Delmas et al. have shown that Ostwald ripening of nanoemulsions, can be completely stopped even at very high

temperatures by adding wax to the normally used oil blend of mono-,di-, and triglycerides **[48]**. The trapped species method though effective in reducing Ostwald ripening, limits size reduction possibility due to presence of an immobile species within the droplet whose size is being reduced. In order to overcome this drawback, another method namely evaporational ripening has been developed. Here, an O/Wemulsion is formulated using an oil phase that typically consists of a polymer dispersed in a highly volatile solvent. When such a system is heated the polymer solvent vaporizes, leaving behind concentrated polymer droplets. Evaporation of volatile solvent provides an infinite sink to overcome internal osmotic pressure created by dense polymer droplets. Emulsion thus becomes finer with passage of time and OR can be effectively avoided for years **[49]**. Nam et al. in their efforts to prevent OR demonstrated that O/W nanoemulsions can be successfully stabilized by usage of surfactants which form a physically robust interphase. They used amphiphilic block copolymer poly (ethylene oxide)-poly (ε-caprolactone) (PEO-b-PCL) which is soluble in oil phase at higher temperatures, but recrystallizes as soon as the system is bought back to ambient temperature and prevents size growth due to Ostwald ripening **[50]**.

Coalescence

Another aspect which creates instability in nanoemulsion is coalescence of droplets. Ostwald ripening and coalescence act simultaneously to accelerate destabilization of nanoemulsions. Coalescence is borne out of kinetic phenomena such as creaming, sedimentation and sometimes even random thermodynamic fluctuations which promote segregation, attachment or impingement of dispersed phase droplets. Sedimentation and creaming are reversible as droplets can be re-dispersed by simple shaking but coalescence leads to irreversible emulsion damage. .It can be inferred from Stoke's law that nanoemulsions with smaller radii should be resistant to destabilization phenomena such as sedimentation or creaming. Yet, coalescence is found to be a contributing factor in destabilization of nanoemulsion structure. It can be avoided by using a more hydrophilic surfactant, which tends to form a thicker hydration layer around interface, increasing its elasticity (it resists interface breakage and subsequent droplet attachment), or using a charged surfactant which can provide an electrostatic stabilization apart from the usual steric stabilization.

Thermodynamic, Centrifugation, pHh Dependent and Rheological Stability Studies

Apart from regular stability and accelerated stability studies prescribed by ICH, nanoemulsions are subjected to special thermodynamic stability studies to ensure droplet integrity in case of temperature fluctuations. These tests include consecutive heating and cooling of nanoemulsions where they are exposed to multiple cycles of refrigeration (4 °C) and heat (45 °C). Nanoemulsions may be subjected to freeze- thaw cycle and monitored for size or phase changes. Kinetic instability such as creaming, settling or any other form of phase separation is ruled out by centrifugation of nanoemulsions at 3000–4000 rpm. Long-term deliberations include storage stability studies where nanoemulsions are stored in ambient or refrigerated conditions over a period of 3–6 months and evaluated periodically for their appearance, size, dispersity, zeta potential, drug content etc. Variations in said parameters does not necessarily indicate instability if they are within a prescribed range, however significant differences if present should be reported and used as a guiding principle for framing storage conditions or if required reformulating a stable formulation. Changes in pH often dictate in vivo amicability (painless administration of

intramuscular injections, low irritancy of topical or ophthalmologic nanoemulsions is also dependent on formulation pH) and stability of housed drugs. Ramipril is sensitive to alkaline pH, and consequently its nanoemulsion is prepared and maintained at an acidic pH. Other examples include size variation, charge reversal, droplet fluidization, etc. induced by pH change. Therefore establishing pH requirements of a nanoemulsion system acquires significance. Nanoemulsions with a larger internal phase volume generally have higher viscosities. In order to transfer such systems, application of huge shear is necessitated which sometimes leads to rupturing of droplets. This may increase systemic viscosity even further by jamming smaller droplets in between larger droplets. Viscosity of nanoemulsion therefore requires prior calibration and should be evaluated at different shear rates at different temperatures using rotary or cone and plate viscometers.

SKIN TARGETED NANOEMULSION

Therefore, for exact visualization (globule size, volume fraction, shape,) electron microscopy (SEM, TEM, cryo-TEM, freeze-fracture), neutron and X-ray scattering are applied to substantiate data obtained via PCS **[51]**.

Nanoemulsion in Psoriasis

The existing systemic therapies for psoriasis are both biological and non-biological which are commonly used as mono-therapies. Potential of methotrexate (MTX), cyclosporine and orally administrated retinoids have been explored extensively against psoriasis. Subsequently, the utility of topical MTX administration through nanoemulsion carrier in inhibiting epidermal cell proliferation and inflammation was firstly reported by Pinto et al. in 2014. It avoided the chance of liver toxicity and gastrointestinal side effects usually observed after systemic administration of MTX. During transportation through skin, MTX loaded nanoemulsion improved the topical efficacy after modulating the cellular arrangement and interacting with the membrane components such as enzymes and transporters. The possible reason for enhanced biological activity of MTX is that the positively charged nanoemulsion facilitated enhanced penetration by ionic interactions with the skin cells and by forming hydrogen bonds. Polysorbate 60 (P60) and Polysorbate80 (P80) affected the flux rate of MTX, where P60 based nano lipid carrier (NLC-P60) exhibited higher diffusion rate through the skin than that of P80 based NLC-P80 and pure MTX. Since it has been very clearly stated that the lipid and protein based carriers show better penetration through the severely altered stratum corneum of the psoriatic skin, the skin barrier integrity in the case of psoriasis was still unclear **[52]**. In2014, Somagoni et al. have highlighted penetration profile through the psoriatic skin. Their report reveals the low permeation of the drug through psoriatic skin because of the creation of a strong barrier for the penetration of nanoemulsion owing to the presence of plaque, scaling, epidermal alterations, epidermal thickening and elongation of epidermal ridges. This hypothesis was based on in vitro permeation and microdialysis experiments, where lesser permeation of the aceclofenac and capsaicin was observed into the receptor compartment through the inflammed skin (Imiquimod-induced skin inflammation) as compared to normal skin. Nano formulations NMG [a combination of nanoemulsion (NEM) and nanomicelles (NMI)] improved deposition of aceclofenac and capsaicin into the deeper skin layers (concentration was about 3-fold higher) compared to the marketed formulation due to the formation of drug deposits in the deep skin layers. Vitamin E TPGS (surfactant), which was used in the development of NMI disturbed the arrangement of cells temporarily and facilitated paracellular absorption of aceclofenac and capsaicin. NEM was absorbed through trans-cellular route by virtue of its smaller size and lipophilicity of olive oil present in it. Synergistic

results from NMG are attributed to the addition of individual characteristic of NEM and NMI which followed two possible absorption pathways and therefore, retention of drug in the skin and therapeutic effect against psoriasis model was enhanced **[53]**. Another drug clobetasol propionate (CP), which is a topical corticosteroid, has tremendous utility in the treatment of psoriasis and atopic dermatitis, in parallel causes skin atrophy (irreversible histopathological modification), steroid acne, hypopigmentation and allergic contact dermatitis. Tween 20 and ethanol based eucalyptus oil/water nanoemulsion of very slightly soluble CP have reversibly modulated the skin atrophy and penetrated into deeper skin layers. Nanoemulsion significantly increased the anti-inflammatory activity and nucleoside triphosphate diphospho hydrolases activity of CP in lymphocytes which is responsible for the hydrolysis of extracellular ATP. On account of which, cellular (keratinocytes) proliferation, differentiation and inflammatory processes get stopped. Furthermore, the role of TPGS based nanoemulsion was explored to localize paclitaxel (PCL) into deeper skin layers for longer duration and to get its maximum enhanced efficacy as PCL is a poorly soluble and poorly permeable drug i.e. classified as class IV drug of Biopharmaceutics Classification System (BCS). Due to the lipophilic nature and high molecular weight, PCL was selected as the model candidate to localize in skin layers through nanoemulsion. Increasing concentration of drug locally at the therapeutic target and keeping the systemic drug concentrations at its minimum facilitate efficient treatment of psoriasis. TPGS based o/w nanoemulsion showed tremendous utility in accumulating PCL locally (improved dermal bioavailability) with minimum systemic availability. Distribution of PCL into stratum corneum, epidermis and dermis of skin showed regional pharmacokinetic advantage of nanoemulsion following topical administration. Upon dermal application, maximum local concentration in dermis layer reached to $10\mu g/cm^2$, whereas systemic concentration was about 100 ng/ml. However, after intravenous administration of the same formulation, maximum local concentration in dermis layer was reached to $1\mu g/cm^2$ **[54]**.

Nanoemulsion in Dermatitis

Atopic dermatitis is characterized as a dry skin condition due to trans-epidermal water loss where impaired stratum corneum senses loss of barrier function and hence the major concern with atopic dermatitis is to restore the skin moisture and maintain the homeostasis. Lipids such as cholesterol, free fatty acids especially ceramides are well recognized for maintaining skin barrier homeostasis. In an approach, topical administration of positively charged nano-emulsified ceramide had restored the skin hydration and elasticity. Ceramides are practically insoluble in water and their intrinsic functionality could not achieve unless its solubility was improved. Efficient penetration through stratum corneum was obligatory to be available to the lipid lamellae therefore, surface of nanoemulsion of ceramide was modified using positively charged phytosphingosine (PS) which allowed ceramide to stay for long duration in contact with the skin cells. On account of which, the level of ceramide was maintained in to the stratum corneum and hence homeostasis of skin barrier was maintained. Subsequently, PS which plays a vital role in the preparation of ceramides and influences a broad variety of cellular functions like inflammation of the skin, was found to show tremendous utility in increasing ceramide levels in stratum corneum. Therefore, using PS in the development of nanoemulsion increases the ceramide level in the skin and develops positive charge on the surface of nanoemulsion. Baspinar and Borchert in 2012, have used these properties of PS to develop nanoemulsion of an anti-inflammatory corticosteroid namely prednicarbate which

showed dual mechanism of treatment against atopic dermatitis. Inflammatory condition was improved and level of ceramide in stratumcorneum was restored to the normal level which ultimately recovered the skin homeostasis. Clobetasol propionate (CP) loaded nanoemulsion (CP-NE) which was prepared for the dermatological application improved the in vivo efficacy against contact dermatitis after topical administration in a rat model. CP is clinically effective in the treatment of atopic dermatitis due to its vasoconstrictor, antiinflammatory, immunosuppressive, and antiproliferative effects. However, it is also having adverse effects. CP-NE (0.05%) improved nucleoside triphosphate diphosphohydrolase (NTPDase) activity inlymphocytes of rats in a preclinical study with contact dermatitis. The nanoencapsulation of CP led to a better control of the drug release from prepared formulation and provided better in vivo dermatological efficacy by improving benefit-risk ratio, reducing the frequency of drug administration, and improving dermatokinetics. Nanoemulsion of rice bran oil prepared by low energy emulsifycation method showed tremendous utility in the treatment of atopic dermatitis and improved hydration of skin **[55]**.

Nanoemulsion in Cancer

Nanoemulsion enhances drug delivery into and across the skin primarily by modulating concentration gradient across skin and skin barrier function by virtue of the presence of emulsifiers and proved its utility in chemotherapy. O/W nanoemulsion of dacarbazine (DAC) comprising soybean oil, polysorbate-80, DAC and water improved anti-tumor efficacy of DAC significantly in epidermoid carcinoma xenograft mice. Although DAC is mutagenic to prokaryotic/eukaryotic cells, it is unstable in solution form due to the photosensitivity issue. Tween 80 emulsified nanoemulsion ensured reduced oxidation and degeneration of DAC into 4-diazoimidazole-5-carboxamide in water dispersion. This strategy ensured local availability of DAC and maximum possibility to be taken up by tumor tissue after topical application. DAC nanoemulsion circumvented its side effects viz. skin discoloration and ulcers. Zn(II) phthalocyanine (ZnPc) and foscans which is a known photosensitizer were incorporated in to the magnetic nanoemulsion for photodynamic therapy (PDT) of skin cancer topically. Phosphate-coated magnetite (g-Fe2 O3), soy phospholipids Epikuron 170, and Poloxamer 188 (non-ionic surfactant) constituted biodegradable o/w magnetic nanoemulsion of foscans administered locally at tumor site. Visible light (between 600 and 800 nm) exposed to the tumor site promoted foscans from the ground singlet state to the triplet-excited state via a singlet excited state resulted to generate re- active oxygen species (oxygen superoxide O2−, hydroxyl radical) and caused irreversible destruction of the target tissues. Magnetic nanoemulsion improved retention of foscans in stratum corneum and epidermis and destructed neoplastic cell in specific tissue regions **[56]**

NANOEMULSION IN TRANSDERMAL DRUG DELIVERY

Advantages of transdermal drug delivery systems include termination of therapy (having lower therapeutic window) whenever required and avoidance of hepatic metabolism of the drugs that have rapid First pass metabolism. Ideally, nanoemulsion that favors transdermal route may demonstrate minimum interaction with different layers of the skin **[57]**, hold neutral/negative charge over the surface, elastic nature that may restore the spherical shape, ability in altering cellular arrangement for paracellular transportation etc. Nanoemulsion delivery through transdermal route is intended to administer the drug with rapid first pass metabolism, secondary metabolite of highly toxic nature, very low aqueous solubility (BCS class II drugs).

Cancer

Despite remarkable progress in chemotherapy during last couple of decades, cancer still remains one of the most sickening diseases due to its almost inevitable futility. It is the second leading cause of mortality all over the world **[58]**. Chemotherapeutics have been specifically targeted to the tumor site so as to avoid apoptosis of normal cell. This has been achieved by ligand mediated site specificity (in the influence of albumin, folate, glycyrrhetinic acid receptors) following intravenous route of administration **[59, 60]** however, transdermal route of administration is required for long-term management of cancer pain which have been extensively studied and very well documented **[61]**. Nanoemulsion technology has been proved as an important tool inchemotherapeutic applications. Properties like optical clarity, bio compatibility, non-immunogenic, ease of preparation and thermo- dynamic stability place them to the new epoch. Caffeine o/w nanoemulsion (surfactant Transcutol-HP and co-surfactant Isopropyl alcohol)offered significant increment in permeability as compared to its aqueous formulation which was attributed to small particle size (20.14 nm) and negative charge over the droplets to facilitate paracellular transportation of caffeine **[62]**. Moreover, nanoemulsion of 5-fluorouracil was formulated to deliver by transdermal route owing to its very low oral bioavailability. Due to the presence of low HLB surfactant (Tanscutol-HP), flux of 5-fluorouracil was improved through skin barriers that portrayed the enhanced permeation of nanoemulsion through skin and finally higher apoptosis in SK-MEL-5 cancer cells **[63]**. Further- more, transdermal application of an antioxidant synergy nanoemulsion formulation was found to be effective in reducing neuroblast growth rate in mouse model **[64]** which was also attributed to the smaller particle size for better penetration of nanoemulsion.

Rheumatoid Arthritis

It is characterized by a symmetric, peripheral polyarthritis that often results in severe joint damages. Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used drugs to reduce pain and inflammation of rheumatoid arthritis. NSAIDs commonly cause gastrointestinal ulcers, bleeding and adverse cardiovascular events after oral administration. Transdermal gel of etoricoxib nanoemulsion has been found to be active in maintaining the effective drug plasma concentration without portraying the adverse effects associated with its oral delivery. Unlike the etoricoxib conventional gel, nanoemulsified etoricoxib gel increased permeation and flux through the skin, transdermally. This transdermal gel was credited due to the smaller droplet size and fluidic nature that has improved the anti-inflammatory efficacy of etoricoxib **[65]**. Anti-inflammatory potential of piroxicam and ketoprofen are well exploited against rheumatism. Frequent oral intake of these drugs is not comfortable as they cause discomfort to the gastricmucosa. However, their transdermal nanoemulgel was prepared to improve their availability in to the systemic circulation. Oleic acid that is used in the development of nanoemulsion and constituted oil phase is thought to disrupt the cellular arrangement of the stratum corneum irreversibly and thereby allowed better penetration through the different skin layers **[66]**.

Hypertension

It is a chronic medical condition in which the blood pressure in the arteries is persistently elevated. Several classes of medications vizthiazide-diuretics, calcium channel blockers, angiotensin converting enzyme inhibitors and angiotensin receptor blockers have been used in the treatment of hypertension. However, the drugs like carvedilol (α1and β-blocker), have several limitations like extensive first pass metabolism and unpredictable or low bioavailability. Carvedilol nanoemulsion based

nanogel formulation for transdermal delivery is an alternative to its low oral bioavailability. The results revealed that nano size range of oil globules crossed the skin barriers and achieved effective drug concentration in blood. Numerous calcium channel antagonists i.e. nicardipine hydrochloride **[67]**, amlodipine **[68]** and felodipine **[69]** have poor oral bioavailability, short elimination half-life and extensive hepatic first pass metabolism. Water in oil nanoemulsion of nicardipine hydrochloride and o/w nanoemulsion of amlodipine and felodipine have been developed for transdermal delivery and have been successfully assessed against the treatment of hypertension. The better penetration of these calcium channel antagonists through the skin was attributed to the higherflux (due to the presence of surfactant and co-surfactant) and small droplet size of nanoemulsion.

Diabetes

It is a consequence of defective or impaired insulin secretion and is treated by administering insulin and oral hypoglycemic **[70]**. Developing transdermal systems overwhelmed the shortcomings related with oral hypoglycemic like glibenclamide (GCL). Mohammed et al. 2013 have investigated GCL efficacy when it was emulsified in nanocarrierfor transdermal delivery **[71, 72]**. GCL transdermal nanoemulsion gel exhibited better control of hyperglycemia and more effectively reversed the diabetes mellitus complications than oral GCL administration in experimental animals. Better result of the gel is attributed to the many factors such as larger amount of surfactant (labrafac) and cosurfactant (triacetin) carrying drug, high penetration into the skin, nanosized droplets, high solubilization of drug in nanoemulsion, high thermodynamic activity providing significant driving force for its release and permeation and lastly hydration of the stratum corneum due to external water phase of the nanoemulsion results in high diffusivity of lipophilic drug as droplet size approaches to molecular dispersion. Subsequently in 2015, Dina et al. developed the nanoemulsion of essential oil of fennel which is highly recommended for diabetes owing to the presence of trans-anethole as one of the major constituent. This nanoemulsion showed high potential in reducing plasma glucose levels of rats when delivered via transdermal route due to the presence of oleic acid and PG (propylene glycol; co-surfactant) as both of them acts as permeation enhancer for dermal delivery since they would increase the fluidity of the liquid portion of the stratum corneum. Moreover, Tween 20 (surfactant) enhanced the flux of the materials permeating through biological membranes resulted in better penetration of oil and hence improved activity **[73]**.

REFERENCES

[1] K. Tanaka, S. Kurotsu, T. Asano, N. Yamakawa, D. Kobayashi, Y. Yamashita, H. Yamazaki, T. Ishihara, H. Watanabe, T. Maruyama, H. Suzuki, T. Mizushima, Superiority of pulmonary administration of mepenzolate bromide over other routes as treatment for chronic obstructive pulmonary disease. Sci. Rep. 4, 4510, 2014.

[2] N. Bhattarai, J. Gunn, M. Zhang, Chitosan-based hydrogels for controlled, localized drug delivery. Adv. Drug Deliv. Rev. 62, 83–99, 2010.

[3] L.M. Ensign, R. Cone, J. Hanes, Oral drug delivery with polymeric nanoparticles: the gastrointestinal mucus barriers. Adv. Drug Deliv. Rev. 64, 557–570, 2012.

[4] P. Desai, R.R. Patlolla, M. Singh, Interaction of nanoparticles and cell-penetrating peptides with skin for transdermal drug delivery. Mol. Membr. Biol. 27, 247–259, 2010.

[5] Ajazuddin, A. Alexander, A. Khichariya, S. Gupta, R.J. Patel, T.K. Giri, D.K. Tripathi, Recent expansions in an emergent novel drug delivery technology: Emulgel. J. Control. Release, 171, 122–132, 2013.

[6] K.S. Paudel, M. Milewski, C.L. Swadley, N.K. Brogden, P. Ghosh, A.L. Stinchcomb, Challenges and opportunities in dermal/transdermal delivery. Ther. Deliv. 1, 109–131, 2010.

[7] B. Sutradhar Kumar, L. Amin Md, Nanoemulsions: increasing possibilities in drug delivery. Eur. J. Nanomed. 97, 2013.

[8] Global Topical Drug Delivery Market Research Report, http://www. micromarketmonitor.com/market-report/topical-drug-delivery-reports- 4242454697.html.

[9] S. Sajeesh, K. Bouchemal, V. Marsaud, C. Vauthier, C.P. Sharma, Cyclodextrin
complexed insulin encapsulated hydrogel microparticles: an oral delivery system for insulin. J. Control. Release 147, 377–384, 2010.

[10] S. Mutalik, N. Udupa, S. Kumar, S. Agarwal, G. Subramanian, A.K. Ranjith, Glipizide matrix transdermal systems for diabetes mellitus: preparation, in vitro and preclinical studies. Life Sci. 79, 1568–1577, 2006.

[11] N. Ghalandarlaki, A.M. Alizadeh, S. Ashkani-Esfahani, Nanotechnology-applied curcumin for different diseases therapy. BioMed Res. Int. 23, 2014.

[12] T.G. Mason, J.Wilking, K. Meleson, C. Chang, S. Graves, Nanoemulsions: formation, structure, and physical properties. J. Phys. Condens. Matter 18, R635, 2006.

[13] R. Aboofazeli, Nanometric-Scaled Emulsions (Nanoemulsions). Iran. J. Pharm. Res. 9, 325–326, 2010.

[14] M.E. Aulton, K.M. Taylor, Aulton's Pharmaceutics: The Design and Manufacture of Medicines. Elsevier Health Sciences, 2013.

[15] D.J.McClements, Nanoemulsions versus microemulsions: terminology, differences, and similarities. Soft Matter 8, 1719–1729, 2012.

[16] S.L. Tan, J. Stanslas, M. Basri, R.A. AbediKarjiban, B.P. Kirby, D. Sani, H.B. Basri,Nanoemulsion-based parenteral drug delivery system of carbamazepine: preparation,characterization, stability evaluation and blood-brain pharmacokinetics. Curr. Drug Deliv. 795–804, 2015.

[17] N. Sharma, S.Mishra, S. Sharma, R.D. Deshpande, R.K. Sharma, Preparation and optimizationof nanoemulsions for targeting drug delivery. Int. J. Drug Dev. Res. 5 (4), 37–48, 2013.

[18] S. Al-Edresi, S. Baie, Formulation and stability of whitening VCO-in-water nanocream. Int. J. Pharm. 373, 174–178, 2009.

[19] P.E. Makidon, S.S. Nigavekar, A.U. Bielinska, N. Mank, A.M. Shetty, J. Suman, J.Knowlton, A. Myc, T. Rook, J.R. Baker Jr., Characterization of stability and nasal deliverysystems for immunization with nanoemulsion-based vaccines. J. Aerosol.Med. Pulm. Drug. Deliv. 23, 77–89, 2010.

[20] R.R. Lala, N.G. Awari, Nanoemulsion-based gel formulations of COX-2 inhibitors for
enhanced efficacy in inflammatory conditions. Appl. Nanosci. 4, 143–151, 2014.

[21] A. Hussain, A. Samad, S.K. Singh, M.N. Ahsan, M.W. Haque, A. Faruk, F.J. Ahmed,Nanoemulsion gel-based topical delivery of an antifungal drug: in vitro activity and in vivo evaluation. Drug Deliv. 23, 642–647, 2016.

[22] M. Nasr, S. Nawaz, A. Elhissi, Amphotericin B lipid nanoemulsion aerosols fortargeting peripheral respiratory airways via nebulization. Int. J. Pharm. 436, 611–616, 2012.

[23] A. Amani, P. York, H. Chrystyn, B.J. Clark, Evaluation of a nanoemulsion-based formulationfor respiratory delivery of budesonide by nebulizers. AAPS PharmSciTech, 11, 1147–1151, 2010.

[24] D. Tamarkin, A. Besonov, M. Eini, J. Danziger, Foam prepared from nanoemulsionsand uses. Google Patents, 2007.

[25] D. Mou, H. Chen, D. Du, C. Mao, J. Wan, H. Xu, X. Yang, Hydrogel-thickenednanoemulsion system for topical delivery of lipophilic drugs. Int. J. Pharm, 353, 270–276, 2008.

[26] S. Khani, F. Keyhanfar, A. Amani, Design and evaluation of oral nanoemulsion drug delivery system of mebudipine. Drug Deliv. 23, 2035–2043, 2016.

[27] V.K. Pawar, S.B. Panchal, Y. Singh, J.G. Meher, K. Sharma, P. Singh, H.K. Bora, A.Singh, D. Datta, M.K. Chourasia, Immunotherapeutic vitamin E nanoemulsion synergiesthe antiproliferative activity of paclitaxel in breast cancer cells via modulating Th1 and Th2 immune response. J. Control. Release, 196, 295–306, 2014.

[28] R.S. Bhanushali, M.M. Gatne, R.V. Gaikwad, A.N. Bajaj, M.A. Morde, Nanoemulsionbased intranasal delivery of antimigraine drugs for nose to brain targeting. Indian J. Pharm. Sci. 71, 707–709, 2009.

[29] H.O. Ammar, H.A. Salama,M. Ghorab, A.A. Mahmoud, Nanoemulsion as a potentialophthalmic delivery system for dorzolamide hydrochloride. AAPS PharmSciTech, 10, 808, 2009.

[30] M.N. Yukuyama, D.D.M. Ghisleni, T.J.A. Pinto, N.A. Bou-Chacra, Nanoemulsion: processselection and

application in cosmetics – a review. Int. J. Cosmet. Sci. 38, 13–24, 2016.

[31] L.Wang, X. Li, G. Zhang, J. Dong, J. Eastoe, Oil-in-water nanoemulsions for pesticideformulations. J. Colloid Interface Sci. 314, 230–235, 2007.

[32]. Kreilgaard, M.; Pedersen, E.J.; Jaroszewski, J.W. NMR characterisation and transdermal drug delivery potential of microemulsion systems. J. Control. Release, 69, 421–433, 2000.

[33]. Paolino, D.; Ventura, C.A.; Nistico, S.; Puglisi, G.; Fresta, M. Lecithin microemulsions for the topical administration of ketoprofen: Percutaneous adsorption through human skin and in vivo human skin tolerability. Int. J. Pharm. 244, 21–31, 2002.

[34]. Hua, L.; Weisan, P.; Jiayu, L.; Ying, Z. Preparation, evaluation, and NMR characterization of vinpocetinemicroemulsion for transdermal delivery. Drug Dev. Ind. Pharm. 30, 657–666, 2004.

[35]. Lopes, L.B. Overcoming the Cutaneous Barrier with Microemulsions. Pharmaceutics 6, 52–77, 2014.

[36] J.M. Aguilera, D.W. Stanley, Microstructural Principles of Food Processing and Engineering. Springer Science & Business Media, 1999.

[37] J.-U.A. Junghanns, R.H. Müller, Nanocrystal technology, drug delivery and clinical applications. Int. J. Nanomedicine 3, 295, 2008.

[38] S. Mahdi Jafari, Y. He, B. Bhandari, Nano-emulsion production by sonication and microfluidization—a comparison. Int. J. Food Prop. 9, 475–485, 2006.

[39] P.R. Gogate, A.M. Kabadi, A review of applications of cavitation in biochemical engineering/ biotechnology. Biochem. Eng. J. 44, 60–72, 2009.

[40] T.S. Leong, T.J. Wooster, S.E. Kentish, M. Ashokkumar, Minimising oil droplet size using ultrasonic emulsification. Ultrason. Sonochem. 16, 721–727, 2009.

[41] R. NeslihanGursoy, S. Benita, Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. Biomed. Pharmacother. 58, 173–182, 2004.

[42] K. Bouchemal, S. Briançon, E. Perrier, H. Fessi, Nano-emulsion formulation using spontaneous emulsification: solvent, oil and surfactant optimization. Int. J. Pharm. 280, 241–251, 2004.

[43] L. Bachmann, Freeze-etching of dispersions, emulsions and macromolecular solutions of biological interest, in: R. Steinbrecht, K. Zierold (Eds.), Cryotechniques in Biological Electron Microscopy. Springer, Berlin Heidelberg, pp. 192–204, 1987.

[44] C. Grapentin, S. Barnert, R. Schubert, Monitoring the stability of perfluorocarbon nanoemulsions by cryo-TEM image analysis and dynamic light scattering. PLoS One, 10, e0130674, 2015.

[45] A.A. Kislukhin, H. Xu, S.R. Adams, K.H. Narsinh, R.Y. Tsien, E.T. Ahrens, Paramagneticfluorinated nanoemulsions for sensitive cellular fluorine-19 magnetic resonance imaging. Nat. Mater. 15, 662–668, 2016.

[46]T.J. Wooster, M. Golding, P. Sanguansri, Impact of oil type on nanoemulsion formation and Ostwald ripening stability. Langmuir, 24, 12758–12765, 2008.

[47] M.M. Fryd, T.G. Mason, Advanced nanoemulsions. Annu. Rev. Phys. Chem. 63, 493–518, 2012.

[48] T. Delmas, H.L.N. Piraux, A.C. Couffin, I. Texier, F.O. Vinet, P. Poulin,M.E. Cates, J.R.M.

Bibette, How to prepare and stabilize very small nanoemulsions. Langmuir, 27, 1683–1692, 2011.

[49] M.M. Fryd, T.G. Mason, Time-dependent nanoemulsion droplet size reduction by

evaporative ripening. J. Phys. Chem. Lett. 1, 3349–3353, 2010.

[50] Y.S. Nam, J.W. Kim, J. Shim, S.H. Han, H.K. Kim, Nanosized emulsions stabilized by semisolid polymer interphase. Langmuir 26, 13038–13043, 2010.

[51] Y. Wu, Y.H. Li, X.H. Gao, H.D. Chen, The application of nanoemulsion in dermatology: an overview. J. Drug Target. 2, 321–327, 2013.

[52] M.F. Pinto, C.C. Moura, C. Nunes, M.A. Segundo, S.A. Costa Lima, S. Reis, A new topical formulation for psoriasis: development of methotrexate-loaded nanostructured lipid carriers. Int. J. Pharm. 477, 519–526, 2014.

[53] J. Somagoni, C.H. Boakye, C. Godugu, A.R. Patel, H.A. MendoncaFaria, V. Zucolotto, M. Singh, Nanomiemgel—a novel drug delivery system for topical application—in vitro and in vivo evaluation. PloS One, 9, e115952, 2014.

[54] S. Khandavilli, R. Panchagnula, Nanoemulsions as versatile formulations for paclitaxel delivery: peroral and dermal delivery studies in rats. J. Invest. Dermatol. 127, 154–162, 2007.

[55] D.S. Bernardi, T.A. Pereira, N.R. Maciel, J. Bortoloto, G.S. Viera, G.C. Oliveira, P.A. Rocha-Filho, Formation and

stability of oil-in-water nanoemulsions containing rice bran oil: in vitro and in vivo assessments. J. Nanobiotechnology, 9, 44, 2011.

[56].F.L. Primo, L. Michieleto, M.A.M. Rodrigues, P.P. Macaroff, P.C. Morais, Z.G.M. Lacava, M.V.L.B. Bentley, A.C. Tedesco, Magneticnanoemulsions as drug delivery system for Foscan®: skin permeation and retention in vitro assays for topical application in photodynamic therapy (PDT) of skin cancer. J. Magn. Magn.Mater. 311, 354–357, 2007.

[57] G. Sonavane, K. Tomoda, A. Sano, H. Ohshima, H. Terada, K. Makino, In vitro permeation of gold nanoparticles through rat skin and rat intestine: effect of particle size. Colloids Surf. B 65, 1–10, 2008.

[58] Cancer, Fact Sheet, Media Centre, World Health Organization, 2017.

[59] S. Ganta, M. Talekar, A. Singh, T.P. Coleman, M.M. Amiji, Nanoemulsions in translational research—opportunities and challenges in targeted cancer therapy. AAPS PharmSciTech, 15, 694–708, 2014.

[60] S. Ganta, A. Singh, N.R. Patel, J. Cacaccio, Y.H. Rawal, B.J. Davis, M.M. Amiji, T.P. Coleman, Development of EGFR targeted nanoemulsion for imaging and novel platinum therapy of ovarian cancer. Pharm. Res. 31, 2490–2502, 2014.

[61] C. Peptu, R. Rotaru, L. Ignat, A.C. Humelnicu, V. Harabagiu, C.A. Peptu, M.M. Leon, F. Mitu, E. Cojocaru, A. Boca, B.I. Tamba, Nanotechnology approaches for pain therapy through transdermal drug delivery. Curr. Pharm. Des. 21, 6125–6139, 2015.

[62] F. Shakeel, W. Ramadan, Transdermal delivery of anticancer drug caffeine from water-in-oil nanoemulsions. Colloids Surf. B, 75, 356–362, 2010.

[63] F. Shakeel, N. Haq, A. Al-Dhfyan, F.K. Alanazi, I.A. Alsarra, Chemoprevention of skin cancer using low HLB surfactant nanoemulsion of 5-fluorouracil: a preliminary study. Drug Deliv. 22, 573–580, 2015.

[64] F. Kuo, T. Kotyla, T. Wilson, L. Kifle, T. Panagiotou, I. Gruverman, J.B. Tagne, T. Shea, R. Nicolosi, A nanoemulsion of an anti-oxidant synergy formulation reduces tumor growth rate in neuroblastoma-bearing nude mice. J. Exp. Ther. Oncol. 6, 129–135, 2007.

[65] R.R. Lala, N.G. Awari, Nanoemulsion-based gel formulations of COX-2 inhibitors for enhanced efficacy in inflammatory conditions. Appl. Nanosci. 4 , 143–151, 2014.

[66] B. Dhawan, G. Aggarwal, S. Harikumar, Enhanced transdermal permeability of piroxicam through novel nanoemulgel formulation. Int. J. Pharm. Investig. 4, 65–76, 2014.

[67] K.B. Singh BhuwaneshPratap, S.K. Jain, ShafaatKausar, Development and characterization of Ananoemulsion gel formulation for transdermal delivery of carvedilol. Int. J. Drug Dev. Res. 4, 151–161, 2012.

[68] P.C. Wu, Y.H. Lin, J.S. Chang, Y.B. Huang, Y.H. Tsai, The effect of component of microemulsion for transdermal delivery of nicardipine hydrochloride. Drug Dev Ind. Pharm. 36, 1398–1403, 2010.

[69] D. Kumar, M. Aqil, M. Rizwan, Y. Sultana, M. Ali, Investigation of a nanoemulsion as vehicle for transdermal delivery of amlodipine. Die Pharmazie, 64, 80–85, 2009.

[70] M. Trotta, S. Morel, M.R. Gasco, Effect of oil phase composition on the skin permeation of felodipine from o/w microemulsions. Die Pharmazie, 52, 50–53, 1997.

[71] V.K. Rai, N. Mishra, A.K. Agrawal, S. Jain, N.P. Yadav, Novel drug delivery system: an immense hope for diabetics. Drug Deliv. 1–20, 2015.

[72] A.S.M. Wais, I. Nazish, A. Khale, M. Aqil, M. Khan, Formulation development exvivo and in-vivo evaluation of nanoemulsion for transdermal delivery of glibenclamide. Int. J. Pharm. Pharm. Sci. 5, 747–754, 2013.

[73] D.M. Mostafa, S.H. Abd El-Alim, M.H. Asfour, S.Y. Al-Okbi, D.A. Mohamed, G. Awad, Transdermal nanoemulsions of foeniculum vulgare mill. essential oil: preparation, characterization and evaluation of antidiabetic potential. J. Drug Deliv. Sci. Technol. 29, 99–106, 2015.