Review Article

Nanoemulsion: A Pharmaceutical Review

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ABSTRACT

Nanoemulsions are submicron sized emulsions that are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. They are by far the most advanced nanoparticle systems for the systemic delivery of biologically active agents for controlled drug delivery and targeting. Nanoemulsions are the thermodynamically stable isotropic system in which two immiscible liquid (water and oil) are mixed to form a single phase by means of an appropriate surfactants or its mix with a droplet diameter approximately in the range of 0.5-100 um. Nanoemulsion droplet sizes fall typically in the range of 20-200 nm and show narrow size distributions. Nanoemulsion show great promise for the future of cosmetics, diagnostics, drug therapies, and biotechnologies. In this review, the attention is focused to give brief regarding nanoemulsion formulation aspect, method of preparation, characterization techniques with special emphasis on various applications of nanoemulsion in different areas such as in cancer treatment, in drug targeting, as a mucosal vaccine, as a vehicle for transdermal drug delivery and lipophilic drug, as a self-nanoemulsifying and solid self-nanoemulsifying drug delivery system, etc.

Introduction

Nanotechnology comprises technological developments on the nanometer scale, usually 0.1-100 nm. The use of nano technology in pharmaceuticals and medicine has grown over the last few years. The pharmaceuticals developed on the basis of nanotechnology are termed as "NANOPHARMACEUTICALS." The various nanopharmaceuticals currently being used or in the process of development are[1] Nanoemulsions (NE) (submicron sized emulsions), nanosuspensions (submicron sized suspensions), nanospheres (drug nanoparticles in polymer matrix), nanotubes (sequence of nanoscale C60 atoms arranged in a long thin cylindrical structure), nanoshells (concentric sphere nanoparticles consisting of a dielectric core and a metal shell), nanocapsules (encapsulated drug nanoparticles), lipid nanoparticles (lipid monolayer enclosing a solid lipid core) and dendrimers (nanoscale three-dimensional macromolecules of polymer). NEs are a group of dispersed particles used for pharmaceutical and biomedical aids and vehicles that show great promise for the future of cosmetics, diagnostics, drug therapies, and biotechnologies. NEs can be defined as oil-in-water (o/w) emulsions with mean droplet diameters ranging from 50 to 1000 nm. Usually, the average droplet size is between 100 and 500 nm. The particles can exist as water-in-oil and oil-in water forms, where the core of the particle is either water or oil, respectively. NEs are made from surfactants approved for human consumption and common food substances that are "Generally Recognized as Safe" (GRAS) by the FDA. These emulsions are easily produced in large quantities by mixing a water-immiscible oil phase into an aqueous phase with a high-stress, a mechanical extrusion process that is available worldwide[2] [Figure 1].

The NEs are also referred as miniemulsions, ultrafine emulsions and submicron emulsions. Phase behavior studies have shown that the size of the droplets is governed by the surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point induced

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Figure 1: Picture of a nanoemulsion (left) and a macro-emulsion right) with droplet diameters of 35 nm and 1 μm, respectively

by either temperature or composition. Studies on NE formation by the phase inversion temperature method have shown a relationship between minimum droplet size and complete solubilization of the oil in a microemulsion bicontinuous phase independently of whether the initial phase equilibrium is single or multiphase. Due to their small droplet size NEs possess stability against sedimentation or creaming with Ostwald ripening forming the main mechanism of NE breakdown. The main application of NEs is the preparation of nanoparticles using a polymerizable monomer as the disperse phase (the so-called miniemulsion polymerization method) where NE droplets act as nanoreactors. Another interesting application which is experiencing an active development is the use of NEs as formulations, namely, for controlled drug delivery and targeting.[3]

NEs posses various advantages such as[4]

- NEs have a much higher surface area and free energy than macro emulsions that make them an effective transport system.
- NEs do not show the problems of inherent creaming, flocculation, coalescence, and sedimentation, which are commonly associated with macroemulsions.
- NEs can be formulated in variety of formulations such as foams, creams, liquids, and sprays.
- NEs are non-toxic and non-irritant, hence can be easily applied to skin and mucous membranes.
- Since NEs are formulated with surfactants, which are approved for human consumption (GRAS), they can be taken by enteric route.
- NEs do not damage healthy human and animal cells, hence are suitable for human and veterinary therapeutic purposes.

Formulation aspects for nanoemulsion

Apart from drug, other formulation additives for NE are shown in Table 1. A typical formulation is given in Table 2.

Methods of preparation of nanoemulsions[5-8]

High-pressure homogenization: This technique makes use of high-pressure homogenizer/piston homogenizer to produce NEs of extremely low particle size (up to 1nm) [Figure 2].

Microfluidization: Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer. This device uses a high-pressure positive displacement pump (500-20000 psi), which forces the product through the interaction chamber, which consists of small channels called "microchannels." The product flows through the microchannels on to an impingement area resulting in very fine particles of submicron range. The two solutions (aqueous phase and oily phase) are combined together and processed in an inline homogenizer to yield a coarse emulsion. The coarse emulsion is into a microfluidizer where it is further processed to obtain a stable NE. The coarse emulsion is passed through the interaction chamber of the microfluidizer repeatedly until desired particle size is obtained. The bulk emulsion is then filtered through a filter under nitrogen to remove large droplets resulting in a uniform NE.

Other method used for NE preparation is the phase inversion temperature technique.

Table 1: Formulation additive for a nanoemulsion

Components of nanoemulsion formulations[9]	
Oils	**Emulsifiers**
Castor oil, coconut oil, corn oil, cottonseed oil, evening primrose oil, fish oil, jojoba oil, lard oil, linseed oil, mineral oil, olive oil, peanut oil, PEG-vegetable oil, perflurochemicals, pine nut oil, safflower oil, sesame oil, soybean oil, squalene, sunflower oil, wheatgerm oil	Natural lecithins from plant or animal sources, phospholipids, PEG- phospholipids, poloxamers (e.g. F68), polysorbates, polyoxyethylene castor oil derivatives, polyglycolized glycerides, stearylamine, oleylamine
	Additives
	Antioxidants (α-tocopherol, ascorbic acid)
	Tonicity modifiers (glycerol, sorbitol, xylitol)
	pH adjustment agents (NaOH or HCl)
	Preservatives

Characterization of nanoemulsion

Different characterization parameters for NE include transmission electron microscopy, NE droplet size analysis, viscosity determination,

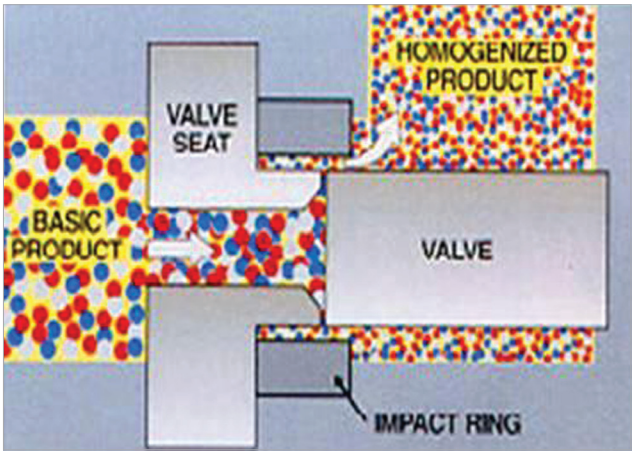

Figure 2: High-pressure homogenization technique for nanoemulsion preparation

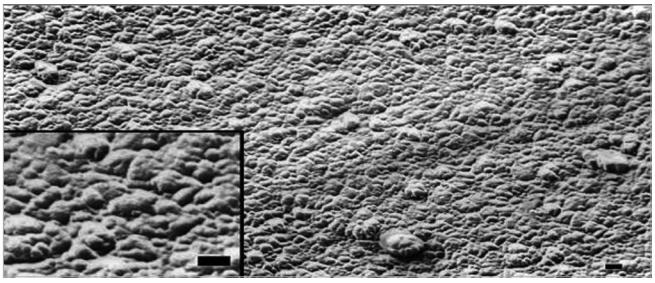

Figure 3: Freeze fracture electron microscopy image of paclitaxel nanoemulsions formulated using 20% oil phase (pine nut oil) and egg phosphatidylcholine as surfactant and deoxycholic acid as co-surfactant

Table 2: Examples of a nanoemulsion formulation

Ingredient	Function	Weight (% m/m)
Evening primrose oil	Lipid	25,0
Tocopherol	Antioxidant	5,0
Lecithin	Emulsifying agent	4,0
Water	Diluent	ad 100,0

refractive index, *in vitro* skin permeation studies, skin irritation test, *in vivo* efficacy study, thermodynamic stability studies, and surface characteristics.

The surface charge of the NE droplets has a marked effect on the stability of the emulsion system and the droplet *in vivo* disposition and clearance [Figure 3].

The inset [Figure 3] shows microscopy image at a higher magnification. NE droplets were in the size range of 25-40 nm with some particle aggregates in the size range of 100-150 nm.[9]

Applications of nanoemulsions

Use of nanoemulsions in cosmetics

NEs have recently become increasingly important as potential vehicles for the controlled delivery of cosmetics and for the optimized dispersion of active ingredients in particular skin layers. Due to their lipophilic interior, NEs are more suitable for the transport of lipophilic compounds than liposomes. Similar to liposomes, they support the skin penetration of active ingredients and thus increase their concentration in the skin. Another advantage is the small-sized droplet with its high surface area allowing effective transport of the active to the skin. Furthermore, NEs gain increasing interest due to their own bioactive effects. This may reduce the trans-epidermal water loss (TEWL), indicating that the barrier function of the skin is strengthened. NEs are acceptable in cosmetics because there are no inherent creaming, sedimentation, flocculation, or coalescence that are observed with macroemulsions. The incorporation of potentially irritating surfactants can often be avoided by using high-energy equipment during manufacturing.[10]

New Jersey-based TRI-K Industries and its parent company Kemira have launched a new nano-based gel aimed at enhancing the efficacy of a wide range of skin care products. Kemira NanoGel is said to be a unique NE Carrier system that has been designed around easy formulation, combined with the added benefits brought about by its nanotechnology properties.

NanoGel technology provides a simple process and system to create submicron emulsions from an easy-to-use, oil-in-water concentrate. The formula is particularly suited to minimizing transepidermal water loss, enhanced skin production, and penetration of active ingredient. These characteristics suggest that it would be particularly useful for sun care products as well as moisturizing and anti-aging creams—particular areas where nanotechnology is already being incorporated into a host of products currently on the market. Likewise, it is also highlighted that it helps to give skin care formulations a good skin feel, an increasingly important characteristic for formulators.

NEs have attracted considerable attention in recent years for application in personal care products as potential vehicles for the controlled delivery of cosmetics and the optimized dispersion of active ingredients in particular skin layers.[11]

Antimicrobial nanoemulsions

Antimicrobial NEs are oil-in-water droplets that range from 200 to 600 nm. They are composed of oil and water and are stabilized by surfactants and alcohol. The NE has a broad-spectrum activity against bacteria (e.g. *E. coil,* Salmonella, *S. aureus*), enveloped viruses (e.g. HIV, *Herpes simplex*), fungi (e.g. Candida, Dermatophytes), and spores (e.g. anthrax). The NE particles are thermodynamically driven to fuse with lipid-containing organisms.

This fusion is enhanced by the electrostatic attraction between the cationic charge of the emulsion and the anionic charge on the pathogen. When enough nanoparticles fuse with the pathogens, they release part of the energy trapped within the emulsion. Both the active ingredient and the energy released destabilize the pathogen lipid membrane, resulting in cell lysis and death. In the case of spores, additional germination enhancers are incorporated into the emulsion. Once initiation of germination takes place, the germinating spores become susceptible to the antimicrobial action of the NE. A unique aspect of the NEs is their selective toxicity to microbes at concentrations that are non-irritating to skin or mucous membrane. The safety margin of the NE is due to the low level of detergent in each droplet, yet when acting in concert, these droplets have sufficient energy and surfactant to destabilize the targeted microbes without damaging healthy cells. As a result, the NE can achieve a level of topical antimicrobial activity that has only been previously achieved by systemic antibiotics.[12]

Prophylactic in bio-terrorism attack[13]

Based on their antimicrobial activity, research has begun on use of NEs as a prophylactic medication, a human protective treatment, to protect people exposed to bio-attack pathogens such as anthrax and ebola. A broad-spectrum NE was tested on surfaces by the US Army (RestOps) in December 1999 for decontamination of anthrax spore surrogates. It was tested again by RestOps in March 2001 as a chemical decontamination agent. All tests were successful.

The technology has been tested on gangrene and *Clostridium botulism* spores and can even be used on contaminated wounds to salvage limbs. The NE technology can be formulated into a cream, foam, liquid, or spray to decontaminate a variety of materials marketed as NANOSTAT™ (Nanobio Corp.)

Nanoemulsions as a mucosal vaccines[14]

NEs are being used to deliver either recombinant proteins or inactivated organisms to a mucosal surface to produce an immune response. The first applications, an influenza vaccine and an HIV vaccine, can proceed to clinical trials. The NE causes proteins applied to the mucosal surface to be adjuvanted and it facilitates uptake by antigen-presenting cells. This results in a significant systemic and mucosal immune response that involves the production of specific IgG and IgA antibody as well as cellular immunity. Initial work in influenza has demonstrated that animals can be protected against influenza after just a single mucosal exposure to the virus mixed with the emulsion. Research has also demonstrated that animals exposed to recombinant gp120 in NE on their nasal mucosa develop significant responses to HIV, thus providing a basis to examine the use of this material as an HIV vaccine. Additional research is ongoing to complete the proof of concept in animal trials for other vaccines including Hepatitis B and anthrax. The University of Michigan has exclusively licensed this technology to NanoBio®. Epidemiological and experimental data suggested that both robust neutralizing antibodies and potent cellular responses play important roles in controlling primary HIV-1 infection. In this study, they have investigated the induction of systemic and mucosal immune responses to HIV gp120 monomer immunogen administered intranasally in a novel, oil-in-water NE adjuvant. Mice and guinea pigs intranasally immunized by the application of recombinant HIV gp120

antigen mixed in NE demonstrated robust serum anti-gp120 IgG, as well as bronchial, vaginal, and serum anti-gp120 IgA in mice. The serum of these animals demonstrated antibodies that cross-reacted with heterologous serotypes of gp120 and had significant neutralizing activity against two clade-B laboratory strains of HIV (HIVBaL and HIVSF162) and five primary HIV-1 isolates. The analysis of gp120-specific CTL proliferation, INF-γ induction, and prevalence of anti-gp120 IgG2 subclass antibodies indicated that nasal vaccination in NE also induced systemic, Th1-polarized cellular immune responses. This study suggests that NE should be evaluated as a mucosal adjuvant for multivalent HIV vaccines.[15] Hepatitis B virus infection remains an important global health concern despite the availability of safe and effective prophylactic vaccines. Limitations to these vaccines include requirement for refrigeration and three immunizations thereby restricting use in the developing world. A new nasal hepatitis B vaccine composed of recombinant hepatitis B surface antigen (HBsAg) in a novel NE adjuvant (HBsAg-NE) could be effective with fewer administrations. Comprehensive pre-clinical toxicology evaluation demonstrated that HBsAg-NE vaccine is safe and well tolerated in multiple animal models. Our results suggest that needle-free nasal immunization with HBsAg-NE could be a safe and effective hepatitis B vaccine, or provide an alternative booster administration for the parenteral hepatitis B vaccines. This vaccine induces a Th1 associated cellular immunity and also may provide therapeutic benefit to patients with chronic hepatitis B infection who lack cellular immune responses to adequately control viral replication. Long-term stability of this vaccine formulation at elevated temperatures suggests a direct advantage in the field, since potential excursions from cold chain maintenance could be tolerated without a loss in therapeutic efficacy.[16]

A novel technique for vaccinating against a variety of infectious diseases—using an oil-based emulsion placed in the nose, rather than needles—has proved able to produce a strong immune response against smallpox and HIV in two new studies. The two studies showed that the NE platform is capable of developing vaccines from very diverse materials. The technology is licensed to NanoBio Corp.[17] Researchers have published results from a preliminary test of a NE vaccine's effectiveness against HIV. The HIV NE vaccine tested in the noses of mice in the study represents a different approach in the way it produces immunity and the type of immunity produced. Vaccines administered in the nose are also able to induce mucosal immunity in the genital mucosa. Evidence is growing that HIV virus can infect the mucosal immune system. Therefore, developing mucosal immunity may be very important for protection against HIV. In the study, the NE HIV vaccine showed that it was able to induce mucosal immunity, cellular immunity, and neutralizing antibody to various isolates of HIV virus. A protein used by the team, gp120, is one of the major binding proteins under study in other HIV vaccine approaches. A patent has been granted and assigned for the NE vaccine technique, which has been exclusively licensed to NanoBio Corp.[17]

Nanoemulsion as non-toxic disinfectant cleaner[18,19]

A breakthrough nontoxic disinfectant cleaner for use in commercial markets that include healthcare, hospitality, travel, food processing, and military applications has been developed by EnviroSystems, Inc. that kills tuberculosis and a wide spectrum of viruses, bacteria and fungi in 5-10 min without any of the hazards posed by other categories of disinfectants. The product needs no warning labels. It does not irritate eyes and can be absorbed through the skin, inhaled, or swallowed without harmful effects. The disinfectant formulation is made up of nanospheres of oil droplets ≤106 μm that are suspended in water to create a NE requiring only miniscule amounts of the active ingredient, PCMX (parachlorometaxylenol). The nanospheres carry surface charges that efficiently penetrate the surface charges on microorganisms' membranes—much like breaking through an electric fence. Rather than "drowning" cells, the formulation allows PCMX to target and penetrate cell walls. As a result, PCMX is effective at concentration levels 1-2 orders of magnitude lower than those of other disinfectants; hence, there are no toxic effects on people, animals, or the environment. Other microbial disinfectants require large doses of their respective active ingredients to surround pathogen cell walls, which cause them to disintegrate, fundamentally "drowning" them in the disinfectant solution. The formulation is a broad-spectrum disinfectant cleaner that can be applied to any hard surface, including equipment, counters, walls, fixtures, and floors. One product can now take the place of many reducing product inventories and saving valuable storage space. Chemical disposal costs can be eliminated, and disinfection and cleaning costs can be reduced. It is marketed as a EcoTru ™ (EnviroSystems, Inc.).

Nanoemulsions in cell culture technology[20]

Cell cultures are used for *in vitro* assays or to produce biological compounds, such as antibodies or recombinant proteins. To optimize cell growth, the culture medium can be supplemented with a number of defined molecules or with blood serum. Up to now, it has been very difficult to supplement the media with oil-soluble substances that are available to the cells, and only small amounts of these lipophilic compounds could be absorbed by the cells. NEs are a new method for the delivery of oil-soluble substances to mammalian cell cultures. The delivery system is based on a NE, which is stabilized by phospholipids. These NEs are transparent and can be passed through 0.1 μm filters for sterilization. NE droplets are easily taken up by the cells. The encapsulated oil-soluble substances therefore have a high bioavailability to cells in culture. The advantages of using NEs in cell culture technology are better uptake of oil-soluble supplements in cell cultures; improve growth and vitality of cultured cells, and allowance of toxicity studies of oil-soluble drugs in cell cultures.

Nanoemulsion in cancer therapy and in targeted drug delivery

The effects of the formulation and particle composition of gadolinium (Gd)-containing lipid NE (Gd-nanoLE) on the biodistribution of Gd after its intravenous (IV) injection in D1-179 melanoma-bearing hamsters were evaluated for its application in cancer neutron-capture therapy. Biodistribution data revealed that Brij 700 and HCO-60 prolonged the retention of Gd in the blood and enhanced its accumulation in tumors. Among the core components employed, soybean oil yielded the highest Gd concentration in the blood and tumor, and the lowest in the liver and spleen. When each Gd-nanoLE was IV injected once or twice at a 24-h interval, the Gd concentration in the tumor correlated well with the total dose of Gd, and it reached a maximum of a 189 μg/g wet tumor. This maximum Gd level was greater than the limit required for significantly suppressing tumor growth in neutron-capture therapy.[21] In order to achieve penetration

of Paclitaxel (PCL) into deeper skin layers while minimizing the systemic escape, a NE (NE) was formulated and its *in vivo* pharmacokinetic performance was evaluated. Further, the same formulation was explored for peroral bioavailability enhancement of PCL. Upon dermal application, the drug was predominantly localized in deeper skin layers, with minimal systemic escape. This has amounted to an absolute bioavailability of 70.62%. Inhibition of P-glycoprotein efflux by D-tocopheryl polyethyleneglycol 1000 succinate and labrasol would have contributed to the enhanced peroral bioavailability of PCL. This investigation provides direct evidence on the localization of high-molecular-weight, lipophilic drug, PCL, in dermis. Further, the NE formulation has enhanced the peroral bioavailability significantly to more than 70%. The developed NE formulation was safe and effective for both peroral and dermal delivery of PCL.[22]

Camptothecin is a topoisomerase I inhibitor that acts against a broad spectrum of cancers. However, its clinical application is limited by its insolubility, instability, and toxicity. The aim of the present study was to develop acoustically active NEs for camptothecin encapsulation to circumvent these delivery problems. The NEs were prepared using liquid perfluorocarbons and coconut oil as the cores of the inner phase. These NEs were stabilized by phospholipids and/or Pluronic F68 (PF68). The NEs were prepared at high drug loading of approximately 100% with a mean droplet diameter of 220-420 nm. Camptothecin in these systems showed retarded drug release. Camptothecin in NEs with a lower oil concentration exhibited cytotoxicity against melanomas and ovarian cancer cells. Confocal laser scanning microscopy confirmed NE uptake into cells. Using a 1 MHz ultrasound, an increased release of camptothecin from the system with lower oil concentration could be established, illustrating a drug-targeting effect.[23]

The scientists have investigated the NE containing risperidone (RSP) to accomplish the delivery of drug to the brain via nose. Risperidone NE (RNE) and mucoadhesive NE (RMNE) were characterized for drug content, pH, percentage transmittance, globule size, and zeta potential. Biodistribution of RNE, RMNE, and risperidone solution (RS) in the brain and blood of Swiss albino rats following intranasal (i.n.) and intravenous (i.v.) administration was examined using optimized technetium-labeled [(99 m) Tc-labeled] RSP formulations. Gamma scintigraphy imaging of rat brain following i.v. and i.n. administrations were performed to ascertain the localization of drug in brain. Higher drug transport efficiency (DTE%) and direct nose to brain drug transport (direct transport percentage, DTP%) for mucoadhesive NEs indicated more effective and best brain targeting of RSP amongst the prepared NEs. Studies conclusively demonstrated rapid and larger extent of transport of RSP by RMNE (i.n.) when compared to RS (i.n.), RNE (i.n.), and RNE (i.v.) into the rat brain.[24]

The advantages of formulating various lipophilic anti-cancer drugs in submicron O/W emulsion are obvious. The oil phase of the emulsion systems can act as a solubilizer for the lipophilic compound. Therefore, solubility of lipophilic drugs can be significantly enhanced in an emulsion system, leading to smaller administration volumes compared to an aqueous solution. In addition, because lipophilic drugs are incorporated within the innermost oil phase, they are sequestered from direct contact with body fluids and tissues. Lipid emulsions can minimize the pain associated with intravenously administered drugs by exposing the tissues to lower concentrations of the drug or by avoiding a tissue-irritating vehicle. This has been demonstrated with propofol, diazepam, methohexital, clarithromycin, and etomidate.[9]

Another study reported the formulation of filter sterilizable emulsion formulation of paclitaxel using α-tocopherol as the oil phase and α-tocopherylpolyethyleneglycol-1000 succinate (TGPS) and poloxamer 407 as emulsifiers. The formulation exhibited better efficacy and was more tolerable when studied in B16 melanoma tumor model in mice.[25]

Emulsion formulations also show promise in cancer chemotherapy as vehicles for prolonging the drug release after intramuscular and intratumoral injection (W/O systems) and as a means of enhancing the transport of anti-cancer drugs via the lymphatic system.[26]

Positively charged NEs systems are expected to interact with negatively charged cell surfaces more efficiently, and this aspect of the positively charged NEs has been explored for possibility of oligonucleotide delivery to cancer cells.[27-30] Photodynamic therapy (PDT) of cancer is based on the concept that certain photosensitizers can be localized in the neoplastic tissue, and subsequently, these photosensitizers can be activated with the appropriate wavelength (energy) of light to generate active molecular species such as free radicals and singlet oxygen (1O2) that are toxic to cells and tissues.[31-33] Various PDT therapies have reported two different vehicles for photosensitizers, a cremophor oil emulsion and DPPC (dipalmitoylphosphatidylcholine) liposomal vesicles. The reported pharmacokinetic studies clearly indicate that the former vehicle yields a significantly larger selectivity of tumor targeting, mainly as a consequence of an enhanced accumulation in the malignant lesion. Neutron Capture Therapy (NCT) is a binary radiation therapy modality that brings together two components that when kept separate have only minor effects on the cells. The first component is a stable isotope of boron or gadolinium (Gd) that can be concentrated in tumor cells by a suitable delivery vehicle. The second is a beam of low-energy neutrons. Boron or Gd in or adjacent to the tumor cells disintegrates after capturing a neutron, and the high energy heavy charged particles produced through this interaction destroy only the cancer cells in close proximity to it, leaving adjacent normal cells largely unaffected.[34] The success of NCT relies on the targeting of boron and Gd-based compounds to the tumor mass and to achieve desirable intracellular concentrations of these agents. At the present time, there are two targets with NCT, namely glioblastoma (malignant brain tumor) and malignant melanoma.

Lu and co-workers developed and evaluated a very low-density lipoprotein (VLDL), resembling phospholipid-submicron emulsion as a carrier system for new cholesterol-based boronated compound, BCH (anti-cancer boron neutron capture therapy compound), for targeted delivery to cancer cells.[35] Perfluorochemicals are hydrophobic and are not miscible with water. Perfluorochemicals have to be emulsified for intravenous use. To mimic the natural oxygen carrying cells (RBCs), the droplet size of perfluorocarbon emulsions is maintained in submicron range (median diameter $<$0.2 mm). Egg phospholipid has been used as an emulsifier of choice in these formulations. The examples of the commercial perfluorocarbon emulsions are oxygente (Alliance Pharmaceutical Corporation, San Diego, CA, USA), oxyfluorw (Hemagen Inc., St Louis, MO, USA), and fluosol-DA (Alpha Therapeutic Corp., Los Angeles, CA, USA).

The perfluorochemical NEs (PFCE) have opened interesting opportunities in cancer therapy. It is suggested that fluorocarbon emulsions might find a role in photodynamic therapy, both as carriers for sensitizing dyes and to maintain tissue oxygenation in hypoxic regions of solid tumors. The high solubility of oxygen in fluorocarbon emulsions maintains solution oxygen tension, optimizing photo-oxidative damage. The hydrophobic anti-cancer

drugs can be delivered to the tumor mass by dissolving them in a hydrophobic core of the emulsion. Furthermore, PFCE can be used as an adjuvant to radiation therapy and/or chemotherapy in the treatment of solid tumors.[36,37]

The preclinical studies have shown very positive effects with single dose and fractionated radiation in several rodent solid tumor models. Many widely used anticancer drugs, including anti-tumor alkylating agents and doxorubicin, have shown improved response by PFCE coadministration.[38] Also, local application of toxic doses of PFCEs resulted in the necrosis of cancer cells. This is especially promising in the treatment of cancers of the head and neck regions that are currently difficult to treat.[39]

Nanoemulsion in the treatment of various other disease conditions

Pharmos' (US-based company) has developed the nanoemulsion topical diclofenac cream as a potential treatment for osteoarthritis (OA) pain. OA is a painful condition affecting more than 30 million people in the USA and is the most frequent cause of physical disability among adults, mainly elderly. Topical diclofenac is also being considered as treatment for soft tissue injuries, sprains, and strains. It is estimated that 20% of OA patients are not receiving treatment, mainly due to gastrointestinal side effects of oral NSAIDs and cardiovascular risk of COX-2 inhibitors. A topical NSAID offering adequate pain relief targeted to the site of injury with an improved safety profile could become a treatment alternative for these patients. In the USA, there are no approved topical NSAIDs for the treatment of OA. Pharmos' NE technology consists of an efficient solvent-free topical vehicle based and drug entrapment in stable, submicron particles of oil-in-water emulsions with a mean droplet size between 100 and 200 nm that are uniformly dispersed in an aqueous phase. One of the unique characteristics of the NE technology is the relatively high percentage of total particle volume occupied by the internal hydrophobic oil core of the droplets. This provides high solubilization capacity for lipophilic compounds compared to other lipoidal vehicles such as liposomes. Viscosity-imparting agents are used for nanoemulsion thickening to produce creams with the desired semisolid consistency for application to the skin. The skin penetrative properties of the solvent-free NE delivery technology and its low irritancy make this novel topical nanovehicle a promising candidate for effective transcutaneous delivery of lipophilic drugs. Pharmos owns a family of patents covering novel nanoemulsion formulations as vehicles for localized delivery of lipophilic drugs. A topical application of the nanotechnology has already demonstrated excellent targeted delivery of lipophilic drugs to muscle and joints in animal models. Preclinical data using a paw edema animal model showed enhanced anti-inflammatory activity with NSAIDs encapsulated in nanoemulsion creams compared to commercial formulations. Pharmacokinetic studies using nanoemulsion topical creams containing radiolabeled diclofenac and ketoprofen were performed to assess drug penetration through skin and to determine local tissue (muscle and joint) and plasma levels of drugs following topical administration. Compared to oral administration, diclofenac and ketoprofen administered via nanoemulsion topical creams demonstrated 4- to 6-fold lower drug concentration in plasma, 60-to 80-fold more drug in muscle tissue, and about 9-fold more drug in joints. The Company's NE containing diclofenac was also tested in a previous Phase 1 human skin irritancy study involving 25 healthy volunteers. No irritation or allergic responses were observed after topical application. A second lipid-based drug delivery system developed by Pharmos is the nanoemulsion (NE) technology. The NE technology consists of spheric oily droplets (in the range of 100-200 nm) uniformly dispersed in an aqueous medium. The emulsion droplet size reduction is essential to generate drug formulations with high stability. The NE technology has been successfully applied in the formulation of ophthalmic preparations showing improved drug delivery and reduced ocular irritation in humans in Phase I/II clinical studies.

Preclinical data using the carrageenan-induced paw edema animal model showed enhanced anti-inflammatory activity with NSAID encapsulated in NE creams compared to conventional commercial formulations. A pharmacokinetic study using NE creams containing radiolabeled diclofenac and ketoprofen was performed to assess drug penetration through skin and to determine local tissue (muscle and joint) and plasma levels of drugs following topical administration. Compared to oral administration, NE-diclofenac and NE-ketoprofen topical creams showed 4- to 6-fold less drug in plasma, 60- to 80-fold more drug in muscle tissue, and about 9-fold more drug in joints.

An NE topical cream product containing diclofenac has been tested in a 48-h human skin irritancy study involving 25 healthy volunteers. No irritation or allergic responses were observed after topical application. The NE diclofenac cream was further evaluated in a Phase I study in 16 healthy male and female volunteers during 2006. The study evaluated the safety, tolerability, and pharmacokinetic profile of NE diclofenac topical cream following 14 days of three daily administrations. The NE diclofenac product was found to be safe and well tolerated with no severe or serious adverse events; subject compliance with the 14-day treatment period was excellent. The pharmacokinetic analysis demonstrated low systemic exposure of diclofenac with no drug accumulation following repeated daily administrations. The improved skin penetrative properties of the NE delivery technology, its low irritancy, and excellent human acceptance make this novel topical vehicle very promising to achieve increased transcutaneous drug penetration of lipophilic drugs. The site specificity of the NE technology results in a superior safety profile compared to oral administration while maintaining efficacy, thus improving patient compliance with the treatment.

Primaquine (PQ) is one of the most widely used antimalarial and is the only available drug till date to combat relapsing form of malaria especially in case of *Plasmodium vivax* and *Plasmodium ovale*. Primaquine acts specifically on the pre-erythrocytic schizonts that are concentrated predominantly in the liver and causes relapse after multiplication. However, application of PQ in higher doses is limited by severe tissue toxicity including hematological and GI-related side effects that are needed to be minimized. Lipid NE has been widely explored for parenteral delivery of drugs. Primaquine when incorporated into oral lipid NE having a particle size in the range of 10-200 nm showed effective antimalarial activity against *Plasmodium bergheii* infection in Swiss albino mice at a 25% lower dose level as compared to conventional oral dose. Lipid NE of primaquine exhibited improved oral bioavailability and was taken up preferentially by the liver with drug concentration higher at least by 45% as compared to the plain drug.[40]

Nanoemulsion formulations for improved oral delivery of poorly soluble drugs

NE formulations were developed to enhance oral bioavailability of hydrophobic drugs. Paclitaxel was selected as a model hydrophobic drug. The oil-in-water (o/w) NEs were made with pine nut oil as the

internal oil phase, egg lecithin as the primary emulsifier, and water as the external phase. Stearylamine and deoxycholic acid were used to impart positive and negative charge to the emulsions, respectively.

The formulated NEs had a particle size range of 90-120 nm and zeta potential ranging from +34 mV to −45 mV. Following oral administration, a significantly higher concentration of paclitaxel was observed in the systemic circulation when administered in the NE relative to control aqueous solution. The results of this study suggest that NEs are promising novel formulations that can enhance the oral bioavailability of hydrophobic drugs.[41]

Coenzyme Q10 (CoQ10), also known as ubiquinone, is used for energy production within cells and acts as an anti-oxidant. Since CoQ10 is highly lipophilic, the topical and oral bioavailability is very low. Several attempts have been made to improve absorption. Latest technical developments reveal that encapsulation of CoQ10 in NEs results in a significantly enhanced bioavailability. The application of CoQ10 has been further improved by the development of novel CoQ10 double NEs containing tocopherol and CoQ10 in individual nanodroplets. In addition, the CoQ10 concentration in these NEs could be increased by the development of a supersaturated CoQ10 NE.[42]

Nanoemulsions as a vehicle for transdermal delivery

From *in vitro* and *in vivo* data, it was concluded that the developed NEs have great potential for transdermal drug delivery of aceclofenac.[43] The NEs of the system containing ketoprofen evidenced a high degree of stability. Ketoprofen-loaded NEs enhanced the *in vitro* permeation rate through mouse skins as compared to the control.[44]

The study was developed to evaluate the potential of NEs for increasing the solubility and the *in vitro* transdermal delivery of carvedilol. The prepared NEs were subjected to physical stability tests. Transdermal permeation of carvedilol through rat abdominal skin was determined with the Keshary-Chien diffusion cell. Significant increase ($P < 0.05$) in the steady state flux (Jss) and permeability coefficient (Kp) was observed in NE formulations as compared to control or drug-loaded neat components. The irritation studies suggested that the optimized NE was a non-irritant transdermal delivery system.[45]

Celecoxib, a selective cyclo-oxygenase-2 inhibitor, has been recommended orally for the treatment of arthritis and osteoarthritis. Long-term oral administration of celecoxib produces serious gastrointestinal side effects. Skin permeation mechanism of celecoxib from NE was evaluated by FTIR spectral analysis, DSC thermogram, activation energy measurement, and histopathological examination. The optimized NE was subjected to pharmacokinetic (bioavailability) studies on Wistar male rats. Photomicrograph of a skin sample showed the disruption of lipid bilayers as distinct voids and empty spaces were visible in the epidermal region. The absorption of celecoxib through transdermally applied NE and NE gel resulted in 3.30- and 2.97-fold increase in bioavailability as compared to oral capsule formulation. Results of skin permeation mechanism and pharmacokinetic studies indicated that the NEs can be successfully used as potential vehicles for enhancement of skin permeation and bioavailability of poorly soluble drugs.[46]

Self-nanoemulsifying drug delivery systems

Self-nanoemulsifying drug delivery systems (snedds) for oral delivery of protein drugs: Formulation development, *in vitro* transport study and *in vivo* oral absorption study.[47-49]

The research project was done to develop a self-nanoemulsifying drug delivery system (SNEDDS) for non-invasive delivery of protein drugs. An experimental design was adopted to develop SNEDDS. Fluorescent-labeled beta-lactamase (FITC-BLM), a model protein, was loaded into SNEDDS through the solid dispersion technique. The experimental design provided 720 compositions of different oil, surfactant, and co-surfactant at various ratios, of which 33 SNEDDS prototypes were obtained. A SNEDDS was developed to load FITC-BLM into the oil phase that can spontaneously form O/W NE upon the addition of water. Fluorescently labeled BLM (FITC-BLM), a model protein, formulated into 16 SNEDDS preparations through a solid dispersion technique were studied for transport across monolayer. All the SNEDDS NEs resulted in higher transport rate than the free solution. The transport rate by SNEDDS depends on the SNEDDS composition. The SNEDDS significantly increased the transport of FITC-BLM across MDCK monolayer *in vitro*. SNEDDS may be a potential effective delivery system for non-invasive protein drug delivery. The oral absorption of BLM in rats when delivered by such a SNEDDS was investigated and showed significantly enhance in the oral bioavailability of BLM. So the SNEDDS has a great potential for oral protein delivery.

Solid self-nanoemulsifying delivery systems as a platform technology for formulation of poorly soluble drugs

New drug discovery programs produce molecules with poor physico-chemical properties, making delivery of these molecules at the right proportion into the body, a big challenge to the formulationscientist. The various options available to overcome the hurdle include solvent precipitation, micronisation/nanonization using high-pressure homogenization or jet milling, salt formation, use of microspheres, solid dispersions, cogrinding, complexation, and many others. Self-nanoemulsifying systems (SNES) form one of the most popular and commercially viable approaches for delivery of poorly soluble drugs exhibiting dissolution rate limited absorption, especially those belonging to the Biopharmaceutics Classification System II/IV. SNES are essentially an isotropic blend of oils, surfactants, and/or cosolvents that emulsify spontaneously to produce oil in water NE when introduced into aqueous phase under gentle agitation. Conventional SNES consist of liquid forms filled in hard or soft gelatin capsules, which are least preferred due to leaching and leakage phenomenon, interaction with capsule shell components, handling difficulties, machinability, and stability problems. Solidification of these liquid systems to yield solid self-nanoemulsifying systems (SSNES) offer a possible solution to the mentioned complications, and that is why these systems have attracted wide attention.[50]

Patented nanoemulsions[51-53]

Some important patents related to NEs:

1. Patent name: Method of Preventing and Treating Microbial Infections. Assignee: NanoBio Corporation (US). US Patent number:6,506,803.
2. Patent name: NE based on phosphoric acid fatty acid esters and its uses in the cosmetics, dermatological, pharmaceutical, and/or ophthalmological fields. Assignee: L'Oreal (Paris, FR). US Patent number:6,274,150.
3. Patent name: NE based on ethylene oxide and propylene oxide

Table 3: Commercial nanoemulsion (sub-micron emulsion) formulations[9]

Drug therapeutic	Brand	Manufacturer	Indication
Propofol	Diprivan	Astra Zeneca	Anesthetic
Dexamethasone	Limethason	Mitsubishi Pharmaceutical, Japan	Steroid
Palmitate Alprostadil	Liple	Mitsubishi Pharmaceutical, Japan	Vasodilator platelet inhibitor
Flurbiprofen axetil	Ropion	Kaken Pharmaceuticals, Japan	Nonsteroidal analgesic
Vitamins A, D, E, K	Vitalipid	Fresenius Kabi, Europe	Parenteral nutrition

block copolymers and its uses in the cosmetics, dermatological and/or ophthalmological fields. Assignee: L'Oreal (Paris, FR). US Patent number: 6,464,990.

4. NE of 5-aminolevulinic acid (6,559,183). Assignee: ASAT AG Applied Science and Technology (Zug, CH). PCT number: PCT/EP99/08711.
5. NEs of poorly soluble pharmaceutical active ingredients and methods of making the same. Patent no.: WO/2007/103294.

Some commercially available NE formulations are shown in Table 3.

Conclusion

NE formulations offer several advantages for the delivery of drugs, biologicals, or diagnostic agents. Traditionally, NEs 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 also reached the marketplace. Although NEs are chiefly seen as vehicles for administering aqueous insoluble drugs, they have more recently received increasing attention as colloidal carriers for targeted delivery of various anticancer drugs, photosensitizers, neutron capture therapy agents, or diagnostic agents. Because of their submicron size, they can be easily targeted to the tumor area. Moreover, the possibility of surface functionalization with a targeting moiety has opened new avenues for targeted delivery of drugs, genes, photosensitizers, and other molecules to the tumor area. Research with perflurochemical NEs has shown promising results for the treatment of cancer in conjugation with other treatment modalities and targeted delivery to the neovasculature. It is expected that further research and development work will be carried out in the near future for clinical realization of these targeted delivery vehicles.

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