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Lipid nanoemulsions for anti-cancer drug therapy

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Multifunctional lipid nanoemulsions have shown to combine several advantages e.g. tissue targeting, cell targeting, imaging analysis, barrier permeability enhancement, and therapeutic purposes. Depending on the choice of lipid composition, surfactants and additional surface modifiers ratio, different drug loadings may be achieved and exploited for drug delivery in cancer chemotherapy. However, a safe and effective delivery system for cancer therapy should also be able to overcome the major impediment of multidrug resistance. Several strategies have been tested in nanoemulsions including P-glycoprotein-mediated drug resistance. The present review focuses on a comprehensive discussion of the use of nanoemulsions in anti-cancer therapy, reporting the technological aspects of pharmaceutical formulation of these carriers, and exploiting their advantages in siRNA therapy.

1. Introduction

Although several types of therapies have been designed to treat cancer, the success achieved so far is very limited. Among the different types of therapies (geneherapy, chemotherapy, immunotherapy, and radiotherapy) systemic chemotherapy is the main treatment available for disseminated malignant disease (Aditya et al. 2010). Chemotherapy requires multiple cycles of treatment which unfortunately render toxic side effects to the host cell. In addition to this, many chemotherapeutics yielded from drug discovery processes have low aqueous solubility. Here it comes the new technology – nanotechnology –, which encompasses technological developments on the nanometer scale, usually between 0.1 and 99 nm. The use of this robust technology in health care has developed tremendously since last few years, towards the development of the so-called nanomedicine. Various types of nanodelivery systems have been developed to improve the therapeutic efficiency of drugs, including nanoemulsions, nanosuspensions, nanospheres (drug nanoparticles in polymer matrix), nanotubes (sequence of nanoscaled C_{60} 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 (nanoscaled three-dimensional macromolecules of polymer). Among these nanocarriers, nanoemulsions are under extensive investigation as drug carriers for improving the delivery of drugs in general, and for chemotherapeutics in particular. Nanoemulsions are dispersions of oil and water where the dispersed phase droplets are in the nanosized range and stabilized with a surface active film. The terms sub-micron emulsion and mini-emulsion are used as synonyms. Usually, these contain 10 to 20% oil stabilized with 0.5 to 2% egg or soybean lecithin. Nanoemulsions for human use (either for medicines or for food industry) should be composed of surfactants of generally recognized as safe (GRAS) status (McClements and Li 2010). The nanosizes of the droplets prevent creaming or sedimentation form occurring on storage and droplet coalescence. Nanoemulsions provide much larger oil-in-water contact area due to the nanosized droplet compared to classical emulsions, which facilitates drug release from the dispersed droplets. They are the most advanced nanoparticle systems for the systemic delivery of biologically active agents for controlled drug delivery and targeting. Nanoemulsion us have shown great promise for the future of cosmetics, diagnostics, drug therapy and in the area of biotechnology (Sarker 2005; Tiwari and Amiji 2006).

The advantages of formulating lipophilic drugs in o/w nanoemulsion are obvious. The oil phase of the emulsion systems can act as a solubiliser for the lipophilic compound. Therefore, solubility of lipophilic drugs can be significantly enhanced in a nanoemulsion 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 which help to overcome the pharmacokinetic mismatch associated with that particular chemotherapeagent. Lipid emulsions can also 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 which helps to increase the patient compliance (Cevc and Vierl 2010).

2. What are nanoemulsions?

Nanoemulsions are thermodynamically stable isotropic systems, in which two immiscible liquids (water and oil) are mixed to form a single phase, by means of appropriate surfactants or

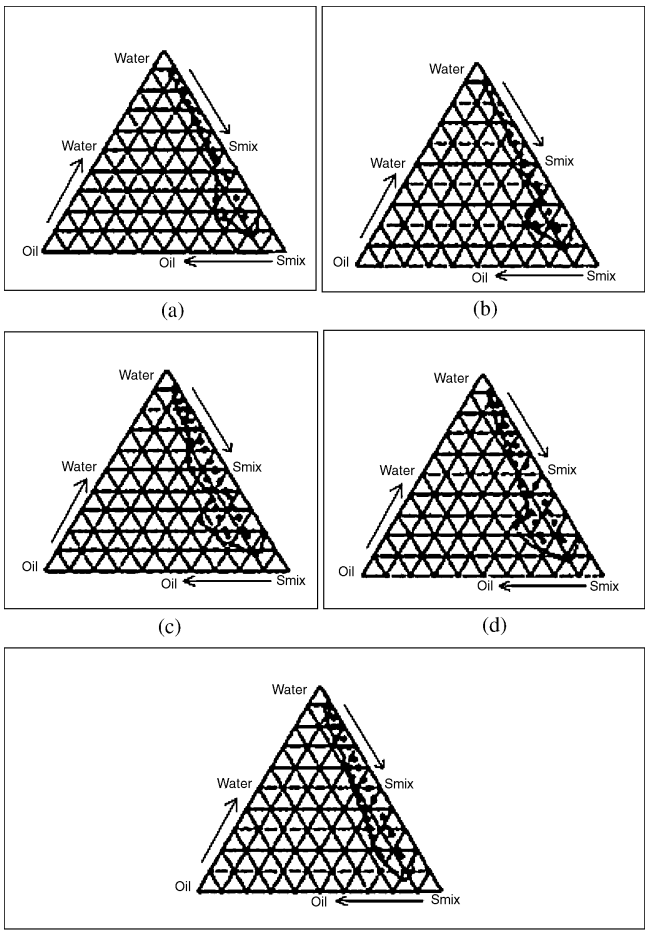

Fig.: Pseudoternary phase diagrams indicating oil-in-water nanoemulsion (shaded area) region of labrafil and triacetin (oil), Tween 80 (surfactant), and Transcutol P (co-surfactant) at different S_{mix} ratios: (a) S_{mix} 1:0; (b) S_{mix} 1:1; (c) S_{mix} 2:1; (d) S_{mix} 3:1; (e) S_{mix} 4:1. (Adapted from (Shakeel et al. 2007)).

its mix, with a droplet diameter approximately in the range of 0.5–100 μm. 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. Since it is a stable isotropic system, careful balance of the three phases is essential for the system to achieve a thermodynamically state. The proportion of the three phases is determined using three phase diagram which denotes the ratio of constituents that exist in balance to form nanoemulsion.

2.1. Pseudoternary phase diagram study

Constructing phase diagrams is time-consuming, particularly when the aim is to accurately delineate a phase boundary. Care has to be taken to ensure that observations were not made on metastable systems, although the free energy required to form an emulsion is very low, the formation is thermodynamically spontaneous (Boonme et al. 2006). The relationship between the phase behaviour of a mixture and its composition can be captured with the aid of a phase diagram. Pseudoternary phase diagrams are usually generated separately for each S_{mix} ratio, to allow that o/w nanoemulsion regions are identified and nanoemulsion formulations optimized.

In the pseudoternary phase diagram shown above, the S_{mix} ratio 1:0 (Fig. 1A) has a low nanoemulsion area and is confined to the water-rich apex of the phase diagram where o/w nanoemulsion system would form (Shakeel et al. 2007). Here, the maximum concentration of oil that could be solubilised in the phase diagram was only 16% (wt/wt) using 67% (wt/wt) of S_{mix}. With increase in the surfactant concentration up to the S_{mix} ratio 1:1 (Fig. 1B), a higher nanoemulsion region was observed, perhaps because of further reduction of the interfacial tension, increasing the fluidity of the interface, thereby increasing the entropy of the system. There may be greater penetration of the oil phase in the hydrophobic region of the surfactant monomers (He et al. 2010; Kawakami et al. 2002). The maximum concentration of oil that could be solubilised in the phase diagram was only 16% (wt/wt) using 67% (wt/wt) of S_{mix}. By increasing surfactant concentration S_{mix} 2:1 (Fig. 1C), the nanoemulsion region increases as compared with the region in 1:0 and 1:1. The maximum concentration of oil that could be solubilised by this ratio was 22% wt/wt using 52% wt/wt of S_{mix}. When the S_{mix} ratio of 3:1 was studied (Fig. 1D), the nanoemulsion region decreased slightly as compared with 1:1, which may have been due to the increased concentration of the surfactant, although the maximum oil that could be solubilised by this ratio of S_{mix} was 22% (wt/wt) with 52% (wt/wt) of S_{mix}. Similarly, when the S_{mix} ratio of 4:1 was studied (Fig. 1E), the nanoemulsion area further decreased as compared with 3:1 and 2:1 but increased as compared with 1:0 and 1:1. The maximum concentration of oil that could be solubilised by this ratio of S_{mix} was 17% (wt/wt) with 67% (wt/wt) of S_{mix}. When surfactant concentration increased as compared with co-surfactant, the nanoemulsion area increased up to 2:1 ratio, but in 4:1 ratio the nanoemulsion region decreased again, thus there was no need to try S_{mix} ratio of 5:1. No nanoemulsion regions were found in S_{mix} ratios of 1:2 and 1:3. The above phase diagrams show that the free energy of nanoemulsion formation is dependent on the extent to which the surfactant lowers the surface tension of the oil-water interface and the change in dispersion entropy (Ansari et al. 2008; Talegaonkar et al. 2008). Thus, a negative free energy of formation is achieved when a large reduction in surface tension is accompanied by significantly favourable entropic changes. In such cases, nanoemulsion formation is spontaneous and the resulting dispersions are thermodynamically stable (Izquierdo et al. 2004). The surfactant or S_{mix}, which are able to increase the dispersion entropy, reduce the interfacial tension, increase the interfacial area, and thus lower the free energy of the system to a very low value with the minimum concentration which is thermodynamically stable.

2.2. Ostwald ripening

The formation of stable transparent nanoemulsions poses two challenges, namely, the ability to initially generate an emulsion where the entire droplet size distribution is below 80 nm, and the subsequent stabilization of the system against Ostwald ripening. Although nanoemulsions have extreme Laplace pressures, of order 10–100 atm, the droplets can remain stable against Ostwald ripening if the inner phase (droplets) has very low solubility in the continuous outer phase. The strong Brownian motion of the tiny droplets in nanoemulsions makes them ideal for products in which gravitational creaming must be prevented to ensure a long shelf life (Teo et al. 2010; Wooster et al. 2008).

The physical properties of the oil phase and the nature of the surfactant layer were found to have a considerable impact on nanoemulsion formation and stabilization (Wooster et al. 2008). Nanoemulsions made with highly viscous oils, such as long chain triglycerides (LCT), were considerably larger, with a mean diameter of ca. 120 nm, than nanoemulsions prepared with oils of lower viscosity, such as hexadecane with a mean diameter of approx. 80 nm. The optimization of surfactant architecture, and differential viscosity, has led to the formation of remarkably small nanoemulsions. LCT nanoemulsions do not undergo Ostwald ripening and are physically stable for over 3 months.

Ostwald ripening is prevented by the large molar volume of LCT oils, which makes them insoluble in water thus providing a kinetic barrier to Ostwald ripening. Examination of the Ostwald ripening of mixed oil nanoemulsions found that the entropy gain associated with oil demixing provided a thermodynamic barrier to Ostwald ripening. Some of the smallest nanoemulsions reported are also thermodynamically stable to Ostwald ripening when at least 50% of the oil phase is an insoluble triglyceride (Tadros et al. 2004). Tadros et al. prepared nanoemulsion of virgin coconut oil added with a non-soluble oil like squalene in the ratios of 10:0, 9.8:0.2, 9.6:0.4, 9.4:0.6, 9.2:0.8, 9:1 and 8:2 and Emulium Kappa® is an ionic emulsifier. The results showed that higher molecular weight of squalene (above critical molecular weight) resulted in low polarity and insolubility in the continuous phase. The continuous partitioning between the droplets result in the decline of Ostwald ripening. Furthermore, flocculation may occur due to the instability of nanoemulsion, especially for the preparations with little or without squalene. The stability of the nanoemulsion was evaluated by the electrophoretic properties of the emulsion droplets. The zeta potential values for the emulsion increased as the percentage of squalene increased (Tadros et al. 2004).

3. Advantages of nanoemulsions in cancer therapy

Nanoemulsions have many advantages as drug delivery systems, such as the (i) higher surface area and free energy than macroemulsions that make them an effective transport system; (ii) do not show the problems of inherent creaming, flocculation, coalescence and sedimentation, which are commonly associated with macroemulsions; (iii) they can be processed in a variety of formulations such as foams, creams, liquids and sprays; (iv) nontoxic, non-irritant thus can be easily applied to skin and mucous membranes; (v) since nanoemulsions are formulated with surfactants, which are approved for human use (GRAS), they can be administered by the enteric route; (vi) nanoemulsions do not damage healthy human and animal cells being therefore suitable for human and veterinary purposes.

Estimatiens conclude that 90% of new chemical entities discovered in recent days are poorly soluble or lipophilic compounds (Kommuru et al. 2001). Relative to compounds with high solubility, poor drug solubility often pose host of *in vivo* consequences like decreased bioavailability, increased chance of food effect, more frequent incomplete release from the dosage form and higher interindividual variability. Poorly soluble compounds also present many *in vitro* formulation development hindrances, such as complex dissolution testing with limited or poor correlation to the *in vivo* absorption. However, important advances have been made towards improving the bioavailability of poorly soluble compounds adopting techniques such as micronization, salt formation, complexation. The recent trends for the enhancement of solubility/bioavailability comprise lipid based systems such as microemulsions, nanoemulsions, solid dispersions, solid lipid nanoparticles and liposomes. These approaches have gained commercial importance, as formulation scientists increasingly turn to a range of nanotechnology-based solutions to improve drug solubility and bioavailability.

Nanoemulsions have been reported to make the plasma concentration profiles and bioavailability of poorly soluble drugs more reproducible (Chen et al. 2010). Nanoemulsions have also been reported as one of the most promising techniques for enhancement of transdermal permeation and bioavailability of poorly soluble drugs (Kong and Park 2011; Sarker 2005). Many formulation scientists have investigated the skin permeation mechanism of many drugs using the microemulsion technique (Changez et al. 2006; Dreher et al. 1997; Klang et al. 2010). Shakeel et al. (2008a, b) prepared nanoemulsions is containing celecoxib and undertook pharmacokinetic (bioavailability) studies on Wistar male rats. The absorption of celecoxib from a transdermally applied nanoemulsion and nanoemulsion gel resulted in 3.30 and 2.97 fold increase in bioavailability as compared to an oral capsule formulation. Nanoemulsion formulations were also developed to enhance the oral bioavailability of hydrophobic drugs like paclitaxel (Tiwari and Amiji 2006). The o/w nanoemulsions 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 nanoemulsions had a particle size range of 90–120 nm and a zeta potential ranging from + 34 mV to 245 mV. Following oral administration, relative to control aqueous solution, nanoemulsions showed a significantly higher concentration of paclitaxel in the systemic circulation. The results of this study suggest that nanoemulsions are promising formulations that can enhance the oral bioavailability of hydrophobic drugs (Tiwari and Shenoy 2006). Coenzyme Q10 (CoQ10) is a highly lipophilic anti-oxidant with very low topical and oral bioavailability. Several attempts have been made to improve its absorption. Latest technical developments reveal that loading CoQ10 in nanoemulsions results in a significantly enhanced bioavailability. Double nanoemulsions containing tocopherol and CoQ10 in individual nanodroplets were prepared for further improvement of its application. In addition, the CoQ10 concentration in these nanoemulsions could be increased by the development of a supersaturated CoQ10 nanoemulsion (Beg et al. 2010; Junyaprasert et al. 2009).

Tagne et al. (2008) prepared water-soluble nanoemulsion of the highly lipid-soluble drug tamoxifen. The nanoemulsion demonstrated a greater zeta potential (increased negative charge) which is associated with increasing drug/membrane permeability. The study reported that relative to suspensions of tamoxifen with particle sizes greater than 6000 nm, nanoemulsions of tamoxifen, having mean particle sizes of 47 nm, inhibited cell proliferation 20-fold greater and increased cell apoptosis 4-fold greater in the HTB-20 breast cancer cell line.

In order to achieve penetration of paclitaxel into deeper skin layers while minimizing the systemic escape, a nanoemulsion was formulated and the *in vivo* pharmacokinetic performance was evaluated. 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 (TPGS) and labrasol would have contributed to the enhanced peroral bioavailability of paclitaxel. These results provide direct evidence on the localization of high-molecular-weight, lipophilic drug, paclitaxel, in dermis. Furthermore, the nanoemulsion has significantly enhanced the peroral bioavailability by more than 70%. The developed nanoemulsion was safe and effective for both peroral and dermal delivery of paclitaxel (Khandavilli and Panchagnula 2007).

Another study reported the formulation of a filter sterilizable emulsion formulation of paclitaxel using α-tocopherol as the oil phase and TPGS and poloxamer 407 as emulsifiers. The formulation exhibited highes efficacy and was better tolerable when studied in a B16 melanoma tumour model in mice (Constantinides et al. 2004).

Nanoemulsions may also be suitable alternatives in cancer chemotherapy as vehicles for prolonging the drug release after intramuscular and intratumoural injection (w/o systems) and as a means of enhancing the transport of chemotherapeutics via the lymphatic system (Eccleston 1995). Positively charged nanoemulsion systems are expected to interact with negatively charged cell surfaces more efficiently, and this aspect of the

positively charged nanoemulsions has been explored for the possibility of oligonucleotide delivery to cancer cells (Fraga et al. 2008; Hagigit et al. 2010, 2008).

Photodynamic therapy and neutron capture therapy are two derived therapeutic approaches based on the concept of triggering the activity of the otherwise inactive/very low active photosensitize or a stable isotope by using light energy of suitable wavelength or low energy neutron respectively, after they have accumulated in or in the vicinity of tumour tissue (Primo et al. 2008). Various photodynamic therapies have reported two different vehicles for photosensitizers, a cremophor oil emulsion and dipalmitoylphosphatidylcholine (DPPC) liposomal vesicles to accumulate them in tumour tissues. The reported pharmacokinetic studies clearly indicate that the former vehicle yields a significantly larger selectivity of tumour targeting, mainly as a consequence of an enhanced accumulation in the malignant lesion. In case of neutron capture therapy, the first component used is a stable isotope of boron or gadolinium (Gd) that can be concentrated in tumour cells by a suitable delivery vehicle. The second is a beam of low-energy neutrons. Boron or Gd in or adjacent to the tumour cells disintegrates after capturing a neutron, and the high energy heavy charged particles produced through this interaction destroy the cancer cells in close proximity (Broerse and Barendsen 1967; Svensson and Landberg 1994). Thus, the success of photodynamic and neutron capture therapies relies on the targeting of the inactive component to the tumour mass and to achieve desirable intracellular concentrations of these agents. At the present time, there are two targets developed with nanoparticles, namely glioblastoma (malignant brain tumour) and malignant melanoma. Shawer et al. (2002) developed and evaluated a very low-density lipoprotein (VLDL), resembling phospholipid-submicron emulsion as a carrier system for a new cholesterol-based boronated compound, for targeted delivery to cancer cells. Hydrophobic perfluorochemicals are emulsified in an nanoemulsion for intravenous use. Egg phospholipid has been used as an emulsifier of choice in these formulations. The examples of the commercial perfluorocarbon nanoemulsions 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).

A Gd-loaded nanoemulsion was prepared for further evaluation of the biodistribution of Gd after its intravenous injection in D1–179 melanoma-bearing hamsters. The systems were evaluated for their 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 tumours. Among the core components employed for nanoemulsion preparation, soybean oil yielded the highest Gd concentration in the blood and tumour, and the lowest in the liver and spleen. When each nanoemulsion was injected intravenously once or twice during a period of 24 h interval, the Gd concentration in the tumour correlated well with the total dose of Gd, and it reached a maximum of a 189 mg/g wet tumour. This maximum Gd level was greater than the limit required for significantly suppressing tumour growth in neutron-capture therapy (Ichikawa et al. 2007).

The perfluorochemical nanoemulsions have opened interesting opportunities in cancer therapy. Hydrophobic chemotherapeutics can be delivered to the tumour mass by dissolving them in a hydrophobic core of the emulsion. These types of nanoemulsions can be used as adjuvants to radiation therapy and/or chemotherapy in the treatment of solid tumours, particularly located in difficultly accessible domains in the body (Teicher 1992; Teicher et al. 1992). Many widely used chemotherapeutics, including anti-tumour alkylating agents and doxorubicin, have shown improved response by co-administration with perfluorochemical nanoemulsions (Teicher et al. 1992). Camptothecin is a topoisomerase I inhibitor that acts against a broad spectrum of cancers but its clinical application is limited by its insolubility, instability, and toxicity. Fang et al. (2009) developed acoustically active nanoemulsions for camptothecin to circumvent these delivery problems. The nanoemulsions were prepared using liquid perfluorocarbons and coconut oil as oil cores of the inner phase. These nanoemulsions were stabilized by phospholipids and/or poloxamer 188. The nanoemulsions showed very high drug loading capacity (up to $\sim$ 100%) with a mean droplet diameter of 220–420 nm. Camptothecin in nanoemulsions with a lower oil concentration exhibited cytotoxicity against melanomas and ovarian cancer cells. Confocal laser scanning microscopy confirmed nanoemulsion uptake into cells. Hemolytic studies to assess the interaction between erythrocytes and the nanoemulsions showed that formulations with phosphatidylethanolamine as the emulsifier showed less hemolysis than those with phosphatidylcholine. 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. Also, local application of toxic doses of perfluorochemical nanoemulsions 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 (Clasen 1990; Rockwell et al. 1986).

4. Role of nanoemulsions in siRNA therapy

Even if chemotherapeutics are made soluble with the available technology and delivered to the tumour region, efficacy of these drugs may be affected by the development of the so-called Multi Drug Resistance (MDR) effect, which is one of the most common reason for recrudescence or failure of the treatment. To overcome these hurdles, in the functional genomics era gene therapy is playing an important role in cancer treatment (Bumcrot et al. 2006; Jain 2006). Small interfering Ribose Nucleic Acids (siRNA) molecules can be used to silence the specific disease associated alleles. siRNA molecules can knock-down their cognate targets specifically and effectively based on direct homology dependent post-transcriptional gene silencing. These short double-stranded RNAs are recognized by the endonuclease, Dicer, and cleaved into two fragments called siRNA. The antisense strand of the two strands becomes associated with a complex of proteins, designated as the RNA-induced silencing complex, which targets messenger RNA (mRNA). Next, Argonaute 2 (Ago2), a RNA endonuclease within the complex cleaves the target mRNA and leads to its degradation, shutting down protein expression (Khurana et al. 2010; Ozpolat et al. 2010).

Even though this technology is exciting and looks to be promising it is quite new and needs to overcome several pragmatic drawbacks. These siRNAs with high molecular weight, anionic charge, and hydrophilic nature are susceptible to degradation by serum nucleases and are associated with poor cellular uptake, rapid renal clearance following systemic administration (Semple et al. 2010). In addition, it is of great importance to consider possible off-target effects in the siRNA therapy to reduce the concentration (ED50) and toxicity. Thus, the relevance of developing novel drug delivery systems which can overcome the aforementioned drawbacks is anticipated. Combination of both siRNA and nanotechnology has proven to be a competent pathfinder to decipher the practical problems associated with siRNA therapy. Within a short span of time, nanoparticle based siRNA delivery moved from academic to industrial research (clinical trials). Recent advances in this field has led

to creation of a wide variety of nanosystems which are useful in delivering siRNA via desired routes of administration, reducing toxicity and increasing the therapeutic competence (Semple et al. 2010). Even though nanoemulsions are extensively used for anticancer drug delivery, till date only few attempts have been made to use this novel delivery system for siRNA therapy. One of the most important reasons is the negative charge of the nanoemulsions which will reduce the transfection efficiency due to charge hindrance (both carrier and cells will have negative charge on the surface). However, this drawback was partially solved by inducing a positive charge on the surface on the emulsion. This positive charge on the surface is obtained by various cationic surfactants or cationic lipids. The common cationic surfactants include DC-Chol, stearylamine, and 1,2-dioleoyl-sn-glycero-3-trimethylammonium-propane (DOTAP) (Khurana et al. 2010; Tros de Ilarduya et al. 2010). In this regard, recently nanoemulsions have been reported as vehicles for siRNA therapy by Kaneda et al. (2010). They developed a cationic DOTAP nanoemulsion coated with surfactant which has a size of approx. 300 nm with 304–421 positive charges per nanoparticles. Transfection complexes were formed through electrostatic interactions between the negatively charged phosphate backbone of the siRNA and the positive head group of the DOTAP lipid within the surfactant layer of the nanoparticles. These perfluorocarbon nanoemulsions were internalized into the cytoplasm by the lipid raft mediated transport.

5. Conclusions

Since the last couple of decades technology transfer has been playing an important role for the success of any new technology. In this regard, within a short span of time, nanoemulsion based delivery has moved from academic to industrial research. Recent advances in this field have led to creation of a wide variety of nanoemulsion formulations which are useful in delivering therapeutic agents via desired routes of administration, reducing the toxicity and increasing the therapeutic competence. As reviewed above, nanoemuslsions are playing a very important role cancer therapy. Till date, these nanoemulsions were investigated in terms of their ability to deliver anticancer drugs with respect to their physicochemical properties, drug loading and entrapment efficiency, and *in vitro* and *in vivo* efficiency and toxicity in animal models. But still more important issues, such as the role of these carriers in siRNA therapy, stability and other safety issues, need to be addressed before seeing them in the market.

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