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REVIEW ARTICLE

NOVEL DRUG DELIVERY SYSTEM-NANOEMULSIONS: A RECENT REVIEW

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ABSTRACT

Nanoemulsions are the emulsions of O/W type, having the size range of several microns. Nanoemulsions are clear, thermodynamically stable, isotropic liquid mixtures of oil, water, surfactant and co-surfactant. They are prepared by using the surfactants which are considered safe for the human use and approved by the FDA. These types of emulsions have higher surface area and hence can easily penetrate through the skin. They are also non toxic and non irritant in nature and can be used in the animals and veterinary purpose. Nanoemulsions can be prepared by the high pressure homogenization and microfluidisation technique. Nanoemulsions have been reported for the delivery of drugs to cell culture, cancer therapy and as disinfectants. Nanoemulsion show great promise for the future of cosmetics, diagnostics, drug therapies and biotechnologies.

KEY WORDS: Nanoemulsions, O/W type, High-pressure equipment, Submicron

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INTRODUCTION:

Nanoemulsions are submicron sized emulsions that are under extensive investigation as drug carriers for improving the delivery of therapeutic agents. The Nanoemulsions are also referred as miniemulsions, ultrafine emulsions and submicron emulsions. The small size of the particles in these kinds of delivery systems (r < 100 nm) means that they have a number of potential benefits for certain applications: enhanced long-term stability, high optical clarity and increased bioavailability. Nanoemulsions are increasingly being utilized in food and pharmaceutical industries to encapsulate, protect, and deliver lipophilic bioactive components[1].

Nanoemulsions are formed when the interfacial tension at the oil/water interface is brought to a very low level and the interfacial layer is kept highly flexible and fluid. These two conditions are usually met by a careful and precise choice of the components and of their respective proportions and by the use of a "co-surfactant" which brings flexibility to the oil/water interface[2].

Nanoemulsions are one of the most interesting fields of application, once they can act as carriers or delivery systems for lipophilic compounds, such as nutraceuticals, drugs, flavors, antioxidants, and antimicrobial agents. Another interesting application which is experiencing an active development is the use of nanoemulsions as formulations, namely for controlled drug delivery and targeting[1, 2].

ADVANTAGES OF NANOEMULSIONS[1]:

1. Increases bioavailability.
2. Increase the rate of absorption.
3. Provides aqueous dosage form for water insoluble drugs.
4. Eliminates variability in absorption.
5. Rapid and efficient penetration of the drug moiety.
6. Helpful in taste masking.
7. Less amount of energy requirement.
8. Various routes like tropical, oral and intravenous can be used to deliver the product.
9. Provides protection from hydrolysis and oxidation as drug in oil phase in O/W.
10. Nanoemulsion is not exposed to attack by water and air.
11. Liquid dosage form increases patient compliance.
12. Nanoemulsion has a transparent and fluidy property which improves the formulation patient compliance and safe for administration due to the absence of any thickening agent and colloidal particles.
13. Nanoemulsion formulation required low amount of surfactant compared to microemulsion. For example about 20- 25 % surfactant is required for the preparation of microemulsion but 5-10 % surfactant is sufficient in case of nanoemulsion.
14. Nanoemulsions are thermodynamically stable system and the stability allows selfemulsification of the system.

LIMITATIONS OF NANOEMULSIONS[1]:

Even though nanoemulsions provide great advantages as a delivery system, but sometimes the reduced size of droplets are responsible for the limited use of nanoemulsion formulation. Some limitations o nanoemulsions are as follows:

1. Nanoemulsion stability creates a big problem during the storage of formulation for the longer time period. Ostwald

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ripening is the main factor associated with unacceptability of nanoemulsion formulations. This is due to the high rate of curvature of small droplet show greater solubility as compared to large drop with a low radius of curvature.

2. The manufacturing process of nanoemulsion formulation is expensive, because size reduction of droplets is very difficult as it required a special kind of instruments and process methods. For example, homogenizer arrangement, microfluidization &ultrasonification require high financial support.

3. Less availability of surfactant and co-surfactant required for the manufacturing of nanoemulsion is another factor which marks as a limitation to nanoemulsion manufacturing.

4. Limited solubilizing capacity for high-melting substances.

FORMULATION ASPECTS FOR NANOEMULSIONS[3]:

Since nanoemulsions have very small particle size range, they can be most effectively produced using high-pressure equipment.

1. HIGH-PRESSURE HOMOGENISATION:

This technique makes use of high-pressure homogenizer/piston homogenizer to produce nanoemulsions of extremely low particle size (up to 1nm). In a high-pressure homogenizer, the dispersion of two liquids (oily phase and aqueous phase) is achieved by forcing their mixture through a small inlet orifice at very high pressure (500 to 5000 psi), which subjects the product to intense turbulence and hydraulic shear resulting in extremely fine particles of emulsion. Homogenizers of varying design are available for lab scale and industrial scale production of nanoemulsions. This technique has great efficiency, the only disadvantage being high energy consumption and increase in temperature of emulsion during processing.

2. MICROFLUIDIZATION:

Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer. This device uses a highpressure 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 nanoemulsion. 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 uniform nanoemulsions. Other method used for nanoemulsions preparation is the phase inversion temperature technique.

TECHNIQUES FOR THE IDENTIFICATION AND CHARACTERIZATION OF NANOEMULSIONS:

Detection, identification, and characterization of nanodelivery systems are essential for the understanding of the benefits as well as the potential toxicity of these systems. In this section, the analytical techniques that can be used for the identification and characterization of nanoemulsions are described. The analytical approaches have been subdivided into three groups: separation, characterization, and imaging techniques. The principles of the techniques together with their advantages and drawbacks are reported.

SEPARATION TECHNIQUES:

Some analytical techniques can be used for in situ identification of nanoemulsions; nevertheless, in most cases it is not possible to detect them in the food matrixes. Therefore, separation techniques are necessary to isolate the nanoemulsions from food prior to their characterization. This section describes the most important separation techniques for isolation of nanoemulsions.

1. CHROMATOGRAPHY:

Because size and/or charge are typical characteristics of nanoemulsions, size-exclusion chromatography (SEC) and/ or ion exchange chromatography (IEC) are the most suitable types of liquid chromatography for the separation of nanoemulsions from the food matrix. In the case of SEC, compounds are separated on the basis of size; larger molecules elute faster than smaller ones. However, elution could be affected by the shape of the compound. In the case of IEC, compounds are separated on the basis of charge; low-charged compounds elute faster than highly charged ones.

HPLC is a form of liquid chromatography to separate, analyse, and quantify compounds that are dissolved in solution. Compounds are separated by injecting the sample mixture (carried by mobile phase) onto a column. Depending on the type of stationary phase, compounds can be separated based on their charge (weak/strong cation or anion exchange chromatography), molecular mass (size-exclusion chromatography), hydrophobicity/polarity (reversed-phase HPLC, hydrophobic interaction chromatography), and specific

characteristics (affinity chromatography). The most common detectors for HPLC are ultraviolet–visible (UV–Vis) light absorbance, fluorescence, electrochemical, and diffraction detectors. HPLC has been used in food analysis for measuring numerous compounds, for example, carbohydrates, vitamins, additives, mycotoxins, amino acids, proteins, tryglycerides in fats and oils, lipids, chiral compounds, and pigments. It is a straightforward, robust, and reproducible technique. Sensitive selective detectors are available for HPLC, and their selection depends mostly on the compound to be analyzed. HPLC can also be used to quantify functional compounds encapsulated in the nanoemulsions: β-carotene, α-tocopherol and thalidomide[2, 4, 5].

2. FIELD FLOW FRACTIONATION:

FFF is a flow-assisted technique for the separation of analytes, from macromolecules, such as proteins (nanometer range), to micrometer-sized particles, such as whole cells. In a one single run, very broad ranges of molecular sizes can be separated. FFF can also separate particles due to their Stokes radius. In this technique, smaller particles are transported faster and elute earlier if a parabolic flow is used; if a cross flow is used with particles of the same volume and different shape, the isometric particles will be eluted first than the asymmetric particles. FFF has advantages over other separation techniques such as higher biocompatibility, reduction of sample carry-over, and simple sterility issues. The separation times typically range from a few minutes up to 30 min. Despite these advantages, the FFF has the disadvantage of being easily overloaded by higher concentrations of the compounds. In order to overcome this, dilutions should be made. Nevertheless, the detection of trace amounts of compounds is limited by the sensitivity of an appropriate detector system[2, 4].

PHYSICAL CHARACTERIZATION TECHNIQUES:

This section describes the techniques that are used to characterize nanoemulsions from a physical perspective (e.g., size, size distribution, zeta potential, and crystallinity of the nanoemulsions).

1. DYNAMIC LIGHT SCATTERING:

DLS also known as photon correlation spectroscopy or quasi-elastic light scattering is a technique used for rapid determination of the size distribution profile of small particles in suspensions or polymers in solution. DLS measures Brownian motion and relates this to the size of the particles through Stokes–Einstein equation. Through the illumination of the particles with a laser and analyzing the intensity fluctuations in the scattered light, DLS allows calculating the size of the particles. DLS provides a fast and adequate evaluation of the size of nanoemulsions and is often used to evaluate the size distribution of nanoemulsions, as well their size stability through storage[4, 6].

2. ZETA POTENTIAL:

Zeta potential is a scientific term for electrokinetic potential in colloidal systems. In colloidal chemistry literature, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. A value of 30 mV (positive or negative) can be taken as the arbitrary value that separates low-charged surfaces from highly charged surfaces. Zeta potential value can be related to the stability of colloidal dispersions, indicating the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e., the solution or dispersion will resist to aggregation. When the potential is low, attraction exceeds repulsion and the dispersion will break and flocculate. So, colloids with high zeta potential (negative or positive) are electrically stabilized while colloids with low zeta potentials tend to coagulate or flocculate. Briefly, zeta potentials from 0 to ±30 mV indicate instability, while zeta potentials higher than ±30 mV indicate stability. The zeta potential of nano-scaled particles is influenced by many factors, such as the source of particles and the treatment with different surfactants, electrolyte concentration (ionic strength), particle morphology and size, pH of the solution and state of hydration. For instance, Preetz et al., evaluated the zeta potential of nanoemulsions: the information given by the zeta potential allows stating that the nanoemulsions with highly charged surfaces are stable and will resist to droplet aggregation[6, 7].

3. DIFFERENTIAL SCANNING CALORIMETRY:

DSC is a thermo-analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned. DSC can be used to detect phase transitions including the melting of crystalline regions, and to analyse the proportion of solid fat or the proportion of ice crystals

in emulsions. Thanasukarn et al. shows that fat crystallization affects the emulsion stability depending on the emulsifier used. They showed that the thermal decomposition follows the melting of the drug encapsulated have shown that DSC can be used to determine the crystallization temperature of a mixture of surfactants[2, 8].

4. FOURIER TRANSFORM INFRARED:

FTIR spectroscopy is based in an infrared radiation that passes through a sample where it is mostly absorbed by the sample and some of it is transmitted. The resulting spectrum represents themolecular absorption and transmission, creating a molecular fingerprint of the sample. Each sample fingerprint presents its characteristic absorption peaks that correspond to the frequencies of vibrations between the bonds of the atoms of the material. Because each different material is a unique combination of atoms, no two compounds produce the exact same infrared spectrum. Therefore, infrared spectroscopy can result in a positive identification of different materials. In addition, the size of the peaks in the spectrum is a direct indication of the amount of material present in the sample. The major advantages of FTIR are the fact that it can determine the amount of components in a mixture, it can determine the quality or consistency of a sample, the small time required for analyses (because all frequencies are measured simultaneously), and the fact that it is a very sensitive method, relatively simple to work with and internally calibrated. These advantages make measurements made by FTIR extremely accurate and reproducible. Araújo et al., report the encapsulation of thalidomide in nanoemulsions by spontaneous emulsification, where the crystallization process was studied through FTIR analysis of the crystals; when present in a nanoemulsion, these crystals were found to be in a different polymorphic form than that found before nanoemulsions preparation[2, 6].

5. NUCLEAR MAGNETIC RESONANCE:

NMR is a powerful and complex analytical tool that allows the study of compounds in either liquid or solid state and serves equally in quantitative as in structural analysis. It is very efficient in gathering structural information regarding molecular compounds. It can be used as a complementary technique to methods of optical spectroscopy and mass spectrometry leading to precise information concerning the structural formula, stereochemistry, information about the preferred conformation of molecules; it may also be used to identify the compound in study. The application of NMR to nanoemulsions characterization has been only slightly exploited. Jenning et al., successfully incorporated medium-chain triglycerides oil in a matrix of a solid long-chain glyceride (glyceryl behenate) and the structure of the liquid lipids inside the matrix of solid lipid nanoparticles was characterized through 1H NMR. Information about the mobility, the arrangement and the environment of the oil molecules was obtained[2, 9].

6. X-RAY DIFFRACTION:

XRD techniques are a family of non-destructive analytical techniques that reveals information about the crystallographic structure, chemical composition and physical properties of materials. XRD is based on observing the scattered intensity of an X-ray beam hitting a sample as a function of incident and scattered angles, polarization and wavelength or energy. XRD is mostly used for the identification of crystalline compounds by their diffraction pattern.

Nevertheless, it covers a lot of specific uses: identification of single phase materials, determination of the crystal structure, identification and structural analysis of samples, recognition of amorphous materials in partially crystalline mixtures, determination of crystallite size from analysis of peak broadening, determination of crystallite shape from the study of peak symmetry and the study of thermal expansion in crystal structures using in situ heating stage equipment. Mulik et al. showed that the diffraction pattern of curcumin is significantly different from the diffraction pattern of solid lipid nanoparticles loaded with curcumin. The diffraction pattern of loaded solid lipid nanoparticles indicates that curcumin is entrapped in the lipid core of the nanoparticles, while the diffraction pattern of the unloaded solid lipid nanoparticle indicates that the addition of curcumin has not changed the nature of the solid lipid nanoparticles[10].

7. SMALL-ANGLE X-RAY SCATTERING:

Small-angle X-ray scattering (SAXS) is a technique for the study of structural features of colloidal size particles where the elastic scattering of X-rays by a sample that has in homogeneities in the nanometric range, is recorded at very low angles (typically 0.1–10°). This angular range contains information about the shape and size of macromolecules, characteristic distances of partially ordered materials, pore sizes, etc. SAXS is capable of delivering structural information on macromolecules between 5 and 25 nm and on repeating distances in partially ordered systems of up to 150 nm. Through the scattering patterns, information on the structure, shape, and size of macromolecules can be achieved. This method

is non-destructive and requires a minimum sample preparation. One of the disadvantages of SAXS data analysis is that only a one-dimension scattering pattern can be obtained. Jenning et al. studied the influence of the oily constituent of their particles on the subcell parameters and long spacings of solid Compritol nanocyrstals through SAXS, which was used to confirm the polymorphism behavior found through DSC measurements. Also, through SAXS, Venturini et al. showed that sorbitan monostearate is interacting with the oily phase, in the core of the solid–lipid nanoparticle. Imaging Techniques Microscopy can be used as a direct imaging technique for nanoemulsions; nevertheless the type of microscopy used depends on the kind of matrix to be analyzed. This technique enables information regarding the size, shape, and aggregation state of the nanoemulsions. Some of the imaging methods that are used for the characterization of nanoemulsions systems are presented below[2, 9].

8. TRANSMISSION ELECTRON MICROSCOPY:

TEM is a technique capable of a resolution on the order of the 0.2 nm. It is widely used in the study of materials for science/metallurgy and biological sciences; in both cases the samples must be very thin and able to withstand the high vacuum present inside the instrument. Nevertheless, this technique has some drawbacks. Some materials require extensive sample preparation to produce a sample thin enough to be electron transparent, which makes TEM analysis a relatively time-consuming process with a low throughput of samples. The structure of the sample may be changed during the preparation process; the field of view is relatively small and the sample may be damaged by the electron beam. Bouchemal et al. studied the morphology and structure of the nanoemulsions using TEM; the combination of bright field imaging at increasing magnification and of diffraction modes were used to reveal the form and size of the emulsions and to determine the amorphous or crystalline character of the components. They direct observation enabled the possibility to perform selected area electron diffraction in order to check the crystallinity of the emulsion core components. Chu et al. observed the microstructure and the particle-size distribution in their nanoemulsions, concluding that β-carotene particles exhibited spherical morphology with a mean diameter of 20 nm, confirming the results obtained by DLS[2, 4].

9. SCANNING ELECTRON MICROSCOPY:

SEM is capable of producing high-resolution images of a sample surface. SEM images have a characteristic three-dimensional appearance and are useful for judging the surface structure. Generally, TEM resolution is about an order of magnitude higher than SEM resolution; however, because the SEM image relies on surface processes rather than transmission, it is able to image bulky samples and has a much greater depth of field and so can produce images that are a good representation of the 3D structure of the sample. In particular, the significantly large depth of field available in SEM allows a large amount of the sample to be in focus at one time. It is also capable to produce high-resolution images at higher magnification. The combination of higher magnification, larger depth of field, greater resolution, and ease of sample observation makes SEM one of the most heavily used methods in research areas today. Nevertheless, SEM is also an expensive technique and requires high vacuum and relatively high sample conductivity. The presence of surfactants during nanoemulsions preparation can sometimes inhibit their characterization via SEM due to the formation of a smooth camouflaging coating on the particle surfaces[2, 4].

10. ATOMIC FORCE MICROSCOPY:

AFM is a more recently developed microscopy technique. The high resolution (±0.1 nm) achieved by AFM has been used to directly view single atoms or molecules that have dimensions of a few nanometers. AFM relies on the raster scanning of a nanometer-sized sharp probe over a sample that has been immobilized onto a carefully selected surface (mica or glass), resulting in a high-resolution three dimensional profile of the surface under study. AFM has the advantage of imaging almost any type of surface, including polymers, ceramics, composites, glass, and biological samples. AFM allows biomolecules to be imaged not only under physiological conditions but also during biological processes. The advantage of directly observing biomolecular systems in their native environment opens the possibility of analyzing their structural and functional properties at the submolecular level. Surface irregularities observed by SEM are absent in AFM inspection. AFM's disadvantages are related with the analysis of surfaces which are soft, sticky, or have loose particles floating, as the tip is in direct contact with the actual surface and will run into difficulties in those cases. Nevertheless, AFM works on most materials and has been used in a range of applications from biology and chemistry, to electronics. AFM images are complementary to other established techniques. AFM can be used for the structural characterization of, e.g., proteins, polysaccharides, and liposomes. Preetz et al. showed the differences between nanoemulsions and nanocapsules by studying the shape, morphology and mechanical properties of the emulsion

and capsule shell through AFM, and was able to demonstrate that the shell around an oil droplet solidified with increasing amounts of polyelectrolytes[2, 4, 7].

APPLICATION:

1. APPLICATION OF NANOEMULSIONS IN COSMETICS:

Nanoemulsions 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, nanoemulsions are more suitable for the transport of lipophilic compounds than liposomes. Nanoemulsions are acceptable in cosmetics because there is no inherent creaming, sedimentation, flocculation, or coalescence that is observed with macro emulsions. The incorporation of potentially irritating surfactants can often be avoided by using high-energy equipment during manufacturing. Nanoemulsions 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[3, 11].

2. ANTIMICROBIAL NANOEMULSIONS:

Antimicrobial nanoemulsions are oil-inwater droplets that range from 200 to 600 nm. They are composed of oil and water and are stabilized by surfactants and alcohol. The nanoemulsionhas a broad-spectrum activity against bacteria (e.g. *E. coli,* Salmonella s, *S. aureus*), enveloped viruses (e.g. HIV, *Herpes simplex*), fungi (e.g. Candida, Dermatophytes), and spores (e.g. anthrax). The nanoemulsion particles are thermodynamically driven to fuse with lipidcontaining 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 nanoemulsion. As a result, the nanoemulsion can achieve a level of topical antimicrobial activity that has only been previously achieved by systemic antibiotics[12, 13].

3. AS A MUCOSAL VACCINE:

Nanoemulsions 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 nanoemulsion causes proteins applied to the mucosal surface to be adjuvant and it facilitates uptake by antigen-presenting cells. Additional research is ongoing to complete the proof of concept in animal trials for other vaccines including Hepatitis B and anthrax. Mice and guinea pigs intranasally immunized by the application of recombinant HIV gp120 antigen mixed in nanoemulsion 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-g induction, and prevalence of anti-gp120 IgG2 subclass antibodies indicated that nasal vaccination in nanoemulsion also induced systemic, Th1- polarized cellular immune responses. This study suggests that nanoemulsion should be evaluated as a mucosal adjuvant for multivalent HIV vaccines. 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 nanoemulsion adjuvant (HBsAgnanoemulsion) could be effective with fewer administrations. Comprehensive pre-clinical toxicology evaluation demonstrated that HBsAg- nanoemulsion 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. Longterm 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. A novel technique for vaccinating against a variety of infectious diseases-using an oilbased 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. Developing mucosal immunity may be very important for protection against HIV. In the study, the nanoemulsion 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[3, 14].

4. NANOEMULSION AS NON-TOXIC DISINFECTANT CLEANER:

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 mm 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[3, 15].

5. NANOEMULSIONS IN CELL CULTURE TECHNOLOGY:

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. The advantages of using nanoemulsions in cell culture technology are better uptake of oilsoluble supplements in cell cultures; improve growth and vitality of cultured cells, and allowance of toxicity studies of oil-soluble drugs in cell cultures[16].

6. NANOEMULSION IN CANCER THERAPY AND TARGETED DRUG DELIVERY:

The effects of the formulation and particle composition of gadolinium (Gd)-containing lipid nanoemulsion (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. 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 nanoemulsion formulation has enhanced the peroral bioavailability significantly to more than 70%. The developed nanoemulsion formulation was safe and effective for both peroral and dermal delivery of PCL. 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 nanoemulsions for camptothecin encapsulation to circumvent these delivery problems. The nanoemulsions were prepared using liquid perfluorocarbons and coconut oil as the cores of the inner phase. These nanoemulsions were stabilized by phospholipids and/or Pluronic F68 (PF68). The nanoemulsions 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 nanoemulsions with a lower oil concentration exhibited cytotoxicity against melanomas and ovarian cancer cells. Confocal laser scanning microscopy confirmed nanoemulsion 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. The scientists have investigated the nanoemulsion containing risperidone (RSP) to accomplish the delivery of drug to the brain via nose. Risperidone nanoemulsion (RNE) and mucoadhesive nanoemulsion (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) Tclabeled] 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 nanoemulsions indicated more effective and best brain targeting of RSP amongst the prepared nanoemulsions. 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. Another study reported the formulation of filter sterilizable emulsion formulation of paclitaxel using á-tocopherol as the oil phase and á-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. 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. Positively charged nanoemulsions systems are expected to interact with negatively charged cell surfaces more efficiently, and this aspect of the positively charged nanoemulsions has been explored for possibility of oligonucleotide delivery to cancer cells. 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 had 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. 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. The perfluorochemical nanoemulsions (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. 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. 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[3].

7. NANOEMULSION FORMULATIONS FOR IMPROVED ORAL DELIVERY OF POORLY SOLUBLE DRUGS:

Nanoemulsion formulations were developed to enhance oral bioavailability of hydrophobic drugs. Paclitaxel was selected as a model hydrophobic drug. The oil-inwater (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 zeta potential ranging from +34 mV to 245 mV. Following oral administration, a significantly higher concentration of paclitaxel was observed in the systemic circulation when administered in the nanoemulsion relative to control aqueous solution. The results of this study suggest that nanoemulsions are promising novel formulations that can enhance the oral bioavailability of hydrophobic drugs.

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 nanoemulsion s results in a significantly enhanced bioavailability. The application of CoQ10 has been further improved by the development of novel CoQ10 double nanoemulsions containing tocopherol and CoQ10 in individual nanodroplets. In addition, the CoQ10 concentration in these nanoemulsions could be increased by the development of a supersaturated CoQ10 nanoemulsion[3, 17].

CONCLUSION:

Nanoemulsions are submicron (range of 20-200 nm) sized emulsion that is under extensive investigation as drug carriers for improving the delivery of therapeutic agents. These are by far the most advanced nanoparticle systems for the systemic delivery of active pharmaceutical for controlled drug delivery and targeting. Nanoemulsions constitute one of the most promising systems to improve solubility, bioavailability, and functionality of hydrophobic compounds. Food industry seeks to use these systems for the incorporation.

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