Rajalakshmi R. et al. / Vol 1/ Issue 1/ 2011/ 1-8.

International Journal of
Innovative Drug Discovery
www.ijidd.com

A CRITICAL REVIEW ON NANO EMULSIONS

R. Rajalakshmi*, K. Mahesh, C.K. Ashok Kumar

ABSTRACT

Nanoemulsions are only kinetically stable. However, the long term physical stability of nanoemulsions (with no apparent flocculation or coalescence) makes then unique and they are sometimes referred to as 'Approaching thermodynamic stability. The inherently high colloid stability of nanoemulsions can be well understood from a consideration of their stearic stabilization (when using non-ionic surfactants and /or polymers) and how this is affected by the ratio of the adsorbed layer thickness to droplet radius. This present review focused on preparation, physical property, characterization and advantages of nanoemulsions. It concluded Nanoemulsions are proposed for numerous applications in pharmacy as drug delivery systems because of their capacity of solubilizing nonpolar active compounds. Thus the use of Nanoemulsions as formulations for active delivery and targeting is also an active and interesting application of nanoemulsion.

Keywords: Nanoemulsions, Stability, Physical property.

*Sree Vidyanikethan College of Pharmacy, Tirupati, Andhra Pradesh - 517102.

INTRODUCTION

Nanoemulsions are transparent or translucent systems mostly covering the size range 50-200nm [1]. Unlike micro emulsions (which are also transparent or translucent and thermodynamically stable). Nano emulsions are only kinetically stable. However, the long term physical stability of nanoemulsions (with no apparent flocculation or coalescence) makes then unique and they are sometimes referred to as 'Approaching thermodynamic stability. The inherently high colloid stability of nanoemulsions can be well understood from a consideration of their stearic stabilization (when using non-ionic surfactants and /or polymers) and how this is affected by the ratio of the adsorbed layer thickness to droplet radius. Unless adequately prepared (to control the droplet size distribution) and stabilized against Ostwald ripening (that occur when the oil has some finite solubility in the continuous medium), nanoemulsions may use their transparency with time as a result of increase in droplet size [2-5].

Advantages of nanoemulsions:

1. Nanoemulsions have a much higher surface area and free energy than micro emulsions.
2. That makes them an effective transport system.
3. Nano emulsions do not show the problems of inherent creaming, flocculation, coalescence and sedimentation, which are commonly associated with macro emulsion
4. Nanoemulsions can be formulated in variety of formulations such as foams, creams, liquids and sprays
5. Nano emulsions are non-toxic; non-irritant hence can be easily applied to skin and mucous membranes.
6. Since nano emulsions are formulated with surfactants, which are approved for human consumptions (GRAS), they can be taken by enteric route.
7. Nano emulsions do not damage healthy humans and animal cells hence are suitable for human and veterinary therapeutic purposes.

Corresponding Author**: - R. Rajalakshmi** Email:- rrajalakshmi2001@yahoo.com

The attraction of nano emulsions for application in pharmaceuticals is due the following advantages

(i) The very small droplet size causes a large reduction in the gravity force and the Brownian motion may be sufficient for overcoming gravity. This means that no creaming or sedimentation occurs on storage.

(ii) The small droplet size also prevents any flocculation of the droplets. weak flocculation is prevented and this enables the system to remain dispersed with no separation.

(iii) The small droplets also prevent their coalescence, since these droplets are non-deformable and hence surface fluctuations are prevented, in addition the significant surfactant film thickness(relative to droplet radius) prevents any thinning are disruption of the liquid film between the droplets.

(iv) Nanoemulsions are suitable for efficient delivery of active ingredients through the skin. The large surface area of the emulsion system allows rapid penetration of actives.

(v) Due to their small size, nano-emulsions can penetrate through the rough skin surface and this enhances penetration of activities.

(vi) The transparent nature of the system, their fluidity (at reasonable oil concentrations) as well as the absence of any thickeners may give them a pleasant aesthetic character and skin feel.

(vii) Unlike micro emulsions (which require a high surfactant concentration, usually in the region of 20% and higher), nano-emulsions can be prepared using reasonable surfactant concentration. For a 20% O/W nano-emulsion, a surfactant concentration in the region of 5-10% may be sufficient.

(viii) The small size of the droplets allows them to deposit uniformly on substrates. Wetting, spreading and penetration may be also enhanced as a result of the low surface tension of the whole system and the low interfacial tension of the O/W droplets.

(ix) Nano-emulsions can be applied for delivery of fragrant, which may be incorporated in many personal care products. This could also be applied in perfumes. Which are desirable to be formulated alcohol free.

(x) Nano-emulsions may be applied as a substitute for liposomes and vesicles (which are much less stable) and it is possible in some cases to build lamellar liquid crystalline phases around the nano-emulsion droplets [6].

Types of emulsions

Single emulsions

Emulsions are dispersed, multiphase systems consisting of at least two insoluble liquids. The dispersed phase is present in the form of droplets in a continuous phase. Depending on the emulsification process, the diameter of the droplets lies between 0.1m and 0.1mm. Emulsions of this kind are thermodynamically unstable, which means that there is a tendency to reduce the interface (as a result of a relatively high interfacial tension), causing the droplets to coalesce and therewith decreasing the total amount of interface.

Double Emulsions

A double emulsion is an emulsion in an emulsion. Two main types of double emulsions can be distinguished: water-in-oil-in-water (W/o/W) emulsions, in which a W/O emulsion is dispersed as droplets in an aqueous phase, and oil-in-water-in-oil (O/W/O) emulsions, in which an O/W emulsion is dispersed in oil phase. W/O/W emulsions are more common than O/W/O emulsions. Double emulsions contain more interface and are even more thermodynamically unstable than single emulsions.

Usually double emulsions are prepared in a two-step emulsification process using two surfactants, a hydrophobic one designed to stabilize the interface of the W/O internal emulsion and a hydrophilic one for the external interface of the oil globules (for W/O/W emulsions). The primary W/O emulsion is prepared under high-shear conditions to obtain small droplets while the secondary emulsification step is carried out with less shear to avoid rupture of the internal droplets [7].

Physical properties of nanoemulsions

Nanoemulsions have many interesting physical properties that are different from or are more extreme than those of larger micro scale emulsions.

- The relative transparency of nanoemulsions
- Their response to mechanical shear or ‘rheology’ and
- The enhanced shelf stability of nanoemusions against gravitationally driven creaming [8].

The relative transparency of nanoemulsions

Nanoemulsions appear visibly different from microscale emulsions since the droplets can be much smaller than optical wavelengths of the visible spectrum. Microscale emulsions multiply scatter visible light, and unless the refractive index of the continuous and dispersed phases is matched by specifically altering the composition to achieve this, they appear white. By contrast, nanoemulsions can appear nearly transparent in the visible spectrum and exhibit very little scattering despite significant refractive index contrast. Quantitative measurements of the optical

transparency of nanoemulsions in the visible and ultraviolet wavelengths are shown through transmission measurements.

Their response to mechanical shear or 'rheology'

Some mechanical shear or 'rheological' properties of nanoemulsions are also affected by the Nano scale size of the droplets. As with micro scale emulsions, the rheological properties depend strongly on whether the droplets interact repulsively or attractively. For very dilute, the shear viscosity of repulsive nanoemulsions resemble that of micro scale emulsions or even hard spheres. The rheology of attractive Nanoemulsions systems is only now being explored, so little information is available compared to that of microscopic emulsions [9]

The enhanced shelf stability of nanoemulsions against gravitationally driven creaming

Nanoemulsions exhibit enhanced shelf stability against gravitationally driven creaming over micro scale emulsions at the same. Brownian motion, caused by entropic driving forces, keeps the droplets suspended even over very long periods of time.

Stability

The small droplet size of Nano-emulsions confer stability against sedimentation (or creaming) because the Brownian motion and consequently the diffusion rate are higher than the sedimentation (or creaming) rate induced by the gravity force. Ostwald ripening or molecular diffusion, which arises from emulsion poly dispersity and the difference in solubility between small and large droplets, is the main mechanism for Nano-emulsion destabilization[9]. The Lifshitz-Slezov and Wagner theory predicts a linear relationship between the cube of the radius (r3), and time (t), with the slope being the Ostwald ripening rate. The (LSW) theory assumes that the droplets of the dispersed phase are spherical, the distance between them is higher than the droplet diameter and the kinetics are controlled by molecular diffusion of the dispersed phase in the continuous phase. According to this theory, the Ostwald ripening rate in O/W emulsions is directly proportional to the solubility of the oil in the aqueous phase. In fact, Taylor has suggested that Ostwald ripening might be used as a tool to the LSW theory. The discrepancy was attributed to factors not taken into account in this theory such as oil transport due to the presence of micelles and/or micro emulsion droplets in the aqueous phase increase in droplet Brownian motion and lowering of the interfacial Gibbs elasticity. Experimental Ostwald ripening rate values about three orders of magnitude higher than the calculated have been also reported in W/O Nano-emulsions stabilized with fluorinated surfactants. The reduction of the Ostwald ripening process by the adition to the system of a small amount of second oil with low solubility in the aqueous phase. It has been recently reported that, for an ethoxilated nonionic surfactant system, the Ostwald ripening rate can also be decreased by adding a second surfactant with the same alkyl chain length and higher degree of ethoxylation than the primary surfactant [10-12].

Preparation of Nano-emulsions

Three methods may be applied for the preparation of Nano-emulsions (covering the droplet radius size range 50-200nm). Use of high pressure homogenizers (aided by appropriate choice of surfactants and co surfactants), use of low energy emulsification method at constant temperature or application of the phase inversion temperature (PIT) concept.

1. Use of high pressure homogenizer

This technique makes use of high-pressure homogenizer/piston 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 production of nanoemulsions. This technique has great efficiency, the only dis advantage being high energy consumption and increase in temperature of emulsion during processing [13,14].

2. Low energy emulsification methods

A study of the phase behavior of watery oil surfactant systems demonstrated that emulsification can be achieved by three different low energy emulsification methods (a) stepwise addition of oil to a water surfactant mixture; (b) stepwise addition of water to a solution of a surfactant in oil; and (c) mixing all the components in the final composition, pre-equilibrating the samples prior to emulsification Nano-emulsions with droplet sizes of the order of 50nm were formed only when water was added to mixtures of surfactant and oil [15].

Phase inversion temperature (PIT) principle

Phase inversion in emulsion can be one of two types: Transitional inversion induced by changing factors which effect the HLB of the system Eg: Temperature and/or electrolyte concentration. Catastropic inversion which is induced by increasing the volume fraction of the disperse phase. Transitional inversion can also be induced by changing the HLB no. of the surfactant at constant temperature using surfactant mixtures.

When an O/W emulsion is prepared using a non-ionic surfactant of the electrolyte type is heated, then at a critical temperature (the PIT), the emulsion inverts to a W/O emulsion. At the PIT the droplet size reaches minimum and the interfacial tension also reaches a minimum. However, the small droplets are unstable and they coalesce very rapidly.

By rapid cooling of the emulsion that is prepared at a temperature near the PIT, very stable and small emulsion droplets could be produced [16].

CHARACTERIZATION OF A NANOEMULSION:

Droplet size analysis

Droplet size distribution of the Nano emulsion was determined by photon correlation spectroscopy (PCS), Light scattering was monitored at 25^0C at a scattering angle of 90^0

Viscosity determination

The viscosity of the nanoemulsion was determined using Brookfield DV ultra V6.0 cone and plate rheometer at 25 ± 0.3^0C.

Refractive index

Refractive index of nanoemulsions formulation was determined using an abbes type refractometer.

Transmission electron microscopy

The morphology and structure of the Nanoemulsion were studied using transmission electron microscopy (TEM). A combination of bright field imaging at increasing magnification and diffraction modes was used, to reveal the form and size of the Nanoemulsions. To perform the TEM observations, the nanoemulsion formulation was diluted with water (1/100). A drop of the diluted nanoemulsions was directly deposited on the holey film grid and observed after drying [17,18] .

Scanning electron microscopy (SEM)

Samples were fixed on SEM stub using conductive double sided tape and then made electrically conductive by coating in a vaccum with a thin layer of gold or palladium. An accelerating voltage of 15KV was used.

Stability studies

Stability studies on optimized Nanoemulsions were performed by keeping the sample at refrigerator temperature (4^0C) and room temperature (25^0C). These studies were performed for the specific period of time. The droplet size, viscosity and RI were determined using methods described above during storage. From different batches formulation were taken in glass vials were kept at accelerated temperature of 30^0C, 40^0C, 50^0C and 60^0C at ambient humidity. The samples were withdrawn at regular intervals of particular time and were analyzed for drug content by stability indicating HPLC method at a particular wave length. Zero time samples were used as controls. Analysis was carried out at each time interval by taking 50µl of each formulation and diluting in to 5ml with methanol and injecting in to HPLC system at 250nm. In addition, samples of pure oil pure surfactant and core surfactant were run separately to check there was no interference of the excipients used in the formulations. The amount of drug degraded and the amount remaining at each time interval was calculated. Order of degradation was determined by the graphical method. Degradation rate constant (K) was determined at each temperature. Arrhenius plot was constructed between log K and 1/T to determine the shelf life of optimized Nanoemulsions formulation. The degradation rate constant at 25^0C (K25) was determined by extrapolating the value of 25^0C from arrhenius plot. The shelf life ($T_{0.9}$) for each formulation was determined by using the formulae [18].

$$T_{0.9} = \frac{0.1052}{K_{25}}$$

APPLICATIONS OF NANOEMULSIONS

1. Use of Nanoemulsion in Cosmetics

Nanoemulsions have recently become increasingly important as potential vehicles for the controlled delivery of cosmetics and for the optimized dispersion of active ingredient in particular skin layers.

Due to their lipophilic interior, Nanoemulsions are more suitable for the transport of lipophilic compounds than liposomes. Similar to liposomes, they support the skin penetration of the active ingredient and thus increase their concentration in the skin. Another advantage of the small-sized droplet with its high surface area allowing effective transport of the active to the skin. Furthermore, Nanoemulsions 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.

Nanoemulsions are acceptable in cosmetics because there is no inherent creaming, sedimentation, flocculation or coalescence observed with in macro emulsions. The incorporation of potentially irritating surfactants can often be avoided by using high energy equipment during manufacturing.

2. Antimicrobial Nanoemulsions

Antimicrobial nanoemulsions are oil-in-water droplets that range from 200-600 nm. They are composed of oil and water and are stabilized by surfactants and alcohol. The Nanoemulsion has a broad spectrum activity against bacteria (eg E.coli, Salmonella, S.aureus), enveloped viruses (eg HIV, Herpes simplex), fungi (eg Candida, Dermatophytes) and spores (eg anthrax). The Nanoemulsion 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 change of the pathogen. When enough nano particles fuse with the pathogens, the released part of the energy trapped within the emulsion. Both the active ingredient and the energy released destabilize the pathogen lipoid membrane, resulting in cell lysis and death [19].

In the case of spores, additional germination enhancers are incorporated in to the emulsion. Once initiation of germination takes place, the germinating spores Become susceptible to the antimicrobial action of the nanoemulsion.

A unique aspect of the nanoemulsion is their selective toxicity to microbes at concentrations that are non-irritating to skin or mucous membrane. The safety margin of the Nanoemulsions 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 Nanoemulsions can achieve a level of topical antimicrobial activity that has only been previously achieved by systemic antibiotics. The nanoemulsion technology can be formulated into a cream, foam, liquid or spray to decontaminate a variety of materials. Marketed as NANOSTAT (Nano bio Corp).

3. Nanoemulsions as Mucosal Vaccines

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 the adjuvant, 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 and 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 emulsion. Research has also demonstrated that animals exposed to recombinant gp120 in nanoemulsion on their mucosa develop significant responses to HIV, thus providing a basis to examine the use of this material as an HIN vaccine. Additional research is ongoing to complete the proof of concept in animal's trials for other vaccines including Hepatitis B and Anthrax. The University of Michigan has exclusively licensed third technology to Nanobio.

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. kills tuberculosis and a wide spectrum of viruses, bacteria and fungi in five to ten minutes 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 Nanospores of oil droplets <=106m which are suspended in water to create a nanoemulsion requiring only miniscule amounts of the active ingredient, PCMX (parachlorometaxylenol). The Nanospheres carry surface charges that efficiently penetrate the surface charges on microorganism's membranes – much like breaking through an electric fence.

Rather than 'drowning' cells, the formulation allows PCMX to target and penetrate cell walls. As s result, PCMX is effective at concentration levels one-to-two 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 wall, which cause them to disintegrate, fundamentally 'drowning' them in the disinfectant solution.

The disinfectant is nonflammable and therefore safe to store most anywhere and also to use in unstable conditions. It is nonoxidizing, nonacidic & nonionic. It does not corrode plastic, metals or acrylic, making the product ideal for use on equipment and instruments. It is environmentally safe hence the costs and health risks associated with hazardous chemicals disposal are eliminated.

The formulation is a broad-spectrum disinfectant cleaners 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. Chemicals disposal costs can be eliminated and cleanings costs can be reduced. Marketed as EcoTru (Envirosystems, Inc)

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 stream. 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.
Nanoemulsions are new method for the delivery of oil-soluble substances to mammalian cell cultures. The delivery system is based on a nanoemulsion, which is stabilized by phospholipids. The nanoemulsions are transparent and can be passed through 0.1-1m filters for sterilization. Nanoemulsion 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 nanoemulsions in cell culture technology are

- Better uptake of oil-soluble supplements in cell cultures.
- Improve growth and vitality of cultured cells.
- Allows toxicity studies of oil-soluble drugs in cell cultures.

6. Nanoemulsions as drug delivery systems:

Nanoemulsions are used drug delivery systems for administration through various systemic routes. Parenteral (or injectable) administration of Nano-emulsions is employed for a variety of purposes, namely, nutrition (e.g. administration of fats, carbohydrates, vitamins, etc), controlled drug release and targeting of drugs to specific sites in the body, delivery of vaccines or as gene carriers. Nano-emulsions are advantageous for intravenous administration, due to the strict requirements of this route of administration, particularly the necessity for a formulation droplet size lower than 1µm. The benefit of Nano-emulsions in the oral administration of drugs has been reported and the absorption of the emulsion in the gastrointestinal tract has been correlated to their droplet size. Nano-emulsion is also used as ocular delivery systems to sustain the pharmacological effect of drugs in comparison with their respective solutions. Cationic Nano-emulsions were evaluated as DNA vaccine carriers to be administered by the pulmonary route. They are also interesting candidates for the delivery of drugs or DNA plasmids through the skin after topical administration [19,20].

Fig.1 : Types of emulsions and preparation of single and double emulsion.

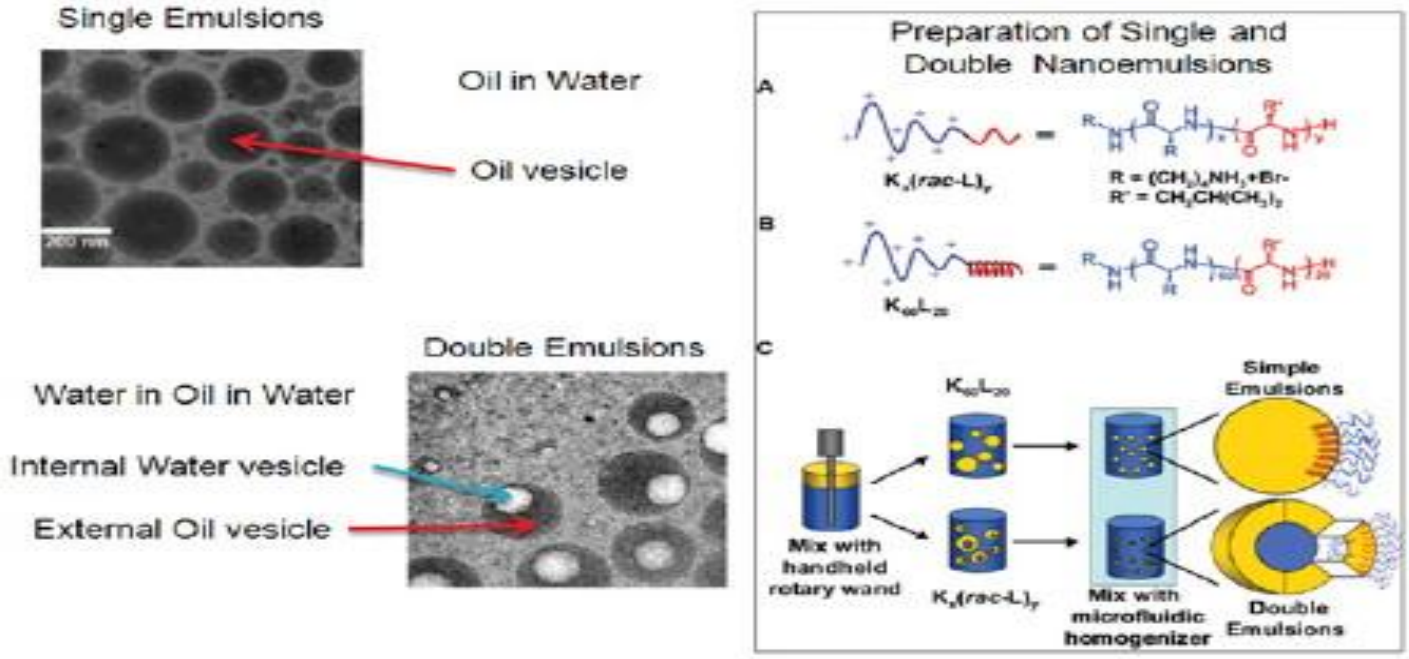
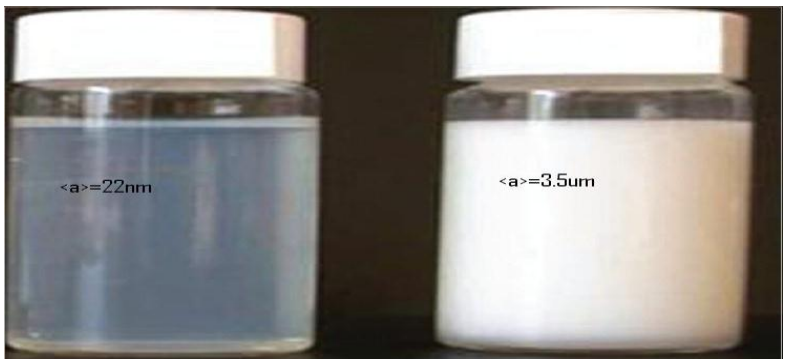


Fig.2: Difference between Micro scale emulsion and Nanoemulsion



Nanoemulsion **Microscale Emulsion**

Fig 3: The production small droplets (submicron) requires application of high energy.

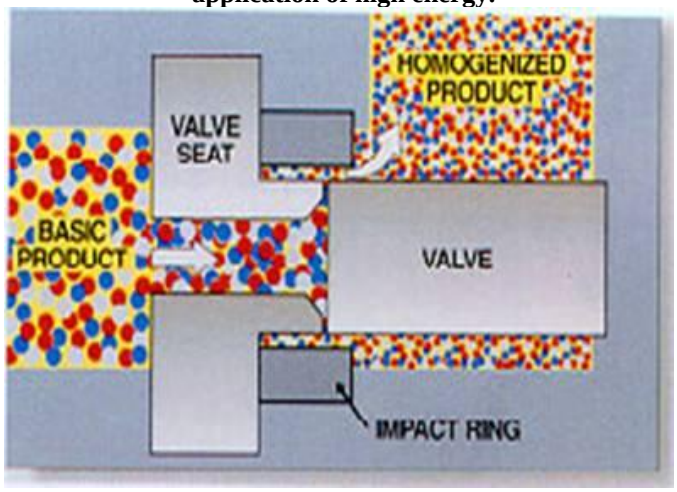


Fig:4 Low energy emulsification methods

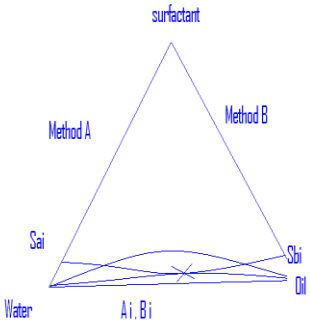


Fig.5: SEM Photograph of Nanoemulsion

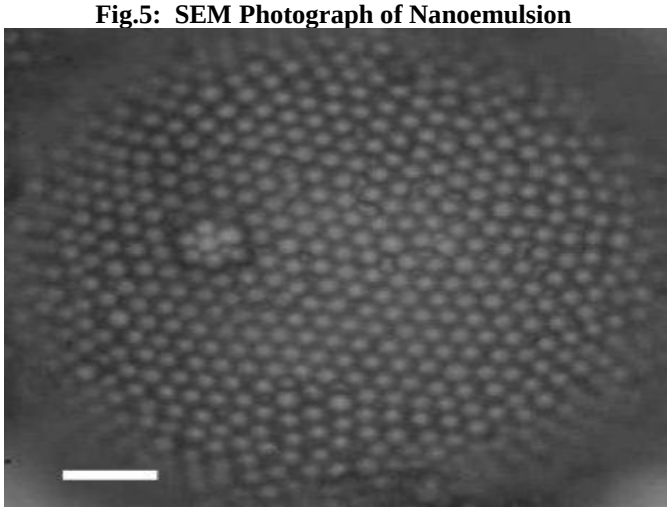


Fig: 6 Nanoemulsions against microorganism

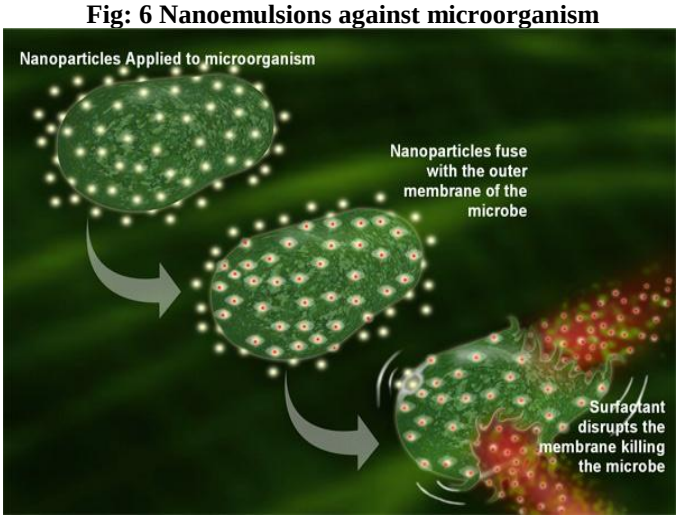
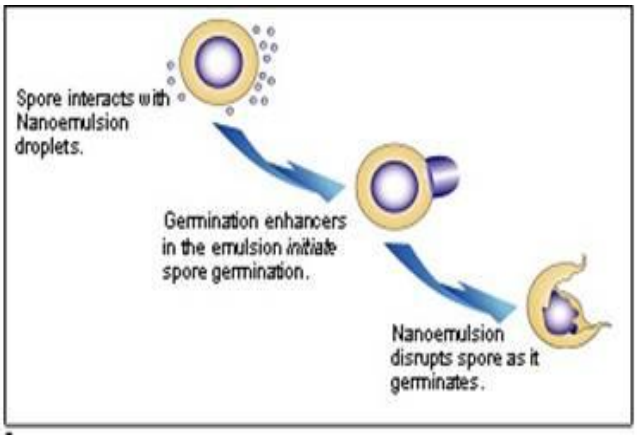
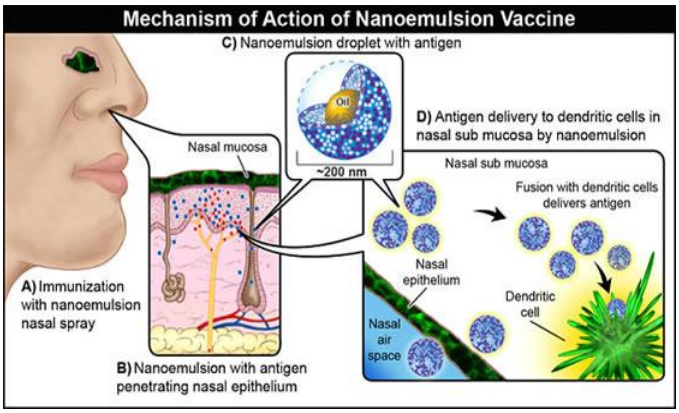


Fig.7: Antimicrobial action by spore germination





CONCLUSION

The study of basic and applied aspects of Nanoemulsions is receiving increasing attention in recent years. Dispersion of high energy emulsification methods are traditionally used for Nanoemulsions formation. Nanoemulsions are proposed for numerous applications in pharmacy as drug delivery systems because of their capacity of solubilizing nonpolar active compounds. Thus the use of Nanoemulsions as formulations for active delivery and targeting is also an active and interesting application of nanoemulsion.

REFERENCES

1. Hee Jung Yang, Wan Goo Cho, Soon AM Park. Stability of oil-in-water nanoemulsion prepared using the phase inversion composition method. *Journal of industrial and engineering chemistry*, 15, 2009, 331-335
2. Nakajima H, Tomomossa S, Okabe M. First Emulsion Conference, Paris,1993.
3. El-Aassar M. Ausa JM. (Ed), polymeric Dispersions, Kluwer Academic Publications, the Netherlands, 1997.
4. Tharwat Tadros, Izquierdo, Esquena Solans; Formation and stabilt of Nano-emulsions. *Advances in Colloid and Interface Science*, 108, 2004, 303-318.
5. Van Der Graff F, CGPH Schro En, RM Boom. Preparation of double emulsions by membrane emulsification-a review. *Journal of Membrane Science*, 251, 2005, 7-15
6. Mason TG, Wilking JN, Melesn K, Chang CB and Graves SM. Nanoemulsions: formation, structure, and physical properties. *J. Phys.: condens. Matter*, 18, 2006, R635-R666.
7. Tadros THF, Izquierdo P, Esquena J, Solans C. Formation and stability of nano-emulsions. *Adv. Colloid Interface Sci.*, 108, 2004, 303-318.
8. Lifshitz IM, Slyozov VV. The kinetics of precipitation from supersaturated solid solutions. *J. Phys. Chem. Solids*, 1961, 19, 35-50.
9. Wagner C. Theorie der Alterung von Niederschlagen dursh Umlosen (Ostwald-Reifung), *Z Elektrochem*, 65, 1961, 581-591.
10. Taylor P, Ostwald. Ripening in emulsions: estimation of solution thermodynamics of the disperse phase. *Adv.Colloid Interface Sci*. 106, 2003, 261-285.
11. Radhakrishna T, Satyanarayana J, Satyanarayana A. LC Determinationof rosiglitazone in bulk and pharmaceutical formulation. *J Pharm Biomed Anal*, 29, 2002, 873-80.
12. Sheikh Shafiq-Un-Nabi, Faiyaz Shakeel et al. Formulation development and optimization using nanoemulsion technique, a technical note. *APPS Pharm sci tech*, 8(2), 2007, E1-E6.
13. Shakeell F, Baboota S, Ahuja A, Ali J, Faisal MS and Shafiq S. Stability evaluation of celecoxib nanoemulsion containing Tween 80. *Thai J.Pharm*. Sci, 32, 2008, 4-9.
14. Eddington ND, Rekhi GS, Lesko LJ, Augsburger LL. Scale-up effects on dissolution and bioavailability of propranolol hydrochloride and metoprolol tartrate tablet formulations. *AAPS Pharm SciTech*., 1(2), 2000, 14-16.
15. Baboota S, Shakeel F, Ahuja A, Ali J, Ahmed S and Shafiq S. Devolopment and validation of a stability-indicating HPLC method for analysis of celecoxib in bulk drug and microemulsion formulation. *Acta Chroat*., 18, 2007, 116-129.
16. Gascon AR, Cuadrado A, Solinis MA, Hernandez RM, Ramirez E, Dalmau R, Pedraz JL. Comparative bioavailability of two immediate release tablets of lisinopril/hydrochlorothiazide in healthy volunteers. *Int J Clin Pharmacol Ther.*, 41(7), 2003, 309- 15.
17. Aulton E. Micheal. Modified release per oral dosage forms, Pharmaceutics – The Science of Dosage form Design , Churchill LivingSton New York, pp. 575.
18. Banker S. Gilbert, Rhodes T. Christopher. Mordern Pharmeceutics, Marcel Dekker, Inc., New York, pp. 575
19. Bourne DWA and Dittert LW. Chapter 3 in Modern Pharmaceutics 3rd ed., Banker GS and Rhodes.CT. ed. Dekker. New York, 1996.
20. Buignies V, Leclerc B and Evesque. Quantitative measurements of localized density variations in cylindrical tablets using X-ray icrotomography. *Eur J Pharm Biopharm*., 64(1), 2006, 38-50.