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Delivery of lipophilic bioactive compounds derived from food through nanoemulsions

Tapan Kumar Giri [1, *]
[1] NSHM College of Pharmaceutical Technology, NSHM Knowledge Campus, Kolkata Group of Institutions, 124 BL Saha Road, Kolkata-700053, West Bengal, India.
*corresponding author e-mail address: ***tapan_ju01@rediffmail.com***

ABSTRACT
In the food industry, numerous food-derived bioactive compounds exhibit considerable health benefits when consumed in relatively high concentrations. Unfortunately, most of these compounds demonstrate poor solubility and bioavailability in aqueous-based foods. Recently the development of nanoemulsions loaded with lipophilic food components has demonstrated the potential as a carrier to deliver lipophilic food actives. This nanoformulation strategy extensively improves the cell uptake and bioavailability of various hydrophobic food components by increasing their solubility and dissolution rate, maintaining concentration within the therapeutic range by controlling the food components release rate and reducing systemic side effects by targeting to specific site, thus offering a better patient compliance. Hence, it may be used as a new alternative and cheaper carrier in therapy for increased bioavailability, reduction in dose and thereby dose related systemic toxicities. This review provides an outline of the materials used (functional ingredients, emulsifiers, and solvents), manufacturing techniques and analytical methods for the characterization and identification of nanoemulsions prepared from food. Finally, delivery approach of different food constituents also demonstrated that is suitable for utilization within the food industry.

Keywords: *nanotechnology, bioavailability, nanoemulsion, antioxidants, antimicrobials, anticancer agents.*

1. INTRODUCTION

Nanotechnology is a promising technology that holds prospective in the food industry [1, 2, 3]. Nanotechnology involves technological research development with 1-100 nm size ranges [4]. Scientific, technological, and commercial advantages for the industry can be obtained through the technological development of nanosize materials [2]. Several macroscale characteristics of food, such as taste, texture, processability, coloring strength, and stability may change through nanotechnology leading to a number of innovative products.

Nanoemulsions are nonequilibrium, heterogeneous systems consisting of two immiscible liquids in which one liquid is dispersed in another liquid as droplets with diameters of tens to a few hundred nanometers. Owing to their small droplet size nanoemulsions possess stability against sedimentation or creaming with Ostwald ripening forming the main mechanism of nanoemulsion breakdown. Nanoemulsion droplet sizes drop normally in the range of 20-200 nm. Diameter and surface properties of droplets of nanoemulsion plays a significant role in the biological activities of the formulation. Nanoemulsions have a number of possible advantages over conventional emulsions for particular applications within food and beverage products.

Nanoemulsions have been augment the bioavailability of encapsulated hydrophobic bioactive compounds [5], which may be helpful for escalating the bioactivity of some nutraceuticals. The o/w nanoemulsion is an effective approach for the poorly water soluble food constituents. Depending on the ingredients, composition, and preparation methods of formulation the bioactive compounds localized in the different position within o/w nanoemulsion. The bioactive molecule can be present in the interior oil phase or can distribute into the external stabilizer film (Figure 1). Localization of the bioactive molecule influences the release, bioavailability, and stability of the encapsulated ingredient. The movement of the bioactive compounds of o/w nanoemulsion from the interior oil phase to the external surfactant layer due to high pressure homogenization. The entrapment of the bioactive compounds in the external emulsifier layer is probable to decrease the opportunity of controlled release mechanisms. As more and more apprehension is drawn to the application of nanotechnology in food systems, biopolymers have been the most investigated materials used for food nanoemulsions.

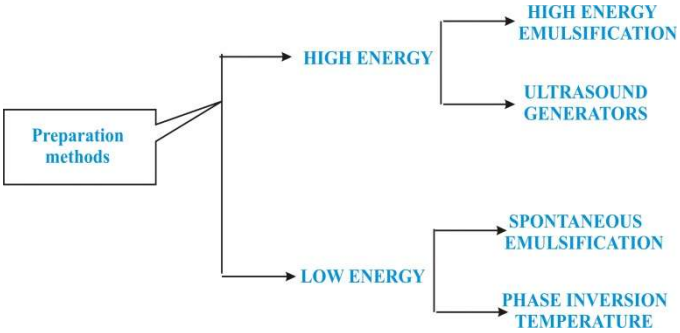

Figure 1. Localizations of bioactive molecules into o/w nanoemulsions.

Since biopolymers are derived from living organisms, particularly from edible plants and animals, they are biocompatible, biodegradable, and non-toxic by oral consumption [6-11]. This article provides an outline of the present condition of nanoemulsion formulation, manufacture, properties, biological fate, applications, and toxicity, with importance on edible systems that are applicable for application within the food industry.

1.1. Advantages nanoemulsion.

1. Nanoemulsions give particularly low interfacial tension and tremendously large surface area that could considerably progress the food absorption in GI tract.

2. Lipophilic neutraceutical antineoplastic agents have been encapsulated in nanoemulsions to augment cytotoxicity and to conquer multi drug resistance.

3. Owing to their higher ratio of surface area to volume, with the intestine providing high concentration gradient and enhanced pharmacokinetics and biodistribution of therapeutic agents and thus reduce toxicity by their better buildup at the target site.

4. The small droplet size of nanoemulsion prevents flocculation, coalescence, and gravity-induced sedimentation or creaming, phase separation and surface fluctuation during storage.

5. Nanoemulsions have been reported to make the plasma concentration profiles and bioavailability of drugs more reproducible.

6. Fine oil droplets empty quickly from the stomach and promote extensive distribution of the drug during the intestinal tract and thus minimizing irritation normally encountered with extensive contact of the drug and gut wall.

7. The structures in the nanoemulsions are much smaller than the noticeable wavelength, so most nanoemulsions become visible optically transparent, even at large loading.

1.2. Instability of nanoemulsion.

Various types of chemical and physical process may destabilize the nanoemulsion. Physical stability problems for example flocculation, creaming, phase inversion, and coalescence results in a change of the spatial allotment or structural association of the molecules, while chemical instability, for example oxidation and hydrolysis, results in the change of the type of molecules present. Instability during nanoemulsion creation occurs if there is inadequate surfactant to coat the whole oil-water interface formed by the homogenizer. The surfactant has a significant role to avoid instantaneous coalescence and surfactant properties determine the quality of the nanoemulsion. Creaming will occurred when larger droplets suspended in low viscosity aqueous medium. Rate of creaming depends on droplet diameter and required perfect homogenization. Destabilization of emulsion does not occurred through creaming, but the creamed layer containing oil droplets with high concentration accelerates contacts that lead to flocculation, coalescence or aggregation. Inability of the surfactant to prevent close approach of the droplets results aggregation and flocculation of the emulsion oil droplets. Flocculation is weaker than aggregation and flocculation is easily reversed by stirring or shaking. The mechanisms of emulsions physical instability was highlighting that flocculation and creaming can frequently be inverted by mild agitation, whereas coalescence is an irretrievable phenomenon (Figure 2).

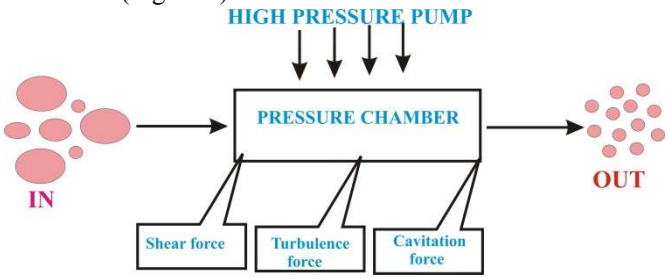

Figure 2. Mechanism of physical instability in nanoemulsions.

Physical and chemical stability of o/w emulsions was studied that contain citrus oils flavor component limonene [12]. Limonene is generally added to o/w emulsion due to its lipid soluble nature. On the other hand, limonene loaded o/w emulsions are inclined to both oxidative degradation and physical instability resulting creation of off-flavors due to loss of aroma. Limonene obtained from whey protein stabilizes the o/w emulsion more effectively than limonene obtained from gum Arabic. Emulsion droplet with cationic interface was formed by limonene obtained from whey protein and diminishes the oxidative degradation of emulsion. Influence of surfactant type, surfactant concentration, temperature, and homogenization pressure on the quality and stability of β-carotene loaded o/w nanoemulsions was studied [13]. The stability of nanoemulsions diminished with an increase of temperature but increased with increasing homogenization pressure.

2. MATERIALS USED IN NANOEMULSION PREPARATION

2.1. Oil Phase.

Various types of hydrophobic components include free fatty acids, triacylglycerols, diacylgycerols, monoacylglycerols, flavor oils, mineral oils, essential oils, waxes, fat substitutes, oil soluble vitamins, weighting agents, and a variety of lipophilic nutraceuticals are used as oil phase to prepare nanoemulsion. Different types of triacylglycerol oils such as soybean, corn, safflower, sunflower, flaxseed, olive, or fish oils are commonly used for the preparation of nanoemulsions due to their low cost and nutritional attributes. Most of these oils principally consist of long-chain triacylglycerols, though medium-chain triacylglycerols and short-chain triacylglycerols are being used in some food applications. The creation of nanoemulsions using medium-chain triacylglycerols and long-chain triacylglycerols oils is repeatedly difficult owing to their comparatively low polarity, high viscosity, and high interfacial tension. Preparation of nanoemulsion using these oils by phase inversion temperature technique is difficult due to exceedingly high hydrophobic nature of these oils [14]. The properties of the oil phase in a nanoemulsion formulation have direct influence on 1) toxicity and biocompatibility, 2) loading ability and stability of active ingredient in the system, 3) effectiveness of nanoemulsion creation, 4) release control of the active ingredient from the matrix, 5) stability of the end product.

2.2. Aqueous Phase.

Mostly water is the aqueous phase used for the preparation of nanoemulsion, but aqueous phase may contain other polar ingredients such as alcohols, proteins, carbohydrates, minerals, bases, and acids. The nature and concentration of polar ingredients influences the interfacial tension, polarity, refractive index, density, rheology, phase behavior, ionic strength, and pH of the aqueous phase and which ultimately affecting the physicochemical properties and stability of the nanoemulsion.

2.3. Stabilizers.

If aqueous and oil phase are combinedely homogenized the system will quickly breakdown by various mechanisms include coalescence, flocculation, gravitational separation, and Ostwald ripening. Therefore, stabilizers are added to nanoemulsions to improve the stability. The choice of suitable emulsifiers is the most vital factors for the appropriate design of a nanoemulsion. Emulsifier is a surface active molecule adsorbed by the droplet surface and prevents the aggregation of droplets [15, 16]. Emulsifier facilitates droplet breakdown in high energy homogenizer through reduction of interfacial tension and produced small droplets. Emulsifier facilitates creation of small droplets in low energy homogenizer due to its ability to produce very low interfacial tensions under certain environmental and solution conditions.

The concentration of surfactant used to manufacture a nanoemulsion affects not only the amount of oil that can be included into the formulation, but also the droplet size of nanoemulsion. In general, an augment in surfactant concentration will lead to a superior concentration of oil to be solubilized in the system and/or a reduce in droplet size. The creation and stability of nanoemulsions can often be enhanced by using combinations of emulsifiers, rather than using a single emulsifier. For instance, employing a lipophilic and hydrophilic surfactant in combination can aid the creation of small particles in both high energy homogenization and low energy homogenization. On the other hand, nanoemulsion stability can be improved by combined emulsifier systems. Cosolvents and cosurfactants are required for a few techniques of forming nanoemulsions by low-energy methods [17, 18]. The function of a cosurfactant in nanoemulsion formation is 1) to fluidize the interface, provide flexibility of the interface between oil and aqueous phase and introducing suitable interfacial curvature, 2) to improve the capability of surfactant to solubilize active ingredient, 3) to augment the hydrophilic surfactant solubility in the oil phase, 4) to optimize the viscosity ratio of disperse-to-continuous phase.

2.4. Others.

There are numerous other ingredients that can be included into a nanoemulsion formulation. Texture modifiers can be added to the formulation to thicken or gel the aqueous phase. Weighting agents are often included into the oil phase to match their density to that of the adjoining aqueous phase. Ripening retarders are used to stabilize oils which are prone to Ostwald ripening.

3. PREPARATION OF NANOEMULSION

Nanoemulsions can be made-up using various approaches; however, according to the basic principle these can usually be broadly categorized as either high-energy or low-energy method (Figure 3).

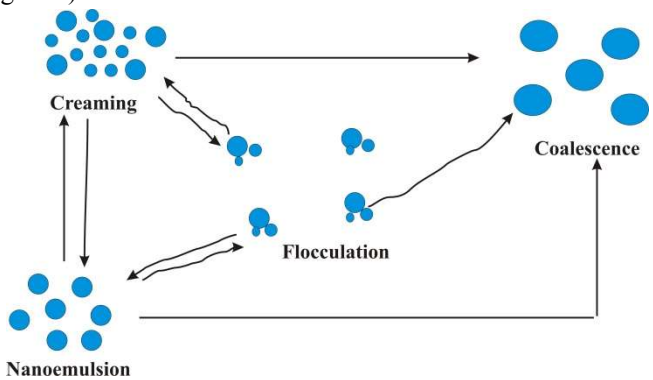

Figure 3. Various methods of preparation of nanoemulsion.

High-energy methods are generating strong disruptive forces that are able to disrupting and intermingling the oil and aqueous phases into tiny oil droplets. The high energy methods include microfluidizers, high pressure valve homogenizers, and sonication techniques are used for the preparation of nanoemulsion.

3.1. High-energy approaches.

High-energy approaches are one of the most flexible ways of producing food-grade nanoemulsions since they can be used with a broad range of diverse oil and emulsifier types. Nanoemulsion prepared by high energy methods using flavor oils, triacylglycerol oils, and essentials oils as the oil phase and polysaccharides, proteins, and phospholipids as emulsifiers the homogenization conditions should be optimized.

3.2. High pressure valve homogenizer.

High pressure valve homogenizers are most likely the general method of preparing small droplet sizes emulsions in the food industry [19, 20]. High pressure valve homogenizer efficiently reducing the droplet size of pre existing coarse emulsions than emulsions produced from two separate liquids. Coarse emulsion produced by high shear mixture is fed into the high pressure valve homogenizer. The homogenizer has a pump that pulls the coarse emulsion into a chamber and then forcefully passes the emulsion through a narrow valve to the other chamber. During the movement of the coarse emulsion through narrow valve the larger droplets are breakdown into small one by strong disruptive forces. To increase the effective breakdown of emulsion droplets various nozzle designs are available in high pressure valve homogenizer. Emulsion droplet size produced by this method decreases by increasing the homogenization pressure.

Very recently, researcher are investigated the creation of β-carotene nanoemulsions by using high pressure homogenization [13, 21, 22]. In the studies, modified starches, why protein isolates, Tween 20 and blend of Tween 20 and why protein isolates were tested to stabilize nanoemulsions. Medium chain triglyceride was used as the solvent to dissolve β-carotene. The outcome showed that nanoemulsions stabilized with Tween 20 had the smallest droplet size, whereas nanoemulsions stabilized with a blend of why protein isolates and Tween 20 were most stable. Moreover, response surface methodology was employed to optimize the creation of nanoemulsions. 10% w/w medium chain triglyceride was used as a carrier and Tween 20 as surfactant. The optimum preparation conditions were suggested to be: pressure 129 MPa, homogenization temperature 47 °C, load of β-carotene 0.82%, and Tween 20 8.2%.

3.2.1. Microfluidizers.

A microfluidizer is forces premixing emulsion by using high pressure through a fine orifice to disrupt the emulsion droplet. Generally, microfluidizers are used to prepare emulsion based products in the pharmaceutical industry. However, microfluidizers are also used in the beverage and food industries for the preparation of nutraceutical emulsions, flavor emulsions,

and homogenized milk. In microfluidizer emulsion flowing through a channel divided into two streams that are flowing through separate narrow channel and finally the two streams are directing into the interaction chamber. When two streams intrude upon each other strong forces are produced resulting highly proficient droplet disruption.

Chen and Wagner reported vitamin E nanoemulsions stabilized by modified starch prepared using a Microfludizer [23]. Vitamin E was homogenized and then the prepared nanoemulsions were spray dried to yield a powder. The outcome showed that higher pressure and a greater number of passes formed smaller droplets. Particle size did not extensively augment upon rehydration in water. Furthermore, during a 6-month storage study of the fortified juice, no ringing or creaming occurred, and the turbidity of juice was highly stable.

3.2.2. Ultrasonic homogenizers.

Ultrasonic homogenizers use high-intensity ultrasonic waves to make the strong disruptive forces required to crumble up water and oil phases to smaller sizes droplets. The requirement of energy is supplied by sonicator probes that include piezoelectric quartz crystals that enlarge and contract in response to an alternating electrical voltage. The tip of the sonicator probe is positioned in the liquids being homogenized and it produced strong vibrations resulting cavitation effects. The cavitation effects produced strong disruptive forces near the sonicator probe resulting droplet disruption. For proficient and consistent homogenization it is vital to make certain that the emulsion spends enough time within the region where droplet disruption occurs. In recent times, batch and flow-through ultrasonic homogenizers have been developed that have unique cells intended to optimize the effectiveness of droplet disruption [24].

3.2.3. Low-energy approaches.

Low energy approaches are often more efficient at producing smaller sizes droplet in comparison to the high energy approaches. Polysaccharides and proteins as emulsifiers are not used in the majority of the low energy approaches used to form nanoemulsions. As an alternative, it is often essential to use comparatively high concentrations of synthetic surfactants to form nanoemulsions via these approaches, which may limit their use for many food applications.

3.2.4. Spontaneous emulsification.

In this method an emulsion or nanoemulsion is instinctively produced when two liquids are mixed together. Systems prepared using this method are typically referred to as either self emulsifying drug-delivery systems or self-nano-emulsifying drug delivery systems in the pharmaceutical industry. Droplet size of the emulsion can be reduced by changing the compositions of water phase and oil phase, pH, temperature, ionic strength, and stirring speed.

3.2.5. Phase-inversion methods.

Numerous techniques have been used to prepare nanoemulsions through inversion of emulsions from a o/w to w/o or w/o to o/w e.g., phase-inversion temperature, phase-inversion composition, and emulsion-inversion point methods. The phase inversion temperature method is based on altering in the relative solubility or molecular geometry of non-ionic surfactants by means of varying temperature [25, 26, 27]. This kind of phase inversion usually involves the constrained change of an emulsion from o/w to w/o or w/o to o/w through a biocontinuous microemulsion or transitional liquid crystalline phase.

The phase-inversion composition technique is to some extent analogous to the phase inversion temperature technique, however the best curvature of the surfactant is altered by altering the formulation strategy [28]. Phase inversion of ionic surfactant stabilized emulsion from o/w to w/o be take place by the addition of salt. On the other hand, a w/o emulsion containing a high salt concentration can be changed into an o/w emulsion by diluting it in water so as to decrease the ionic strength below some critical level. Differently to prepare nanoemulsions using the phase-inversion composition method is by altering the pH to change the electrical charge and stability of emulsions.

In the emulsion inversion point methods the alteration from o/w to w/o or w/o to o/w is in the course of a catastrophic phase inversion, more willingly than a transitional phase inversion as with the phase-inversion composition or phase-inversion temperature methods [28, 29]. A transitional-phase inversion occurs when the surfactant properties are changed by adjusting a formulation variable, such as the pH, temperature, or ionic strength. On the other hand, when the oil/water ratio is changed a catastrophic phase inversion will occur whereas the surfactant properties remain constant. Basically, catastrophic-phase inversion is typically induced by either escalating (or declining) the volume fraction of the dispersed phase in an emulsion above (or below) some critical level.

4. PHYSICOCHEMICAL PROPRIETERS OF NANOEMULSIONS

4.1. Optical properties.

For the design of food products the overall look is very important that is impacted by droplet characteristics [30, 31]. A number of food products should be clear or slightly opaque (soft drinks, clear fortified waters, juices, jams, jellies, deserts, sauces, and dressings) and adding up of any delivery system should not produce opaque or cloudy. Remaining food products are cloudy (mayonnaise, opaque or turbid dressings, sauces, yogurts, dips, and creams) and oil droplets scattered the light in a delivery system may make a significant contribution to their general appearance.

Generally, the particle concentration, refractive index, and particle size distribution influenced the optical properties of nanoemulsions. The turbidity of nanoemulsions was increased by increasing particle concentration and refractive index contrast. The turbidity value was optimum when light scattering is strongest at particular particle concentration. The color strength is inversely related to the extent of light scattering. The color strength of an emulsion may reduce when the droplet concentration is increased [32, 33, 34].

4.2. Rheological properties.

Lipid droplets contained in foods may show diverse rheological characteristics such as viscoelastic liquids, viscous liquids, viscoelastic solids, elastic or plastics solids) depending on their structure and composition. Nanoemulsions showed different

rheological properties than conventional emulsions with same amount of oil owing to the comparatively tiny size of the droplets. For the design of food products the rheology of the food products is very important that is impacted by the droplet characteristics. Some food products should have a relatively low viscosity and the droplets present should not noticeably augment the overall viscosity. Other products should be extremely viscous or gel-like and the viscosity of the nanoemulsion is influenced by droplets. It may be feasible to prepare viscous nanoemulsions containing high fat content with similar rheological properties like conventional emulsions.

4.3.Stability.

Various physicochemical parameters such as coalescence, flocculation, gravitational separation, Ostwald ripening, and chemical degradation influenced the breakdown of nanoemulsion over time like conventional emulsions. Gravitational separation such as sedimentation or creaming is the instability in conventional emulsions. This type of emulsion instability is occurred through the alteration of relative densities of the continuous and dispersed. The upward movement of droplets is known as creaming and that is occurred when the droplets have a lower density in comparison to the adjoining liquid. The downward movement of droplets is known as sedimentation and that is occurred when the droplets have a higher density in comparison to the adjoining liquid. Liquid oils like flavor oils or triacylglycerol have lower densities in comparison to the water and creaming is more favorable o/w emulsions. However, sedimentation is more widespread in w/o emulsions. On the other hand, if an o/w emulsion contains crystalline lipids then they might be inclined to sedimentation since the density of lipids typically increases when they crystallize.

Nanoemulsions normally have much improved stability to particle aggregation in comparison to the conventional emulsions due to their small particle size [35]. The colloidal interactions in an nanoemulsion depends on the radius of the particle, physicochemical properties of the phases (refractive index and dielectric constant), properties of the fluid (ionic strength, pH, temperature, and osmotic pressure), and shell characteristics (charge, thickness, packing, hydrophobicity, and rheology). The size of emulsion droplets increases over time in Ostwald ripening due to movement of oil molecules from small to large droplets [36, 37]. The dynamic force for the movement of oil molecule is the verity that the water-solubility of oil restricted within a spherical droplet increases because the droplet size diminishes resulting elevated concentration of oil molecules solubilized in the aqueous phase neighboring a small droplet than adjacent a larger one. Due to this concentration gradient the oil molecules solubilized in aqueous phase is moved from adjacent the smaller droplets to nearer the larger droplets.

The tiny size of the droplets in o/w nanoemulsions impacted the degradation of encapsulated components due to hydrolysis or oxidation. More of the oil phase will be exposed to the adjacent aqueous phase due to augment in surface area since size of the droplet decreases. The size reduction of the oil droplets increases the reaction rate because chemical degradation occurs mainly at the oil water boundary. Small droplets containing transparent nanoemulsion may degrade quickly in comparison to the opaque conventional emulsion in presence of visible or UV light since they accelerate the degradation rate.

5. CHARACTERIZATION OF NANOEMULSION

5.1. Chromatography.

Ion exchange chromatography and size-exclusion chromatography are suitable for the partition of nanoemulsions from the food matrix. Big molecules elute quickly than small molecules in size-exclusion chromatography. Small charged molecules quickly in comparison to the high charged molecules in ion exchange chromatography [3, 38]. High performance liquid chromatography has been used for the quantification of a variety of compounds such as vitamins, carbohydrates, mycotoxins, additives, amino acids, triglycerides, proteins, lipids, pigments, and chiral compounds in food analysis. High performance liquid chromatography is a simple, reproducible, and robust technique. Susceptible discriminating detectors are accessible in high performance liquid chromatography, and detectors selection depends on characteristics of the compound [3].

5.2. Dynamic light scattering.

Dynamic light scattering is also known as quasi-elastic light scattering or photon correlation spectroscopy. Dynamic light scattering is used for the determination of droplet size and size distribution in nanoemulsion. Stokes–Einstein equation is used to relate the Brownian motion and size of the droplets that is measured by dynamic light scattering technique. The size of the droplets was calculated by dynamic light scattering through the illumination of the droplets by means of a laser and measurement of strength fluctuations in the scattered light.

5.3. Zeta potential.

Stability of colloidal dispersions is measured through zeta potential value. Zeta potential represented the extent of repulsion between similarly charged droplets in dispersion. High zeta potential with smaller sizes particles will oppose aggregation and confer stability. The dispersion will flocculate and break when attraction exceeds repulsion and zeta potential is low. Nanoemulsions are stabilized with high zeta potential (positive or negative) but nanoemulsions are flocculate or coagulate with low zeta potentials.

5.4. Differential scanning calorimetry.

Differential scanning calorimetry measured the difference in the heat necessary to augment the temperature of a test and reference as a function of temperature. Melting of crystalline region, amount of solid fat, and amount of ice crystals in nanoemulsions are detected by the use of differential scanning calorimetry [39]. Depending on the emulsifier used fat crystallization affects the stability of nanoemulsion [39].

5.5. Fourier transform infrared.

Fourier transform infrared study was subjected to guarantee the compatibility amongst its ingredients. Araújo et. al., 2011 studied the crystallization process of thalidomide encapsulated in nanoemulsions by Fourier transform infrared analysis. Crystals were found in a different polymorphic form in nanoemulsion than that found prior to nanoemulsions preparation [40].

5.6. Nuclear magnetic resonance.

Nuclear magnetic resonance spectroscopy, usually known as NMR spectroscopy, is a research method that exploits the magnetic properties of definite atomic nuclei. It determines the physical and chemical properties of atoms or the molecules in which they are enclosed. It relies on the occurrence of nuclear magnetic resonance and can give exhaustive information about the structure, dynamics, reaction state, and chemical environment of molecules. Jenning et. al., 2000b effectively included triglycerides oil in solid lipid nanoparticles prepared from namoemulsion. The structure of the liquid lipids in the solid lipid nanoparticles was characterized through nuclear magnetic resonance spectroscopy. Location and mobility of the oil molecules in the nanoemulsion was determined by this method. Casadei et. al., 2006 prepared ibuprofen loaded solid lipid particles from nanoemulsion and characterized through NMR analysis [41].

5.7. X-Ray diffraction.

X-ray diffraction is a fast analytical method mainly used for phase identification of a crystalline material and can give information on unit cell dimensions. Mulik et. al., 2010 studied the x-ray diffraction of curcumin and solid lipid nanoparticles containing curcumin prepared from nanoemulsion. They found the significant difference in diffraction pattern of curcumin nanoparticles [42]. The diffraction pattern also showed that curcumin is entrapped in the lipid core of the nanoparticles,.

5.8. Transmission electron microscopy.

Transmission electron microscopy is a microscopy method in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is produced from the interaction of the electrons transmitted through the specimen; the image is exaggerated and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a camera. Bouchemal et. al., 2004 examined the surface morphology and organization of the nanoemulsions by Transmission electron microscopy. The form and size of the nanoemulsion was determined by using the combination of diffraction modes and bright field imaging at rising magnification. Crystaline or amorphous character of the components in nanoemulsion was also determined.

5.9. Scanning electron microscopy.

A scanning electron microscope is a kind of electron microscope that produces images of a sample by scanning it with a focused beam of electrons. The electrons interrelate with atoms in the sample, producing different signals that can be detected and that contain information about the sample's surface topography and composition. Scanning electron microscopy differentiated the nanodispersed and coarse samples containing β-carotene [43]. The investigation of coarse emulsion containing β-carotene showed irregular sizes and shapes, as opposite to the nanodispersions.

6. BIOAVAILABILITY IMPROVEMENT OF FOOD CONSTITUENTS THROUGH NANOEMULSION

The health promoting benefits of food bioactive components (e.g.; catechins, phytosterols, curcumin, lycopene, ω-3 fatty acids, and carotenes) have paying more attention in recent years since their biological and pharmacological effects including antioxidation, anticancer, and chronic disease prevention properties have been established in frequent studies. On the other hand, one of the main challenges of these actives is their poor solubility and low bioavailability. There are a variety of formulation approaches that can be used to resolve the problems related with the low solubility and low bioavailability of poorly aqueous soluble drugs [44, 45]. Some of the approaches to increase solubility comprise solubilisation using co-solvents, oily solutions, micronization, surfactant dispersions, use of permeation enhancers, and salt formation and precipitation techniques [46, 47, 48]. Most of these methods for solubility enhancement have advantages as well as some limits and therefore have limited usefulness in solubility enhancement. Other methods used for solubility enhancement comprise emulsions, solid dispersion, microparticles, microemulsions, and inclusion complexes show sensible achievement but they lack in general applicability to all drugs [50-56]. These techniques are not applicable to the drugs, which are not soluble in both aqueous and organic Media.

Nanoemulsions were reported to progress the solubility, stability and bioactivity of various oil-soluble phytochemicals owing to their small droplet size and high kinetic stability. Analytical data [2, 57] recommended epigallocatechin gallate encapsulated in nanoemulsions resulted in less oxidation than that of epigallocatechin gallate in aqueous solution. It was reported that nanoemulsions could progress stability and oral bioavailability of epigallocatechin gallate and curcumin in a mouse model. The bioactivity was further improved when the droplet size of nanoemulsions were further diminished to below 100 nm [56]. Latest research also has shown that nanoemulsions loaded with coenzyme Q10 considerably improved the bioavailability [57]. There is no question that nanoemulsions as delivery carrier for lipophilic food components will maintain to be one of the frontiers of food nanotechnology due to possible commercial applications and health benefits.

Curcumin despite being an effectual chemotherapeutic agent in opposition to diverse type of cancer, suffer from the problem of low systemic bioavailability owing to low aqueous solubility, widespread intestinal metabolism and first-pass metabolism when administered using the oral route. Kumar et.al., evaluated the prospective of nano globules based nanoemulsion formulation for the solubility improvement of curcumin [58]. The nano globules based formulation was developed using Labrafac Lipophile WL 1349, Unitop FFT 40, PEG 400 and distilled water as an oil, surfactant, co-surfactant and aqueous phase correspondingly using aqueous titration method. The optimized formulation had small average globule diameter of 58 nm with zeta potential of -32 mv which indicated long-term dispersion stability. The release of curcumin from nanoemulsion was 96.21% and 98.1% in 6 h and 12 h respectively while curcumin suspension was release up to 28.2% at the end of 12 h. This indicated the improvement of solubility of curcumin in aqueous solution which

is the rate limiting step in the absorption of curcumin in the intestine.

Breviscapine nanoemulsion was formulated and evaluated in vitro and in vivo [59]. The globule size and polydispersity index of the nanoemulsion was 45.6 nm and 0.105, and the entrapment efficiency was 95.2%. The Caco-2 cell transport experiments showed that the breviscapine nanoemulsion facilitated the enhancement of the noticeable permeability coefficient from the apical side to basilar side compared with the free drug. In vivo, the relative bioavailability of breviscapine nanoemulsion reached to 249.7%. All the studies implicated that the nanoemulsion carrier contributed to the augmentation of the oral absorption of breviscapine owing to the better stability and permeation in the gastrointestinal tract.

The problem of poor bioavailability and clinical efficacy of curcumin can be arranged following converting crystalline curcumin into amorphous nanocurcumin. Amorphous nanocurcumin was prepared by converting into nanoemulsion using water titration method. The nanocurcumin was converted to gel using Cabopol 934. The steady state flux, permeability coefficient and enhancement ratio of nanocurcumin gel was determined and compared with crystalline curcumin gel [60]. Anti-inflammatory effects of the formulations were evaluated in carrageenan-induced paw edema method in rats using diclofenac as a reference. These ant-inflammatory effects of nanocurcumin was highly significant ($p<0.001$) compared to crystalline curcumin and significantly ($p<0.05$) comparable with standard diclofenac. The histology of the formulation treated skin showed insignificant changes in the integrity except in the group treated with nanocurcumin. The small disruption in the integrity of skin may be because of surfactant present in the nano formulations. Short term storage stability showed insignificant changes in the droplet size and zeta potential, proving its high shelf-life.

Quercetin is a dietary flavonoid with prospective chemoprotective effects, but has low bioavailability because of poor aqueous solubility and low intestinal absorption. A quercetin-containing self-nanoemulsifying drug delivery system was developed to form oil-in-water nanoemulsions in situ for improving quercetin oral bioavailability [61]. Upon mixing with water, quercetin-containing self-nanoemulsifying drug delivery system formed a nanoemulsion. The optimized quercetin-containing self-nanoemulsifying drug delivery system considerably enhanced quercetin transport across a human colon carcinoma (Caco-2) cell monolayer. Subsequent oral administration of quercetin-containing self-nanoemulsifying drug delivery system in rats, the area under the concentration curve and maximum concentration of plasma quercetin after 24 h augmented by about double and threefold compared with the quercetin control suspension.

Organogel-based curcumin nanoemulsion was prepared and showed enhanced bioavailability by oral route [62]. Curcumin organogel is the oil phase in nanoemulsion formulation. To get maximum bioavailability Tween 20 was used as the emulsifier for the preparation of nanoemulsion containing curcumin. Digestion of nanoemulsion was noticeably quicker than the organogel that is shown by in vitro lipolysis study. The key absorption mechanism of curcumin from nanoemulsion is the digestion and diffusion that is shown by permeation study on Caco-2 cell. The oral bioavailability of nanoemulsion containing curcumin was increased 9 times compared to pure curcumin that is confirmed by *in vivo* pharmacokinetics study on mice.

7. DELIVERY OF FOOD CONSTITUENTS

The bioavailability of hydrophobic food components is increased by the reduction of droplet size in nanoemulsion [64, 65]. Small droplets digested rapidly by digestive enzyme due to large surface area and absorption of components are takes place more easily. Small droplets increased the small intestine residence time that facilitates the absorption. The aqueous solubility of hydrophobic components enhanced as the droplet size decreases, which may improve absorption. Right now there is no excellent perceptive of the relative significance of these diverse mechanisms for nanoemulsions with opposed droplet sizes, compositions, and surface characteristics.

In recent times, it has been shown that the oral availability of curcumin can be augmented by incorporating it within nanoemulsions [2, 57]. Nanoemulsions have also been shown to progress the bioavailability of a variety of lipophilic nutraceuticals and pharmaceuticals. It has also been projected that nanoemulsions can be used to efficiently deliver nutraceuticals to precise sites within the human body, which may lead to improvements of their effectiveness. Numerous studies propose that nanoemulsions are the proficient way for incorporation and delivery of antimicrobial compounds. Nanoemulsions containing antimicrobial agent have been formulated for the sanitization of food packaging material and for other application [66]. Nanoemulsions were prepared using non-ionic surfactants such as tributyl phosphate and soybean oil. The developed nanoemulsions was effective against numerous pathogens include bacteria, spores, fungi, and viruses [67].

7.1. Delivery of antimicrobials.

The use of natural essential oils as antimicrobials is becoming more and more popular owing to the consumers demand of food free from synthetic additives. Essential oils contain a complex mixture of non-volatile and volatile compounds created by aromatic plants as secondary metabolites. Particularly, the antimicrobial action of essential oils has been recognized to their phenolic compounds and their interaction with microbial cell membranes, causing the leakage of cytoplasmatic constituents and, therefore, the loss of cell viability [68]. Essen-tial oils included in nanoemulsions seem to enter quicker in the microbial membranes owing to the enlarged area per weight unit [69, 70, 71]. This would permit reducing the concentration to achieve an comparable or even greater bacterial effect over conventional emulsions. On the other hand, processing method to get essential oil nanoemulsions determine their antimicrobial activity. Salvia-Trujillo et. al., reported that ultrasound processing diminished the antimicrobial prospective of lemon grass oil–alginate nanoemulsions against Escherichia coli in contrast with microfluidized nanoemulsions [72].

Nonionic surfactant stabilized o/w nanoemulsions containing thyme oil as oil components were prepared for the delivery of antimicrobial compounds [73]. The nanoemulsions

were exceedingly unstable to phase separation and droplet growth. This was attributed to Ostwald ripening owing to the higher solubility of thyme oil in water. Incorporation of more than 75% of corn oil in nanoemulsion droplets repressed Ostwald ripening. Anionic surfactant or cationic surfactants were used after homogenization in nanoemulsion to change the electrical characteristics of the droplets. Saccharomyces cerevisiae, Zygosaccharomyces bailli, Brettanomyces naardenensis, and Brettanomyces bruxellensis strains were used in the antifungal activity study of nanoemulsions containing negative, positive, or neutral thymol droplets. Similarly, sodium dodecyl sulfate, lauric arginate, and Tween 80 were also tested for antifungal properties in the nonexistence of thymol oils. Strong antifungal activity was reported by both ionic surfactants in the absence thyme oil. This effect was attributed to partitioning of the surfactants between the microbial surface and oil droplet.

Donsi et al., studied the effect of the nanoemulsion delivery systems on the antimicrobial activity of dissimilar essential oil components [73]. Carvacrol, limonene and cinnamaldehyde were encapsulated in the sunflower oil droplets of nanoemulsions prepared by high pressure homogenization. The calculated antimicrobial activity was considerably affected by the formulation of the nanoemulsion, where the dissimilar bioactive compounds were encapsulated. Particularly, the result of the delivery systems on the antimicrobial activity was connected to the concentration of the essential oil components in the aqueous phase in equilibrium with the nanoemulsion droplets, signifying that the capability of the active molecules to interrelate with cell membranes is linked to their dissolution in the aqueous phase.

Ultrasound cavitation technique was used to prepare o/w nanoemulsion by using sesame oil and Tween 20 or Tween 80 [74]. To obtain higher stability and reduced droplet size the surfactant concentration, emulsification time, and surfactant type was optimized in nanoemulsion. Reduction of droplet size was more efficient by using Tween80 as surfactant in comparison to the Tween20 as surfactant. Nanoemulsion containing eugenol was prepared by means of 13 nm droplet size with more than one month stability. Nanoemulsion containing eugenol with sesame oil showed superior stability and lesser droplet diameter in comparison to the simply nanoemulsion containing eugenol. Nanoemulsion containing eugenol showed antibacterial activity in opposition to Staphylococcus aureus. Staphylococcus aureus showed concentration and time dependent killing of bacteria upon treatment with nanoemulsion.

Zhang et. al., studied the effects of nisin on the antimicrobial activity of d-limonene and its nanoemulsion and developed a novel antimicrobial delivery system by combining the positive effect of these two antibacterial agents at the same time [75]. By the checkerboard method, both the synergistic and additive property of d-limonene and nisin were found against four selected food-related microorganisms. Then, d-limonene nanoemulsion with or without nisin was prepared by catastrophic phase inversion method, which has shown good droplet size and stability. The positive effects and exceptional antimicrobial activity of d-limonene nanoemulsion with nisin were established by MICs comparison, scanning electron microscopy and determination of cell constituents released.

7.2. Delivery of essential vitamins.

Vitamin E is used in cosmetic preparation as anti-oxidant. Oral administration of Vitamin E prevents peroxidation of lipid membrane since it is lipid soluble. Application of Vitamin E to the skin neutralizes free radicals, act as a humectants, and decrease sunburn cells after UV exposure. Nanoemulsion containing palm oil esters was prepared for cosmetic applications [76]. Numerous methods were used to test the stability of the resulting formulation. Both Pluronic F-68 and vitamin E were institute the emulsification and stability of the formulations. The formulation containing 10% vitamin E, 10% Palm Oil Esters, 2.4% Pluronic F-68, 24% Tween 80, and 53.6% deionised water is the best. These compositions produced the emulsion with small particle size, low Ostwald ripening and stable at diverse storing temperatures for four weeks.

A novel natural vitamin E-loaded nanoemulsion was designed to progress the oral bioavailability in rats [77]. The optimal formulation of the natural vitamin E nanoemulsion was effectively developed. The nanoemulsion is effortlessly dispersed in water, forming nanosized emulsion with a diameter of 20–400 nm. Pharmacokinetic studies in rats discovered considerable increases of oral bioavailability and antioxidative efficacy compared with the soft capsule.

β-Carotene was included into oil-in-water nanoemulsions stabilized by either a globular protein (β-lactoglobulin) or a non-ionic surfactant (Tween 20) [78]. Nanoemulsions were then stored at neutral pH and their physical and chemical stability were monitored under accelerated stress storage conditions (55°C). The rate of β-carotene degradation decreased upon addition of water-soluble (EDTA and ascorbic acid) or oil-soluble (vitamin E acetate or Coenzyme Q10) antioxidants. EDTA was more effective than ascorbic acid, and Coenzyme Q10 was more effective than vitamin E acetate. The utilisation of water-soluble and oil-soluble antioxidants in combination (EDTA and vitamin E acetate) was less effective than using them individually. Emulsions stabilized by β-lactoglobulin were more stable to color fading than those stabilized by Tween 20.

7.3. Delivery of antioxidants.

Tocopherol is stronger antioxidant than tocopherol acetate but owing to its viscous form, poor water solubility, instability to light and skin irritation issues it is not used in the current marketed formulations. To conquer the drawbacks, tocopherol was formulated as nanostructured lipid carriers and nanoemulsion. Nanostructured lipid carriers and nanoemulsion were prepared by homogenization technique [79]. In vitro release study showed that 30% of tocopherol was released from nanostructured lipid carriers in the first 2 h of the study as compared to only 4% from nanoemulsion. Permeation study from human skin showed that 762.3 ng/mL of tocopherol was delivered into the epidermis when formulated as nanostructured lipid carriers as compared to 182.3 ng/mL from nanoemulsion. It was seen that both formulations were able to retain the antioxidant activity. Skin irritation testing showed that nanostructured lipid carriers were non-irritant to the skin. Nanostructured lipid carriers and nanoemulsion were also able to protect tocopherol from UV degradation.

Palm kernel oil esters based nanoemulsions were loaded with P. urinaria extract using a spontaneous method and characterized with respect to particle size, zeta potential and

rheological properties [80]. The release profile of the extract was evaluated using in vitro Franz diffusion cells from an artificial membrane and the antioxidant activity of the extract released was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl method. The P. urinaria extract was effectively integrated into a palm kernel oil esters based nanoemulsion delivery system. In vitro release of the extract from the formulations showed 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity. These formulations can neutralize reactive oxygen species and counter oxidative injury induced by ultraviolet radiation and thereby improve skin aging.

Inhibiting or boosting the endogenous levels of antioxidants is one of the ways for the treatment of oxidative stress associated diseases. Nanoemulsion containing quercetin is used as one of the approaching antioxidants for the reduction of oxidative stress. Nanoemulsion containing quercetin was prepared by solvent evaporation method using hyaluronic acid, poly (lactic-co-glycolic acid), Tween-20 [81]. The efficacy of the nanoemulsion was evaluated in the presence or absence of chemical permeation enhancer. Interaction between polymer and quercetin was studied by Fourier transform infrared spectroscopy and showed excellent compatibility. In vitro release and ex-vivo permeation study was conducted to evaluate the transdermal delivery ability. The release of drug from the formulations was found to be diffusion controlled and zero order kinetics. In vitro toxicity was conducted by the electrical cell-substrate impedance sensing method in L929 cells treated with nanoemulsion containing quercetin. The resultant nanoemulsion showed less toxicity, high entrapment efficiency, controlled delivery and effective scavenging of free radicals.

Amri et. al., developed and characterized a resveratrol self-emulsifying drug delivery system, and to compare the uptake of resveratrol by bovine aortic endothelial cells, and the protection of these cells against hydrogen peroxide-mediated cell death, versus a control resveratrol ethanolic solution [82]. Pre-incubation of bovine aortic endothelial cells for 180 min with 25 μM resveratrol in the nanoemulsion obtained from the chosen self-emulsifying drug delivery system considerably augmented the membrane and intracellular concentrations of resveratrol. Resveratrol nanoemulsion appreciably enhanced the endothelial cell defense from H_2O_2-induced injury in comparison with incubation with the control resveratrol ethanolic solution. Formulation of resveratrol as a self-emulsifying drug delivery system drastically enhanced its cellular uptake and potentiated its antioxidant properties on bovine aortic endothelial cells.

7.4. Delivery of enzymes and protein.

The efficiency of a cationic nanoemulsion containing antisense oligonucleotide was studied by mouse retinopathy and rat corneal neovascularization models [83]. The maximum considerable corneal neovascularization reticence effectiveness was observed in the groups administered with nanoemulsion containing antisense oligonucleotide (subconjunctivally and topically). On the other hand, phosphate buffer saline containing antisense oligonucleotide induced a 34% reticence of retinal neovascularization in comparison to the aqueous vehicle containing antisense oligonucleotide injected into the eyes. However, nanoemulsion containing antisense oligonucleotide treated groups induced a 64% reticence of retinal neovascularization.

Beta-Lactamase was used as a model protein, and formulated into the oil phase of a self-nanoemulsifying drug delivery system through solid dispersion technique [84]. Oral delivery of beta-Lactamase in self-nanoemulsifying drug delivery system nanoemulsion resulted in the relative bioavailability of 6.34. Delivery of beta-Lactamase in the aqueous phase of the nanoemulsion resulted in a PK profile similar to that by the free solution. Beta-Lactamase when loaded in oil phase of self-nanoemulsifying drug delivery system, can drastically improve the oral bioavailability of beta-Lactamase. The effect of oil digestibility and droplet size on the bioavailability of a Coenzyme Q10 and heptadecanoic acid was investigated [85]. Small droplets were digested rapidly in comparison to the large droplets that are shown by simulated small intestinal model. Rapid digestion of small droplets was due to the enhanced exposing surface area of lipid to intestinal juices.

7.5. Delivery of fatty acid.

Thiocolchicoside is an effectual therapeutic agent in opposition to the orthopaedic, traumatic and rheumatologic disorders except it suffers from the weakness of poor bioavailability owing to wide first pass metabolism and low permeability through the oral route. Kumar et al., evaluated the prospective of nanoemulsion for bioavailability improvement of thiocolchicoside through the transdermal route [86]. The in vitro skin permeation study was conducted to compare the efficacy of optimized nanoemulsion with aqueous solution of thiocolchicoside. Considerable augment in permeability parameters were observed in nanoemulsion formulation as compared to aqueous solution of thiocolchicoside. The outcome of improved permeation through transdermal route propose that water-in-oil nanoemulsions which are compatible with the lipophilic sebum environment of the hair follicle facilitate the transport of thiocolchicoside, and such transport might be predominantly transfollicular in nature.

Chemically modified DALDA was delivered to the CNS o/w nanoemulsion containing polyunsaturated fatty acid [87]. Nanoemulsion formulation efficiently incorporated the modified peptide analgesic and established efficacy in the pain model in rodents. Preliminary study shows that the nanoemulsion was well tolerated and did not produce any side effects or toxicity.

An impulsive emulsification method was used to manufacture nanoemulsions from polyunsaturated (ω-3) oils [88]. The influence of surfactant-to-oil ratio, oil composition, and cosolvent composition (glycerol, ethanol, propylene glycol, and water) on the formation and stability of the systems was determined. Optically transparent nanoemulsions could be formed by controlling surfactant-to-oil ratio, oil composition, and aqueous phase composition. The spontaneous emulsification method therefore has substantial prospective for fabricating nanoemulsion-based delivery systems for incorporating polyunsatured oils into clear food.

The nanoemulsion loaded with lycobetaine-oleic acid complex and PEGylated lycobetaine-oleic acid-nanoemulsion were prepared by a simple high-pressure homogenization method [89]. A high entrapment efficiency of around 97.32% was obtained for PEGylated lycobetaine-oleic acid-nanoemulsion under optimized conditions. PEGylated lycobetaine-oleic acid-nanoemulsion showed considerably lower lycobetaine

concentration in the heart, liver, and kidney, whilst achieving superior concentration of lycobetaine in the lung when compared to free lycobetaine at the same time. The PEGylated lycobetaine-oleic acid-nanoemulsion exhibited superior growth inhibitory effect and longer survival time than free lycobetaine in both heterotopic and lung metastatic tumor models.

7.6. Delivery of anticancer agents.

The formation of Curcumin-loaded nanoemulsions has been achieved using an ultrasonic bath [90]. Colloidal stability of the ensuing formulations has been measured in terms of particle size distribution and polydispersity index. The mean droplet diameters and PDI of the nanoformulations were about 120 nm and 0.35 respectively. MTS assay test shows encouraging inhibition activity levels on MCF-7 human breast adenocarcinoma cell lines after administration of 20 µl of Curcumin nanoformulation. Around 68% inhibition has been recorded in the cultured cell line after an incubation period of 3 days with the drug formulation.

Tocotrienol rich fraction of vitamin E was shown to have anticancer action in opposition to murine tumor cells in vitro. Tocotrienol rich fraction was also shown to potentiate the anticancer action of statins. Alayoubi et al., prepared and characterized stable parenteral lipid nanoemulsions as a novel platform for the simultaneous delivery of tocotrienol rich fraction and simvastatin for following use in combination chemotherapy, and to evaluated the antiproliferative activity of the nanoemulsions against MCF-7 and MDA-MB-231 human mammary tumor cells [91]. Approximately 20% of simvastatin was released in sink conditions after 24h. The stability of the nanoemulsions was monitored over 6 months of storage. No oxidation or degradation products were detected and no loss in simvastatin loading was observed during this period. The antiproliferative activity of the nanoemulsions was also retained after storage. The IC50 of the tocotrienol rich fraction nanoemulsions against MCF-7 and MDA-MB-231 was 14 and 7 µM, respectively, which decreased to 10 µM and 4.8 µM when simvastatin was added to the nanoemulsions.

Ganta et. al., examined augmentation of therapeutic effectiveness upon co administration of paclitaxel and curcumin, an inhibitor of nuclear factor kappa B as well as a potent down-regulator of ABC transporters, in wild-type SKOV3 and drug resistant SKOV3(TR) human ovarian adenocarcinoma cells [93]. Paclitaxel and curcumin were encapsulated in flaxseed oil containing nanoemulsion formulations. The results showed that the encapsulated drugs were efficiently delivered intracellular in both SKOV3 and SKOV3(TR) cells. Curcumin administration was shown to reduce nuclear factor kappa B activity and down regulate P-glycoprotein expression in resistant cells. Combination paclitaxel and curcumin therapy, particularly when administered in the nanoemulsion formulations, was very effectual in enhancing the cytotoxicity in wild-type and resistant cells by promoting the apoptotic response. In general, this cotherapy strategy has major guarantee in the clinical management of refractory diseases, especially in ovarian cancer.

Nanoemulsion containing caffeine was prepared and evaluated for transdermal delivery [94]. Oil phase titration technique was used to prepare various w/o nanoemulsion containing caffeine. Various parameters such as droplet size, morphology, refractive index, and viscosity was studied to characterize the nanoemulsions. Franz diffusion cell with rat skin as permeation enhancer were used for in vitro skin permeation studies. The permeation parameter of optimized formulation was compared with aqueous solution containing caffeine. Permeability profiles nanoemulsion containing caffeine was increase significantly in comparison to the aqueous solution containing caffeine.

8. CONCLUSION

Nanoemulsions are possible to become increasingly the focus of research and development efforts because of their prospective advantages over conventional emulsions for improving bioavailability and physical stability. On the other hand, there are a number of challenges that need to be overcome before nanoemulsions are more extensively used. Appropriate food-grade ingredients must be recognized for formulating food nanoemulsions. Apposite processing operations must be identified to economically and robustly make food-grade nanoemulsions on an industrial scale. There may be some safety concerns associated in the employment of tiny lipid droplets in foods. These small droplets may change the absorption pattern of lipophilic components. Various challenges still remain for the commercialization of the nanoemulsions to food systems. Production process including cost should be addressed clearly. Product acceptance and safety of the nanoemulsion and food system is also being addressed that is still lacking.

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