

A REVIEW ON NANOEMULSIONS: FORMULATION, COMPOSITION, AND APPLICATIONS

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ABSTRACT

Nanoemulsions are sub-micron sized emulsions that are undergoing detailed assessment as potential drug carriers for enhancing the delivery of therapeutic agents. These are to date the most developed nanoparticulate systems for the systemic delivery of active pharmaceutical for controlled drug delivery as well as targeting. These are the thermodynamically durable isotropic system, in which two incompatible liquids (water and oil) are blended to form a single homogenous phase by utilizing a required quantity of surfactants to achieve mixing with a droplet diameter approaching roughly in the range of 0.5–100 μm . They find applications in various fields such as cosmetics as well as are adopted in various routes of administration.

Keywords: Nanoemulsion, Nanoparticle, Droplet size, Cosmetics.

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INTRODUCTION

Nanoemulsions (NEs) are known as oil-in-water (o/w) emulsions with average droplet sizes falling from 50 to 1000 nm. Normally, the mean droplet size is between 100 and 500 nm, for which terminologies such as sub-micron emulsion (SME) and mini-emulsion are adopted instead. The NEs play the role as a base for manufacturing polymer latex particles, nonporous polymeric solids, etc. In addition, the NEs with pharmaceutically established excipients are used in the manufacturing of drug formulations for oral drug delivery. The NEs are also called by other names such as mini emulsions, ultrafine emulsions as well as SMEs. Phase behavior studies have demonstrated that the dimensions of the droplets are decided by the surfactant phase structure (bicontinuous microemulsion or lamellar) at the inversion point brought about by either temperature or composition. Investigations on NE formation by employing the phase inversion temperature method have conveyed a relationship between minimum droplet size and full solubilization of the oil in a microemulsion bicontinuous phase irrespective of even if the primitive phase equilibrium exists in single or multiphase. Due to their small droplet size, NEs provide stability against sedimentation or creaming with Ostwald ripening being the pivotal pathway of NE degradation. The principal application of NEs in the formulation of nanoparticles by employing a polymerizable monomer serving as the disperse phase (the so-called miniemulsion polymerization method) where NE droplets function as nanoreactors. Another fascinating application is formulating NEs containing active pharmaceutical ingredients (APIs), namely, for controlled drug delivery as well as targeting [1].

Formulation of microemulsions

Many different methods have been utilized for the formulation of NEs examples being high-pressure homogenization, microfluidization, phase inversion, spontaneous emulsification, solvent evaporation as well as hydrogel formation each having unique process [2-5]. Multiple emulsions are normally prepared by utilizing the double emulsion solvent evaporation method. NEs are prepared chiefly using two key methods, (a) the persuasion method and (b) the Brute force method.

Persuasion methods/phase inversion techniques

NE formulation by persuasion method proceeds in the absence of external force, but as a substitute, it requires the emergence of fine dispersions when phase transitions occur by swapping either the temperature or composition while maintaining the other parameter constant. The persuasion method can be widely categorized into, (i)

phase transition from the near-optimum state through a change in a single variable, which involves changing one variable of formulation such as temperature or salinity close to the ideal value. Hydrophilic-lipophilic deviation (HLD) for ideal value is near to core level for a system, for example, adopting higher temperature to microemulsion. (ii) The phase transition from a near-ideal state through a change in multiple variables, which means that by changing multiple variables of the formulation. For example, employing higher temperature and including a new salt in a microemulsion. (iii) Catastrophic inversion, wherein an inversion of the internal phase occurs at low temperature within the emulsion resulting in the transformation of the internal phase to the external phase. (iv) Phase transition stabilized by the establishment of liquid crystals, where nanodroplets stabilization takes place from a condition close to HLD-0 by liquid crystal formation [6].

Brute force method

This method involves the adoption of brute forces for breaking down the oil droplets into the nano range. Instruments that have been employed for the preparation of NEs are high-pressure homogenizer, high-speed mixer, small pore membrane, and high-frequency ultrasonic device. NE properties such as its small size, optical transparency, and high kinetic stability rely not only on the make-up of variables in addition to the processing variables such as emulsification time, degree of mixing, energy input, and emulsifying path. High-pressure homogenization and microfluidization methods are adopted at industrial as well as laboratory scale for achieving a very small size of NE by employing high-pressure equipment. Many other methods are also being used for the formulation of NE such as ultrasonication and *in situ* emulsification.

High-pressure homogenization

For the formulation of NE high shear force is needed; hence, in this procedure high-pressure homogenizer or piston homogenizer is adopted for the manufacturing of NEs with extremely small particle size (up to 1 nm) as shown in Fig. 1. In this method, a mixture is pushed forcefully through an orifice at exorbitant pressure ranging from 500 up to 5000 psi. The final product is further exposed to powerful turbulence and hydraulic shear ultimately forming an emulsion with extremely fine particles. This has been demonstrated to be the most promising method for NE formulation but the only limitation accompanying this technique is greater energy utilization and an elevation in temperature of the emulsion during preparation. For getting lesser particle size, there is also a need for more runs of homogenization cycles. Yilmaz et al. prepared phytosphingosine O/W NEs using a high-pressure

homogenization technique and found out that droplet size was lessened after going through eight homogenization cycles and the NE so formed was stable for more than 6 months when stored [24].

Microfluidization

This technique adopted a device called as microfluidizer that makes use of a high-pressure positive displacement pump (500–40,000 psi) that propels the product out through the interaction chamber made up of stainless steel microchannels on the impaction area which resulted in the formation of very small particles of sub-micron range as shown in

Fig. 2. The mixture is again and again flowed through the microfluidizer until the desired particle size is obtained. The final product is also made to proceed through the filter to divide smaller droplets from larger ones and to attain a homogenous NE. Uluata et al. prepared octadecane O/W NEs by utilizing a microfluidizer and saw that on enhancing the number of passes and homogenization pressure; the droplet size became reduced [26]. Goh et al. formulated tocotrienol-rich fraction NEs by two-step homogenization wherein a key coarse emulsion was made by employing a stirrer, which was further underwent processing utilizing a microfluidizer. They announced that the droplet size fell from 120 to

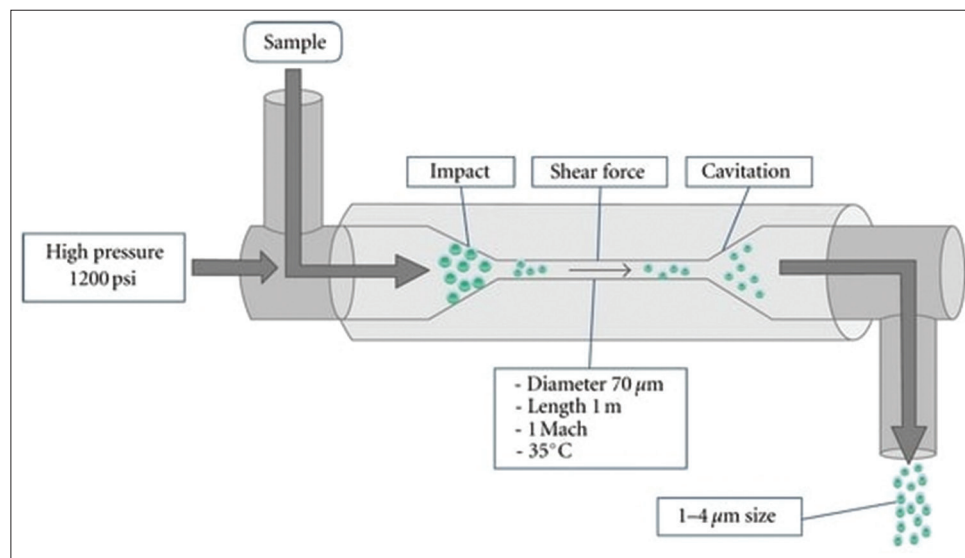


Fig. 1: High-pressure homogenization method. (i) Adapted from Reference [25]. (ii) Copyright obtained under Creative Commons attributed 3.0 (<https://creativecommons.org/licenses/by/3.0/>)

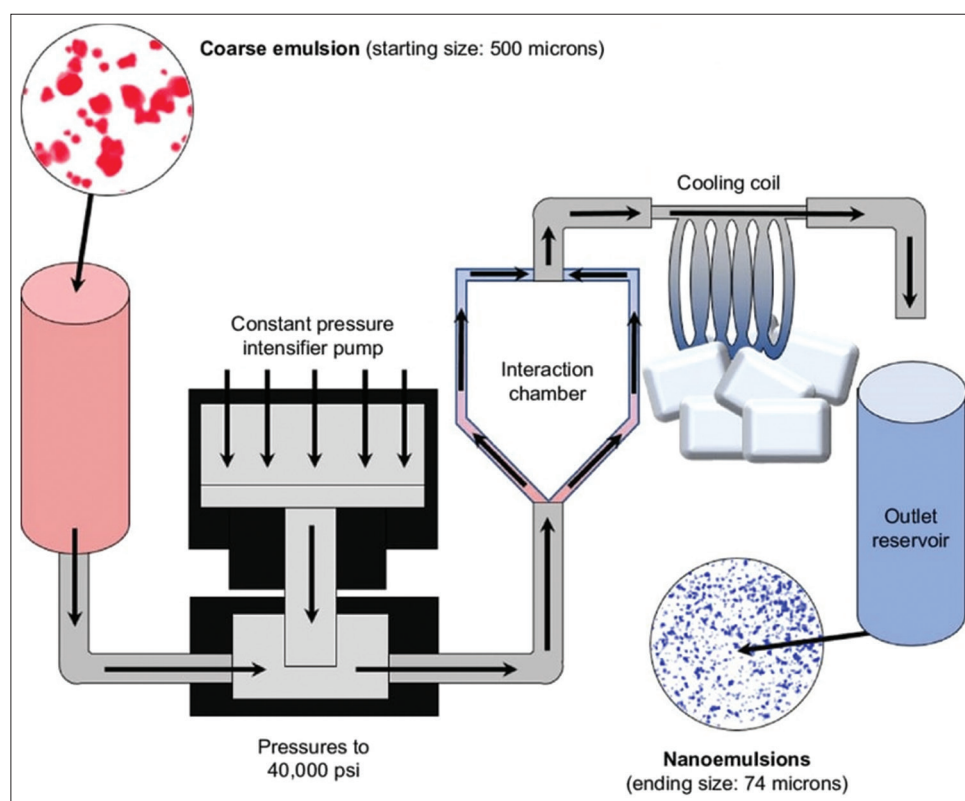


Fig. 2: Microfluidization process. (i) Adapted from reference [28]. (ii) Copyright obtained under Creative Commons Non-Commercial unported 3.0 (<https://creativecommons.org/licenses/by-nc/3.0/>)

65.1 nm after making the formulation pass through ten homogenization cycles at elevated pressure [27].

Ultrasonication

In this method, the premixed emulsion is subjected to agitation at an ultrasonic frequency of 20 kHz which shrinks the droplets to nanosize. The emulsion so formed is then made to pass through the high shear area to make droplets with the same size distribution. A water jacket is adopted in this technique to control the temperature. Sonotrodes which are also known as the sonicator probe are constructed of piezoelectric quartz crystals which serve as the energy suppliers during ultrasonic emulsification. On the passage of alternating electric voltage through these sonotrodes, they shrink and expand. Mechanical vibrations are emanated due to this mechanism at the sonicator tip which when touches the liquid results in cavitation, which further leads to the breakdown of vapor cavities formed within the liquid. This method is chiefly used when the droplet size requirement is less than 0.2 μ . Shi et al. prepared emodin-loaded NE by adopting ultrasonic emulsification method with a frequency set at 25 kHz and attained an average diameter of 10–30 nm [29].

Spontaneous emulsification

This method requires the formulation of NE in three steps. The first step involves the preparation of an organic solution, consisting of oil, and lipophilic surfactant dissolved in a water-soluble solvent as well as hydrophilic surfactant after which the O/W emulsion is prepared by introducing this organic phase into the aqueous phase under magnetic stirring by means of a syringe. The organic solvent is then abolished in the third stage by evaporation. Sugumar et al. prepared stable eucalyptus oil NEs by employing the spontaneous emulsification method after which the average droplet size was discovered to be in the range of 50–100 nm [30].

Following are the types of NEs formed depending on the composition

1. O/W NE: Here, the oil droplets are dispersed in the continuous aqueous phase
2. W/O NEs: Here, the water droplets are dispersed in the continuous oil phase
3. Bi-continuous NEs: Here, the microdomains of oil and water are inter-dispersed within the system

In all three categories of NEs, the interface is made stable by a required blend of surfactants and/or co-surfactants [31].

COMPONENTS OF NES

Three pivotal ingredients are necessary for NE preparation which are

1. Oil
2. Surfactant
3. Co-surfactant.

NEs are basically colloidal systems that consist of three phases namely oil phase, aqueous phase, surfactant, and cosurfactant at acceptable ratios. As contrast to coarse emulsions which are micronized with the aid of outside energy source, NEs are prepared on the basis of low interfacial tension. This is attained by incorporating a cosurfactant, which leads in the voluntary development of a thermodynamically stable NE. The droplet diameter in the dispersed phase is very less, normally below 140 nm in diameter, which is responsible for transparency in NE, NEs can be employed to administer drugs to the patients through many routes, but the topical application of NEs has shown increasing promise [32]. The three key parameters influencing the transdermal penetration of drugs are the flexibility of the drug in the vehicle, release of the drug from the vehicle, and penetration of the drug into the skin. NEs enhance the transdermal delivery of many drugs over the traditional topical formulations such as emulsions [33,34] as well as gels [35,36]. The flexibility of drugs in NEs is easier [34,36,37] when compared against the NE containing gel former which will elevate its viscosity and further reduce the penetration in the skin [35]. The better transdermal

flux from NEs has been demonstrated to be chiefly due to their great solubilization ability for hydrophobic as well as hydrophilic drugs. This creates an enhanced thermodynamic activity toward the skin [36-38]. NEs can influence the penetration of the drug into the skin. In this case, the ingredients of NEs play the role of permeation enhancers. Many compounds employed in NEs have been announced to elevate the transdermal penetration by changing the configuration of the stratum corneum. For example, short-chain alkanols are extensively adopted as permeation enhancers [39-41]. It is recognized that oleic acid, a fatty acid with a single double bond in its chain structure, disturbs the lipid barrier in the stratum corneum by forging different domains which act as a barrier with the continuity of the multilamellar stratum corneum and can bring about vasty permeable alleys in the stratum corneum [42-44]. Isopropyl myristate (IPM) is employed as a permeation enhancer in transdermal preparations, but the way it acts is not well understood [45]. Nonionic surfactants are extensively employed in topical preparations which also serve as solubilizing agents but some current conclusions demonstrate that they may also influence the skin barrier function [46]. It is noteworthy to inspect the consequences of these ingredients in the organized NE structures. A quirky venture was taken up to emulsify coconut oil with the aid of polyoxyethylene 2-cetyl ether (Brij 52) as well as isopropanol or ethanol, resulting in the emergence of stable isotropic dispersion thus setting up the stage for adoption of the plant as well as vegetable oil to be employed as oil phase in NE preparations [47].

The surfactants adopted to stabilize such systems can be:

1. Non-ionic
2. Zwitterionic
3. Cationic
4. Anionic surfactants.

A blend of these, specifically ionic, and nonionic, can be extremely useful at elevating the expansion of the NE region. Examples: (i) Non-ionic surfactants are polyoxyethylene such as Brij 35 (C12E35) or sugar esters, namely, sorbitan monooleate (Span 80). (ii) Zwitterionic surfactants list includes phospholipids are an important example and demonstrate outstanding biocompatibility. (iii) Cationic surfactants list includes Lecithin grades from many different sources such as soybean and egg are accessible commercially and comprise of diacylphosphatidylcholine as its key ingredient [48-51]. Quaternary ammonium alkyl salts are one of the exceptional appreciated classes of cationic surfactants, where hexadecyltrimethylammonium bromide (CTAB) and the double-tailed surfactant didodecyltrimethylammonium bromide (DDAB) are among the most recognized. (iv) Anionic surfactant examples are sodium bis-2-ethyl hexyl sulfosuccinate (AOT) is a double-tailed surfactant that is mainly utilized as a successful stabilizer for w/o microemulsions [52]. Endeavors have been taken to justify surfactant behavior with respect to the hydrophile-lipophile balance (HLB) [53], and the critical packing parameter (CPP) [54,55]. Both viewpoints are reasonably real but can serve as a convenient guide towards surfactant selection. The HLB value decides the relative credit of hydrophilic as well as hydrophobic pieces of the surfactant molecule. It is normally recognized surfactants possessing less HLB (3–6) are recommended for the preparation of w/o NEs while surfactants with high HLBs (8–18) are advised for the preparation of o/w NE systems. Ionic surfactants, namely, sodium dodecyl sulfate which carries HLB values higher than 20, often need the existence of a cosurfactant to lessen their functional HLB to a value within the window desired for NE preparation. Conversely, the CPP points to the property of surfactant to make specific aggregates to the configuration of the molecule itself. In nearly all cases, single-chain surfactants when employed singly are not capable to lessen the oil/water interfacial tension to a point to allow a microemulsion to form, an issue highlighted in many existing microemulsions reviews [56-60]. Medium-chain length alcohols which are traditionally incorporated as cosurfactants have the consequences of additionally lessening the interfacial tension while enhancing the mobility of the interface which elevates the entropy of the system [57,58]. Medium-chain length alcohols also enhance the mobility of the hydrocarbon tails as well as also permitting higher permeation of the oil into this region.

Table 1: Various methods adopted in the preparation of nanoemulsions [7-23]

Method	Formulation	Inference	References
High-pressure homogenization	Oral nanoemulsion (primaquine)	Better oral bioavailability, 10–200 nm particle size	[7]
Pseudo ternary phase diagram+spontaneous emulsification method	Ramipril nanoemulsion	Upgraded bioavailability, droplet size 80.9 nm	[8]
High-pressure homogenization	O/W nanoemulsions	Boosted skin hydration as well as elasticity	[9]
Spontaneous emulsification	O/W nanoemulsion (aceclofenac)	Nanoemulsion with a promising likelihood of transdermal delivery of aceclofenac	[10]
Spontaneous emulsification	Celecoxib nanoemulsion	Elevated physical and chemical stability of celecoxib in nanoemulsion	[11]
High-pressure homogenization	Lecithin-based nanoemulsions (progesterone)	Enhanced permeation rates of progesterone offering long-term stability	[12]
High-pressure homogenization	Prednicarbate nanoemulsion	Enhanced chemical stability of the drug in the formulation	[13]
Phase inversion temperature method	Acyclovir-loaded multiple W/O/W nanoemulsions	Very good physicochemical stability for 6 months at RT, mean droplet size of 100 nm	[14]
Spontaneous nano emulsification method	Clotrimazole nanoemulsion	Enhanced solubility of clotrimazole, mean globule size lesser than 25 nm	[15]
Ultrasonic emulsification method	Basil oil nanoemulsion	Nanoemulsions with a droplet size of 29.6 nm, used as food preservatives	[16]
High-pressure homogenizer	Dimethyl silicone dry nanoemulsion inhalation	Useful in acute lung injury, the particle size of 19.8 nm	[17]
Microfluidization method	Pitavastatin-containing nanoemulsions	Boosted permeation	[18]
High-pressure homogenization+ultrasound	Nanoemulsion	Less energy required for emulsification, fewer particle dimensions, and greater stability	[19]
Sonication method	Saponin-stabilized quercetin-loaded o/w nanoemulsion	No instability is seen for 45 days at RT, mean particle size of 52±10 nm	[20]
High-pressure homogenization	Paclitaxel-baicalein nanoemulsion	Scheme to tame multidrug resistance	[21]
Nanoemulsion templating	PLGA nanoparticles	Imaging agents for biomedical applications	[22]
Spontaneous emulsification method	Chitosan films with cinnamaldehyde nanoemulsions	Offer Adequate UV barrier properties	[23]

Table 2: NEs utilized as adjuvants [81-87]

Adjuvant utilized	Ingredients	Uses	References
MF59®	O/W Squalene containing emulsion	Influenza	[81,82]
AS03	SB62 adjuvant and twice diluted form of O/W squalene	H5N1 and H1N1	[83]
AS02	Immune-stimulatory agents, for example, MPL and triterpenoid saponin molecules	Malaria, HIV, and tuberculosis	[84]
MPL®SE	Blend of monophosphoryl lipid and a stable squalene emulsion	Leishmaniasis	[85]
AF03	Thermoreversible O/W nanoemulsions	H1N1 influenza	[86]
DETOX®	Composed of bacterial cell wall and monophosphoryl lipid A (MPL) dissolved in squalene and Tween 8	Melanoma	[87]

APPLICATIONS

Ophthalmic and pulmonary delivery

For the therapy of eye diseases, drugs are mainly administered topically in the form of o/w NEs which have been studied for ocular delivery, to solubilize poorly soluble drugs, in an effort to enhance absorption, and to achieve prolonged release effect. The NEs comprising pilocarpine were prepared using lecithin, propylene glycol, and PEG 200 as

cosurfactant and IPM acting as the oil phase. The preparations were found to be less viscous with a refractive index acceptable toward ophthalmologic applications [31]. Morsi *et al.* formulated a new NE of acetazolamide where the particle size ranged from 23nm to 90 nm [61].

Cosmetics

The advantage of using NEs in cosmetics is the tiny droplet size and omission of creaming as well as flocculation, which pave the way for the uptake by antigen-presenting cells and results into a pure product formulation [62]. Ribeiro *et al.* [63] prepared O/WNEs containing *Opuntia ficus-indica* (L.) Mill hydroglycolic extract as well as xanthan gum with droplet sizes ranging from 92.2 to 233.6 nm. The O/WNEs comprising 1% of *O. ficus-indica* (L.) Mill extract was absence from any stability issues for 60 days. Furthermore, this formulation enhanced the water content of stratum corneum, demonstrating its moisturizing ability to be a superior product for cosmetics. NEs were prepared by Pengon *et al.* [64] by utilizing coconut oil NEs and diversified amounts of surfactants including polyethylene glycol octyl phenyl ether (PGO) as well as polyethylene glycol hydrogenated castor oil (PHC). The droplet size of NEs comprising 5% (w/w) PHC was found to be 0.162 μm. An even lesser size of coconut oil NEs could be obtained by elevating the concentration of PHC. NE formulations were formulated from the hydroalcoholic extracts of *Vellozia squamata* leaves as well as stems with inflated antioxidant activity by utilizing the phase inversion method. Stable formulations were achieved from both extracts from leaves as well as stems. Antioxidants are popular as anti-aging agents and therefore are acceptable for cosmetics formulation [65]. NEs have also been utilized as a vehicle for controlled delivery and as an effective transport vehicle in cosmetics. NEs will lessen trans-epidermal water loss. The Kemira nano gel- made up of NEs serving as a carrier is a patented formulation intended for cosmetic uses; which will enhance skin cell production as well as the penetration of API to give a good skin sensation [66]. Thananya *et al.* formulated a mixture of essential oils in

NEs dosage form for an anti-tyrosinase activity where the droplet size was found to be ranging from 29 nm to 35 nm and showed well *in vitro* anti-tyrosinase activity plus these formulations were also found to be stable even after 3 months of storage [67].

Asthma

Yasser *et al.* formulated NE of montelukast sodium where the mean droplet size ranged from about 65 to 85 nm and drug release from this formulation was found to be 93% after a period of 30 min [68].

Anti-inflammatory

The blending of oils and emulsifiers improved the absorption of two phytochemicals that are now utilized in treating two major chronic inflammatory diseases which are periodontitis [68] and bowel disease (Crohn's disease and ulcerative colitis) [69]. Periodontitis is a long-term inflammatory disease that attacks the supporting structures of the teeth with many factors such as microbial, genetic, environmental, and host factor play a role in the beginning of that condition. A state of long-term inflammation will take place with the release of oxygen-free radicals by inflammatory cells (polymorphonuclear lymphocytes). Enzymes and toxic by-products given out by the periodontal pathogenic microflora further enhance this destructive activity by breaking down affected cell membranes and extracellular matrices to get nutrients very important for their sustenance [70]. In Crohn's disease, the entire intestinal wall may be attacked by inflammation and is normally transmural and non-continuous, meanwhile in ulcerative colitis, it is many a times continuous, primarily affecting only on the mucosal lining of the colon and rectum [71]. Multiple reasons might reside behind the origin of the diseases including complicated interplays between oxidative stress, immunoregulation, altered inflammatory mediator levels, microbial pathogens, as well as genetic reasons [72]. Phytochemicals have been announced by fresh investigations to be handful in recovering both diseases. Quercetin, a class of bioflavonoids that have treated periodontitis [73], and diterpenoid extracted from *Andrographis paniculata* to remedy bowel diseases [74], have shown possible antimicrobial activity, lessening inflammatory markers, as well as demonstrating anti-inflammatory, antioxidative [75], as well as anticancer activities [76]. But, both ingredients have less aqueous solubility (0.07 mg/mL in water), which has resulted in lesser bioavailability, and due to rapid and large scale metabolism, they have lesser oral absorption. Absorption of both phytochemicals can be enhanced with the blending of oils and emulsifiers serving as carriers for the active components [77]. Hence, for lipophilic drug loading, NEs could be a proper drug delivery vehicle. The speed of absorption is good and changes in absorption rates is bypassed completely and NEs also make way in solubilizing lipophilic drug, enhancing bioavailability, and authorize rapid and systematic puncturing of the drug molecule. *In situ* nanoemulsions could be made when NEs are added into the polymer solution, which pertains sustained as well as controlled drug delivery and pave way for administration, thus patient compliance with drug will be further enhanced [69].

Vaccine delivery

NEs being serving as a vaccine carrier are busily being investigated. The present affective and well-organized procedure is to administer an inactivated organism to a mucosal surface so that an immune response will be activated by the body. Studies have demonstrated that genital mucosa immunity can be generated with vaccines that are delivered into the nasal mucosa as required [78]. Proteins were administered to the mucosal surface by utilizing NEs to act as an adjuvant and govern the absorption of antigen-presenting cells. The antigen can be trapped in the nanocarrier by many mechanisms including physical adsorption, encapsulation, encapsulation with coating, encapsulation with targeting, chemical linking, and conjugation along with a targeting mechanism. The physical adsorption of the antigen onto a nanocarrier can be attained either by a charge or hydrophobic interaction, which demonstrates a weak interaction that leads to the braking of the antigen and nanocarrier bond in the body thus releasing the antigen. As far as the encapsulation is concerned, antigens are blended with nanocarrier

precursors during their synthesis, which leads in the entrapment of antigen into the nanocarrier [79]. The very first application that is currently getting clinical trials is the nanocarrier for influenza and HIV protein. Furthermore, a recombinant HIV gp 120 antigen mix in NEs has been injected into mice as well as guinea pigs through intranasal immunization that show robust serum anti-gp 120 IgG levels [80]. Another noteworthy example is the pandemic flu vaccine AS03, which has been accepted as a constituent of Prepandrix® [81].

CONCLUSION

As described NEs provide a platform on which drugs that are less water-soluble can be delivered successfully. They utilize oils and surfactants to solubilize the drug in the aqueous phase which then serves as a carrier. The various methods of preparation of NEs along with their composition followed by their subsequent applications were discussed in this review. NEs are a new frontier in novel drug delivery systems and will continue to progress ahead with new advancements in the future.

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AUTHORS CONTRIBUTION

Manuscript framing and the concept has been presented by Khan Mohammad Hamid. Literature search and preparation have been done by Gaurang Sawant. Reviewing and editing have been done by Mohammad Wais.

CONFLICT OF INTERESTS

The authors have declared no conflicts of interest.

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