FORMULATION OF NEEM OIL DERIVED NANO-EMULSION BY LOW ENERGY METHOD AND ITS CHARACTERIZATION

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Contents

Abstract

Nanoemulsions have gained popularity in recent times for their widespread usability in pharmaceuticals, pesticides, cosmetics, food, paint and environmental applications. Nanoemulsions are kinetically stable systems (droplet size range in the order of 100 nm) exhibiting multiphase colloidal dispersion with longer shelf life. Due to their small size, they enhance penetration, spreading and uniform distribution on the targeted area. High energy or low energy dissipation methods are widely known for the formulation of nano-emulsions, differing by the amount of energy utilised. Mechanical devices generate large disruptive forces in high energy method, whereas low energy method modifies the physiochemical characteristics of the system to generate nano-sized particles. High energy methods included various mechanical devices such as high speed/high-pressure homogenisers, ultra-sonicators, and high shear stirrers. Thus, formulation using high energy methods are considered as an energy intensive process. Recently, the low energy methods are widely referred to as an eco-friendly technique which utilizes the internal chemical energy of the system itself. Moreover, it has become an appealing approach because of the requirement of components that are temperature sensitive such as pharmaceutical ingredients. The low energy methods mainly include, phase inversion temperature (PIT), phase inversion composition (PIC), microemulsion dilution and surfactant (D) phase emulsification. Raw neem oil (azadirachtin enriched insecticidal properties) in its raw form depicts poor water solubility, oxidation tendency, and toxicity at high intake levels. To overcome the utility bottlenecks of neem oil, its utilization in the form of nano-emulsion has gained attention by various researchers.

This study aimed to prepare neem oil-based nano-emulsion by using the two different low energy methods, i.e., phase inversion composition (PIC) and phase inversion temperature (PIT). This study also addresses the physio-chemical characterization of formulated nano-emulsion such as particle size, zeta potential and viscosity. The Antimicrobial activity of formulation was also analysed against raw neem oil and Ampicillin drug for human pathogenic bacteria.

Neem oil-based nano-emulsion by using phase inversion composition (PIC) method with surfactant blend of Tween 80/Span 80 (1:1 volume ratio of surfactant to oil) at hydrophilic-lipophilic balance (HLB) value of 10.7 showed promising formulation with a droplet size below 200 nm. The resulting formulation showed zeta potential of -19.6 mV at a pH of 5. After two months storage of formulation, the zeta potential increased to -40 mV at a pH of 6.7, confirmed by observing increment in droplet size to the tune of 579 nm after 2 months duration.

It was observed that Minimum Inhibition Concentration (MIC) of neem-based nano-emulsion formulation was less than raw neem oil for all sets of experiments. It signifies effectiveness of nano-emulsion formulation over raw neem oil for antimicrobial activity against E-coli, S. Aureus and S. Pyogenus. The study indicates that antimicrobial activity of neem oil nano-emulsion against S.Pyogenus is better than raw neem oil, whereas inferior than Ampicillin drug.

Besides this, a study was also carried out to prepare neem oil-in-water nano-emulsions stabilized by Brij 30 surfactant using the phase inversion temperature (PIT) method at three different temperatures, i.e., 60°C, 75°C and 80°C. Compositions of homogenous phase were identified in the pseudo-ternary phase diagram. Among the total seventeen formulations, three formulations (NB1, NB2 and NB3) were short-listed and characterized for emulsion size and viscosity. The selected formulations showed emulsion size of 348-981 nm in diameter. The volume percentage ratio of Brij30 to neem oil showed significant effect on the droplet size of nano-emulsions. Formulations having lower concentration of Brij 30 displayed a smaller emulsion droplet size (348 nm). The NB3 formulation (4% neem oil, 11% Brij 30 and 85% deionized water) exhibited the highest stability after 60 days of storage. Antimicrobial study showed that in contrast to raw neem and Ampicillin (synthetic drug), NB1 exhibited best result in terms of minimum inhibition concentration (MIC) reduction by 100% against E-coli, P. Aeruginosa and S.Aureus and S.Pyogenus.

State of the Art of the Research Topic

Raw neem oil enriched with azadirachtin is a potential source of insecticidal properties. Like all medicinal oils, neem oil in its raw form depicts poor water solubility, oxidation tendency, and toxicity at high intake levels. This reduced the ability to use neem oil in raw and direct form. Hence, its utilization in the form of nano-emulsion has gained attention and requires detailed study to develop the technique [1-4]. Nanoemulsions are kinetically stable systems exhibiting multiphase colloidal dispersion with longer shelf life [5]. Due to their small droplet size, they enhance penetration, spreading and uniform distribution on the targeted area. Numerous research papers have focussed on preparing nanoemulsions through various methods, including high-energy and low-energy methods. Formulation using high energy methods was considered an energy-intensive process. Also, it showed a poor utilization rate of energy in the formulation of nano-emulsion. Various literature showed that only about 0.1% of the total energy supplied gets utilized in producing nano-emulsions [6,7]. Presently, the low energy methods are widely considered as an eco-friendly technique which utilizes the internal

chemical energy of the system itself. It includes, phase inversion temperature (PIT), phase inversion composition (PIC), microemulsion dilution and surfactant (D) phase emulsification.

Phase inversion composition (PIC) method is characterized as formulation of nano-emulsion by dropwise addition of water in a varying proportion to a mixture of surfactant and oil at an ambient temperature along with moderate stirring. PIC provides the change in phase starting from w/o phase, followed by bi-continuous phase and ending to o/w phase. In Phase inversion temperature (PIT) method, the Surfactant, oil and water are stirred and heated gradually at room temperature continuously till PIT. Then the solution is rapidly cooled by transferring the mixture to ice bath leading to formation of o/w nanoemulsions. It has been found that when the PIT of the system is approximately 20-65 ^{0}C higher than the storage temperature, nanoemulsions formed are of o/w type. It is observed that the stability of the system formed by this method is sensitive at the temperature near the PIT, so another way of stabilizing this system is addition of co-surfactants. Use of non-ionic surfactants (whose molecular geometry changes with temperature) in the systems prepared by PIT method showed stabilized nanoemulsions.

In line with low energy methods, neem oil-based nano-emulsion was formulated by PIC method using surfactant blend Tween 80 and Span 80 (60:40) at a 1:1 vol ratio with hydrophilic-lipophilic balance (HLB) value of 10.7. The single-phase formulation of neem oil nano-emulsion was characterized for droplet size, polydispersity index, stability, zeta potential and viscosity. In addition to this, neem oil nano-emulsion was also prepared by PIT method using Brij 30 surfactant. With the use of pseudo-ternary phase diagram, stable formulations were determined and characterised for droplet size, polydispersity index, stability and viscosity. Beside this, comparison of both the methods were evaluated for particle size, polydispersity index, stability and viscosity. The study also addresses antimicrobial characteristics of prepared formulations against human pathogenic bacteria. Moreover, antimicrobial activity of both the formulations were compared with raw neem oil and Ampicillin antibiotic drug.

Definition of the Problem

Neem oil is a deep yellow extract obtained from the seeds of neem tree (Azadirachta indica). These seeds are popularly used in India and in other South-East countries as versatile medicinal product due to its antibacterial, antifungal, antimalarial and insect growth inhibition properties, and in cosmetics due to its low toxicity in vivo studies [6, 7]. Nevertheless, neem oil in its raw

form exhibited poor water solubility, oxidation tendency and toxicity in high intake levels. In order to improve the effective utilisation of neem oil, the application in the form of nano-emulsion has gained attention by various researchers [8].

Formulation of nano-emulsion prepared using neem oil or Karanj oil with Tween 80/Polyethylene glycol 400 as a surfactant and co-surfactant, respectively by emulsification method produced smallest droplet size of 481 nm. A researcher had reported about the stability of nanoemulsion formulations of neem oil-water-nonionic surfactant (Lutensol® TO 6), prepared using high-speed homogenization [9]. Moreover, many authors demonstrated formulation of neem-based nano-emulsion with Tween 20 using ultrasonication [10]. The effect of different parameters on droplet size of nano-emulsion formulation through the PIC method was reported by various investigators. These were mainly the composition of the mixture, rate of mixing, surfactant to oil ratio, blend ratio of surfactants and addition rate of aqueous phase [11-14]. Formulation with PIC method was reported for Tween 80/polyethylene glycol 400 with neem/karanj oil, and Tween 80 with raspberry seed oil [15,16]. PIT method to prepare nano-emulsion of natural oils such as soyabean oil, clove oil, nutmeg oil, cinnamon oil, retinyl palmitate oil, and sunflower seed oil have been researched widely [17]. Nevertheless, a little report on the phase inversion composition method is available for the formulation of neem oil-based nano-emulsion using a blend of Tween 80-Span 80 as surfactant. Also, to the best of our knowledge a study about preparing formulation of neem oil-based nano-emulsion using Brij 30 as a surfactant employing a low energy PIT method has not yet been reported. Apart from this, it was investigated that nano-emulsion favours pharmaceutical application due to significant reduction in consumption of active ingredients. Typical nature of the nano-emulsion, i.e., high-water solubility and capacity to solubilize hydrophilic and lipophilic compounds credited to such a change in consumption of active ingredients [18]. Thus, a promising delivery system of nano-emulsion has led to its utilization in antimicrobial application. Various authors demonstrated that nano-emulsion to be efficient for oral delivery of lipophilic drugs such as antibiotics, hormones, steroids, cytotoxics, diuretics and antifungals [18,19].

Objectives and Scope of Work:

1. To prepare neem oil-based nano-emulsion formulation by low energy methods, mainly PIC and PIT.

2. To study stability (over the period of two months) of neem oil-based nano-emulsion formulated through PIC and PIT method.
3. To investigate physio-chemical characterization of formulated nano-emulsion (through PIC method) such as droplet size, polydispersity index, stability, zeta potential and viscosity.
4. To determine stable formulation using pseudo-ternary phase diagram in PIT method.
5. To evaluate characteristics of formulated nano-emulsion (through PIT method) such as droplet size, polydispersity index, stability and viscosity.
6. To study antimicrobial characteristics of prepared formulations against human pathogenic bacteria. Moreover, comparison of antimicrobial activity against raw neem oil and Ampicillin antibiotic drug for PIC and PIT derived neem oil-based nano-emulsion formulations.

Original contribution by the Thesis.

A little report on the phase inversion composition method is available for the formulation of neem oil-based nano-emulsion using a blend of Tween 80-Span 80 as surfactant. Also, to the best of our knowledge a study about preparing formulation of neem oil-based nano-emulsion using Brij 30 as a surfactant employing a low energy PIT method has not yet been reported. Apart from this, it was investigated that nano-emulsion favours pharmaceutical application due to significant reduction in consumption of active ingredients. Typical nature of the nano-emulsion, i.e., high-water solubility and capacity to solubilize hydrophilic and lipophilic compounds credited to such a change in consumption of active ingredients. Thus, a promising delivery system of nano-emulsion has led to its utilization in antimicrobial application.

Methodology of Research and Results:

Research Methodology:

1. Materials:

Neem oil (ρ = 0.949 g/mL; azadirachtin 250 ppm; μ = 4 cP) was purchased from Kama Ayurveda, Arya Vaidya Pharmacy, Coimbatore, India. Tween 80 (polysorbate 80) and span 80 (sorbitane monooleate) was purchased from Molychem, Mumbai. Polyoxyethylene (4) lauryl ether (Brij 30) was purchased from LobaChemie, Mumbai, India. De-ionized water was used for all experiments.

2. Preparation method of formulation by PIC and PIT

In PIC method, initially the blend of surfactants was prepared according to the HLB scale described by Griffin [20]. An Appropriate proportion of Tween 80 and Span 80 for preparing

formulation was selected based on homogeneity of the mixture. Among the six calculated HLB, blend of 10.7 HLB showed a homogenous phase, whereas the rest exhibited a heterogeneous phase. The formulation was prepared using neem oil (15% v/v) and a blend of Tween 80 and Span 80 (60:40 having 10.7 HLB) in an equal volume percentage (15%v/v). Continuous phase, i.e., water (70% v/v) was added dropwise to dispersed phase at 27ºC and stirred with the use of magnetic stirrer at 600 rpm for 60 minutes. **Figure 1** showed physical appearances of disperse phase, continuous phase and final nano emulsion formulation prepared by PIC method.

In PIT method, pseudo ternary phase diagram was constructed using ProSim Ternaire software (France, version 1) for different proportions (v/v%) of neem oil and surfactant Brij 30, i.e., 10:0; 9:1; 8:2; 7:3; 6:4; 5:5; 4:6; 3:7; 2:8; 1:9 and 0:10. The prepared mixtures were stirred and heated to 50°C to attain equilibrium, and gradually cooled to room temperature. Next, water (5 v/v%) was added by titrating the mixtures of neem oil and surfactant until a 95% water content was obtained in the emulsion system. The components were stirred at 600 rpm, 27°C for 30 min using magnetic stirrer. Out of more than 200 formulations, based on the transparency and homogeneity of the resulting mixtures, three formulations were selected and denoted as NB1, NB2 and NB3. As shown in Table 1 the nano emulsion formulations of NB1, NB2 and NB3 were prepared by mixing neem oil, Brij 30, and deionized water (volume %) using magnetic stirrer at 600 rpm in a glass beaker and then heated using a water bath. Once the system reached its phase inversion temperature (the temperature at which significant change in conductivity observed), the mixture was rapidly cooled to 20-27°C by keeping it in an ice bath. The emulsion was continuously stirred using a magnetic stirrer during the heating and cooling stages. The preparation of formulation was derived based on the method described by Rao and McClements [17] with correction in PIT, i.e., at PIT instead of above PIT. **Figure 2** showed physical appearances of nano emulsion formulation prepared by PIT method.

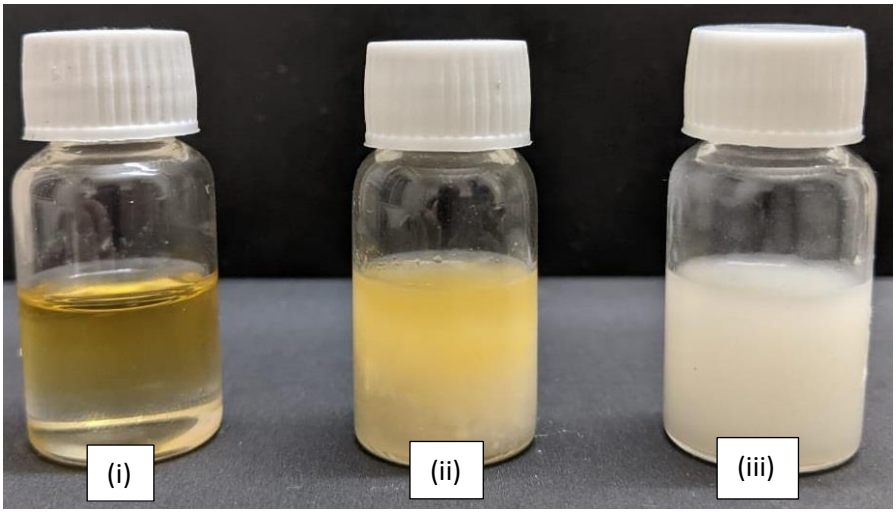

Fig. 1 Appearance of homogeneous stabilized nano-emulsion (i) disperse phase (ii) upon addition of continuous phase (iii) final nano-emulsion

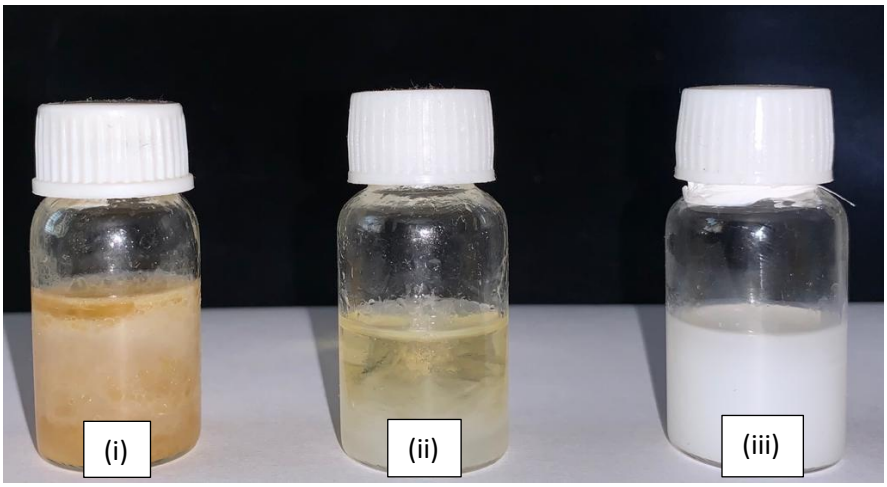

Fig.2 Appearances of nano-emulsion formulation steps (i) Neem oil and Brij 30 (ii) dispersion on water addition (iii) Nano-emulsion by PIT

Table 1. Composition (volume%) of neem oil, Brij 30 and deionized water

Formulation	Neem Oil	Brij 30	Deionized Water	Phase inversion temperature ^{o}C
NB1	10	20	70	80
NB2	10	30	60	75
NB3	4	11	85	60

3. Physicochemical characterization of neem based nanoemulsion:

Particle Size and Zeta Potential Measurement

Droplet size and zeta potential of the formulation were determined using a dynamic light scattering (DLS) instrument (Malvern Mastersizer 2000, UK). For removing multiple scattering effects, the sample was diluted 40 times with deionized water. The nano-emulsion sample was tested again after storage for two months. The readings were taken thrice for each formulation within range of error ±5%.

Measurement of Viscosity

The viscosity of nano-emulsion formulation (non-diluted) was measured by using a digital rotational viscometer (Model: LMDV200; Labman, India) with L0 spindle at 25°C. At 100 rpm, sample reading was noted when equilibrium was reached to zero shear after 2 minutes.

Antibacterial activity of nano-emulsion

The antibacterial activity of the nanoemulsion formulations was measured with the use of the broth dilution method. The Broth dilution method determines minimum inhibition concentration (MIC). MIC defines the lowest concentration of antimicrobial agent and synthetic drugs that prevents visible growth of microorganism under defined conditions as observed by the unaided eye. In the current study, raw neem oil, synthetic drug(ampicillin) and neem oil nano-emulsions were tested for their antibacterial activity against bacterial strains, i.e., E. coli, P.Aeruginosa, S. aureus, and S. Pyogenus. The bacterial strains were procured from the Institute of Microbial Technology, Chandigarh. One set of broth solution containing no antibacterial agent was used as a standard medium and the second set was prepared by taking identical volumes of broth in antibacterial solution (neem oil, ampicillin, nanoemulsion). Both sets of test tubes were inoculated with a defined quantity of bacterial strains i.e., 10^8 organisms/ml. After incubation, the growth of bacteria was analyzed by observing the presence of turbidity or sediment.

4. Results and Discussion

4.1. Neem based nano-emulsion formulation by PIC method:

Nano-emulsion droplet size and Polydispersity Index

Figure 3 showed DLS of new **formulated neem oil nano-emulsion by Tween 80-Span 80 blend** displaying a profile of particle size distribution. The Z-average or cumulant mean size

obtained was 196 nm which was the most stable parameter produced by the technique as defined by ISO:22412 and its intensity mean calculated from the signal intensity. The polydispersity index (PDI) is a dimensionless value which provides width parameter. For a uniform size sample, PDI value is considered zero. As shown in Figure 3 (A) PDI observed was 0.428 which represented broad polydisperse system. As shown in graph, polydisperse characteristics was also confirmed by peak value of number mean size of 88 nm, nearly half the size of Z-average (196 nm). As shown in Figure 3 (B) DLS analysis of formulation after storage of two months showed that the Z-average was 579 nm with a PDI of 1. The second peak at about 1000 nm was observed due to PDI value of 1. Such a value of PDI indicates formulation of larger size of particles in the system due to agglomeration of droplets. Soles et.al.2012 also reported that higher value of PDI favors ripening rate as referred in ostwald ripening phenomenon. Higher laplace pressure of small droplets allow their diffusion into bulk size droplets, leading to formulation of a larger droplet size in the system. Thus, over the period, droplet size distribution reported a higher value [22].

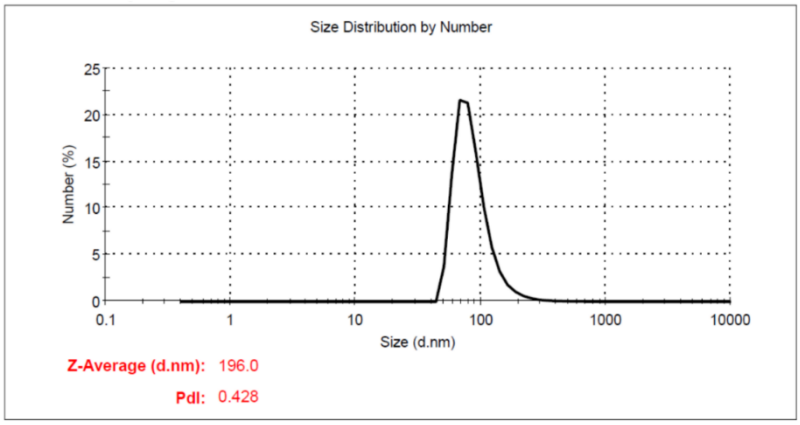

Fig. 3(A) Droplet Size distribution of neem oil nano-emulsion in formulation at t=0

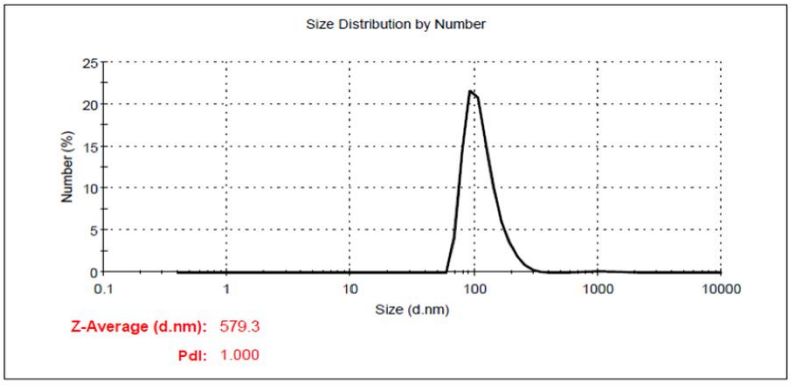

Fig. 3(B) Droplet Size distribution of neem oil nano-emulsion in formulation after two-month storage

Viscosity and zeta potential

To characterize neem oil nano-emulsion formulation, the study of flow behavior in terms of viscosity was reported. In current study, the viscosity of neem oil nano-emulsion formulation showed 2.11cP. The observed viscosity of nanoemulsion was about half the viscosity of neem oil (4 cP). The significant reduction in viscosity was attributed to less volume of neem oil and surfactant in the prepared formulation. Similar results had been observed by Sheikh Shafiq-un-Nabi, et al.2007 which explained that nanodroplets increased the interfacial areas allowing solubility of water insoluble ingredients (e.g., azadiractin) and improving the transport properties of these ingredients. [23,24].

The PIC formulation showed a zeta potential of -19.6 mV at a pH of 5. After two months of storage of formulation, the zeta potential increased to -40mV at a pH of 6.7. The zeta potential of -30mV is reported to be optimum for good stabilization of a nanoemulsion formulation [25,26]. Effect of zeta potential and pH on stability of droplet size has been widely reported. Figure 2 showed stability of formulation in a declining order after storage of two months. The presence of a negative charge on oil-water interface strongly depends on the pH value. The negative charge at the oil water interface due to adsorption of hydroxyl ions was reported by I. B. Ivanov et al. 1996. Ethylene oxide groups of Tween 80 created hydrogen bonds with hydroxyl ions to provide a higher negative charge, which lead to reduced stability and increment in zeta potential.

Bioassay studies

Table 2 shows the effectiveness of raw neem oil, nanoemulsion formulation and synthetic drug as an antimicrobial agent represented in terms of Minimum Inhibition Concentration (MIC). It was observed that MIC of nano-emulsion formulation was less than raw neem oil for all sets of experiments. It signifies effectiveness of nano-emulsion formulation over raw neem oil for antimicrobial activity against E-coli, S. Aureus and S. Pyogenus. It was also observed that MIC of nano-emulsion formulation was less than Ampicillin drug for antimicrobial activity against E-coli and S.Aureus, whereas it was seen high against S.Pyogenus. The study indicates that antimicrobial activity of neem oil nano-emulsion against S.Pyogenus is better than raw neem oil, whereas inferior than Ampicillin [27,28].

Table 2. Antibacterial activity of Raw Neem oil, Nano-emulsion formulation, and Ampicillin

Minimum Inhibition Concentration (µg/ml)			
Sample	E.Coli	S.Aureus	S.Pyogenus
Raw Neem Oil	100	125	250
Neem oil / T80-S80 nano-emulsion	50	100	125
Ampicillin (synthetic drug)	100	250	100

4.2. Neem based nanoemulsion formulation by PIT method:

Effect of Surfactant and PIT on droplet size of nano-emulsion

Table 1 shows the effect of volume ratio of surfactant to oil on the size of emulsions in the selected **neem oil nano-emulsion formulation made by PIT method**. It can be seen that as the ratio of Brij30 to neem oil in the formulation increases from 2 to 3, there is a gradual increase in the size of emulsion formed thereby. Liu et al. studied the size of emulsion formed in paraffin oil, water and chremophor (surfactant) system and observed a similar trend of increasing emulsion size upon increasing the surfactant to oil ratio [29]. Table 1 shows the effect of concentration of surfactant on size of emulsion formed. As the concentration of Brij 30 present in the nano-formulation increased from 11 vol% to 20 vol%, the droplet size of emulsion decreased from 555 nm to 348 nm. Then after, upon further increasing the surfactant concentration to 30% the droplet size of emulsion increased to 981 nm. Concentration of surfactant beyond critical micellar concentration might be attributed to such increment [30]

Figure 4, showed the stability of three formulations examined after 60 days. All the formulations were observed to be visually stable at 27°C with no phase separation, creaming and sedimentation. Nevertheless, the DLS study showed increment in droplet size for NB1, NB2 and NB3 by 116%, 15% and 12%, respectively after 60 days. Ostwald ripening/coalescence phenomenon might be attributed for the visible growth rate of droplet size over the period of storage time [29].

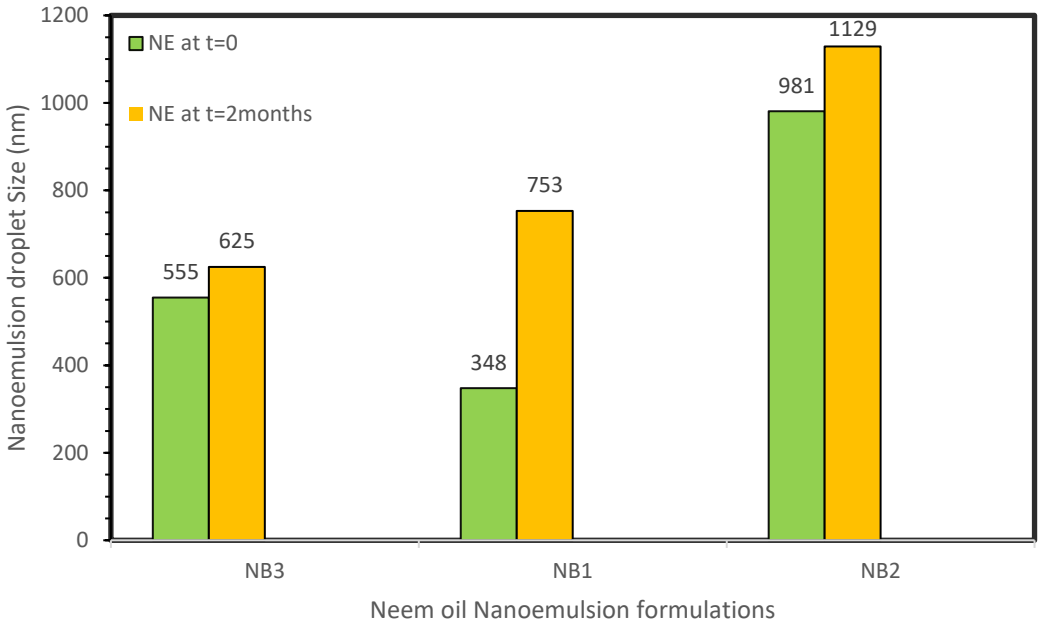

Fig. 4 Stability of droplet size over the period of 60 days

Viscosity

In order to characterize fluidity of neem oil nano-emulsion formulation, viscosity was reported. In this study, viscosity of three formulations, NB1, NB2 and NB3 were found to be 6.83 cP, 6.89 cP and 6.75 cP respectively. The observed viscosity of nano-emulsion was about 72% higher than that of viscosity of raw neem oil (4 cP). Such an increment in viscosity was attributed to typical characteristics of Brij 30 surfactant (highly viscous) in the composition of nano-emulsion formulation. The non-ionic surfactant attracts water molecules into its cross-linked chain, leading to such an increment in viscosity of nano-emulsion formulations [2,28].

Antibacterial Activity

In the PIT study, the MIC of the screened nano-emulsion formulation (NB1, NB2 and NB3) were measured and then compared with raw neem oil and Ampicillin that are popularly used as antimicrobial agents. The results are shown in Table 3. It was observed that MIC of nano-emulsion formulation NB1, NB2 and NB3 were lesser than that of raw neem oil and synthetic drug for all strains. It was also observed that droplet size of nano-emulsion formulations significantly affects the MIC for all strains studied here (E-coli, P. Aeruginosa and S.Aureus and S.Pyogenus). It was observed that, greater the emulsion droplet size of the prepared emulsion a larger amount of MIC had been registered for that particular formulation. The formulation, NB1 (having lowest emulsion droplet size) observed to be best suited as an antimicrobial agent as it had the lowest MIC. In regards to MIC, NB1 showed reduction in

MIC by 100% against E-coli, P. Aeruginosa and S.Aureus and S.Pyogenus when compared with that of raw neem. Similar findings were reported by Choupanian et al. They found that efficacy of azadirachtin, a main ingredient in the neem oil extract (Azadirachta indica) demonstrated a significant improvement in mortality rate of two serious pest species sitophilus oryzae (L.) and tribolium castaneum (Herbst) as compared to raw neem oil [28].

Table 3 Minimum inhibition concentration of raw neem oil, NB3, NB2, NB1 and synthetic drug for various bacterial strains

Minimum Inhibition Concentration (µg/ml)				
Sample	E.Coli	P.Aeruginosa	S.Aureus	S.Pyogenus
Raw Neem Oil	100	125	125	250
NB1 (348nm)	50	62.5	62.5	125
NB2 (981 nm)	62.5	125	125	200
NB3 (500nm)	62.5	100	100	150
Ampicillin (synthetic drug)	100	--	250	100

Achievements with respect to objectives

1. Nano-emulsion with droplet size below 200 nm was prepared at 27^{o}C by stabilizing neem oil-in-water with surfactant blend (Tween 80/Span 80) at a 1:1 vol ratio with hydrophilic-lipophilic balance (HLB) value of 10.7. Moreover, neem oil-in-water nano-emulsions stabilized by Brij 30 surfactant using the phase inversion temperature (PIT) method at three different temperatures, i.e., 60°C, 75°C and 80°C showed promising result in terms of droplet size of nano-emulsion in the range of 348-981 nm in diameter.
2. The ternary phase diagram classified in two regions. A single phase characterized by slightly transparent and stable, whereas rest of the area refers to creaming and gel-like nature.
3. PIC method imparted reasonable stability of the droplets, confirmed by observing increment in droplet size to the tune of 579 nm after 2 months duration. Dynamic light scattering (DLS) study of PIT method showed increment in droplet size for NB1, NB2 and NB3 by 116%, 15% and 12%, respectively after 60 days.
4. The PIC derived formulation showed polydispersity index (PDI) 0.428 which represented broad polydisperse system. The viscosity of neem oil nano-emulsion formulation (2.11cP) was observed to be half that of the viscosity of raw neem oil. The resulting formulation showed zeta potential of -19.6 mV at a pH of 5. The formulation obtained through PIT showed PDI 0.708. After two months storage, both formulations showed one PDI. The viscosity of formulations was about 72% higher than that of viscosity of raw neem oil, i.e., 4 cP.

5. The antibacterial study showed that in contrast to raw neem oil and ampicillin antibiotic drug, a significant reduction in consumption of nanoemulsion formulations were observed. Thus, formulation showed more effectiveness against human pathogenic bacteria.

Conclusion

Neem oil-based nano-emulsion by using phase inversion composition (PIC) method with Tween 80/Span 80 (1:1 volume ratio of surfactant to oil) at HLB value of 10.7 showed promising formulation with a droplet size below 200 nm. The viscosity of neem oil nano-emulsion formulation (2.11cP) was observed to be half that of the viscosity of raw neem oil. Thus, neem oil nano-emulsion formulation improves the potential to be utilized in various product formulation, e.g., cosmetics, drugs, pesticides, food preservatives, etc. In contrast to raw neem oil and ampicillin antibiotic drug, a significant reduction in consumption of nano-emulsion formulation was observed for antibacterial activity. Beside this, the study was carried out to prepare neem oil-in-water nano-emulsions stabilized by Brij 30 surfactant using the phase inversion temperature (PIT) method at three different temperatures, i.e., 60°C, 75°C and 80°C. Compositions of homogenous phase were identified in the pseudo-ternary phase diagram. Among the total seventeen formulations, three formulations (NB1, NB2 and NB3) were short-listed and characterized for emulsion size and viscosity. The selected formulations showed emulsion size of 348-981 nm in diameter. The volume percentage ratio of Brij30 to neem oil showed significant effect on the droplet size of nano-emulsions. Formulations having lower concentration of Brij 30 displayed a smaller emulsion droplet size (348 nm). The NB3 formulation (4% neem oil, 11% Brij 30 and 85% deionized water) exhibited the highest stability after 60 days of storage. Antimicrobial study showed that in contrast to raw neem and Ampicillin (synthetic drug), NB1 exhibited best result in terms of minimum inhibition concentration (MIC) reduction by 100% against E-coli, P. Aeruginosa and S.Aureus and S.Pyogenus.

Future scope of work

1. Improvement in zeta potential leading to formulation of more stable neem-based nano-emulsion. It needs to be explored in future for PIC and PIT method.
2. This study for neem oil-based nano-emulsion formulation is limited to its application as an antimicrobial agent. However, its application can be extended to different areas such as cosmetics, drugs, pesticides, food preservatives, anti-fungal coating for walls of hospitals, etc.

List of Publications:

Journal: (UGC Care)

1. Safaya M, Rotliwala Y C (2019) Nanoemulsions: A review on low energy formulation methods, characterization, applications and optimization technique. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2019.11.267
2. Safaya M, Rotliwala Y C (2021) Neem oil based nano-emulsion formulation by low energy phase inversion composition method: Characterization and antimicrobial activity. Materials Today: Proceedings. https://doi.org/10.1016/j.matpr.2021.12.478
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Conference Proceedings:

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