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DEVELOPMENT OF HIGHLY STABILIZED NEEM OIL MICROEMULSION SYSTEM: A GREEN APPROACH

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ABSTRACT

The aim of this study is to stabilize the neem oil Microemulsion system as well as the active ingredient i.e azadirachtin which is the most reportedly known active ingredient although this is very unstable in water based formulations, by adding 4% lemon grass oil, stability of azadirachtin in neem oil microemulsion system improves significantly. Thermodynamic and kinetic stability data confirms that the system is highly stabilized without using any co-surfactant. The stability of microemusion system is further confirmed by various physio-chemical studies like droplet size, zeta potencial, surface tension measurement, ph, viscosity, transmittance remain same during accelerated temperature storage. HPLC results also shows that the samples having lemon grass azadirachtin content degradation rate is only 4-5% which is higher in neem ME without lemon grass samples. FTIR further verify the presence of lemon grass Phenolic group stretch at 3350 cm^{-1} Which shows that lemon grass is stabilizing agent as separate ingredient and not involved any chemical reaction with the neem.

KEYWORDS: microemulsion, lemon grass, co-surfactant, thermodynamic stability etc.

INTRODUCTION

Microemulsions (ME) are transparent, isotropic dispersed mixture of oil, water, and surfactant, in combination with medium chains of alcohol as cosurfactant.[1,2] Microemulsion systems are thermodynamically stable and transparent due to dispersed droplets (between 5 and 100 nm) and their polydispersity enhances with the magnitude of the droplets (Norazlinaliza et.al., 2012). Structure of microemulsions is as follows: a large liquid cores [oil in oil in water (o/w) microemulsions, water in w/o microemulsions bordered by a surfactant monolayer that stabilizes the dispersion.[3]

Microemulsions have many distinctive features including high solubilisation capacities for both polar and non-polar compounds, low interfacial tensions, fine microstructures, and spontaneous formation.[4] Microemulsions have shown the ability to protect the pesticide, their controlled release and enhances the solubility of pesticides Due to above mentioned unique features microemulsions become a potential system for both hydrophilic and hydrophobic pesticides. Consequently microemulsion systems have been using in numerous applications of pharma as well as agriculture.

Neem oil extracted from the seeds of Azadirachta indica has versatile medicinal properties, including anti-fertility, antifungal, antibacterial, immunostimulant, antipyretic[5] and acaricidal activities.[6] As an acaricide, neem oil is effective against ticks[7,8], poultry red mites[9] and S. scabiei.[10] There are several types of neem formulation available in the market but due to presence of solvent and other inert ingredients make the active ingredient in the formulation unstable. Neem oil microemulsion stability during storage and application is very crucial due to the presence of azadirachtin content and other constituents of insecticidal property. For long period stability different combination of surfactants used by trial and error method. Along with surfactant an alcoholic group containing co-surfactants are added to further reduce the interfacial tension of oil and water. Neem oil in combination with essential oil also gives improved solubility and stability. Lemon grass is the important essential oil which can give not only improve stability but also increase the permeation into the plant tissues. Moreover, the combination of neem oil and lemon grass results synergistic effect.

The aim of this study is to develop neem microemulsion without the use of alcoholic co-surfactact in neem oil in comparision of lemongrass oil in specific proportion. This systematic study involves the preparation of neem oil microemulsion with essential oils which act as a synergist and co surfactant. The prepared formulation is less time consuming and are in great in demand in the pharmaceutical and agrochemical industries. Further, not only oil droplet size is reduced in the prepared microemulsion but also neem oil percentage is more as compared to earlier developed neem microemulsions. this improved properties results into good absorbtion rates and give superior and long lasting results.

MATERIAL AND METHODS

Neem oil, Lemon grass oil, Surfactants and water

Methods

1. Preparation of emulsifier system

Atlox 4896 and nonylphenol 13 were used as compound surfactants. Both surfactants were mixed by glass rod at the weight ratio of 1:3.

2. Formation of neem microemulsion and phase diagrams

Emulsifier system were mixed with distilled water until it get into transparent solution. Neem oil in combination of lemon grass in 60:40 percent ratio were added dropwise under stirring at 30◦C. After addition of all the contents left for stirring till the mixer form homogeneous and stable solution.

Physio-chemical analysis

Chemical analysis

1. Thermodynamic stability

In thermo dynamical stability the formulated emulsion was stored at hot and cool both temperature i.e. 4°C and at 45°C for 48 h and freeze and thaw temperature i.e -21°C and room temperature for 48h. After storage the formulations were centrifugated at 3600 rpm for 30 minutes.

2. Kinetic stability

In kinetical stability emulsion was stored at room temperature for prolonged storage time. Then observed phase separation or creaming or cracking.

3. Droplet Size Measurements and Dilution Stability

Droplet size determination was done by using dynamic light scattering (Malvern Zetasizer Nano S, Malvern, UK). This measurement determines the dilution stability of the Neem microemulsion at various concentration of hard water in 200ppm. The samples were prepared in distilled water and droplet size was performed with various time at 25 ± 0.5 °C. The particle size measurements were done in triplicates.[11]

4. Centrifugation Assay

Microemulsion sealed in centrifuge tube and stored at different temperature i.e. 4,18,20,54°C. These stored samples were then centrifuged for 5 h at 4000 rpm and for 20 min at 10,000 rpm in the centrifuge test.

5. Zeta potential measurements

Zeta potential was determined by zeta sizer (Malvern Zetasizer Nano S, Malvern, UK). Zeta potential of the microemulsion measures electrophoretic mobility of droplets in an electric field. For this measurement samples were diluted in 10mM aqueous NaCl (1:15), mixed homogenously for complete dispersion and then analysed in triplicates.[12]

Physical analysis

1. Appearance

The physical appearance of the microemulsion formulations was checked by visual examination of the formulation under light alternatively against white and black backgrounds and turbidity were checked by turbidity matter.

2. pH measurement

The pH is of the micro emulsion was measured by ph meter at room temperature. For measuring pH of the formulation one gram of formulation is weight and then diluted with 10 ml water and mixed properly. analysis was performed in triplicates without diluting the samples.

3. Viscosity

The viscosity was determined by viscometer (by brookfeild engineering laboratory). Viscosity analysis was done at room temperature. Viscosity measurement was done in triplicates.

4. Surface tension

Surface tension is determined by ring Tensiometer (by eliko marketing ltd, model DST-30). Surface tension measurement was done at room temperature. Three readings of surface tension measurement were recorded with reference to water.

5. Transmittance

% Transmittance was checked against distilled water using UV-visible spectrophotometer at 650 nm (UV-1800 double beam spectrophotometer, Shimadzu, Japan) by dilution of 1 ml of the formulation with distilled water up to 100 ml (100 times) and as such.

6. Functional characterization

FTIR spectra of neem oil, lemon grass and Neem ME were recorded between 4000 and 500 cm^{-1} using Perkin Elmer spectrum-1 FTIR.

7. Chemical characterization

Active ingredient of all the samples of neem ME with and without lemon grass was quantified by the HPLC (model-Perkin Elmer Series 200 HPLC) as approved in BIS method 14299: 1995. The operating conditions were as follows: stationary phase: column C-18, run time-60 min, mobile phase: Acetonitrile/Water (35:65) at 1.2ml/min, detector wavelength, 214nm, and injection volume is 10ul.

RESULTS AND DISCUSSION

1. Thermodynamic and kinetic stability

The neem oil microemulsion samples with lemon grass oil are thermodynamically stable solutions, with no phase sepration, creaming under different temperatures without lemon grass neem oil microemulsion shows phase separation. This shows that prepared microemulsion with 12% neem oil is stabilized in presence of lemon grass as co-solvent and act as stabilizing agent.

Table 1: Appearance of microemulsion at various conditions.

	at freeze and thaw cycle	**at hot and cool cycle**	**at 54 C**	**Centrifuge**	**Dispersion**
FI	Turbid	Turbid	Stable	Sedimentation	Not dispersed
F2	Stable	Stable	Stable	No sedimentation	Fully dispersed

F1. Neem microemulsion without lemon grass

F2. Neem microemulsion with lemon grass

2. Kinetic stability

Samples of ME with lemon grass were stable after 14 days storage at 54 C and without lemongrass sample were turbid and change into macro emulsion having particle size in microns range. This shows that after prolonged storage of ME with lemon grass gives stability and maintains the size of droplets in Nano range which may be act as stabilizing agent also during storage.

3. Droplet size measurement

The droplet size is very important in microemulsion estimation. The stable microemulsion samples with droplet size less than 100 nm are stable as compared to samples with droplet size more than 100nm as large droplets results into creaming.[13] Small droplet size maintain the sufficient Brownian motion of droplets which reduce the gravitational force and the microemulsion remain in the dispersed phase without any separation because the attractive forces are weak in these types of systems.[14]

Table 2: droplet size distribution of different formulations.

S.N	Formulation code	Composition Neem oil+ lemon grass+ surfactants: Co-surfactant	Mean Droplet Size (D Nm) Mean± S.D. (N = 3)
1.	F1	12:0:20:3	24.13 ± 1.4
2.	F2	12:2:20:1	20.24 ± 1.2
3.	F3	12:4:20:0	18.30 ± 1.6

The formulation code F3 of neem oil with lemon grass having particle size is less as compared to F1 and F2 as shown in table. 2. The F3 Formulations with lemon grass as co-solvent which maintain the Brownian motion of ME droplets in dispersed phase and weaken the attractive forces.

4. Zeta potential

Zeta potential is the measure of surface charge over emulsion droplet. Surface charge of ME depends on surfactant and co-surfactants involved in formulation. The zeta potential gives the measurement quantity for micro emulsion stability. The higher zeta potential values signify more repulsion between two droplets. According to DLVO theory electric repulsion of droplets will stabilize microemulsion and for stable micro emulsion it should be in between ±10 or ±30 (Rong, L.2000). The zeta potential values are -10 mv and -2 mv for neem ME with and without lemon grass respectively. These values shows that neem ME with lemon grass has more electric repulsion due to large electrostatic charge i.e -10mv as compared to neem ME without lemon grass i.e. only -2 mv which indicate that these samples particles will remain in deflocculated state and stabilize the sample for longer period of time.

Table 3: Zeta potential values of neem ME with different compositions.

Formulation Code	Composition Neem oil+ lemon grass+ surfactants: Co-surfactant	Zeta potential values	pH
F1	12:0:20:3	**-6.68**	**6.7**
F2	12:2:20:1	**-8.50**	**6.9**
F3	12:4:20:0	**-10.60**	**6.9**

5. Physical appearance

The ME formulation samples with Lemon grass were clear and transparent. The trend of physical appearance is shown in table 4.

Table 4: Variation in physical appearance of neem microemulsion system with different compositions.

Formulations	12 neem oil	12 neem oil + 2 lemon grass	12 neem oil + 4 lemon grass
Appearance	**Turbid**	**Slightly turbid**	**Clear and transpared**
			

6. Transmittance

Clarity of microemulsion was checked by % transmittance. % transmittance of the sample with lemon grass was 98.17%. This transmittance confirms that the ME with lemon grass was clear. The transparency is due to lesser droplet size so, lemon grass act as co-surfactant which will reduce the droplet size and keep the droplet size in Nano range results into clear and transparent ME formulation.

Table 5: Percent transmittance at 650 nm.

Composition Neem oil+ lemon grass+ surfactants: Co-surfactant	Samples	% T at 650 nm	After dilution with water	% T at 650 nm
12:0:20:3	12 neem oil	-	Turbid	-
12:2:20:1	12 neem oil + 2 lemon grass	98.1±0.14	Slightly turbid	97.8±0.12
12:4:20:0	12 neem oil + 4 lemon grass	99.41 ± 0.16	Clear	99.21± 0.13

7. Viscosity

The viscosity of the neem microemulsion system without lemon grass is more as compared to the samples with lemon grass. Viscosity of the microemulsion system is depending upon oils and surfactants. Lemon grass has low fatty acid content and less viscous, due to this it will be reduce the viscosity of the microemulsion as well.

Table 6: Viscosity measurement at 30°C.

Formulation	Composition Neem oil+ lemon grass+ surfactants: Co-surfactant	Viscosity (cp) at 30 0C	pH
F1	12:0:20:3	0.8820 ± 0.02	**Neutral**
F2	12:2:20:1	0.8980 ± 0.016	**Neutral**
F3	12:4:20:0	0.8972 ± 0.02	**Neutral**

8. Surface tension

Surface tension measurement is very important surface active property of liquid formulations. Surface tension governs the wetting and spreading properties of microemulsion system. Low surface tension represents fast spreading of liquid droplets on hydrophobic surfaces.

Table 7: Surface tension measurement of neem ME with lemon grass.

Formulation	Composition Neem oil+ lemon grass+ surfactants: Co-surfactant	Surface tension(in N/m^2)
F1	12:0:20:3	45
F2	12:2:20:1	37
F3	12:4:20:0	30

Surface tension of lemon grass containing microemulsion formulation has lower i.e 30 N/m^2. This shows it has good spreading as compared to Neem ME without lemon grass.

Active ingredient stability by HPLC and FTIR

The amount of azadiractin content analysis by HPLC, degradation is slightly fluctuated i.e. 6-7% from 600 ppm in neem ME with lemon grass as co-surfactant after storage at different temperatures and prolonged duration. The stability of active ingredient in presence of lemon grass oil may be due to the phenolic compounds present in lemon grass oil. So, lemon grass is playing here dual role, act as stabilizer as well as co-surfactant.

FTIR spectra of neem oil as an active ingredient with lemon grass, surfactant and water as an inert ingredient were recorded. Microemulsion was prepared by physical mixing of the neem oil and as an active ingredient along with surfactant, with co-surfactant and water as an inert ingredient.[15] in oil in water Microemulsion formulation. Characteristics sharp band of neem oil (1642, 2928) did not alter in FTIR as in fig.1 there by it indicating absence of chemical interaction between oils and other inert ingredients.

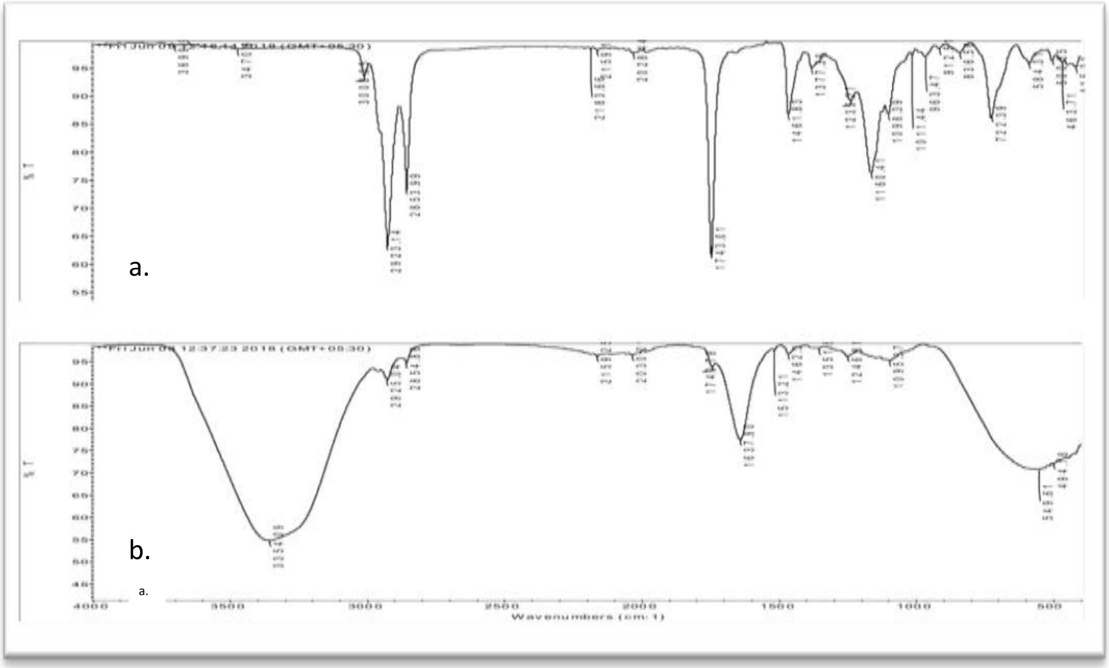

Fig1:a. FTIR of neem oil; B. FTIR on neem oil + lemon grass oil.

CONCLUSION

Microemulsion is the thermodynamically stable system but in neem oil microemulsion system main problem is stability after adding definite proportion of neem oil i.e. after 5%, the microemulsion system will start converting into emulsion form. Co-surfactant is very essential for the stability enhancement of the system but presently used co-surfactants have some carcinogenic properties so, it should be replaced with the some other natural constituents which makes the system completely green. Co-surfactants are basically belongs to alcoholic groups in this study lemon grass is playing the role of co-surfactant as well as for stability enhancement. Because lemon grass oil have lots of phenolic ingredients like borneol, estragole, methyleugenol, geranyl acetate (3,7-dimethyl-2,6-octadiene-1-ol acetate), geraniol (some species higher in this compound than citral). The present study improves the loading capacity of neem oil in ME i.e. upto 20%. This study can be applied for making the other botanical based microemulsion formulation and they will remain green and natural without using any chemical constituents. This formulation will further improve the stability as well as activity and enhances the organic farming output.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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