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Ultrasound assisted citronella oil in water nanoemulsion and comparison with conventional methods

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This study involved the acoustic cavitation aided process intensification of citronella oil-based nanoemulsion with varying process parameters. A citronella oil (10 wt. %) in water emulsion was prepared at optimized parameters such as sonication time of 20 min, surfactant concentration of 7.5 wt. % of the total emulsion with (Hydrophilic-lipophilic balance) HLB value of 12 and power amplitude of 35% (of the total power of 750 W). The prepared emulsions stability was assessed over visual observation and kinetic stability of the emulsion after formulation with 7, 30 and 90 days' time interval term as long-term stability reported as a fraction of phase separation in percentage (f (%)). The ultrasonically prepared emulsion was found to more stable with the mean droplet diameter (MDD) of 22-23 nm, whereas, conventionally prepared emulsion get separated and creamed within the day as well as formulation required more process time and energy dissipation.

Keyword: Citronella Oil, Emulsion Inversion Point, High-speed rotor-stator, Kinetic Stability, Nanoemulsion, Ultrasonication

The emulsion has a natural tendency to decrease the interfacial area among two immiscible phases, and this is the reason why it is treated as a thermodynamically unstable system[1,2]. The emulsion contains immiscible liquids, distributed in each other[3]. Emulsions are classified based on which phase constituent as the disperse phase-in-continuous phase:W/O (dispersed phase: water, continuous phase: oil) and O/W (dispersed phase: oil,continuous phase: water)[4,5]. Also, there aremultiphase emulsions, for example, O/W/O (dispersed phases: oil and water, continuous phase: oil) and W/O/W (dispersed phases: water and oil, continuous phase: water)[6]. An emulsion can be categorized based on the sizeof the dispersed phase. So, they are classified as macroemulsion, the average size of a dispersed liquid ranges from 0.1µm to 50 µm; microemulsion contains droplets of less than 300 nm whereas in nanoemulsions the MDD of dispersed phase range from 10-100 nm[7].

The methods of preparation of emulsions are classified based on energy requirement, low (chemically induced generation) and high (mechanically induced generation) energy methods. The chemically induced method involved a phase inversion method driven by the ouzo effect[2,8]. A mechanically induced method includes microfluidization[9] and high-shear rotor-stator[2,8,10]. The higher energy emulsification method needs high mechanical energy to enhance interfacial area[10], whereas the low energy emulsification method requires more surfactant concentration and appropriate HLB or surfactant and co-surfactant combination.

Although high-shear rotor-stator and microfluidization can be used to supply mechanical energy, these devices are energy-intensive and cannot have control on the size of dispersed phaseas the majority of energy is used for the creation and motion in the continuous phase which is not essential for the creation of the new surface. Cavitation is an alternative and yet effective technique to create a stable emulsion. The mechanical effect of cavity collapse in both acoustic and hydrodynamic cavitation is the creation of local zones of microturbulence. Also, the high-pressure pulse generated by cavity collapse generates pressure shock waves which spread the dispersed phase which further gets stabilized by the Laplace pressure (2γ/r, thus as r, the drop radius, reduces, higher pressures are required to disrupt these smaller drops), as expressed below.

$$\Delta P = \frac{2\gamma}{r} \qquad ...(1)$$

where ΔP is a pressure difference, r is the bubble radius and γ is the surface tension of medium.

In recent years, cavitation has been successfully used for intensification of different physicochemical transformations such as crystallization, extraction, wastewater treatment, de-polymerization, and water disinfection[11]. It is a sequential process of formation, development, and collapses of the voids which release a significant amount of energy (energy density of the order of 1 to 10^{18} kW/m^3) in a small area over an extremely small interval of time i.e. millisecond to microseconds[11,12]. Cavitation occurs at millions of location and creates very high pressure and temperature locally with the overall ambient condition[1]. The key advantages of cavitationally assisted emulsification over the conventional emulsification methods are low energy consumption, less or no use of stabilizers, excellent control on the size ofthe dispersed phase. The efficacy of cavitation-assisted emulsification mainly depends on the optimization of the process parameters (sonication time/ energy input, HLB, surfactant concentration and, oil/water ratio).

Cavitation induced emulsification is done by the disruption of the dispersed phase to form primary droplets in the first step, and in a second step, due to localized intense turbulence and shear forces produced by ultrasonication can result in theviolent and asymmetric collapse of the cavity onto primary droplet surface which results into further breakage of primary droplets into nanoscale droplets[13]. The surfactant stabilizes these nanoscale droplets, surfactant also helps in the breaking process of primary droplets by lowering the interfacial tension, as a result of this, the shear required to break up a drop can be reduced[14].

This work involved the comparative study of the preparation of emulsion by using the high-speed rotor-stator method, emulsion inversion point, and acoustic cavitation-assistedmethod. The objective of this work is to study as well as find out the optimized parameters like energy dissipation (by varying amplitude and time), surfactant concentration and surfactant HLB for the preparation of highly stable nanoemulsion.

Experimental Section

Materials

Citronella oil was purchased from Sigma Aldrich, India, whereas Span 80 (Sorbitan mono oleate) and Tween 80 (Polysorbate 80) was purchased from S. D. Fine, India. Doubled distilled water produced from Millipore system was used in all experiments.

Emulsion preparation method

Acoustic cavitation was used to generate the citronella oil nanoemulsion. Process variables were optimized considering the size of the dispersed phase and the emulsion stability. The similar emulsion was generated using the inversion point method (chemical/ low energy method) and a high-speedrotor-stator method (mechanical/ high energy method).

Acoustic cavitation

The emulsions were generated by acoustic cavitation (20 kHz and Probe model VCX 750, Sonics and Materials) using Span 80 and Tween 80 nonionic surfactant. A titanium probe of 13 mm diameter was used to irradiate 100 mL of the solution inside a glass beaker (Fig. 1). Emulsification was carried out at 20-25°C using a recirculating cooler (Amar equipment, Mumbai). The sonication time (ultrasonic energy input), total surfactant concentration and HLB values were varied in the experiments as shown in Table 1, and the effects were investigated. After sonication, the emulsion, thus generated, was stored in 100 mL glass vials for further analysis of mean droplet diameter, and monitoring of long-term stability. All analysis of the emulsion samples were conducted at 25°C.

Inversion point method

The mixture of Tween 80 and water was added dropwise into a mixture of Span 80 and citronella oil (10 wt. %). The mixing was carried out in 100 mL beaker of inner diameter 50 mm under vigorous agitation at 2000 rpm using a magnetic needle of length 30 mm at room temperature for 12 hr[3]. The concentration of the surfactant and HLB value varied from 5 wt. % to 10 wt. % and 9 to 14 respectively.

High-speed rotor-stator method

The raw pre-emulsion was made by adding a mixture of water and Tween 80 (water phase) into a mixture of citronella oiland Span 80 (oil phase) dropwise under light stirring using a magnetic stirrer. Then, the prepared pre-emulsion was exposed to high-speed rotor-stator (Silverson Machines, MA). The device is equipped with a single rotor of outer diameter 12.3 mm and has four blades that rotate with the same frequency within close-fitting screens. The stator screen of the inner diameter is equal to 13.3 mm and an outer diameter equal to 19 mm and has

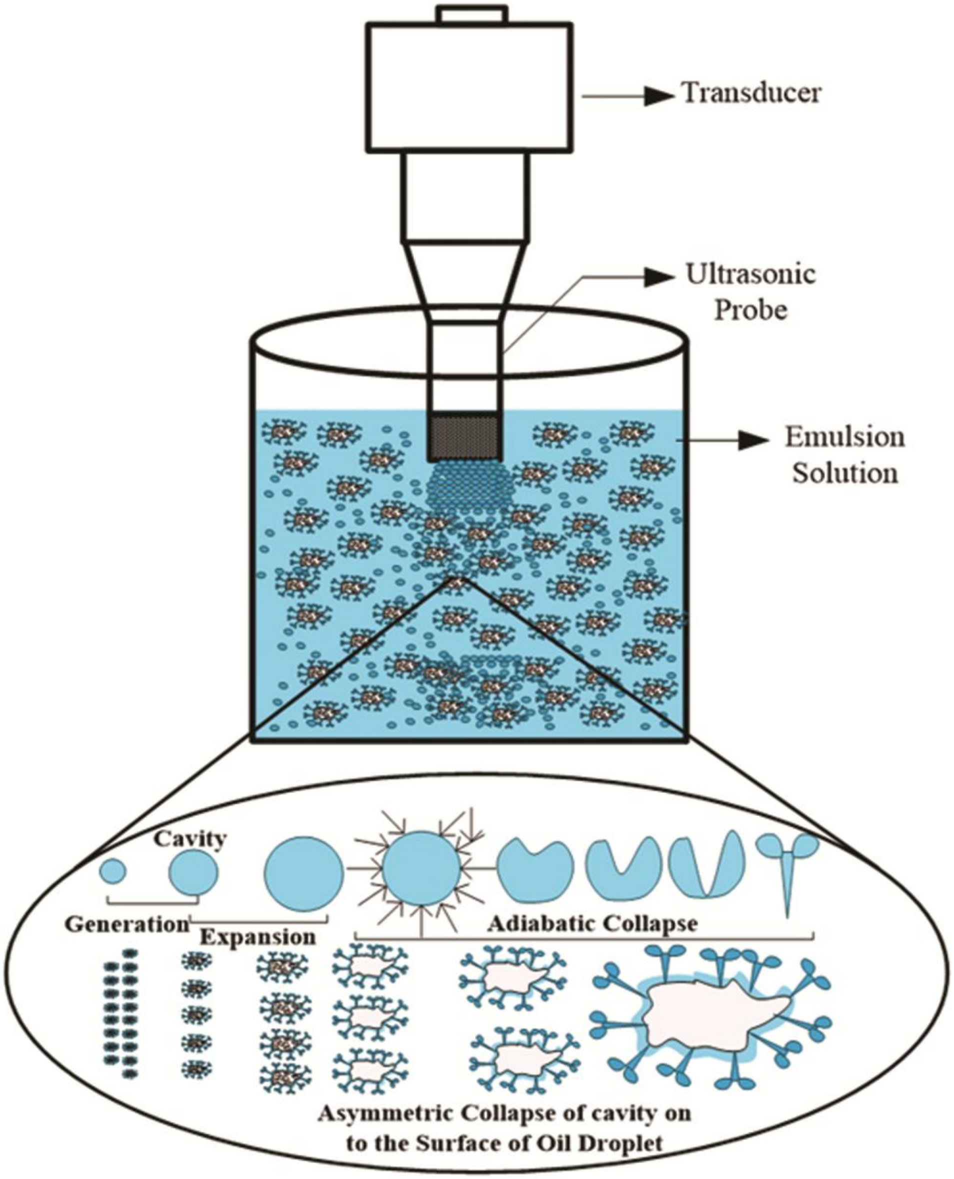

Fig. 1 — The schematic of the experimental set up of ultrasonication assisted emulsification method.

12 cylindrical holes each of thickness 2 mm. The rotor-stator gap is equal to 0.5 mm.The emulsion was processed at a rotor speed of 6000 rpm for a varying time ranging from 15 to 60 min[15] at 20-25°C in a 100 mL jacketed beaker of inner diameter 50 mm. The recirculating cooler ((Amar equipment, Mumbai) was used to maintain the required temperature.The concentration of the surfactant and HLB value varied from 5 wt.% to 10 wt. % and 9 to 14 respectively.

Emulsion characterization

Droplet size characterization

A dynamic light scattering (DLS) method was used to measure the mean droplet diameter and the zeta potential of the emulsion dispersed phase. The electrophoretic mobility at 25°C was measured to find out the zeta potential of oil droplet and expressed in mV.

Emulsion morphology

The surface morphology (size and shape) of the dispersed oil phase was observed using scanning electron microscopy (Quanta 200 ESEM).The emulsion was diluted with distilled water, dried on a silica wafer and sputter-coated with gold before the examination.

Stability of emulsion

Kinetic stability

The kinetic stability of the emulsions was assessed using thermal stress and centrifugation and tests based

Table 1 — Ultrasonication assisted emulsification process parameter optimization (a) Effect of surfactant concentration, (b) effect of sonication time, (c) Effect of power amplitude on the basis of MDD and % *f*.

(a)

Amplitude (%)	Time (min)	Oli Phase	HLB	Surfactant Concentration 5%; Water Concentration 85 %		Surfactant Concentration 7.5%; Water Concentration 82.5 %		Surfactant Concentration 10%; Water Concentration 80 %	
				Droplet Size (nm)	Fraction of Emulsion Phase (R) (%)	Droplet Size (nm)	Fraction of Emulsion Phase (R) (%)	Droplet Size (nm)	Fraction of Emulsion Phase (R) (%)
40	15	10%	14	32.12	100	26.46	100	21.78	100
			13	28.62	100	23.17	92	17.05	85
			12	43.56	100	28.20	86	20.00	80
			11	46.17	92	30.47	65	32.24	32
			10	63.52	90	33.35	52	47.16	21
			9	83.98	81	75.08	23	80.71	15

(b)

Amplitude (%)	Time (min)	Water Phase	Oli Phase	Surfactant	HLB	Droplet Size (nm)	Fraction of Emulsion Phase (R) (%)
40	5	82.5%	10%	7.5%	13	48.50	94
	10					31.50	93
	15					23.17	92
	20					23.10	98
	25					23.50	96
	30					38.10	94

(c)

Amplitude (%)	Time (min)	Water Phase	Oli Phase	Surfactant	HLB	Droplet Size (nm)	Fraction of Emulsion Phase (R) (%)
22	20	82.5%	10%	7.5%	13	82.9	91
25						76.8	91
30						45.1	93
35						22.8	99
40						23.1	99

onthe change in (mean droplet diameter) MDD of the dispersed phase and percentage of phase separation (*f*). The 50 mL of the emulsion was centrifuged in a conical centrifuge tubeat 5000 rpm for 15 min. The thermal stress testwas performed by exposing 50 mL of prepared emulsions to high temperature (at 40°C, 60°C, and 80°C) for 15 min. These samples were tested using DLS to check the effect of the centrifuge and thermal stress on MDD. The percentage phase separation (*f*) of exposed emulsion samples were measured after 24 h using the following formula:

$$f\left(\%\right)=\left(1-\frac{h_c}{h_t}\right)\times 100 \qquad \text{... (2)}$$

Where f (%) is a fraction of the emulsion phase, h_c is a height of the top creamed layer,and h_t is a height of the emulsion system. The f(%) as 100% means no separation which represents the stable emulsion.

Intrinsic stability

The intrinsic stability also called as the long-termstability of the emulsion was estimated by checking the MDD and f (%) with respect to time. The prepared emulsion was stored in calibrated measuring cylinder up to 90 days, and MDD and f (%) were recorded at time intervals (7, 30 and 90 days).

Results and Discussion

Effect of surfactant concentration and HLB

Initially, emulsion samples were generated using acoustic cavitation (sonication time of 20 min; 40 % of 750 W) at various surfactant concentrations and HLBs, and their effects of on MDD and emulsion stability were studied. Emulsions were generated with constant (10 wt.%) oil phase, whereas aqueous phase (85wt.%, 82.5 wt.% and 80wt.%) and surfactant concentration (5wt.%, 7.5 wt.% and 10wt.%) were varied. Similarly, the emulsion samples were generated using various blends of Tween 80 (HLB of 15) and Span 80 (HLB of 4.3) surfactant; and their effect on MDD and f (%) were studied. Results are shown in Fig. 2 in terms of MDD and f (%). HLB value of the mixture was calculated using the following formula:

$$HLB_{mix}=15\times m_T+4.3\times m_S \qquad \text{... (3)}$$

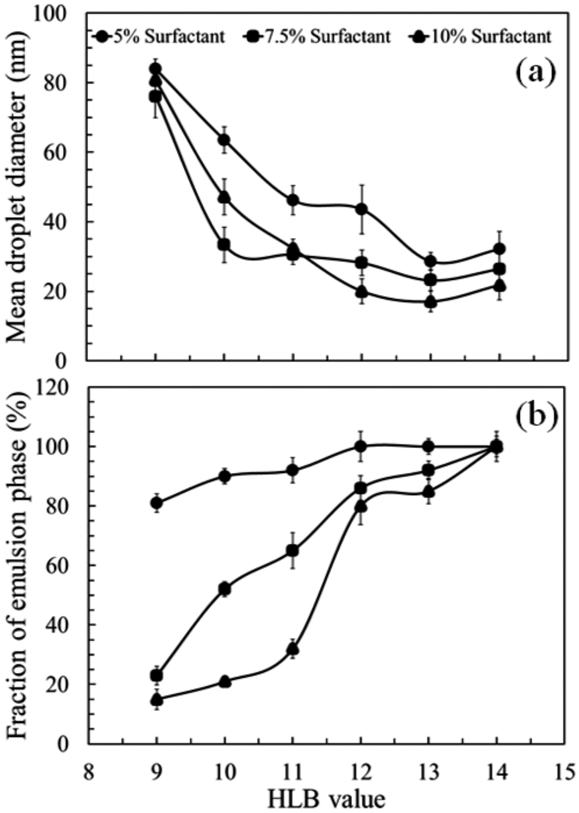

Fig. 2 — Effect of surfactant concentration and HLB (a) on MDD (nm) (after 8 days) (b) on fraction emulsion phase (*f*) (%) (After 6 months) prepared using acoustic cavitation (sonication time of 20 min and 40 % of the maximum power of 750W).

where m_T = mass percentages of Tween 80 and m_S mass percentages of Span 80.

The MDD of the dispersed phase of the emulsion reduces as the surfactant concentration increases as shown in Fig. 2 (a), due to the creation of thick surfactant film around the oil droplet[3,16]. This thick surfactant layer will offer superior steric stabilization against creaming and also enhance the interfacial area and diminishes the interfacial tension between the aqueous phase and oil which results in a reduction in MDD of the dispersed phase of emulsion[8,17–19]. The fraction of the emulsion phase goes on decreasing (unstable emulsion) as a concentration of surfactant increases as shown in Fig. 2 (b). So from these results, it was found that the significant decrease in MDD with the substantial stability of the dispersed phase of the emulsion waso btained at surfactant concentration till 7.5 wt. % of the total emulsion.

It was found that the surfactant mixer having HLB 13 is optimum to prepare a stable emulsion. As discussed earlier, the minimum MDD of the dispersed phase found for the surfactant concentration of 7.5 wt. % of the total emulsion. It can be seen from Fig. 2 (a) that, MDD decreases from HLB 9 to HLB 13 and above HLB 13 it again slightly increases (at all surfactant concentrations). Several researchers have reported that the mixture of surfactant mixtures produces amore stable emulsion having the minimum size as compared to one surfactant[20–23]. The appropriate mixture (optimum HLB) of surfactants can form a stable layer around the droplet of oil and strengthen the interfacial film to maintain thestability of droplets[17,23,24]. In the mixed surfactant (hydrophilic and lipophilic surfactant) system, they align with each other imparting the strength to the surfactant film through hydrogen bonding[21,23]. In this study, a surfactant blend with 81.3 wt. % and 18.7 wt. % with respect to the total surfactant concentration (7.5 wt. % of the total emulsion) of Tween 80 (HLB = 15) and Span 80 (HLB = 4.3) respectively were found tobe optimum for achieving the stable citronella oil in water nanoemulsion.

Effect of ultrasonication parameters on emulsification

Ultrasonic emulsification mainly involved two processes. In the first step, the dispersed phase gets erupted into the continuous phase due to the interfacial waves produced by the acoustic field[25]. In a second step, due to localized strong turbulence and shear forces produced by ultrasonication can result in theviolent and asymmetric collapse of thecavity and causes micro-jets which break the main droplets[13]. These droplets can be stabilized using a surfactant which prevents the agglomeration of freshly formed droplets, it also helps in the breaking process of primary droplets by lowering the interfacial tension between oil and an aqueous phase, so the amount of shear and energy required to break up a drop can be reduced[26].

Optimization of sonication time

To find the optimum sonication time (amount of energy dissipated) for the preparation of stable and nanoscale emulsion, the emulsions were formulated for different sonication time varying from 5 to 30 min at constant power amplitude (40 %), 0.1 oil fractions and optimized surfactant concentration of 7.5 wt. % of HLB 13.Different sonication time effects (energy input in kJ) on the MDD and *f* (%) of the emulsion are shown in Fig. 3 (a). It was observed that as thetime of sonication increases from 5 min. to 20 min., the diameter of emulsion droplet goes on decreasing from 48.5 nm to 26.1 nm and the emulsion phase fraction goes on increasing from 97 to 100%. This is because

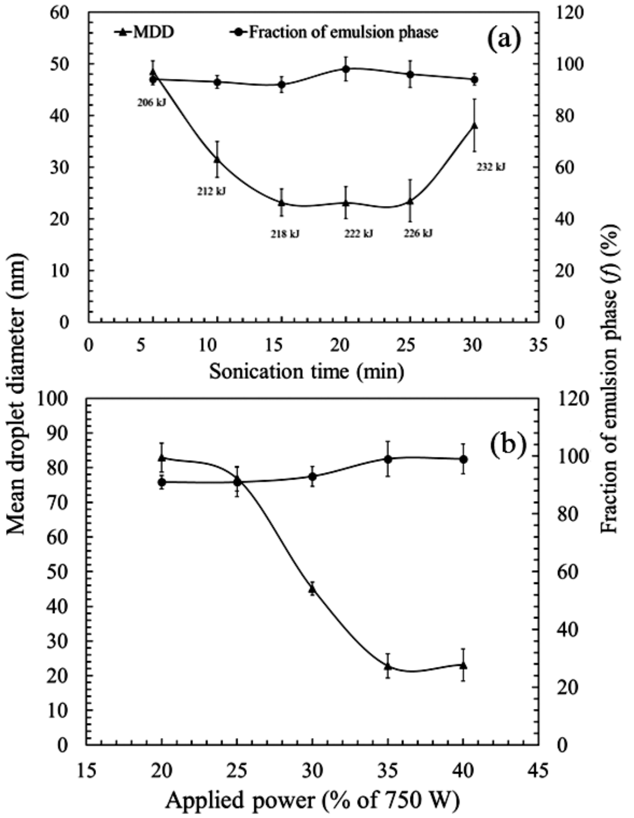

Fig. 3 — (a) Effect of sonication time on MDD (nm) (after 8 days) and a fraction of emulsion phase (f) (%) (after 6 months) at constant power (40 % of the maximum of 750 W); (b) effect of applied power on MDD (nm) (after 8 days) and a fraction of emulsion phase (f) (%) (after 6 months) for a constant sonication time of 20 min.

of the increase in the temperature of the system due to sonication time. The increase in the temperature reduces the interfacial tension and viscosity of the system[27,28]. This reduction in interfacial tension and emulsion viscosity can enhance the cavitational, results in improving the emulsification process[27,29]. It observed that the MDD was reduced with respect to sonication time till 20 min, further, sonication results into the slight increase in the MDD, because of breakage of the surfactant layer due to more sonication, result into coagulation which has also decreased the emulsion phase fraction from 100 to 94% (as shown in Fig. 3 (a)). So, the optimized time of sonication was observed to be 20 min., after whichno further reduction in MDD of the dispersed phase of the emulsion was observed.

Effect of sonication power

The sonication power plays an important role in the stability and MDD of the dispersed phase. So, the emulsions were prepared using different sonication power at optimized sonication time (20 min.), 0.1 oil fractions and 7.5 wt. % of surfactant concentration with an HLB value of 13. From Fig. 3 (b) it was confirmed that as the irradiation power increases from 20 % to 35 % (of 750W), the MDD decrease from 82.9 nm to 27.5 nm and the emulsionphase fraction goes on increasing from 91 to 99%. This is because of that; assonication power increases, the pressure amplitude also increases which enhances the cavitation phenomena that increase the intensity of cavity collapse and a number of events also[3,25]. Also at high sonication power, the energy dissipation is more which increases the temperature of the system and ultimately reduces the interfacial tension and viscosity betweenthe aqueous phase and the oil. As the irradiation power increases above 35 % (ofthe actual power of 750 W), the MDD increases and decreases the emulsion phase fraction. This effect is called “over-processing” produced by the coalescence of emulsion droplet at the higher shear rates[26,30].

Morphology of emulsion

The surface morphology of citronella oil in water emulsion was visualized using scanning electron microscopy (SEM) as shown in Fig. 4. The surface morphology of the prepared emulsion was found to be spherical, diameter ranging from 20-30 nm. The MDD of the dispersed phase of the emulsion observed in SEM analysis matches the data obtained using particle size analyzer.

Stability of emulsion

Emulsion stability evaluation is very important before its applications. In the present study, the stability of the emulsion was assessed using kinetic stability analysis and long-term stability and expressed in *f* (%).

Kinetic stability analysis

The prepared emulsions were exposed to high centrifugal force and high temperature to estimate its kinetic stability. The Brownian motion of the dispersed phase will increase due to centrifugal force and high temperature[31]; which may result in coalescence. The kinetic stability of emulsions was evaluated based on MDD and *f* (%). The ultrasonically (at optimal parameters: surfactant concentration of 7.5 wt. % of the total emulsion with an HLB value of 13, 20 min sonication time, power amplitude of 35%) and conventionally prepared emulsions were exposed to the high temperature (40°C, 60°C, and 80°C) and the centrifugal force of 2800 g (5000 rpm for 15 min.) after

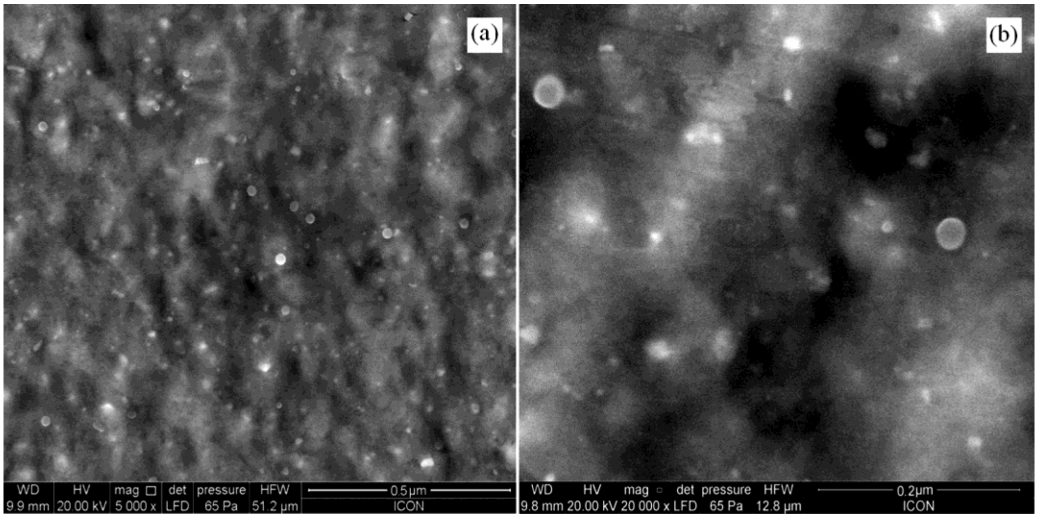

Fig. 4 — SEM image of Citronella oil emulsion at (a) 5000X, (b) 20,000X.

Table 2 — MDD and % *f* of nanoemulsion produced with the optimum condition and after kinetic stability tests.

		MDD (nm)			% *f*	
After Formulation		22.8			99	
Centrifugal Test			5000 rpm for 15 min			
After 7 Days		22.9			99.0	
After 30 Days		23.1			98.8	
After 90 Days		23.4			98.1	
Thermal Stress	40°C	60°C	80°C	40°C	60°C	80°C
After 7 Days	22.8	22.7	22.8	99.0	98.7	99.0
After 30 Days	22.8	22.6	22.6	99.0	98.8	98.8
After 90 Days	22.7	22.5	22.7	98.9	98.8	98.6

7, 30, and 90 days of formulation and their kinetic stability was checked based on variation in MDD and *f* (%).

Centrifuge test

Table 2 shows the variation MDD and *f* (%) of the emulsion prepared at optimal condition after exposure to centrifuge test at 5000 rpm for 15 min (centrifugal force of 2800 g). It was observed thatthere was no creaming or flocculation and no major change in MDD even after 90 days of storage. However, the emulsion prepared below the optimum condition showed some breakdown. From these results, it was confirmed that the combined effect ofoptimal ultrasonication parameters and surfactant (concentration and HLB) retain emulsion stable under centrifugal stress. Similarly, Saharan and Carpenter reported, the effect of the centrifuge on to the ultrasonically prepared Muster oil-in-water emulsion [32]. They have observed that no significant change in MDD and *f* (%) after centrifuge treatment and the emulsion was found to be stable for more than 90 days. Based on the emulsion kinetic stability test, it can be concluded that the emulsion prepared at optimal conditions are stable. This is because of the stress and turbulence created by the cavitation at optimum condition is very much effective for the eruption of the dispersed phase into fine droplets and coverage the newly formed fine droplet with a surfactant respectively, which results into a highly stable emulsion.

Thermal stress test

Similarly, the emulsion formulated at optimum ultrasonic parameters and surfactant was subjected to thermal stress after 7, 30 and 90 days at 40°C, 60°C and 80°C to confirm the stability. From the results,as shown in Table 2, it was confirmed that the emulsion formulated at optimum conditions could sustain high temperature. This confirms that at the optimum condition, the amount of energy dissipated by ultrasonication and surfactant concentration can maintain the dispersion and stability of emulsion under the heating condition.

Long-term stability

Fig. 5 shows the MDD and *f* (%) of the emulsion prepared at optimum condition. The emulsion prepared at optimum parameters was stored for 3 months to check any variation in MDD and *f* (%).

From Fig. 5, the MDD and *f* (%) of the emulsion were unchanged which means that the optimum ultrasonic parameters and surfactant gives excellent stability against coalescence and creaming. Thus, from the present study, it is confirmed that the ultrasonicationis novel and an efficient method for the formulation of stable sustain at high nanoemulsions.

Evaluation of ultrasound-assisted emulsification with conventional methods

The emulsification process involved the simultaneous occurrence of re-coalescence and break-up of the dispersed phase of the emulsion. The intensity of the applied shear between the interface of the aqueous phase and oil decides the frequency of occurrence of these two opposing processes. If the applied shear exceeds the Laplace pressure within the dispersed phase, then the droplet breakup process occurs more than droplet re-coalescence[33]. The efficiency of droplet breakup process depends on the nature of the shear and can be enhanced by using suitable surfactant[34]. The surfactant helps to lower the interfacial tension between two phases, which results in easy deformation of the disperse phase. The droplet re-coalescence can also be minimized if the surfactant forms the film on the surface of freshly formed droplets. The emulsification process that involved the frequency of occurrence of droplet breakup process greater than droplet re-coalescence and high turbulence for complete coverage of the freshly formed surface of the droplet with a surfactant, can resultin formation very stable nanoemulsion[35].

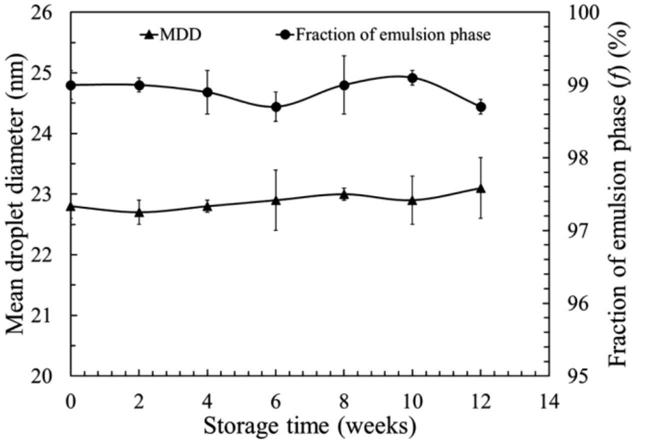

Fig. 5 — Temporal evolution of mean droplet diameter and fraction of the emulsion phase of emulsions produced using optimized conditions.

The similar emulsion has been formulated by conventional methods (emulsion inversion point method and high-speed homogenizer), to compare and find out the best method for formulation of the emulsion. From this study, it was confirmed that the emulsion prepared using optimized parameters having high intrinsic stability (more than 6 months of storage) while the emulsion made by emulsion inversion point method and the high-speed homogenization method gets separated/creamed within one day as shown in Fig. 6.

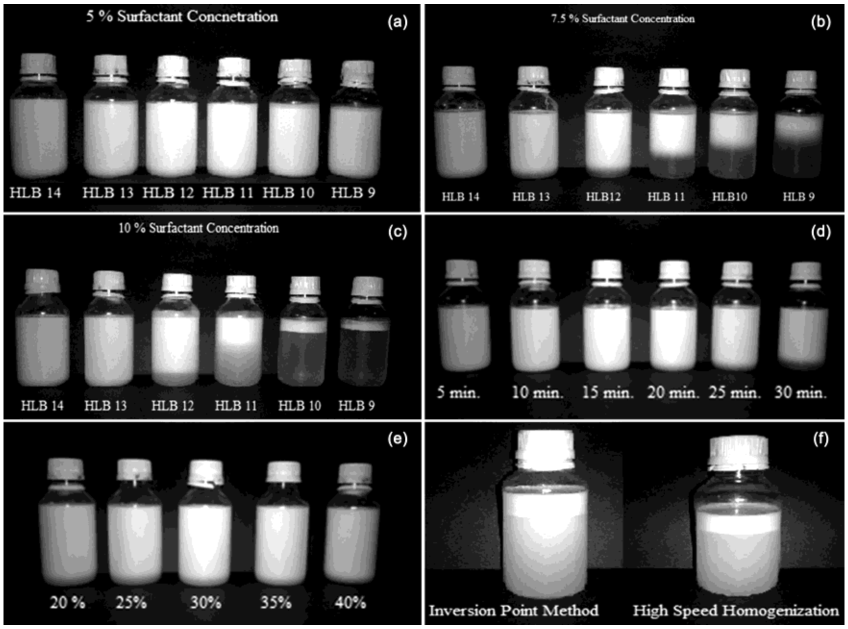

Fig. 6 — The visual appearance of emulsions prepared by sonication method at (a) surfactant conc. 5% (HLB 9-14), (b) surfactant conc. 7.5% (HLB 9-14), (c) surfactant conc. 10% (HLB 9-14), (d) various sonication times, (e) various amplitudes, after 6 months (f) emulsion prepared by inversion point method and high-speed homogenization method, after 1 day.

This is because of the emulsion inversion point and high-speed homogenization methods cannot provide the substantial amount shear for droplet breaking process Also;these methods cannot create high turbulence, which improves the surfactant adsorption on of newly created oil droplet. Due to low shear and turbulence, these methods produce the emulsion with partially covered large oil droplet, which after some time get separated due tocreaming and coagulation, this type of emulsion is very unstable[3].

Conclusion

The work has been performed to intensify the formulation of citronella oil in water nanoemulsion. The process parameters which affect the ultrasonic emulsification like sonication time, ultrasound power, surfactant concentration, and HLB values have been optimized by considering the stability and the droplet sizeof emulsion. The optimized ultrasonication parameters were found to be 20 min of sonication time and35% (of the actual power of 750 W) applied powerat which MDD and the emulsion phase fraction were obtained to be 26 nm and 100 % (for more than 6 months) respectively, at a surfactant concentration of 7.5 % (HLB 13) of the total emulsion. The emulsion has been formulated by other emulsification methods such as high-speed homogenization and inversion point methods and results compared with the ultrasonically prepared emulsion. The conventionally prepared emulsion was very unstable asit getsseparated within one day of storage. Whereas, the emulsion produced at optimized cavitational parameter was found to be very stable for more than 6 months on storage. This process can be scaled up using hydrodynamic cavitation method.

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