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Formation and stability of oil-in-water nano emulsion containing turmeric oil

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ABSTRACT

Nano emulsions have practical applications in a multitude of commercial areas, such as the chemical, pharmaceutical and cosmetic industries. The aim of this study was to employ high energy methods to create oil in water nano emulsion using turmeric oil to evaluate their physical stability, phytotoxicity and antibacterial properties. The nano emulsion developed by phase diagram method was prepared in different ratios ranging from 1:1 to 1:9 using turmeric oil, tween 20 (surfactant) and propylene glycol/ ethanol (co-surfactants). Stable emulsions were formed at acidic pH characterized by a low viscosity and increased zeta potential respectively. The average hydrodynamic size of the formulation was in the range of 65 nm using propylene glycol and 45 nm using ethanol as co-surfactants. The optimized nano emulsion was phytotoxic to corn and cucumber seeds but the extent of toxicity varied with respect to concentration of the formulation. The antibacterial studies revealed that the formulation had selective antibacterial properties on gram positive *S. aureus* characterized by a clear zone of inhibition.

KEYWORDS

Nano emulsion;
Surfactant;
Turmeric oil;
Toxicity;
Anti bacterial.

INTRODUCTION

Nanoemulsion offers enhanced material properties and versatility. It replaces micro scale emulsions in a number of applications. They gain increasing attention in food industry as a novel delivery system for lipophilic materials, cosmetics, medicine and agriculture[1-6]. They are defined as dispersions consisting of oil, surfactant, co surfactant and water[7] with a mean droplet diameter ranging from 20-200 nm. The focus of the present study was to prepare turmeric oil nanoemulsion by mixing different ratios of oil (turmeric oil), surfactant (Tween-20) and co surfactant (propylene glycol/ethanol). Turmeric oil was chosen for this study on account of its excellent therapeutic properties. Surfactants forms a barrier between the nanodroplets and it prevents coalescence in the new droplets[8]. Tween-20 is non-ionic surfactant that is readily miscible in the oil-water interface.

In lieu of adequate reports the primary objective of our current study was to characterize the process variables in the preparation of an uniform-sized nanoemulsion using turmeric oil and to evaluate its antibacterial and phytotoxic properties. The formation and stability of the prepared formulation were monitored

FULL PAPER

by changes in pH and viscosity. Phytotoxicity studies of the nanoemulsion were tested on corn and cucumber seeds. Antibacterial efficacy of the formulated nano emulsions were evaluated on gram positive bacteria (*Staph. aureus*) and gram negative bacteria (*E. coli*).

MATERIALS AND METHODS

Chemicals

Turmeric oil was obtained from AOS products (ISO 9001:2000 and WHO-GMP Accredited Company),Uttar Pradesh. Tween 20 (Polyethylene glycol sorbitan monolaurate) was purchased from Sigma Aldrich and propylene glycol was procured from Merck chemicals. Deionized distilled water was used for all experiments.

Preparation of nano emulsions

Different mass ratios from 1:1-1:9 of Turmeric oil: S_{mix} (surfactant and co-surfactant) were prepared to study the physical properties and phase behavior of the nanoemulsion formulations. These mixtures were stirred for 24 h in a magnetic stirrer at 160 rpm/min. Further they were sonicated for fifty minutes at 60% amplitude using a sonicator (Sonics, USA).

Optimization

The optimization of the process parameters were done using two different co-surfactants such as propylene glycol and ethanol with turmeric oil and tween-20 as surfactant. The nanoemulsions with least size, increased zeta potential and low viscosity was chosen for further characterisational studies.

Characterisation studies

The formulations were checked for phase separation (if any) after centrifugation at 3000 rpm for 10 min. Viscosity of the nano emulsion was checked using a Viscometer (Brookfield LV). Further the stability of the formulations were studied at different pH and subjected to hydrodynamic particle size and zeta potential measurements using Malvern Zeta sizer 3000HS (Malvern Instruments Ltd., UK). Morphological and structural features of turmeric oil nano emulsions were studied by Transmission Electron Microscope (TEM) JEM 1011, JEOL, Japan by placing a drop of the nanoemulsion on the film grid.

Phytotoxicity studies

Corn and cucumber seeds were first checked for their viability by suspending them in deionized water. The viable seeds were selected and soaked for 10 min in a surface sterilizing agent (10% sodium hypochlorite solution) (US-EPA, 1996). Following surface sterilization, the seeds were rinsed thrice in deionized water, soaked and stirred for 2 h in deionized water (control), nano emulsion ratios of 1:6 with propylene glycol and 1:5 with ethanol, tween 20 (negative control). Whatmann No.1 filter paper was placed into each Petri dish (100 mm x 15 mm) and 5 ml of the respective formulations were added. The seeds were then transferred to the Petri dish, placed equidistant from one another. The dishes were sealed with sealing tape and placed in dark condition for 4 days. The end points of the experiment was taken at 80% of the germination in the control seeds[9-11].

Antibacterial activity of nano emulsion

Staphylococcus aureus and Escherichia coli were cultured to study the toxicity of bacterial growth using nano emulsions. The bacteria were inoculated onto two separate nutrient broths and the sub-cultured medium was kept in orbital shaker for twenty-four hours. The growth of bacteria was confirmed by visible turbidity in the nutrient broths. Bacteria were then pour plated onto Luria Bertani agar to get a lawn culture. Sterile filter paper disks of 10 mm diameter were placed on the surface of the agar. 50 µL of the samples containing turmeric oil, Tween 20, propylene glycol/ ethanol in the ratios of 1:6 and 1:5 respectively were slowly pipetted into the sterile disks using a micropipette. Distilled water was used as control. After four days, zone of inhibition was measured[12-14].

RESULTS AND DISCUSSION

Characterization

Excess S_{mix} (surfactant, co surfactant) volume with turmeric oil & tween-20 may lead to the aggregation of nano emulsions by the influence of surface charges, Brownian motion and Ostwald ripening. Stable optimized nano emulsions containing propylene glycol and

ethanol were formed in acidic conditions i.e., at pH 4.3 and 6.7 respectively. The least viscosity of 3.7 and 2.8 were obtained for the nanoemulsion formulations with 1: 6 of propylene glycol and 1:5 of ethanol.

Characterization was done for the formulation containing turmeric oil, tween- 20(surfactant), propylene glycol (co-surfactant) and distilled water. The ratios from 1:1-1:9 indicates oil and S_{mix} (surfactant and co-surfactant) (TABLE 1). Polydispersity index (PDI) is the ratio of standard deviation to mean droplet size, which indicates the uniformity of droplet size within the formulation. The higher the PDI, the lower the uniformity of the droplet size in the formulation. Monodispersity index (MDI) was observed in all ratios except 1:4.

Similarly characterization was done for the formulation containing turmeric oil, tween- 20(surfactant), ethanol (co-surfactant) and distilled water (TABLE 2). The ratios of 1:6, 1:7 and 1:8 showed PDI. Other ratios were monodispersed. The average hydrodynamic diameter of the formulation containing 1:5 (turmeric oil:ethanol) was 45nm and the zeta potential value was – 17Mv. TEM image of nano emulsions revealed the spherical shaped particles (Figure 1). The ratios from 1:3-1:6 with increased zeta potential and low viscosity were more stable for both nanoemulsions viz. with propylene glycol and ethanol.

Stability studies

No phase separation was seen after centrifugation, indicating the emulsion obtained was stable. Nano emulsions were stored at room temperature (25^0C) and cooling temperature (4^0C) for three months[15,16]. Nano emulsions were not formed at 1:1 and 1:2 using both co-surfactants. At 25^0C S_{mix} ratios (1:3-1:6) showed stability for three to four weeks with a gradual increase in size. But 1:6-1:9 ratios were stable for only two weeks. Interestingly at 4^0C 1:3-1:6 ratios were stable for over a month.

TABLE 1 : Characterization results of various formulations using propylene glycol as co-surfactant

Formulation (propylene glycol)	Stability (phase separation)	pH	Viscosity (c.P)	PSA (d.nm)	Zeta Potential (mV)	PDI
1:1	Not stable	-	-	-	-	-
1:2	Not stable	-	-	-	-	-
1:3	Stable	3.8	3.45	86.14	-10.7	0.02
1:4	Stable	4.21	2.61	181.2	-7.2	0.41
1:5	Stable	4.21	4.51	77.27	-16.3	0.15
1:6	Stable	4.27	3.7	65.71	-15.0	0.04
1:7	Stable	4.5	5.03	129.8	-8.6	0.62
1:8	Stable	4.53	10.0	169.2	-2.4	0.05
1:9	Stable	4.6	32.2	125.8	-14.1	0.21

TABLE 2 : Characterization results for various formulations using ethanol as co-surfactant

Formulation (ethanol)	Stability (phase separation)	pH	Viscosity (c.P)	PSA (d.nm)	Zeta potential (mV)	PDI
1:1	Not stable	-	-	-	-	-
1:2	Not stable	-	-	-	-	-
1:3	Stable	4.1	1.86	106.3	-9.04	0.24
1:4	Stable	4.2	2.6	111.7	-5.0	0.17
1:5	Stable	6.7	2.8	45.47	-17.2	0.05
1:6	Stable	4.1	4.6	63.99	-15.2	0.24
1:7	Stable	4.3	6.06	98.16	-7.58	0.35
1:8	Stable	5.6	6.2	70.11	-11.3	0.45
1:9	Not Stable	-	-	-	-	-

Phytotoxicity of turmeric oil nano emulsion

Toxic effects of nano emulsions on corn and cu-

FULL PAPER

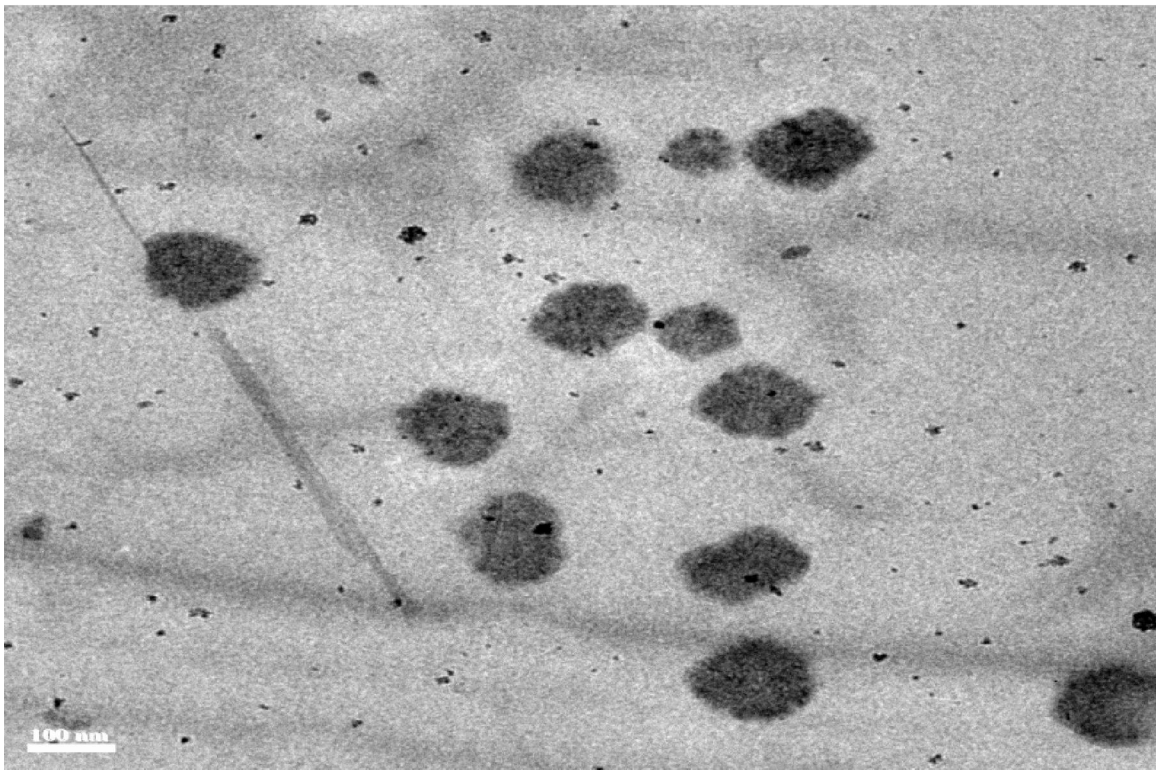

Figure 1 : TEM image of nanoemulsions showing the particles were spherical in shape and monodispersed.

cumber seeds were studied using seven formulations. The root length and germination rates were monitored. Nano emulsions affected the seed germinations of both cucumber and corn seeds in all tested ratios but the diluted concentrations were less toxic. Seed coat exhibit selective permeability and play a very important role in protecting the embryo from harmful external factors (12). This explains that the seed germination in this study was altered by the nanoemulsions.

Antibacterial activity of turmeric oil nano emulsion

Growth studies were done to find out the effects of nanoemulsions on the survival rate of bacteria. In disc diffusion method, it was observed that the bacterial strains exhibited selective susceptibility to the tested formulation. Bacterial susceptibility to each nanoemulsion was determined by the zone of inhibition. The two formulated nanoemulsions containing propylene glycol and ethanol were tested on *Staph. aureus*

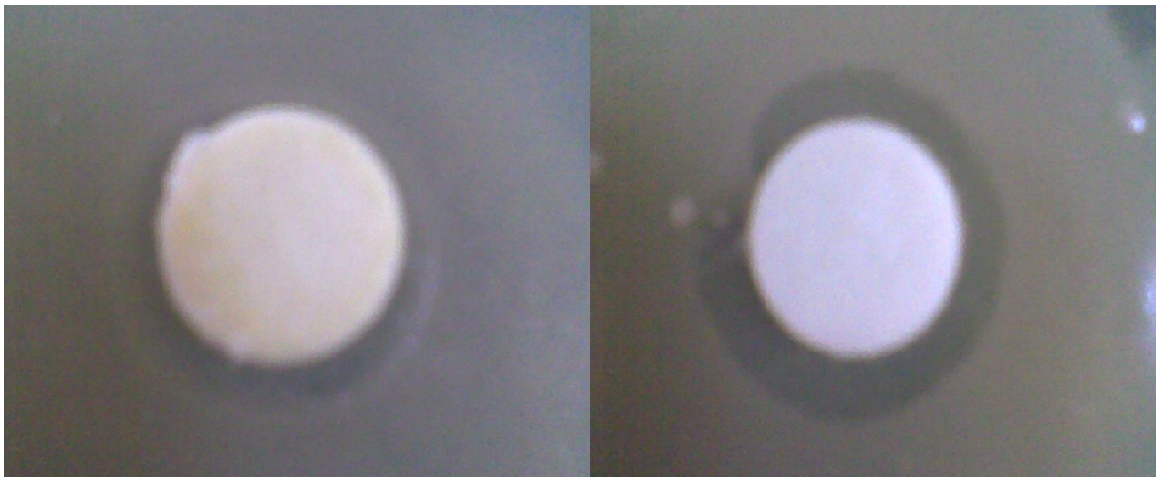

Figure 2 : Zone of inhibition of *S. aureus* using turmeric oil and ethanol

and E. coli individually by pour plate method. Among different concentrations 1:6 ratio of turmeric oil with propylene glycol exhibited zone of inhibition (14 mm zone diameter) with *Staph. Aureus* (Figure 2) while there was no zone of inhibition with *E. coli.* This is because gram negative bacteria like *E. coli* have rich lipopolysaccharides (LPS) which increase the negative charge of cell wall and helps in stabilising the overall structure. This could be the reason for the selective toxicity of the nanoemulsion.

CONCLUSION

Our results revealed that stable turmeric oil nano emulsions were obtained at 1: 6 ratio of tween 20: propylene glycol and 1:5 ratio of tween 20: ethanol. It was concluded that turmeric oil nano emulsions have phytotoxic and antibacterial properties. Nanoemulsions were toxic to corn and cucumber seeds. Antibacterial activity of turmeric oil nanoemulsions were observed on Staph.aureus whereas no antibacterial effect was seen in E.coli. These results clearly indicate that the nano emulsions were more stable and had excellent antibacterial property to Staph.aureus. Hence these nanoemulsions may have promising results in therapeutic applications.

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REFERENCES

[1] K.Pays, G.Kahn, B.Pouligny, J.Bibette, F.Leal-Calderon; J.Control Release., **79**, 193-205 **(2002)**.
[2] N.Garti, Bisperink; Curr.Opin.Colloid Interface.Sci., **3**, 657-667 **(1998)**.
[3] J.Surh, G.T.Vladisavljevic, S.Mun, D.J.McClements; J.Agric.Food Chem., **55**, 175-184 **(2007)**.
[4] N.Garti, A.Aserin; Adv.Colloid Interface, **65**, 37-69 **(1996)**.
[5] M.F.Ficheux, L.Bonakdar, F.Leal-Calderon, J.Bibette; Langmuir, **14**, 2702-2706 **(1998)**.
[6] G.Muschiolik; Curr.Opin.Colloid Interface .Sci., **12**, 213-220 **(2007)**.
[7] S.Hari Kumar, S.Vishal; Journal of drug delivery & therapeutics, **2(4)**, 40-45 **(2012)**.
[8] H.W.Hibbott; Handbook of Cosmetic Science-An introduction to principles and applications, New York, Macmillan, **(1963)**.
[9] M.Apisadyakul, N.Vanittanakam, D.Buddasukh; J.Ethnopharmacol., **49**, 163-169 **(1995)**.
[10] A.Banerjee, S.S.Nigam; Ind.J.Med.Res., **68**, 864-866 **(1978)**.
[11] J.Shankaranarayan, C.I.Jolly; Ind.J.Pharma.Sci., **1**, 6-13; J.Exp.Biol., **6**, 232-256 **(1993)**.
[12] D.H.Lin, B.S.Xing; Phytotoxicity of nanoparticles, inhibition of seed germination and root elongation.Environ.Pollut, **150**, 243-250 **(2007)**.
[13] T.Andrew, Harris, R.BaliJ; On the formation and extent of uptake of silver nanoparticles by live plants.Nanopart Res., **10**, 691–695**(2008)**.
[14] D.H.Lin, B.S.Xing; Environ.Sci.Technol., **42**, 5580–5585 **(2007)**.
[15] B.Vivek, S.Pathakb, S.Sharmab, V.Patravale; International Journal of Pharmaceutics, **431**, 149–160 **(2012)**.
[16] F.Shakeel, S.Baboota, A.Ahuja, J.Ali, M.S.Faisaland, S.Shafiq; Thai J.Pharm.Sci., **32**, 4-9 **(2008)**.