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RESEARCH ARTICLE

FORMULATION AND INVITRO EVALUATION OF MEBENDAZOLE NANOEMULSION CONTAINING OREGANO ESSENTIAL OIL

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ABSTRACT

Aim of the study is to formulate and evaluate Mebendazole nanoemulsion containing Oregano essential oil, for enhancing anthelmintic activity prepared by spontaneous emulsification method followed ultrasonication. A problem stated with the use of synthetic anthelmintics is the development of parasitic resistance, which threaten the success of treatment in humans, use of a herbal alternative may reduce such resistance. Essential oil of *Oreganum vulgare* is taken orally kills intestinal parasites, therefore it can enhance the action potential of anthelmintics. Nanoemulsion was formulated using a Pseudo ternary phase diagram, which was created using design expert software 9.0.5.1. Out of the five phase diagrams ratio of 1:3 was chosen, since it shows maximum area in the overlay plot. Nanoemulsion containing drug (NE1), containing drug and oregano essential oil (NE2) and oregano oil (NE3) were prepared using smix ratio 1:3. Anthelmintic activity, viscosity and globule size were fixed as the parameters for optimisation.NE2 was selected as the optimised formulation having viscosity (18.61±0.45Cp), Globule size (36.24µm), polydispersity index (0.2). NE 2 showed a minimum mean time of paralysis (1.2±0.01min) and mean time of death (2.2±0.02 min). Reduction in time for paralysis and death may be an indication of the synergistic effect of oregano essential oil on anthelmintic activity of Mebendazole. Evaluations of anthelmintic activity was carried out in artificial laboratory conditions by using the earthworms (*Lumbricusterrestris*).This study suggest that nanoemulsion is a promising novel formulation that can enhance the solubility of poorly soluble drug like Mebendazole and thereby enhance its oral bioavailability.

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INTRODUCTION

The various nanopharmaceuticals currently being used or in the process of development are nanoemulsion (NE) which are submicron size emulsion, nanosuspension (submicron sized suspensions), nanospheres (drug nanoparticles in the polymer matrix), etc. In drug discovery, about 40% of the new molecular entities (NMEs) display low solubility in water leading to poor bioavailability; Furthermore, oral delivery of numerous drugs is hindered owing to their high hydrophobicity. Therefore, producing suitable formulations is very important to improve the solubility and bioavailability. Nanoemulsions are defined as isotropic, thermodynamically stable, transparent or translucent dispersions of oil and water stabilized by an interfacial film of surfactant molecules having the droplet size 20-500 nm.

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Major components of nanoemulsion

Oils

Selection of an appropriate oily phase is very important as it influences the selection of other ingredients of nanoemulsions, mainly in case of O/W nanoemulsions (Gouri and Shubrajit, 2013). Usually, the oil which has maximum solubilising potential for selected drug candidate is selected as an oily phase for the formulation of nanoemulsions. This helps to achieve maximum drug loading in the nanoemulsions. The choice of oily phase is often a compromise between its ability to solubilize the drugs and its ability to facilitate formation of nanoemulsion of desired characteristics. Thus mixture of oils can be used to meet both the requirements. For example, a mixture of fixed oil and medium chain triglycerides is used to have good balance between drug loading and emulsification. Apart from the basic component of the nanoemulsion, oregano essential oil is also added, as it has anthelmintic property due to the presence of carvacrol and thymol. Which increases the

cytoplasmic permeability of H^+ and K^+ ions in the worms, and also inhibit the production of ATP, causing the death.

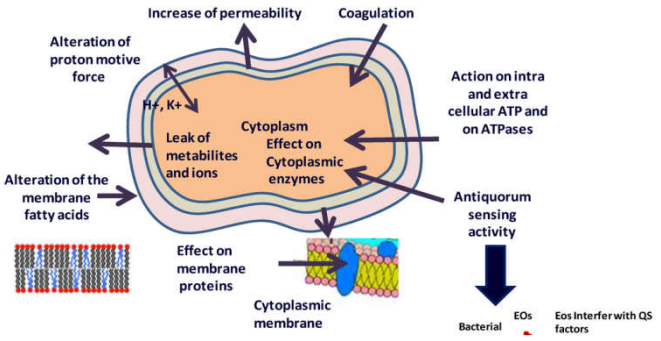


Figure 1. Mechanism of action of Carvacrol

Cosurfactants

Cosurfactants penetrate into the surfactant monolayer providing additional fluidity to interfacial film and thus disrupting the liquid crystalline phases which are formed when surfactant film is too rigid. Usually a very low HLB cosurfactant is used with a high HLB surfactant to modify the overall HLB of the system. Unlike surfactant, the cosurfactant may not be capable of forming self-associated structures like micelles on its own. Hydrophilic cosurfactants preferably alcohols of intermediate chain length such as hexanol, pentanol and octanol, which are known to reduce the oil/water interface and allow the spontaneous formation of nanoemulsion (Rohit and Riyaz, 2014). Organic solvents such as ethanol, glycerol, propylene glycol (PG), polyethylene glycol (PEG) are suitable for oral delivery, and they enable dissolution of large quantity of either the hydrophilic surfactant or the drug in the lipid base by co-solvency and by making the environment more hydrophobic by reducing the dielectric constant of water.

Aqueous Phase

The droplet size and stability of nanoemulsion is influenced by the nature of aqueous phase. Hence, pH and ionic content of aqueous phase should be given due importance while designing. nanoemulsion. The physiological milieu has diverse pH ranges varying from pH 1.2 (pH in stomach) to 7.4 and greater (pH of blood and intestine). In addition, the presence of various ions in the physiological milieu can also have considerable effect on the properties of nanoemulsions.

Methods of formulation

1. High-Pressure Homogenisation

This technique makes use of high-pressure homogenizer /piston homogenizer to produce nanoemulsions of extremely low particle size (up to 1nm). In a high-pressure homogenizer, the dispersion of two liquids (oily phase and aqueous phase) is achieved by forcing their mixture through a small inlet orifice at very high pressure (500 to 5000 psi), which subjects the product to intense turbulence and hydraulic shear resulting in extremely fine particles of emulsion. Homogenizers of varying design are available for lab scale and industrial scale production of nanoemulsions. This technique has great efficiency, the only disadvantage being high energy consumption and increase in temperature of emulsion during processing.

2. Microfluidization

Microfluidization is a patented mixing technology, which makes use of a device called microfluidizer. This device uses a high-pressure positive displacement pump (500 to 20000psi), which forces the product through the interaction chamber, which consists of small channels called 'microchannels'. The product flows through the microchannels on to an impingement area resulting in very fine particles of sub-micron range.

3. Spontaneous Emulsification

Preparation of the homogeneous organic solution composed of oil and a lipophilic surfactant in water miscible solvent. The homogeneous aqueous phase was formed by water, and hydrophilic surfactant. The organic phase was injected in the aqueous phase under magnetic stirring: the o/w emulsion was formed instantaneously by diffusion of the organic solvent in the external aqueous phase leading to the formation of nanodroplets. The magnetic stirring was maintained during 30 min to let the system reach equilibrium. The totality of the water miscible solvent was removed by evaporation during 45 min under reduced pressure. Nano droplets of oil were dispersed in an aqueous solution of water and hydrophilic surfactant (Sunil, 2014).

Applications of nanoemulsion

1. Ocular delivery

Oil in water emulsions are being explored for improved topical lipophilic drug delivery to the eye. Examples: Piroxicam, Pilocarpine, Indomethacin, cyclosporine A.

2. Percutaneous route

Many drugs exhibit low skin permeation, which results in poor efficacy. A solvent free topical vehicle based on drug entrapment in the o/w emulsion droplets nm is more efficacious in terms of percutaneous absorption with possibly devoid of adverse effects. Examples:NSAIDs, Diazepam, α-tocopherol, Miconazole nitrate) EMLA (Eutectic Mixtures of local anaesthetic) have proven to be useful medication by this route.

3. Nanoemulsions and Intranasal Drug Delivery

The olfactory region of the nasal mucosa provides a direct connection between the nose and brain, and by the use of nanoemulsions loaded with drugs, conditions such as Alzheimer's disease, migraine, depression, schizophrenia, Parkinson's diseases, meningitis, etc. can be treated.

4. Use of nanoemulsion in cosmetics

Nanoemulsions are acceptable in cosmetics because there is no inherent creaming, sedimentation, flocculation or coalescence observed with macro emulsions (Shah and Shelat, 2010).

5. Antimicrobial and Anthelmintic Nanoemulsions

Nanoemulsion has broad spectrum activity against bacteria (e.g. E. Coli, Salmonella, S. aureus) enveloped viruses (e.g. HIV, Herpes Simplex), Fungi (e.g. Candida, Dermatophytes)

and spores (e.g. anthrax). Anthelmintic nanoemulsion ranges from 100-400nm, the nanoemulsion shows enhanced solubility and bioavailability, the released drug induces paralysis and death. It has activity against roundworm, hookworm, whipworm and threadworm infestations. It is effective in the treatment of ascariasis.

MATERIALS AND METHODS

Mebendazole and triacetin was purchased from Balaji Chemicals Mumbai, Oregano essential oil from chrysalis essential, Noida. All others chemicals used were of analytical and laboratory standards. The equipments used were UV spectrophotometer (V-630, Jasco), Melting point apparatus (Optic technology), Philip's X'Pert Pro X-ray diffractometer (Almelo, Netherlands), FT-IR model Thermo Nicolet, Avatar 370, Brookfield rheometer viscometer RVDVE, Malvern ZetasizerVer .7.01.

Characterisation of Mebendazole

1.Solubility

100 mg of drug was taken in a test tube containing 10 ml 7.4 phosphate buffer containing 1%w/v Tween 80. Beaker was shaken occasionally for 24 h and maintained at 25°C. The solution was centrifuged and supernatant filtered through No.1 Whatmann filter paper. Diluted suitably and analyzed by UV spectrophotometer (V-630, Jasco) at 234 nm.

2.Melting point

Melting point of Mebendazole was determined using melting point apparatus (Optic technology). The drug sample was taken in a small capillary tube and placed in the melting point determination apparatus. The melting point of the sample was examined visually through the window and the sample melted temperature was noted.

3.UV-Vis spectroscopy

The UV spectrum of Mebendazole was taken in distilled water and methanol (1:1), in a JASCO V-630 spectrophotometer over a wavelength range of 200-400 nm.

4.FT-IR spectroscopy

FT-IR spectrum of Mebendazole was obtained. The sample was made into pellets with KBr and FT-IR model Thermo Nicolet, Avatar 370 was used for obtaining spectra. The spectrum obtained was compared with reported data of Mebendazole.

5.XRPD

XRPD pattern of Mebendazole was obtained. XRPD was performed at room temperature with Philip's X'Pert Pro X-ray diffractometer (Almelo, Netherlands), voltage 40 kV. The diffraction pattern was recorded from 10 to 60° at an angle 2θ and compared with reported data.

Characterisation of triacetin

1. Solubility

Solubility of triacetin was determined qualitatively in various media.

2.Viscosity

Viscosity of triacetin was determined using Brookfield rheometer viscometer RVDVE at 30°C with a CPE 01 spindle at 30 rpm.

3.FT-IR spectroscopy

FT-IR spectrumoftriacetin was taken and compared with reference spectrum for confirmation. FT-IR model Thermo Nicolet, Avatar 370was used for obtaining spectra. The spectrum obtained was compared with reported data of triacetin.

Characterization of oregano essential oil

1. Solubility

Solubility of oregano essential oil was determined qualitatively in various media.

2. Viscosity

Viscosity of Triacetin was determined using Brookfield rheometer viscometer RVDVE at 30°C with a CPE 01 spindle at 30 rpm.

3.GC-MS

GC-MS spectrum of oregano essential oil was taken using GC-MS model Varian 1200 L Single Quadrupole and Fragment ion peaks were obtained. The fragment ion peak m/z obtained was compared with m/z ratio of the standard.

Drug-oil compatability studies

Prior to formulation, to study the chemical compatability of drug with oil phase triacetin and Oregano essential oil were determined, the following studies were conducted on drug and oil mixtures. Ratio of drug and oil (1:1).

1.FT-IR spectroscopy

The FT-IR spectrum of drug-oil mixture was obtained. FT-IR model Thermo Nicolet, Avatar 370was used for obtaining spectra. The spectrum obtained was compared with reported data of drug and with spectra of oil.

2.GC-MS

GC-MS spectrum for drug-oil mixture was taken using GC-MS model Varian 1200 L Single Quadrupole and Fragment ion peaks were obtained. The fragment ion peak obtained was compared with m/z ratio of the individual components.

Formulation - Design and development

Pseudo ternary phase diagram

Based on the partition coefficient value of essential oil oregano oil was selected. Triacetin was selected as the oil phase. Tween 80 was used as the surfactant. The Co-Surfactant Ethanol was selected. The ratio of surfactant to cosurfactant was fixed at different ratios 1:1,2:1,3:1,4:1,5:1 on the volume basis for each phase diagram. The mixture of

surfactant to cosurfactant is refered to as smix in the following discussion. The oil phase was mixed with smix phase in different ratio. An o/w microemulsion technique was employed for the preparation of pseudo ternary phase diagrams. The diagrams were created using Design- expert software. Distilled water was added drop by drop to the mixture of oil and smix after each water addition, the mixture stirred by using vortex mixer until homogenous solution was obtained. The end point was the appearance of turbidity. The quantity of water required to make the mixture turbid was noted. In the Pseudo ternary phase diagram each axis represents aqueous phase, Oil phase and Smix respectivel. The area in each of this phase diagram corresponds to the critical points. The criteria of choosing respective ratio depends on the area of graphs. The critical point was the point at were the turbidity was observed. Five phase diagrams were constructed taking ratios 1:1, 1:2 ,1:3, 1:4, 1:5 (Bahar *et al*., 2013).

Preparation of nanoemulsion by spontaneous emulsification method followed by Ultrasonication

From the pseudo ternary phase diagram, the formulation showing clear nanoemulsion was selected. The formulation was previously analysed at different oil/smix ratios for optimisation. The formulation composition for the respective nanoemulsion was obtained. 1% of oregano essential oil was mixed with Smix (Triacetin and Ethanol). Required quantity of water was added in drop wise manner and mixed using a magnetic stirrer, until transparent emulsion was formed. The emulsion obtained was then ultrasonicated using Ultrasonic bath sonicator 1.5 L(H Pcianalyte) for 60 minutes. For the incorporation of drug, it was dissolved in mixture of oregano essential oil and smix and mixed using magnetic stirrer for 15 minutes until a homogenous solution is obtained followed by the above procedure. The prepared nanoemulsionwere subjected to various studies for determining the optimised nanoemulsion. The studies include:

1. Anthelmintic activity

Preparation of Drug Solutions

Mebendazole was purchased from Balajichemicals, Mumbai. The formulations NE1,NE2,NE3 were assessed for its anthelmintic activity. Mebendazole 100mg/ml in ethanol was used as Standard and ethanol was used as control.

Collection of Earthworms

Earthworms (*Pheretimaposthuma*) were collected from Agricultural college, Vellayani. The assay was performed in vitro using adult earthworms owing to their anatomical and physiological resemblance with the intestinal round worms and parasites of human beings for preliminary evaluation of anthelmintic activity.

Evaluation of Anthelmintic Activity Using Earthworms

Earthworms six number, each of average length of 6 cm, were placed in Petri dishes containing the formulations NE1, NE2, NE3 and Mebendazole (Standard). This was done after pouring the Petri dishes content in to a flat surface and allowing the worms to move freely. Motility of the worms were assessed by tapping the end of each worm with the index finger and applying a bit of pressure. It was also observed for control. The time taken for paralysis, motility activity of any sort, and death time of worms were observed and recorded after ascertaining that the worms did not move neither when shaken vigorously nor when dipped in warm water (50°C). Results were taken in triplicate Mean ± SD(n=3) (Bahar *et al*., 2013).

2.Viscosity

The viscositiy was measured to determine rheological properties of formulations. Brookfield Rheometer viscometer RVDVE at 30°C with a CPE 01 spindle at 30 rpm was used for this purpose. Results were taken in triplicate and the average was taken in to consideration.

3.Globule size analysis

Globule size of the formulations was determined using Photon Correlation Spectroscopy (PCS) using a Malvern Zetasizer Ver .7.01.The formulation with least globule size is considered as optimised.

Table 1. Formulation of nanoemulsion (NE1,NE2,NE3)

Formulation code	NE1	NE2	NE3
Triacetin %v/v in 10 ml	1	1	1
Smix %v/v(tween 80 and ethanol) in 10 ml	3	3	3
Water%v/v	5	5	5
Amount of drug added mg	100	100	NIL
Oil:smix	1:3	1:3	1:3
Smix ratio	1:1	1:1	1:1
Oregano oil %v/v in 10 ml	NIL	0.1	0.1

From the three formulations (NE1, NE2 and NE3) one formulation was selected as the optimized nanoemulsion. The selection was based on the criteria that the formulation with anthelmintic activity, smaller viscosity and globule size.

Characterisation of optimised nanoemulsion

1. Zeta potential

The zeta potentials of the samples were determined at 25^{o}C after suitable dilution with distilled water using by Photon Correlation Spectroscopy (PCS) using a Malvern ZetasizerVer .7.01.

2. Percentage Transmittance

The percent transmittance of the nanoemulsion was measured using in a JASCO V-630 spectrophotometer keeping distilled water as blank at 560nm.

3. Transmission electron microscopy

The morphology of the nanoemulsion was examined using by JEOL Model JSM - 6390LV an electronic transmission microscope at 70 kV. After dilution with the original dispersion medium of the nanoemulsion, the samples were negatively stained with 1% (w/v) phosphotungstic acid for observation (Zhong, 2012).

4. Scanning electron microscopy

The morphology of the nanoemulsion was examined by JEOL Model JSM - 6390LV. The samples were stained with 2%

(w/v) phosphotungstic acid for 30 s and placed on copper grids with films for viewing.

RESULTS

Characterisation of mebendazole

1.Solubility

The solubility of Mebendazole in Phosphate buffer pH7.4 containing 1% tween 80 was found to be 0.632mg/ml.

2.Melting point

The melting point of Mebendazole was found to be 213^{0}C. The reported data had a closer value and confirmed the result.

3.UV-Vis spectroscopy

The UV spectrum of Mebendazole[48]in methanol showed a characteristic peak at 242 nm. The reported data had a closer value and confirmed the result.

4.FT-IR spectroscopy

The FT-IR spectrum of Mebendazole showed characteristic peaks at range of 505-3328 cm^{-1}. The prominent peak at 3328.75 cm^{-1} showed N-H stretching. The results observed were in close agreement with the reported data.

5.XRPD

XRPD pattern of Mebendazole showed different peaks 5.14,11.269,19.342,20.58,25.778, 25.969,28.221,28.88 etc. The results observed were in close agreement with reported data.

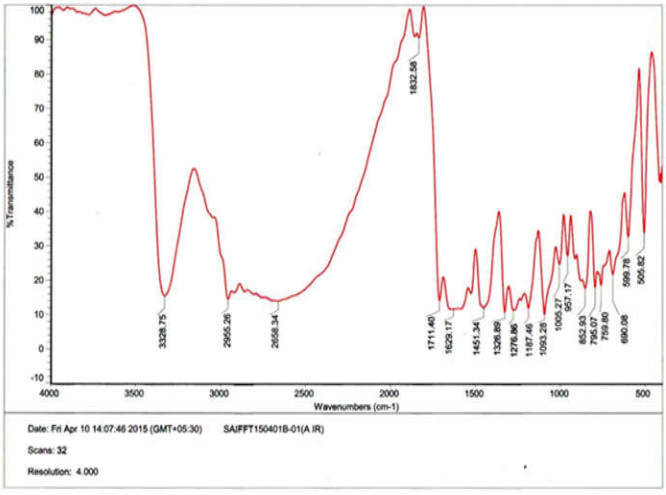


Figure 2. FT-IR of Mebendazole

Characterisation of triacetin

1. Solubility

Triacetin was found to be soluble in water, miscible in acetone, chloroform, ethyl ether, soluble in phosphate buffer pH 7.4.

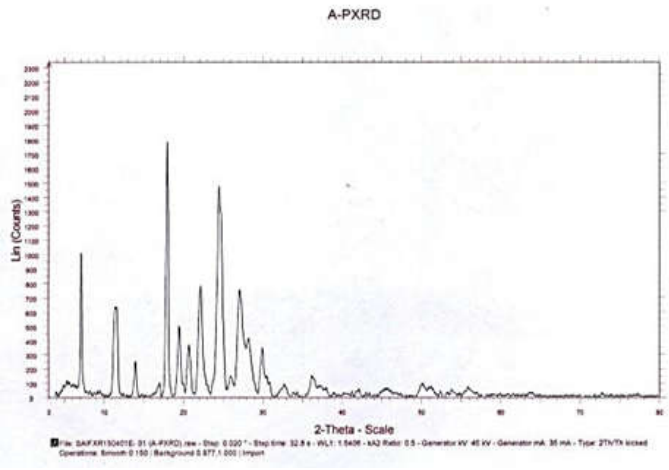


Figure 3. XPRD of Mebendazole

2.Viscosity

Viscosity of triacetin was found to be 23.5 cp.

3.FT-IR spectroscopy

The FT-IR spectrum of triacetin showed characteristic peaks ranging from 426-3636cm^{-1}. The prominent peak at 3636 cm $^{-1}$ showed presence of carbonyl group overtone stretching. Other results are tabulated below. The results observed were in close agreement with the reported data.

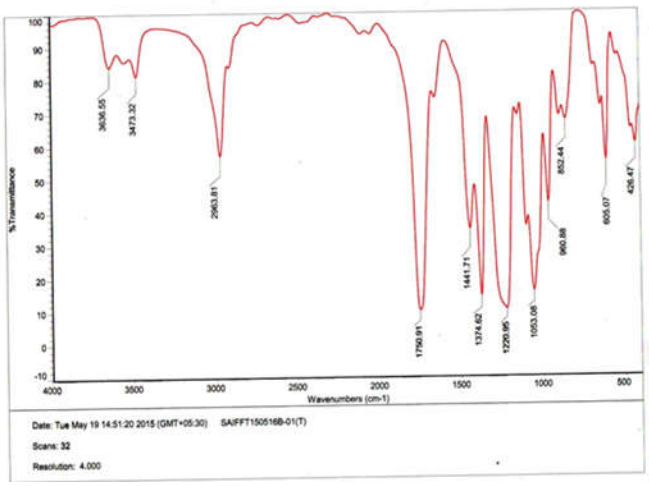


Figure 4. FT-IR of Triacetin

Characterization of oregano essential oil

1.Solubility

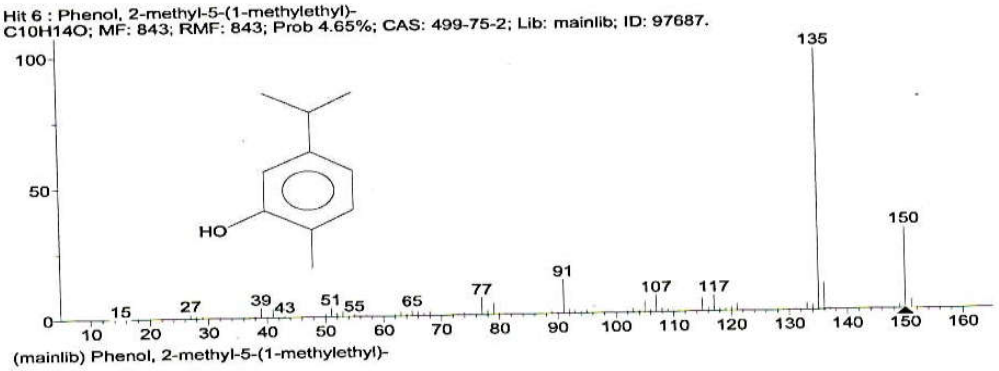
Oregano essential oil was found to be soluble in ethanol, methanol, acetone, slightly soluble in DMSO, Insoluble in petroleum ether, soluble in phosphate buffer pH 6.8.

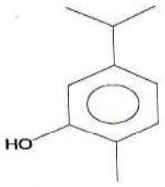
2. Viscosity

Viscosity of Oregano essential oil was found to be 0.8921 Cp.

3.GC-MS

The GC-MS peak for oregano essential oil was obtained. The spectra confirms the presence of phenolic compounds like





Name: Phenol, 2-methyl-5-(1-methylethyl)-
Formula: $C_{10}H_{14}O$
MW: 150 CAS#: 499-75-2 NIST#: 229581 ID#: 97687 DB: mainlib
Other DBs: Fine, TSCA, RTECS, EPA, HODOC, NIH, EINECS
Contributor: Japan AIST/NIMC Database- Spectrum MS-NW- 815
10 largest peaks:
135 999 | 150 314 | 91 131 | 136 102 | 77 68 | 107 67 | 117 64 | 115 50 | 79 44 | 39 39 |
85 m/z Values and Intensities:

15	2	18	2	26	1	27	19	28	7	29	6	38	4	39	39	40	5	41	33
42	2	43	10	50	11	51	36	52	13	53	21	54	2	55	11	56	1	57	6
58	3	59	2	60	2	61	2	62	5	63	16	64	6	65	27	66	16	67	10
68	16	69	3	72	1	73	3	74	9	75	5	76	3	77	68	78	17	79	44
80	4	81	4	82	2	86	1	87	2	89	9	90	5	91	131	92	16	93	9
94	6	95	8	101	1	102	5	103	14	104	4	105	39	106	8	107	67	108	14
109	10	115	50	116	14	117	64	118	8	119	8	120	16	121	27	122	4	127	1
128	2	129	2	131	5	132	2	133	27	134	18	135	999	136	102	137	7	147	2

Figure 5. GC-MS of oregano oil

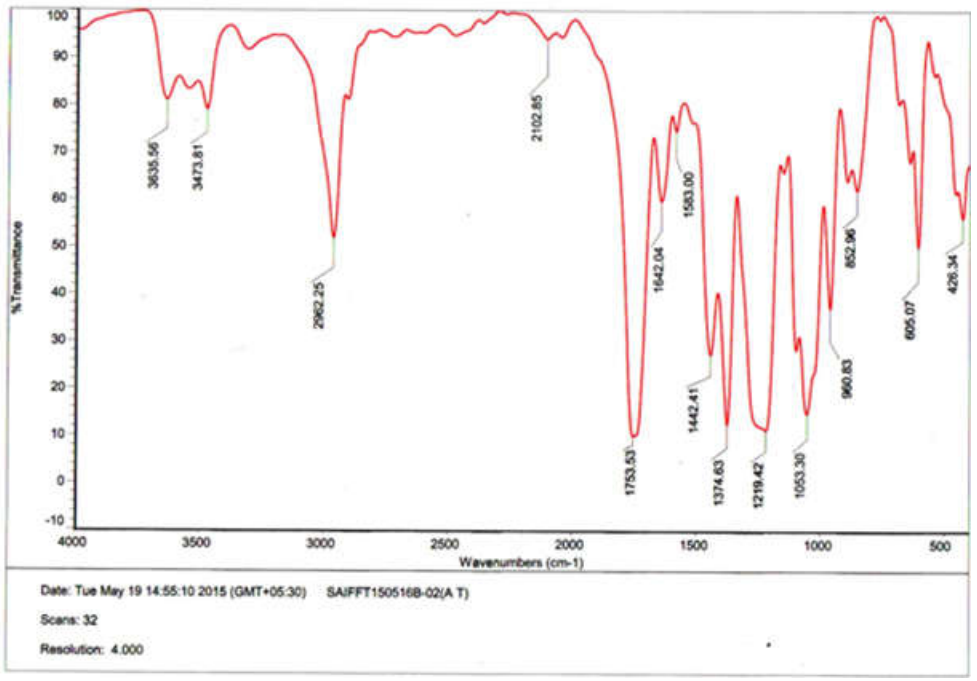


Figure 6. FT-IR of Oil-Drug mixture (1:1)

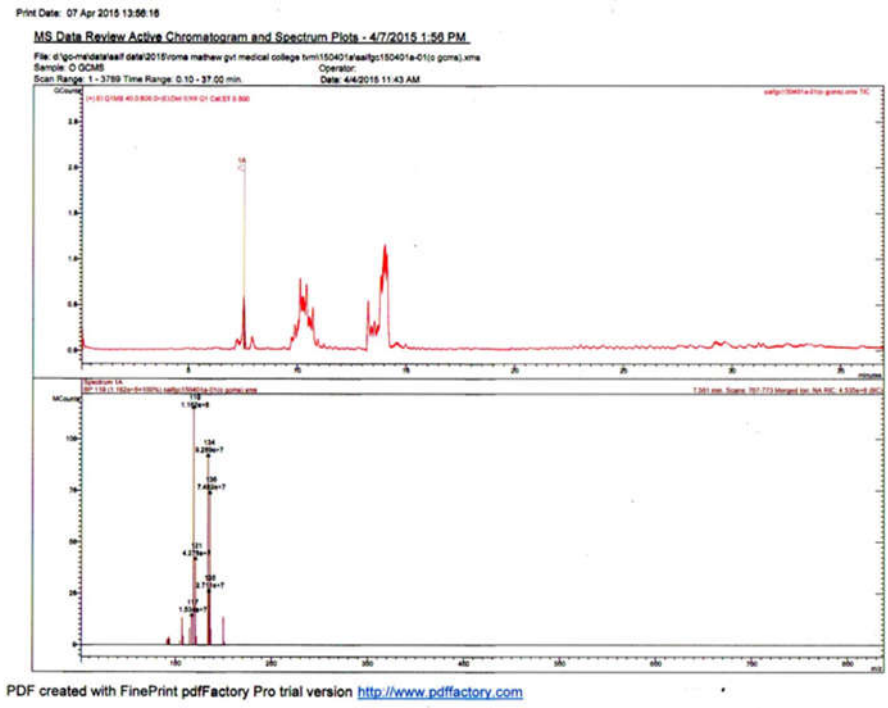


Figure 7. GC-MS of oil-Drug Mixture

thymol and cresol. The m/z value representing these compounds was found to be 83.The presence of carvacrol was confirmed in the spectra having m/z value of 55.The results observed were in close agreement with the reported data (Mahalat and Raja, 2014).

Drug-oil compatability studies

1. FT-IR spectroscopy

The FTIR spectra of drug and oil phase mixture in the ratio 1:1 was obtained. The FT-IR spectrum of mixture showed characteristic peaks at a range of 426-3625cm^{-1}. The prominent peak at 3473.75 cm^{-1} showed N-H stretching. The results observed were in close agreement with the reported data.

2. GC-MS

The GC-MS peak for oregano essential oil and Mebendazole mixture was obtained. The spectra confirms the presence of phenolic compounds like Thymol and Cresol. The m/z value representing these compounds was found to be 83.The presence of carvacrol was confirmed in the spectra having m/z value of 55. The spectra also confirms the presence of Mebendazole structure fragments like methyl carbamate at 70 m/z and 5-methyl 2-(1-methyl ethyl acetate) at m/z ratio of 63.There was no interaction between the oil and the drug.

Formulation - Design and development

1.Pseudo ternary phase diagram

File Version	9.0.5.1		
Study Type	Mixture	Runs	14
Design Type	Simplex Lattice	Blocks	No Blocks
Design Model	Quadratic		

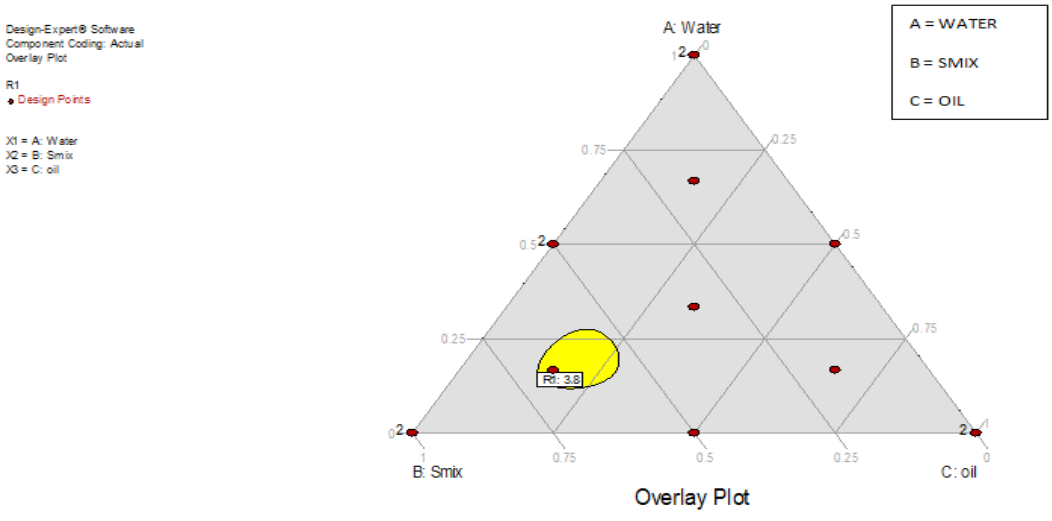


Figure 8. Phase diagram for oil : smix ratio (1:1)

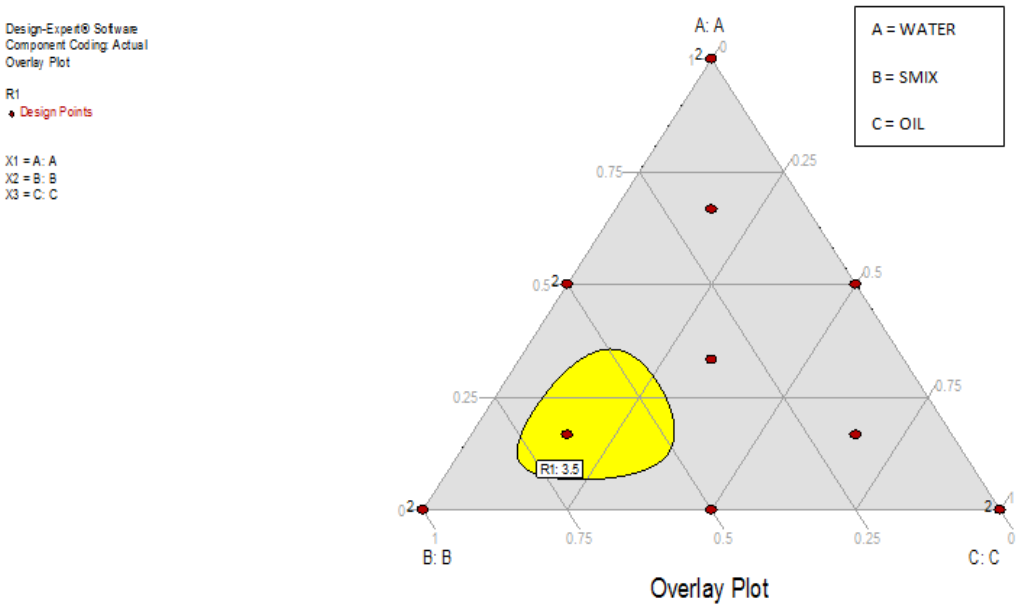


Figure 9. Phase diagram for oil : smix ratio (1:2)

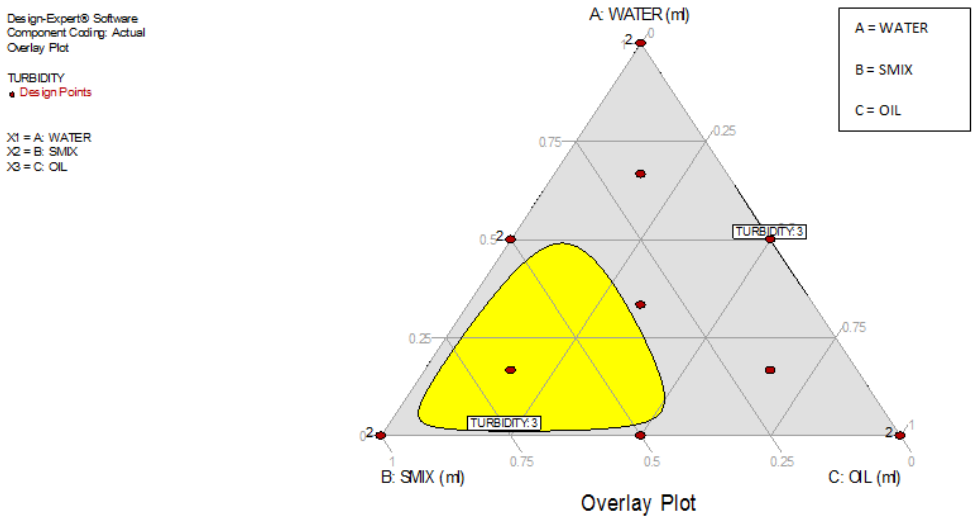


Figure 10. Phase diagram for oil : smix ratio (1:3)

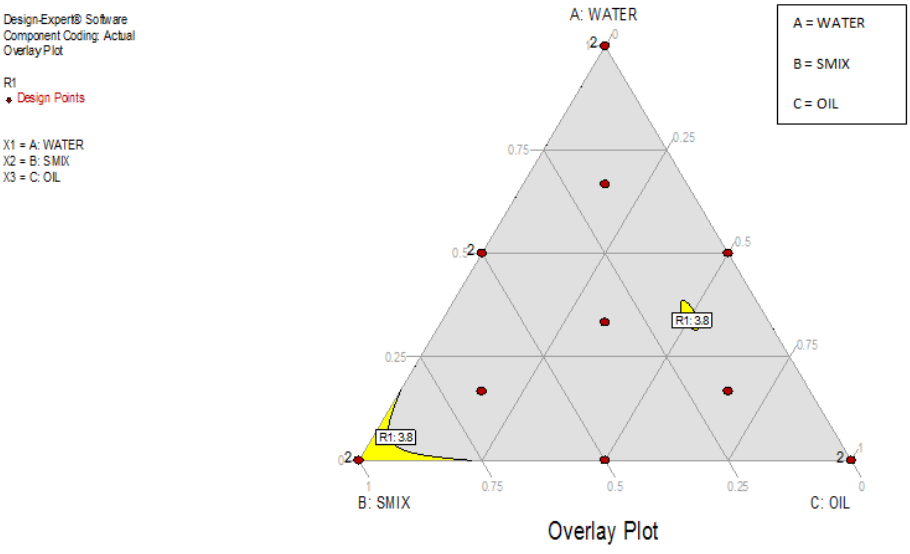


Figure 11. Phase diagram for oil : smix ratio (1:4)

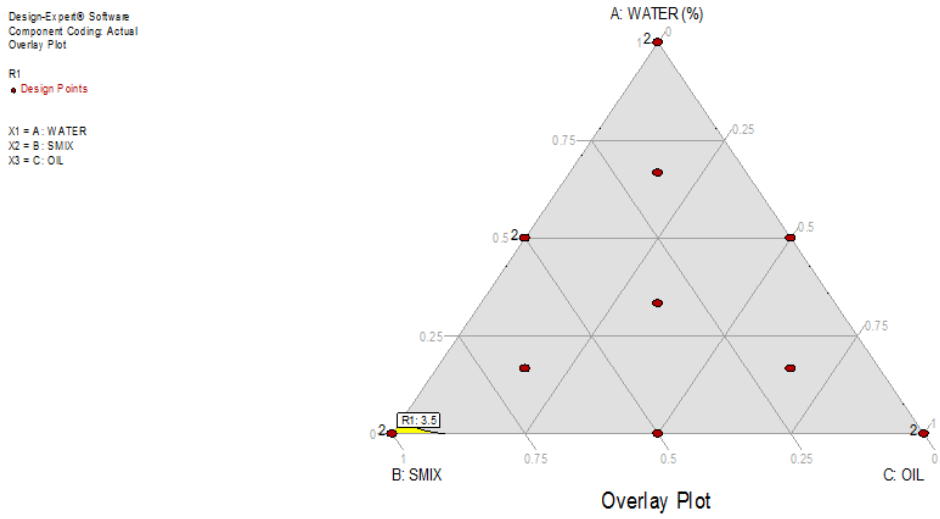


Figure 12. Phase diagram for oil : smix ratio (1:5)

The criteria of choosing respective ratio depends on the area of graphs. The graph showing maximum area is the ratio 1:3 and so was selected.

Optimisation of Nanoemulsion

1.Anthelmintic activity

Table 3. Anthelmintic activity of different nanoemulsion formulation

Formulation code	Mean time taken for paralysis (min)	Mean time taken for death(min)
NE1	2.5±0.04	3.5±0.027
NE2	1.2±0.01	2.2±0.02
NE3	3±0.16	4.6±0.36

The formulation NE2 showed minimum time of paralysis and death as compared to other two formulation, hence NE2 is chosen as optimised formulation (Meher and Giya, 2013).

2.Viscosity

Viscosity of the three formulations NE1,NE2,NE3 were determined and compared.

Table 4. Viscosity of different formulation

Formulation code	Viscosity in Cp
NE1	18.96 ±0.24
NE2	18.61 ± 0.45
NE3	21.8 ± 0.38

The determination of viscosity was performed in triplicate and expressed as Mean ± SD(n=3). The formulation NE2 have the least viscosity of 18.61±0.45 Cp. The minimum viscosity enhances the oral drug absorption, hence NE2 is chosen as the optimised formulation.

3.Globule size

Globule size of the three formulations NE1,NE2,NE3 was determined and compared

Table 5. Observation of globule size and polydispersity index of formulations

Formulation code	Globule size nm	Poly dispersity index
NE1	40.07	0.349
NE2	36..24	0.204
NE3	42.56	0.567

NE2 possess a lower globule size of 36.24 nm and polydispersity index of 0.204 compared to other formulation. Therefore NE2 was selected as optimised formulation.

Characterization of optimized nanoemulsion

1. Zeta potential

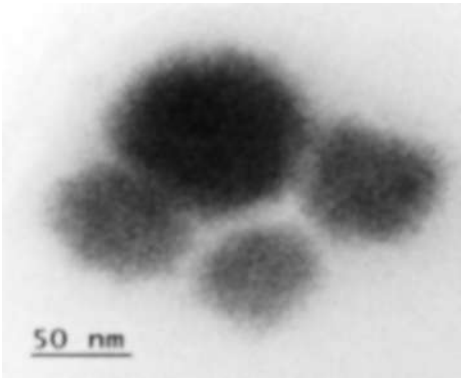
Zeta potential of NE2 was determined. Increased value of zeta potential prevents the coalescence of globules due to electrostatic repulsion and the value was found to be -53.5 mV.

2. Percentage Transmittance

The percentage transmittance of NE2 was found and was compared with NE3.It was found to be 89.98%. The percentage transmittance of NE3 (without drug) was found to be 94.83 %. The decrease in percentage transmittance was found due to presence of drug in NE2.

3. Transmission electron microscopy

Transmission electron microscopy of NE2 was done and spherical structure of globules were confirmed



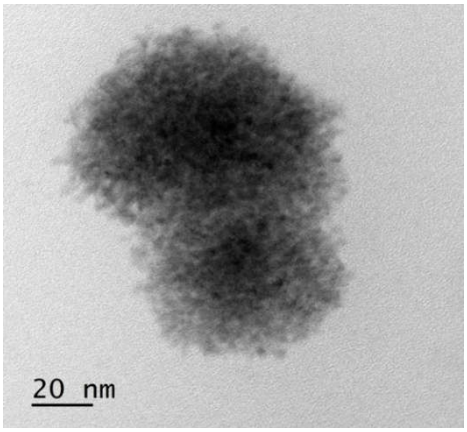


Figure 13. TEM of NE2

4. Scanning electron microscopy

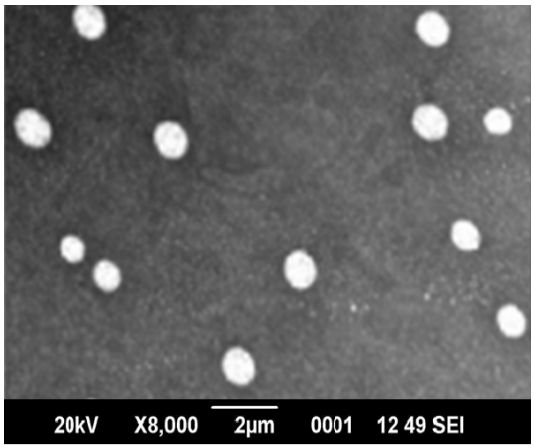


Figure 14. SEM of NE 2

DISCUSSION

As per the plan of work the characterisation of Mebendazole, triacetin, and oregano oil was performed. The result obtained was in close agreement with the reported data. Drug – oil compatibility studies were also performed by FT-IR and GC-MS. There was no significant interaction between the oil and the drug. From the three formulations NE1, NE2 and NE3, NE2 was selected as the optimized nanoemulsion. The selection was based on the criteria that the formulation with higher anthelmintic activity, smaller viscosity and globule size. NE2 showed mean time of paralysis of 1.2±0.01 min and 2.2±0.02 min mean time for death, viscosity of 18.61 ± 0.45 Cp and globule size of 38.24 nm (Piyush and Seema, 2013).

Conclusion

Essential oils are volatile, natural, and complex compounds characterized by a strong odour and formed by aromatic plants as secondary metabolites. Essential oil of *oreganum vulgare* taken by mouth for intestinal parasites, therefore it can enhance the action potential of anthelmintics. The biological activity of these substances has been related to their phenolic compounds content such as Thymol and Carvacrol, which represent between 40 - 50% of oils (Saron and Prabodh, 2014). Nanoemulsion containing drug (NE1), containing drug and oregano essential oil (NE2) and oregano oil (NE3) were prepared using smix ratio 1:3. Anthelmintic activity, viscosity and globule size were fixed as the parameters for optimisation.NE2 was selected as the optimised formulation. NE 2 showed a minimum mean time of paralysis and mean time of death reduction in time for paralysis and death compared with other formulation which is an indication of the synergistic effect of oregano essential oil on anthelmintic activity of Mebendazole. The minimum viscosity of NE2 enhances the oral drug absorption.NE2 possess a lower globule size polydispersity index, which is an indication of stability (Hasika and Remi, 2014). The results of these studies indicated that the Mebendazole nanoemulsion containing oregano essential oil formulations may be used for enhancing anthelmintic activity, and also suggest that this nanoemulsion is a promising novel formulation that can enhance the solubility of poorly soluble drug mebendazole and thereby enhance its oral bioavailability (Rocha and Camargo, 2015).

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