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Effect of Nanoemulsion on Antibacterial Potential of Tinidazole: Preparation and *In-Vitro* Characterization

Vijaya R *, Gurunaath P, Krithiga S, Vijayalakshmi S

Department of Pharmaceutical Technology, University College of Engineering, Anna University, BIT Campus, Trichy, Tamil Nadu, India

Address for Correspondance
Vijaya R,
vrssvrs@gmail.com

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ABSTRACT: The aim of the study was to prepare Tinidazole oil in water Nanoemulsion (NE) (O/W) using Tween 80, soya bean oil and water by high pressure homogenization technique. The formulation was optimized for homogenization speed and sonication time. The tinidazole NE (TNE) was evaluated for size, zeta potential, thermodynamic stability, Fourier transform infrared spectra (FTIR), drug content and antimicrobial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*. The optimized TNE had a droplet size of 187.8nm. Thermodynamic stability studies showed that the storage of TNE at room temperature is favorable than other temperatures. The antimicrobial efficiency of TNE was high upon comparison with Tinidazole. From the above studies it can be concluded that the TNE is stable and elicited improved efficacy in the tested microorganisms and thus suitable for the treatment of local and systemic infections.

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INTRODUCTION

NE is a kind of novel delivery system suitable for most of the therapeutic agents in all routes of administration [1]. In case of antimicrobial therapy, NE has been reported to enhance the efficacy, as NE itself said to possess antimicrobial property. Recent reports showed the delivery of antimicrobials using NE as a carrier [2, 3]. In our study, tinidazole an antibacterial and antiprotozoal drug used in the treatment of bacterial vaginosis, amoebiasis and giardiasis has been chosen to enhance its efficacy so as to reduce the dose and the associated adverse effects of the drug, such as bitter taste in mouth, nausea, vomiting, upset stomach, stomach pain, indigestion, loss of appetite, constipation and diarrhea. Further the therapeutic dose of tinidazole is single dose of 2g or 1g daily for five days for oral route. Tinidazole is available as tablet for oral administration meant for local and systemic infections [4].

Here, the development of tinidazole NE may be an encouraging method for enhanced action against intestinal amoebiasis and vaginal infections. The TNE has been developed and characterized for size, stability and antibacterial efficacy.

MATERIALS AND METHODS

Tinidazole was obtained from Apex laboratory, Chennai. Tween 80 and Ethanol were purchased from S.D fine chemical limited, Mumbai. Soyabean oil used was Fortune refined oil, India. Methanol was purchased from National scientific supplies, Trichy.

Preparation and Optimization of Tinidazole Nanoemusion

2.5ml of soyabean oil and 2.5ml of tween 80 were uniformly mixed using vortex shaker (Hi media, India). This was taken

as an oil phase and 1.5ml of ethanol and 43.5ml of water was taken as the aqueous phase. Oil phase was homogenized with 50mg of tinidazole and was added slowly to the aqueous phase with continuous stirring. The formulation was sonicated using a bath sonicator (ANM industries, India) for three cycles (45min.) to reduce the size of oil globules. The formulation was subjected to different homogenization speed (5000 rpm, 10000 rpm, 12000rpm) using a high pressure homogenizer (Ultra Turrax, IKA homogenizer) resulting in the formation of translucent stable nanoemulsion [5].

***In Vitro* Characterization of Nanoemulsion [6, 7]**

Compatibility: Compatibility study has been done for all the formulation excipients mixed physically with the drug using Jasco FTIR spectrophotometer (Perkin Elmer, India) for detection of any possible chemical interactions.

Droplet size and zeta potential: The droplet size of NE depends on the rate of emulsification process. The formulation (0.1ml) was dispersed in 50ml of water in a volumetric flask and gently mixed by inverting the flask. Measurement was done using the zetasizer (Nanoseries, United Kingdom). Zeta potential is a technique used to measure the surface charge properties and the long term physical stability of nanoemulsion. The measurements were carried out using a diluted nanoemulsion formulation and its values were determined from the electrophoretic mobility of the oil droplets.

Stability Testing

Centrifugation [8]: The optimized NE formulation was centrifuged (Remi equipment pvt.Ltd, Mumbai) at 3500rpm for 30min. Those formulations that did not show any phase separation confirmed the stability of the nanoemulsion.

Heating cooling cycle:[9] Six cycles between refrigerator (Samsung electronics, India) temperature 4ºC & at 45°C with storage at each temperature of not less than 48h was studied.

ICH Guidelines: The TNE formulations were stored at three different temperatures of 45°C, refrigerator of -4°C and room temperature of 25ºC. Those formulations that did not show any phase separation confirmed the stability of the nanoemulsion.

pH: The apparent pH of the formulation was measured by calibrated pH meter (Sartorius, Germany) in triplicate at 25ºC. The pH was determined by dipping the glass electrode into the formulation.

Drug Content [10]: The stock solution (100µg/ml of tinidazole) was prepared by taking 2.5ml of TNE and diluted with methanol for 25ml. Appropriate dilutions were made and the samples were analysed for drug concentration by UV Visible spectrophotometer at λmax of 310nm (Shimadzu (AsiaPacific) Ltd, Japan). A calibration graph was plotted for this purpose.

Antimicrobial Studies [11, 12]: Agar well diffusion method is widely been adapted to evaluate the antimicrobial potential of therapeutic drugs. The log phase bacterial inoculum was uniformly spread using sterile cotton swab on a sterile petri dish containing nutrient agar medium. A well with a diameter of 6mm to 8mm is punched aseptically using a sterile cork borer and volume 20µl of the solution of desired concentration was introduced into the well. The agar plates were then incubated under suitable condition depending upon the test microorganism. The petridishes were then incubated at 36ºC±1ºC for 24h, under aerobic conditions. After incubation, the zone of inhibition of the bacterial growth in mm was measured. Test was performed in triplicate.

RESULTS AND DISCUSSION

Compatibility

The results (Fig. 1) of FTIR revealed the presence of Tinidazole functional group peaks that are compatible with the excipients.

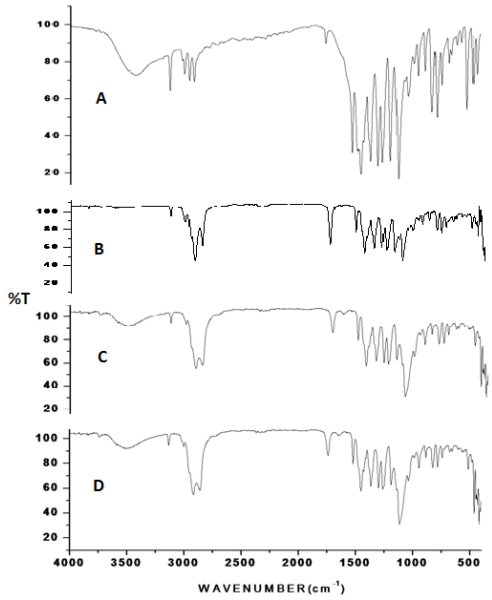

Figure 1: FTIR Spectrum of (a) Tinidazole, (b) Tinidazole + Soyabean Oil, (c) Tinidazole+Tween 80, (d) Tinidazole +Soyabean Oil+ Tween 80

Droplet Size and Zeta Potential

A stable NE was obtained at a centrifugal speed of 10, 000rpm with a small droplet size. The results of the size analysis are given in table 1. The PDI values <1 indicate the homogeneity of internal oil globules dispersion in the emulsion. The size of formulation TNE 2 was the less than 200nm thus has been chosen for further characterization. It has been observed that the speed of homogenization in the preparation of NE has highly influenced the size of the internal phase. The potential value in the range of ±30mv represents the stability of prepared nanoemulsion TNE 2 (Fig.2). The zeta potential of Tinidazole nanoemulsion gets altered due to the association of strongly basic Tinidazole in the acidic groups of soybean oil.

Table 1. Droplet Size of the Formulations TNE 1, TNE 2 and TNE 3

S.NO	FORMULATION	RPM	SIZE(nm)	PDI
1	Plain formulation	10,000	189.6	0.617
2	TNE 1	5000	225.8	0.498
3	TNE 2	10,000	187.8	0.574
4	TNE 3	12,000	225.3	0.732

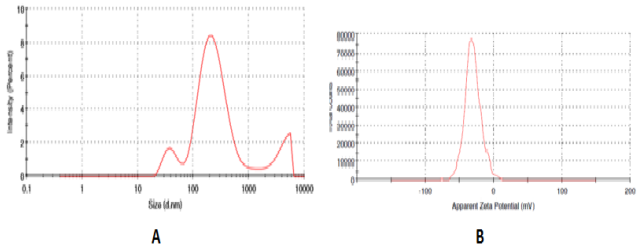

Figure 2: (a) Droplet Size of TNE 2, (b) Zeta Potential of TNE 2

Stability Testing

Centrifugation: No phase separation has occurred when TNE 2 was subjected at high centrifugal speed. This showed the physical stability of TNE.

Heating and cooling cycle: No phase separation has been observed at the end of heating and cooling cycle and the zeta size measured was 213.9nm which is closer to the value of TNE before study. The result (Fig.3) indicates the thermodynamic stability of the formed TNE.

ICH guidelines: There was no separation observed when the samples were stored at room temperature and in refrigerator but the phase separation was observed for samples stored at 40ºC. The zeta size of the formulations stored at room temperature (size 204.5nm) was close to that of initial values (187.8nm) whereas a slightly increase in size was observed for samples stored at 4ºC (232.5nm) (Fig.3). Hence it can be concluded that the formulation can best be stored at room temperature rather than in refrigerator temperature to maintain the physical stability.

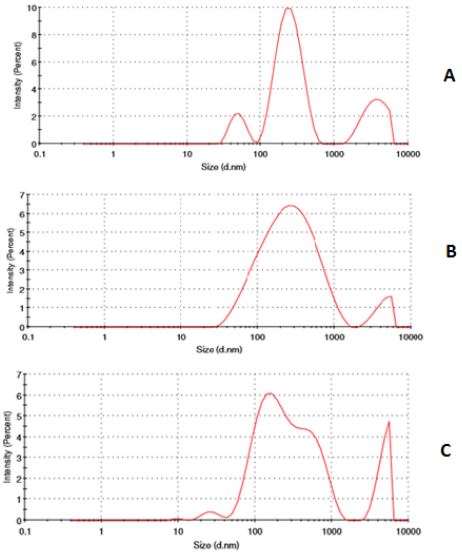

Figure 3: Droplet Size of TNE 2 (a) At the end of Heating and Cooling Cycle, (b) At Room Temperature after 15days, (c) At Refrigerator Temperature after 15days

Physical Characteristics of TNE

The physical properties evaluated for the formulations are tabulated in table 2. All the formulations were appeared to be translucent when examined visually.

Table 2. Physical Characteristics of NE & TNE

PARAMETER	PLAIN NE	TNE 1	TNE 2	TNE 3
pH	8	7.5	7.4	7.7
Conductivity (ohms)	0.0413	0.0413	0.0413	0.0413
Viscosity (cp)	0.8872	0.8872	0.8872	0.8872
Refractive index	1.33	1.33	1.33	1.33

Determination of Drug Content

The formulation for the determination of drug content was diluted with sufficient quantity of methanol and the absorbance was measured at λmax 310nm by UV spectrophotometer. Concentration of tinidazole was calculated using a standard calibration curve having the equation (Y = 0.036x - 0.055) with a regression coefficient value of 0.999. The estimated quantity of tinidazole in the NE was found to be 88%.

Antimicrobial Studies

The efficiency of Tinidazole nanoemulsion against *Pseudomonas aeruginosa, Bacillus subtilis* are tabulated in table 3. The results indicate that the TNE 2 showed maximum antimicrobial activity compared with plain NE, whereas no activity was observed for Tinidazole. The antimicrobial activity was observed at 25 and 50% concentration of preparations against G(+ve) *Bacillus subtilis and* G(-ve) *Pseudomonas aeruginosa* respectively. It might be due to the higher concentration of TNE that may be required by the gram negative bacteria than the gram positive bacteria.

Table 3. Zone of Inhibition of Tinidazole, Plain NE, TNE 2 against the Micro Organisms *Bacillus Subtilis* and *Pseudomonas Aeruginosa*

MICRO ORGANISM	Level of Dilution*	TINIDAZOLE (mm)	PLAIN NE (mm)	TNE 2 (mm)
Bacillus subtilis	0	-	0.8	1
	1	-	0.6	0.7
	2	-	0.5	0.5
Pseudomonas Aeruginosa	0	-	0.6	0.8
	1	-	0.5	-
	2	-	-	-

* Concentration of TNE at Level 0-100%, Level 1- 50%, Level 2- 25%.

STATISTICAL ANALYSIS

The results were represented as mean ± S.D with a significant $p \leq 0.05$.

CONCLUSION

The prepared nanoemulsion containing tinidazole was found to be stable and the technique was reproducible. TNE exhibited suitable physicochemical properties and enhanced antimicrobial potential than tinidazole alone. However, studies are required in future to confirm their in vitro/in vivo performance after converting the TNE into a suitable dosage form for treating vaginal and intestinal infections.

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