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Development, Characterization and Evaluation of Tinidazole Nanosuspension for Treatment of Amoebiasis

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Abstract

Amoebiasis is an infectious disease of gastrointestinal tract caused by the protozoan, Entamoeba histolytica. This disease is widely distributed worldwide and is endemic in most parts of India and other developing countries. The protozoa multiply rapidly in their hosts. Since effective vaccines are as yet unavailable, chemotherapy is the major strategy to treat infected individuals and reduce transmission. Nitroimmidazoles, like Tinidazole (TNZ) form an important class of drugs, which are active against both intestinal and systemic forms of amoebiasis. Most of these drugs have high dose and metallic to bitter taste which result in poor patient compliance. In the present study, nanosuspension was developed by nanoprecipitation method for oral delivery using Tinidazole as model drugs with improved its dissolution rate and hence bioavailability. With improved bioavailability it may be possible to reduce the dose requirement, which would be especially advantageous for paediatric patients. *Ex-vivo* absorption studies performed using the rat intestinal model showed an enhanced absorption from the nanosuspension as compared to micronized drug.

Keywords: Nanosuspension; Bioavailability; Nanoprecipitation; Taste masking; Amoebiasis

Introduction

Solubility is important factor for drug efficacy, independent of route of administration, especially when drug is poorly soluble in both aqueous as well as organic media. This is a major challenge before pharmaceutical companies while developing new pharmaceutical products as very low amount of active substance is either insoluble or poorly soluble in water. Oral administration of such low soluble drugs in humans and laboratory animals frequently leads to incomplete and irregular absorption from the gastrointestinal tract, since the dissolution of the active substance is the major rate limiting step in the absorption process. Enhancement of oral bioavailability by micronization is not sufficient for many of the poorly soluble new chemical entities (NCE), the next step is Nanosization [1].

Some of the methods investigated as potential oral novel systems for poorly and sparingly soluble drugs include microparticles, nanoparticles, solid dispersions, microemulsions etc. Nanosuspensions are defined as ultra-fine suspensions of pure drug nanoparticles stabilized with a surfactant or surfactant mixtures. The poorly soluble and oil soluble compounds can be formulated as nanosuspensions also existing drugs can be reformulated to eliminate the toxicologically less favorable excipients. Such nanosized particles have high crystal energy, indicated by a high melting point, which reduces the tendency of the crystal to dissolve, regardless of the solvent. Different hydrophobic drugs have already been formulated successfully in this way, eg: Naproxen, Clofazimine, Nimesulide, Omeprazole, Mitotane, and Nifedipine [1].

An important advantage of nanosuspensions is the increase in saturation solubility, hence the dissolution rate of compound. This increase in the dissolution rate is may be due to increased surface area of particles. Another important feature of nanosuspensions is altered crystalline structure (increase in the amorphous fraction or may be creating totally amorphous particles) [1]. The drug candidates with poor solubility and dissolution-rate limited absorption generally exhibits low and variable oral bioavailability. Due to poor oral bioavailability such drug candidate would have to be administered in high doses thus making the therapy costly.

Atovaquone and Bupravaquone an orally administered antibiotics are the god examples of this type. Nanosizing of such drugs leads to a drastic increase in their oral absorption and consequently bioavailability. The increase in bioavailability is due to surface area (due to reduction in particle size by 337 fold), increased saturation solubility and increase in dissolution velocity. The drug dose reduction is possible with enhancement in bioavailability making therapy cost effective and demolishing excessive drug dumping in the body [2].

Nanosuspensions as drug delivery systems are advantageous since they have better physical stability due to small size of nanoparticles experiencing less gravitational force as compared to large microparticles, flocculation and ostwald ripening are also absent in case of nanoparticles [3-7].

In the recent studies nanoprecipitation method is widely used to increase solubility and bioavailability of the drug [8-16].

Materials and Methods

Tinidazole (gift sample from Ajanta Pharmaceuticals, Mumbai, India), Pluronic F127 and Cremophor-EL (BASF corporation, India), HPMC K4M (Colorcon Asia Private Ltd.), Soyalecithin Lecimuthin® (Degussa), Tween 80, Lactose Anhydrous, Maltodextrin and Polyethylene Glycol-PEG 600 (S. D. Fine chemicals), Mannitol BP (Sarabhai M Chemicals) and Trehalose (Hyashibara, Japan). All other chemicals and reagents were of analytical grade.

Preparation of nanosuspension

Nanoprecipitation method was attempted, because of

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the simplicity of the method, and feasibility to scale up. Several surfactants (Tween-80, Sodium Lauryl sulphate (SLS), Pluronic F-127, HPMC K4M, SLS, Cremophor-EL, Soyalecithin) and solvents (Dichloromethane (DCM), Ethyl acetate, Acetone) were screened to develop stable nanosuspension formulation. Of these soyalecithin and acetone were found best for TNZ nanosuspension (Table 1).

Nanosuspension was prepared by drop wise addition of organic phase (100 mg of TNZ in 25 ml of acetone) to aqueous phase (50 mg of soyalecithin in 15 ml distilled water) with continuous stirring. The emulsion thus formed was then subjected to evaporation at 50°C using Rotavapor under vacuum. After complete removal of acetone the preformed suspension was then diluted with remaining 5 ml of filtered water to adjust final volume to 20 ml. To mask bitter taste and to improve patient compliance of the formulation different flavoring and coloring agents were evaluated. Selected final optimized batch of nanosupension was subjected to freeze-drying. Different cryoprotectants like maltodextrin, trehalose, lactose and mannitol were evaluated, amongst which trehalose was found to be the best.

Physicochemical characterization

The developed freeze dried formulation was characterized for drug content, particle size, pH, residual solvent, ease of reconstitution, moisture content, in-vitro drug release, saturation soyalecithin, IR Spectroscopy, Differential Scanning Calorimetry (DSC), X-ray diffractometric analysis (XRD), Environment scanning electron microscopy (ESEM) and stability.

Ex-vivo absorption studies

The objective of *ex-vivo* absorption studies was to compare the rate and extent of absorption of the formulated nanosuspensions with that of micronized drug, marketed tablet using the rat intestinal model. *Ex vivo* absorption study was carried out using the selected freeze-dried sample of developed formulation, physical mixture of formulation, free drug (micronized TNZ) and its marketed tablet.

The inner lumen (villi portion) of freshly excised rat intestine tied at one end and through other end dispersed samples of TNZ were injected with help of a tuberculin syringe and then tied. These segments were then tied onto the paddle of dissolution USP apparatus Type II (Electro lab TDT 06 T) and immersed in phosphate buffer (pH 6.8) medium. The study was performed at 50 rpm.

Solvent	Surfactant	Drug: surfactant Ratio	Solvent quantity	Particle Size (nm)
Acetone	Soyalecithin	1: 0.25	93.75 ml	217.0

Table 1: Final batch of nanosuspension prepared by nanoprecipitation method.

Results and Discussion

Nanosuspension with 0.75% TNZ, 0.19% soyalecithin showed a particle size of 217.0 nm and formed a homogenous suspension on reconstitution, with 7% and 10% increase in dissolution rate, compared to micronized drug and marketed tablet respectively. Within 2 hours 95% of drug was released from nanosuspension as compared to micronized drug (88%) and marketed product (85%). About 10% increase in saturation solubility was observed.

Taste masking of the formulation was successful with inclusion of 1.2% Peppermint water and 0.2% Sucralose. *Ex-vivo* absorption studies showed an enhanced absorption from the nanosuspension (94%)

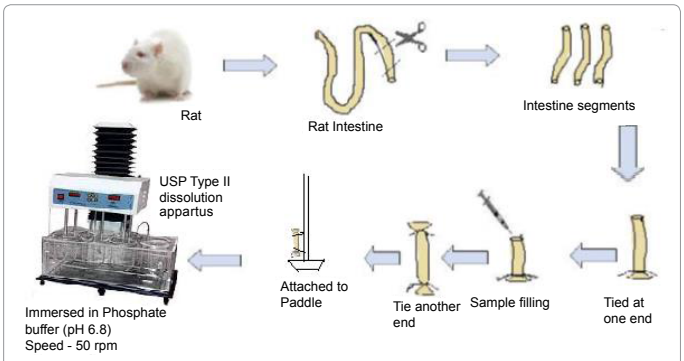

Figure 1: Procedure of *Ex-vivo* absorption studies.

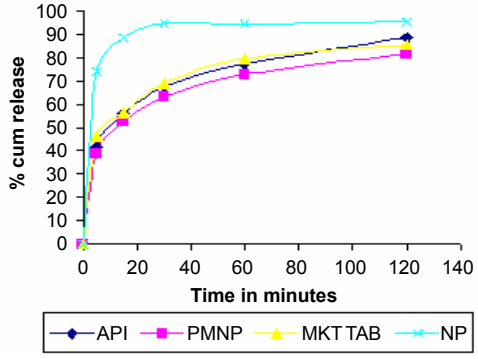

Figure 2: *In-vitro* drug release profile of nanosuspension.

Characteristics	Before freeze drying	After freeze drying
Mean Particle Size, polydispersity index	216.6 nm ± 92.09 nm (0.332)	325.3nm ± 151.95 nm (0.858)
Ease of reconstitution	Homogenous suspension	Homogenous suspension
Drug Content (%w/w)#	98.1 ± 0.5	97.9 ± 0.5
pH	6.4 ± 0.08	6.39 ± 0.05
FTIR spectroscopy	-	No interaction between drug and excipients (Figure 2)
Differential Scanning Calorimetry (DSC)	-	No significant change in crystallinity of the drug; the increase in dissolution rate is thus attributed to an increase in available surface area of the nanoparticles (Figure 3)
X- ray diffractometric analysis (XRD)	-	Nanoprecipitation method does not change the crystalline nature of drug which is in contrast to literature (Figure 4)
Environment scanning electron microscopy (ESEM)	After nanoprecipitation rod shaped crystals of the drug converted to spherical in shape (Figure 6)	-

#Where S.D is calculated for three experiments (n=3)

Table 2: Characterization of nanosuspensions.

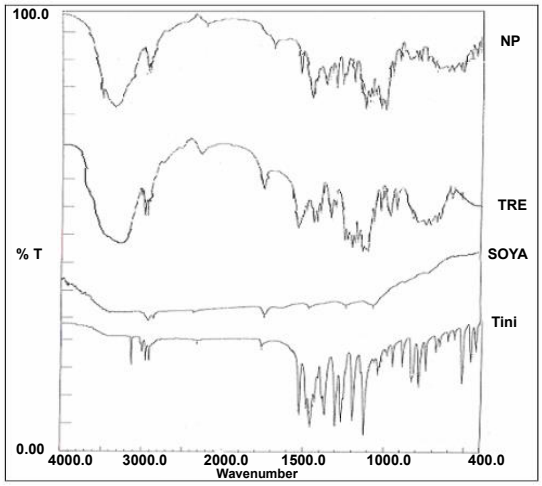

Figure 3: Comparative IR spectra of Tini (micronized Tinidazole), SOYA (soyalecithin), TRE (trehalose), NP (nanosuspension).

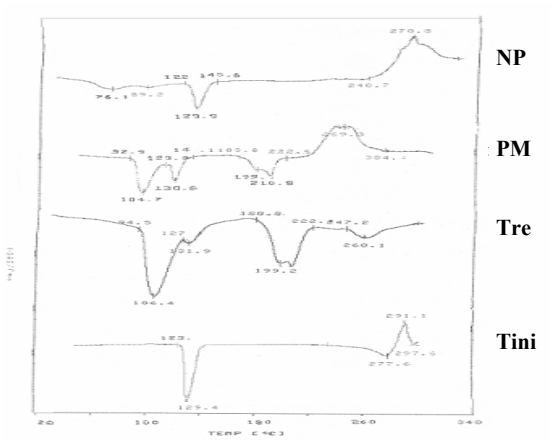

Figure 4: Comparative DSC of Tini (micronized Tinidazole), Tre (trehalose), PM (physical mixture), NP (nanoprecipitation sample).

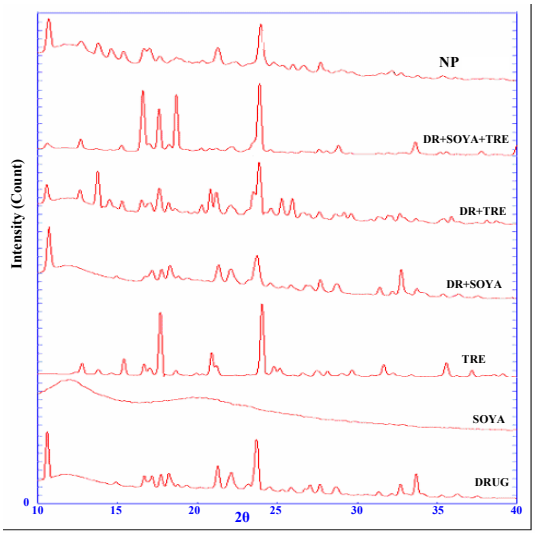

Figure 5: X-ray diffraction (XRD) DR (micronized Tinidazole), TRE (trehalose), SOYA (soyalecithin), NP (Nanosuspension).

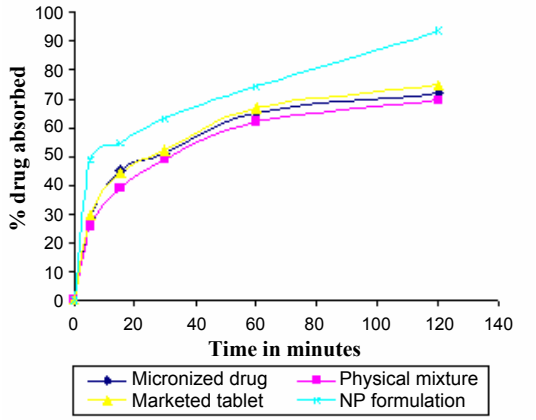

Figure 6: *Ex- vivo* absorption of drug through rat intestine.

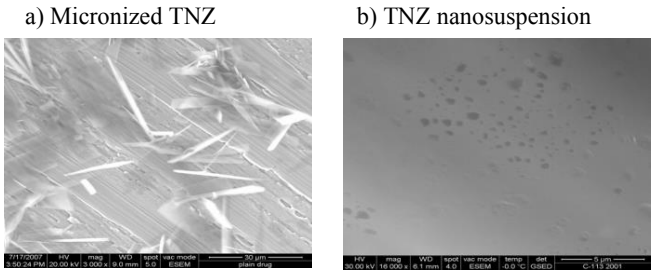

Figure 7: ESEM photograph.

compared to micronized drug (72%), physical mixture (70%) and marketed tablet (75%) within 2 hrs, indicating enhanced bioavailability from the nanosuspension product (Figures 1-7).

API-Micronized drug, PM NP-Physical mixture nanoprecipitation method, MKT TAB-Marketed tablet and NP-nanosuspension.

Conclusion

In conclusion, the present study has led to successful development of a stable, palatable nanosuspension of Tinidazole, with enhanced absorption, which would be suitable for pediatric patients. Differential Scanning Calorimetry (DSC) confirmed that the increase in dissolution rate is attributed to an increase in available surface area of the nanoparticles and conversion of rod shaped crystals of the drug to spherical shape confirmed by Environment scanning electron microscopy (ESEM).

Acknowledgments

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