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Optimization of Metronidazole Benzoate Nanosuspension by Probe Sonication Using a 2^3 Full Factorial Design

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ABSTRACT

Metronidazole benzoate (MB) is a BCS Class II antiprotozoal drug with limited aqueous solubility, which makes dissolution the rate-limiting step in its oral absorption. Nanosuspension strategies represent a practical approach to improving dissolution without compromising drug stability. This study aimed to prepare and optimize MB nanosuspension by probe sonication and assess the effects of three formulation variables. Twelve formulations (F1 - F12) were prepared: eight factorial runs and four center-point replicates. The formulation variables included; Tween 80 (T80) concentration, xanthan gum (XG) concentration, and sonication time. A 2^3 full factorial design was used to assess the effects of these variables on physicochemical properties and *in vitro* drug release. The full factorial design matrix showed excellent reproducibility, with T80 concentration and sonication time significantly affecting particle size, polydispersity index, and zeta potential. The dissolution rate and equilibrium solubility showed a 9-fold improvement at 0.5% T80 compared to the surfactant-free control formulation. XG resulted in low shear viscosity (6,444 – 33,106 cP at 0.3 rpm) without affecting dissolution. All formulations exhibited pseudoplastic behaviour. The optimum formulation was found to be F8 (T80 (0.5%), XG (0.3%), sonication time (7 min), particle size (501 nm), Polydispersity index (PDI) (0.272), zeta potential (−29.7 mV), and 87.4% drug release in 30 min). The 2^3 factorial design have shown the independent contributions of each formulation variable; T80 (0.5%) and 7-min sonication time yielded optimal dissolution, while XG (0.3%) provided the rheological profile required for physical stability. Therefore, F8 is recommended as the optimized formulation for further evaluation.

Keywords: Metronidazole Benzoate, Nanosuspension, Probe Sonication, Factorial Design.

Introduction

Metronidazole benzoate (MB) is a benzoate ester prodrug of metronidazole, a 5-nitroimidazole antibacterial and antiprotozoal agent. It is active against a broad spectrum of pathogens, including *Entamoeba histolytica*, *Giardia lamblia*, *Trichomonas vaginalis*, *Clostridium difficile*, and anaerobic bacteria such as *Bacteroides fragilis*.[1] The benzoate ester form is the preferred choice for oral liquid preparations because it masks the bitter taste of the parent drug. [2,3] Enzymatic or pH-driven hydrolysis in the gastrointestinal tract regenerates metronidazole as the pharmacologically active species.[4]

MB belongs to BCS Class II antiprotozoal drug, its water solubility is 4 mg/100 mL at 25ºC (practically insoluble in water). The partition coefficient (log P = 1.64) indicates moderate lipophilicity. Therefore, the rate-limiting step in the absorption of MB is the dissolution rate. Conventional suspension formulations disperse MB microparticles in an aqueous vehicle, which is stabilized by hydrocolloid polymers. Generally, they provide an acceptable delivery vehicle, but they do not improve solubility. The dissolution rate remains dependent on the particle surface area and wetting efficiency of the preparation.[5,6]

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Nanosuspension technology offers a very interesting strategy to overcome dissolution issues by reducing particle size below 1 µm. The increase in specific surface area improves dissolution kinetics according to the Noyes–Whitney equation (dC/dt = DA{Cs−Ct}/h); the effective diffusion layer thickness (h) decreases substantially at the nanoscale level.[7] Unlike amorphous solid dispersions, nanosuspensions preserve drug crystallinity and avoid the thermodynamic instability associated with amorphous forms.[8] Probe sonication is a widely applied top-down preparation technique that generates acoustic cavitation and intense localized shear to progressively comminute the drug particles. This method of preparation does not need organic solvents, nor high-pressure equipment. Also, probe sonication method, if used intermittently, it becomes suitable for thermolabile compounds.[9]

Two excipients are central to MB nanosuspension formulation, Tween 80 and Xanthan gum. Tween 80 (polysorbate 80) is a nonionic surfactant with a Hydrophilic-Lipophilic Balance (HLB) value of 15, which serves a dual role; it acts as wetting and particle-stabilizing agent. Tween 80 adsorbs at newly generated surfaces during sonication to prevent reaggregation. Tween 80 also acts as a micellar solubilizer that increases apparent drug solubility above its critical micelle concentration (~0.012 mg/mL in water).[10] Xanthan gum on the other hand, is a high-molecular-weight anionic heteropolysaccharide produced by *Xanthomonas campestris* fermentation. At concentrations between 0.1% and 0.3% w/v, it produces a viscoelastic network in the aqueous phase that provides high viscosity at low shear rates. Such rheological profile, increases xanthan gum resistant to sedimentation according to Stokes' law, while exhibiting shear thinning that allows easy pouring and swallowing at higher shear rates.[11]

The combined influence of surfactant concentration, polymer concentration, and sonication energy input on the physicochemical attributes of a nanosuspension is multivariate and potentially interactive. Evaluating one-factor separately is inefficient to detect