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Research Article

PROCESS OPTIMIZATION FOR *FORMULATION* OF AN ORAL PYRAZINAMIDE CUBOSOME

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ABSTRACT

The aim of work was to formulate and evaluate cubic formulation of Pyrazinamideto improve the bioavailability of the drug. Different formulations of Pyrazinamide cubosomes were prepared by Top down approach using GMO as lipid phase vehicle, Poloxamer 407 as stabilizer and distilled water as aqueous phase by varying the concentrations of GMO and Poloxamer 407. The effect of stabilizer concentration was investigated to determine their effects on the morphological and dimensional characteristics of cubosomes. At the optimized homogenization conditions, cubosomes with reproducible, narrow particle size distribution and a mean particle size of 180 nm±4.9 nm were obtained. Resultant formulations were characterized for particle size, surface morphology, encapsulation efficiency, in-vitro dissolution and diffusion studies. Structure characterization was confirmed that loading of Pyrazinamide shows no disturbance in the structure of formed cubosomes. The encapsulation efficiency determined by UV spectroscopy and further confirmed that Pyrazinamide was successfully encapsulated in Cubosomes.

INTRODUCTION

Pyrazinamideis a white crystalline powder belongs to BCS class III and is used in tuberculosis along with Isoniazid, Ethmbutol and Rifampicin, having molecular formula $C_5H_5N_3O$, molecular weight 123.113g/mol and official in Indian Pharmacopoeia 2007, British Pharmacopoeia 2005, and United state pharmacopoeia 2007 (Chenna *et al*, 2011; Block, 2004; Neil, 2011; Sweetmann, 2011). Because of low short-term toxicity and tuberculocidal activity it is used infirst line therapy of TB (Sweetmann, 2011, Joel *et al*, 2004; IP, 2007; BP, 2005). From the literature survey, it was found that Pyrazinamide was estimated by analytical methods such as few UV-Visible methods, Reverse-phase high-performance liquid chromatographic (RP-HPLC) method, gas chromatography and UPLC method (USP, 1995; Khuhawar, 2002; Hector, 1999; Martin, 2006; Calleri, 2002; Mariappan, 2000). The present developed method was simple, precise, specific and accurate.

Cubosomes are bicontinuous cubic liquid crystalline phase, having particle size of 10-500nm, formulated by input of high energy and stabilization using surfactants (Nanjwade *et al*, 2014; Bhosale *et al*, 2013). The prepared preparation can interchange in any dosage form and because of amphiphilic domains having a great potential to increase solubility and permeability of all BCS class drugs (Mateescu *et al*, 2014). The aim of present study is to formulate a capsule formulation of Pyrazinamide to increase its bioavailability as a model drug.

MATERIAL AND METHODS

Instrument used and reagents

The glycerol monooleate RYLO MG 19(GMO) was a giftfrom Danisco Cultor (Grindsted, Denmark). Poloxamer 407, Pluronic F127 was a gift from BASF Corporation (Ludwigshafen, Germany). Pyrazinamide was a gift from Mylon Nashik (MH). All chemicals used in the studywere of analytical grade and used without further purification.

Preparation of Pyrazinamide-Loaded Cubosomes

Cubosomes was prepared by Top-down approach (Esposito *et al*, 2005). Briefly, for each sample, a volume of 15 ml of chloroform was used to completely dissolve GMO and Pluronic F127. GMO, water and alcohol was used in ratio of 60:20:20 according to the phase diagram, followed by the addition of the different amount of Pluronic and 10 mg Prazinnamide with continuous stirring at magnetic stirrer (Barauskas *et al*, 2005; Lai *et al*, 2009). The mixture was then sonicated for 10 minutes, and allow to stand at room temperature for 24-48hr. The mixture then homogenized at high pressure and cycled through HPH (GEA Niro Soavip, Panda, Canada). Aluminium coils were used to cover the sample vials in order to protect

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samples from direct light. The dispersions were then used for future tests and evaluation (Zhang *et al*, 2007; Garg *et al*, 2007; Bei *et al*, 2009)

Standard Calibration Curve

From the stock solution, 1µg/ml, 2, 3, 4, 5, 6µg/ml was prepared and the absorbance was taken at 271nm.The mean value of triplet reading was taken for construction of standard calibration curve. The absorbance of the prepared formulations was measured at 271nm intriplets separately (Hector 1999). The average absorbance value was used for the formulation estimation of Pyrazinamide. The % drug estimated in the formulations.

Particle Size Determination

For the particle size calculation, sample was vortexed and measured through dynamic light scattering (DLS; Brookhaven Instruments Corporation, Austin, TX, USA) at 25°C at wavelength 659.0 nm. The dispersion was observed for particle mean diameter, size distribution, and polydispersity. Based on a reference from National Institute Standard (NIST), a PI <0.05 was considered monodispersed (Meng *et al*, 2011; Hackley and Ferraris, 2011, Beckett and Stenlake, 1988).

Transmission Electron Microscopy

The samples were prepared by putting a 5µl droplet of the cubosomes suspension onto a 300 mesh carbon-coated copper grid and letting the cubosomes settle for 3-5 min. Then, the excess fluid was removed. The air-dried samples were negatively stained in 1% uranyl acetate for 3-5 min (Sri KV *et al*, 2014). The samples were then viewed on a JEOL Model JEM 1400 120KV transmission electron microscope (JEOL-USA, Wil mington, DE, USA) and photographed digitally on a Gatan axis-mount 2kx2k digital camera.

Encapsulation Efficiency

To quantify the drug encapsulated in dispersion after production, 2ml of cubic dispersion containing PYR was added into the centricon reservoir (Optima MAX-XP Ultracentrifuge, Beckman coulter, USA). Cubic 2ml of cubic dispersion centrifuged for 30 min at 30,000 rpm. The supernant dispersion was then taken and analyzed for entrapped Pyrazinamide content using UV spectroscopy.

In-vitro Dissolution and Diffusion Studies

The dissolution and diffusion studies of pure Pyrazinamide and the Cubosomes of PYR performed in 0.1N HCl and Phosphate buffer pH 6.8 respectively and compared.

RESULT

The prepared cubosomes was evaluated for different parameters.

Particle Size Determination

The dispersion was observed for particle mean diameter, size distribution, and polydispersity and summarized in Table 1 & Fig 1. Based on a reference from National Institute Standard (NIST), a PI <0.05 was considered monodispersed [29-31]. The PI of the sample varied from to 0.00568 to 0.074. Dispersion is able to show smallest particle size at optimized concentration of Poloxamer.

Table 1 Particel size, distribution, EE of pyrazinamide loaded Cubosomes (effect of GMO: polymer)

Formulation Batch	GMO/Pluronic F127	Particle Size (nm)	PDI	EE (%)
F1	100/8	101.2	0.068	85
F2	100/10	102.6	0.056	90
F3	100/12	180	0.038	97
F4	100/14	264.2	0.061	100.06
F5	100/16	297	0.074	100

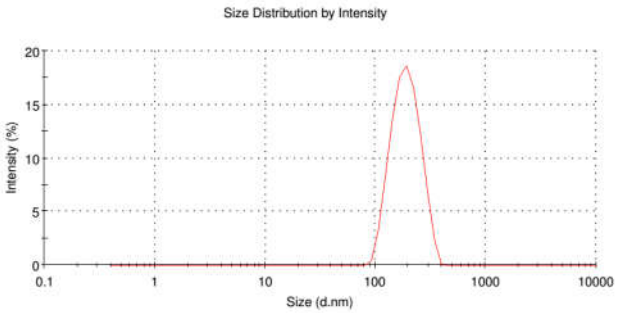

Fig 1 Particle Size Distribution of the optimized batch.

Transmission Electron Microscopy

The TEM confirmed that the cubosomal structure maintained even after the addition of Pyrazinamidewhich shown in Fig 2.

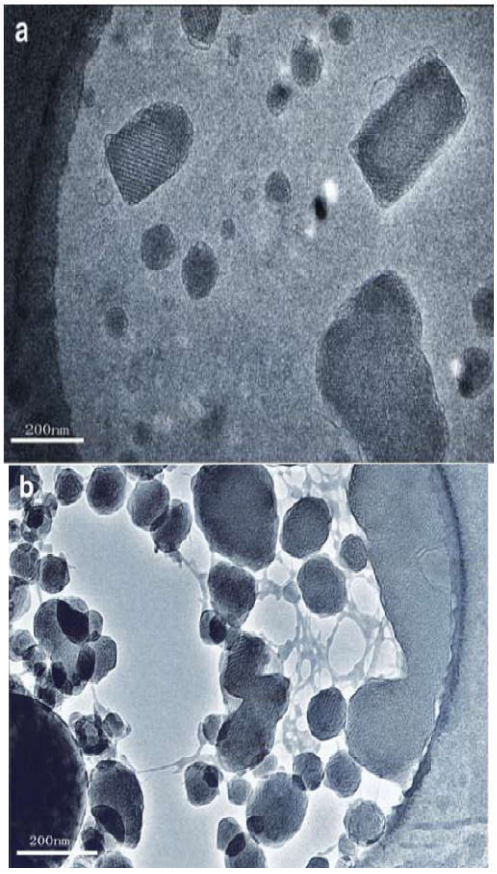

Fig 2 Cryo TEM: a: Blank cubic particles, b: drug loaded cubosomes

In-vitro Dissolution and Diffusion studies

Dissolution and Diffusion of the cubosomes higher than the pure pyrazinamide. It confirmed that the dissolution of pyrazinamide in encapsulated cubosomal shows a prolong release than pure pyrazinamide. Likewise the diffusion incresed in cubosomal formulation which can correlate with the incresed bioavailability. The comparative dissoultiona and diffusion study was shown in Fig 3.

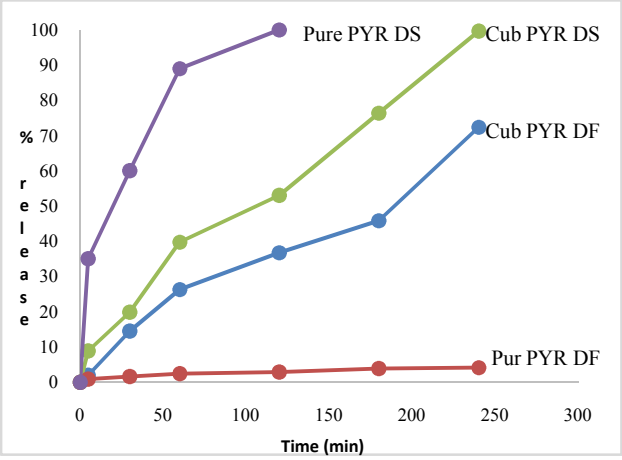

Fig 3 Dissolution and Diffusion Study of pure Pyrazinamide and Cubosomal formulation of Pyrazinamide. (DF: Diffusion, DS: Dissolution)

CONCLUSION

Literature showed that use of poloxamer as a surfactant in LC formulation increase the stability. At a optimized condition, influence of the stabilizer was summarized for the average particle size, the polydispersity index (PI), and the encapsulation efficiency. By using phase diagram of monolein-poloxamer. Cubosomes was prepared by the top-down approach which is economic due to consumption of less time and energy[22,23]. SEM was used to revel the liquid crystalline matrix structure. These data suggested that cubosomes were indeed formed with particle size ranging from 101 to 227 nm. Literature also claims that the incorporation of additives in lipid cubic phase can interact or modify the structure of the cubic phases. However, incorporation of PYR don't affect the structure. Cubic disperse phase with a mean particle size of 110nm and uniform size distributions were obtained in this study. Liquid crystalline system can use for all BCS class molecules due to existence of polar and non-polar domain and can serve as universal carrier than the established one.

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