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Novel Binary Itraconazole-Skimmed milk Solid Dispersion: Preparation and Evaluation

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ABSTRACT

Binary solid dispersions of a poor water soluble drug, itraconazole were prepared by lyophillization technique employing skimmed milk as carrier. Characterization by Differential scanning calorimtry, Scanning electron microscopy suggested partial amorphization of itraconazole in binary solid dispersions. Futhermore, results of X-ray diffractometry showed decrease in magnitude of relative degree of crystallinity (RDC) with increase in skimmed milk content. No chemical interaction was observed between itraconazole and skimmed milk, indicated by FT-IR studies. In addition, significant decrease in surface tension values ($P<0.01$) was observed on increasing the skimmed milk proportion indicating detergent action of milk proteins. The results of solubility studies in Simulated Gastric Fluid (SGF pH 1.2) revealed higher solubility of itraconazole in solid dispersion than bulk drug. Dispersion containing highest skimmed milk proportion (MSD-1) demonstrated 84% drug release in 1 h. The values of % DE $_{20min}$ and Rdr_{5min} were calculated as 53.2% and 4.16 respectively. The dissolution data showed that the release followed the Higuchi model equation suggesting diffusion as main mechanism of drug release from solid dispersion.

Key words Binary solid dispersion · Skimmed milk · Saturation solubility · Dissolution rate · Surface tension

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INTRODUCTION

Itraconazole is, chemically 4-[4-[4-[4-[{2-(2,4-dichlorophenyl)-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxyl]phenyl]-1-piperazinyl]phenyl]-2,4-dihydro-2-(1-methylpropyl) -3H-1,2,4-triazol-3-one with a molecular formula of $C_{35}H_{38}CI_2N_8O_4$ [1, 2]. It is a broad-spectrum antifungal agent with activity against *Candida* species, *Aspergillus* species, *Malassesia furfur*, *Fusarium* species and *Histoplasma capsulatum*. Itraconazole is indicated in the treatment of histoplasmosis, blastomycoses [3], candidiasis [4] , onchomycoses [5] and aspergillosis [6].

It is a weakly basic drug, with pKa of 3.7 and log P of 5.66. As per Biopharmaceutical Classification System (BCS), itraconazole is classified as BCS class II drug *i.e.* has extremely poor solubility, which is pH dependent and higher permeability. Itraconazole dissolves to the extent of 1 ng/mL at pH 7 and 6 µg/mL at pH 1 (0.1 N HCl). Because of the poor dissolution in GIT, it shows large inter-individual variations in its oral bioavailability [7,8]. Thus, poor solubility charecteristic of itraconazole poses a great challenge for formulators.

Earlier, a large number of approaches for enhancing dissolution rate of itraconazole, have been published such as solid dispersions [9-11], salt formation [12], micronization [13], nanonization [14], self microemulsifying drug delivery system [15] and cyclodextrin complexation [16]. Itraconazole is commercially available as a capsule dosage form (Sporanax, Janssen Pharmaceuticals, US), which contains solid dispersion of itraconazole with HPMC. In present study, skimmed milk has been chosen as drug carrier. In the past few years, skimmed milk has been employed for enhancement of solubility of prednisolone [17], meloxicam [18], sulindac [19] and ketoprofen [20]. Milk is reported to contain casein proteins and whey proteins. Casein proteins comprise of alpha casein (alphas1 and alphas2), beta casein, gamma casein, delta casein and kappa casein, while component of whey sproteins are beta-lactoglobulin, alpha-lactalbumin, serum albumin and immunoglobulins. Whey proteins are comparatively more hydrophilic than casein proteins [21, 22]. These proteins are strongly amphipathic, adsorb readily at the interfaces decreasing surface tension by forming surface/interfacial film with variable rheological properties. The surface activity reported for the individual milk proteins can be ranked as: β-casein > monodispersed casein micelle > serum albumin > α-lactalbumin > αs-casein = κ-casein > β-lactoglobulin > euglobulins [23]. Formulation of solid dispersion using organic solvent has long been problematic from safety standpoint. Skimmed milk is a promising alternative to organic solvent for solid dispersion. Moreover, skimmed milk is a cheap, easily available, biodegradable and eco-friendly carrier, which has not yet been explored for enhancing dissolution of itraconazole. Lyophilization technique of solid dispersion affords protection of surface active milk proteins which are responsible for solubility enhancement.

Therefore, itraconazole-milk solid dispersions were prepared, employing lyophilization technique. The solid dispersions were characterized by FTIR, DSC, X-RD and SEM study and evaluated for saturation solubility and *in vitro* release studies.

MATERIALS AND METHODS

Materials

Skimmed milk (fat content 1.5%) was purchased from Hisar-Jind Coop. Milk Producer Union Ltd., Jind (Haryana, India). Itraconazole and metronidazole were obtained as gift samples from Jubilant Organosys Ltd. (Noida, India) and GMH laboratories Pvt Ltd. (Baddi, India) respectively. HPLC grade water, acetonitrile and diethanolamine were procured from Qualigens Fine Chemicals (Mumbai, India). All other reagents and chemicals used in the study were of analytical grade.

Preparation of solid dispersion formulations

Solid dispersion of itraconazole and skimmed milk was prepared employing lyophilization technique [24]. Various batches of solid dispersions were prepared by dispersing an accurately weighed quantity of itraconazole in 25 mL of milk, and stirring at 50^{o} C until homogeneous mixture was formed. The resulting dispersion was frozen to -60^{o} C and lyophilized in a lab model lyophilizer (Alpha-2, 4 LD Plus, Martin Christ, Germany) at –90^{o}C and 0.0010 mbar for 24 h. The composition of different batches of itraconazole skimmed milk solid dispersion is summarized in Table 1.

Preparation of skimmed milk powder

The skimmed milk powder was prepared by lyophilizing 25 mL of skimmed milk for 24 h. The yield was found to be 2.591 g for skimmed milk powder. The product so obtained was sieved through 250-μm mesh.

Preparation of physical mixture

Physical mixture of itraconazole and skimmed milk powder was prepared by homogenous mixing of itraconazole and skimmed milk powder using an agate mortar and pestle.

HPLC Analysis

Assay of itraconazole in samples was carried out by injecting 20 μL of the solution, spiked with metronidazole as internal standard into a chromatographic system equipped with 600 pump controllers (Waters), 2487 dual wavelength absorbance UV detector (Waters), and 7725*i* manual injector (Rheodyne). The chromatographic separation of itraconazole was achieved using mobile phase composition of acetonitrile : water : diethanolamine (70 : 29.95 : 0.05, v/v) at a flow rate of 1 mL/min, in an isocratic run through Kromasil 100 C8 (Flexit Jour Lab. Pvt Ltd., Pune, India), 5μ (150 x 4.6 mm *i.d.*) column. The eluant was monitored for itraconazole at 254 nm.

Characterization and evaluation of solid dispersions

Drug content
An accurately weighed amount of powder equivalent to 10 mg of itraconazole was dissolved in 10 mL acetic acid (50%, v/v). The solution so obtained was appropriately diluted with SGF (pH 1.2), spiked with metronidazole, filtered through 0.2 µm nylon syringe filter and analyzed for the contents of itraconazole by reverse phase HPLC.

FTIR spectroscopy
The samples were subjected to FTIR spectroscopy in a Fourier transformed infrared spectrophotometer (Perkin Elmer, Spectrum B X-II, USA) in the range of 4000-400 cm^{-1} as KBr pellets.

Powder X-ray diffraction analysis
PXRD studies were carried out on a diffractometer (Philips X'PertPRO Panalytical, Germany) using Cu Kα radiations generated at voltage of 45 kV and a current of 40 mA in the area of $5^o < 2\theta < 50^o$ with a step size of 0.0170 ^{o}/step.

Differential Scanning Calorimetry
The thermal characteristics of itraconazole, skimmed milk powder, physical mixture of itraconazole and skimmed milk, and solid dispersion of itraconazole and skimmed milk were determined by Q10 differential scanning calorimeter (TA Systems, USA). Samples in the range of 3-5 mg were heated in an aluminum pan and DSC analysis was carried out at a constant nitrogen flow rate of (50 mL/min) and at heating rate of 10 ^{o}C/min from 40-250 ^{o}C.

Scanning electron microscopy
The morphology of itraconazole, skimmed milk powder, physical mixture of itraconazole and skimmed milk and solid dispersion of itraconazole and skimmed milk were examined using scanning electron microscope (JEOL, JSM-6100). The particles were fixed by mutual conductive adhesive tape on aluminium stubs and covered with gold/palladium using sputter coater prior to analysis. The SEM was operated at an accelerating voltage of 10 kV.

Saturation solubility study
Solubility of itraconazole bulk, physical mixture of itraconazole and skimmed milk, and solid dispersion of itraconazole and skimmed milk was determined using shake flask method. Pure itraconazole or physical mixture or solid dispersion containing 20 mg of itraconazole was placed in a conical flask with 10 mL of SGF (pH 1.2). The samples were shaken on a rotating shaker incubator (Narang Scientific Works, Delhi, India) for 48 h at 25 ^{o}C. The equilibrated suspensions were then filtered through nylon syringe filter (0.22 μm), appropriately diluted and spiked with metronidazole (internal standard) and assayed for drug content by HPLC.

Surface Tension measurement
Surface tension of saturated solutions of itraconazole bulk, physical mixture of itraconazole and skimmed milk powder and solid dispersions in SGF (pH 1.2) were measured by stalagmometer using drop weight method

In vitro drug release study
In vitro dissolution study of itraconazole bulk, physical mixture of itraconazole and milk and solid dispersion of itraconazole and milk was carried out using 900 mL of SGF (pH 1.2) containing (0.5%, w/v sodium lauryl sulfate) as dissolution medium in USP dissolution rate test apparatus (TDT 08L, Electrolab, India) [25]. An accurately weighed quantity of powder equivalent to 10 mg of itraconazole were weighed and placed into 900 mL of dissolution medium, stirred by means of paddle at 100 rpm and maintained at 37 ± 0.05 ^{o}C. Aliquots of 5 mL each were withdrawn at 5, 10, 20, 40, 60 and 120 min intervals and replaced with an equal volume of fresh medium. The samples were immediately filtered through 0.22 μm syringe filter, appropriately diluted, spiked with metronidazole and analyzed for itraconazole contents by HPLC method.

RESULTS AND DISCUSSION

Solid dispersions were successfully prepared by lyophilization technique. The binary solid dispersions thus obtained were found to be white in color, fine and free flowing powder. As shown in Table 1, contents of itraconazole in binary solid dispersions ranged from 98.9 to 100.7%. Thus, the lyophilization technique used for preparing solid dispersion appears to be suitable for preparation of skimmed milk solid dispersion with high content uniformity.

Table 1. Composition and results of drug content and solubility ofbinary solid dispersions

Batch Code	Itraconazole (mg)	Milk(ml)	Drug Content (%)*	Solubility in SGF pH 1.2*	Solubility enhancement factor
Drug	-	-	-	3.12± 0.37	-
MSD-1	100	25	99.6 ± 0.32	11.04± 0.82	3.54
MSD-2	200	25	98.9 ± 0.85	9.98± 0.15	3.20
MSD-3	300	25	101.8 ± 0.46	8.70± 0.25	2.79
MSD-4	400	25	100.7 ± 0.12	7.49± 0.54	2.40
MSD-5	500	25	100 ± 0.78	6.62± 0.32	2.12
PM MSD-1	100	Powder equivalent to 25 ml	-	4.90± 0.28	1.57

** Each value represents mean ± Standard deviation (n= 3)*

Figure 1 exhibits the FTIR spectra of itraconazole, skimmed milk powder and solid dispersion (MSD-1). Spectra of itraconazole showed triazole ring breathing at 1041.80 cm^{-1}, C-H out of plane bending for o-p-benzene substitution at 821.84 cm^{-1}, C-Cl stretching at 734.46 cm^{-1}, alkyl bending R- N) at 1451.6 cm^{-1}, Aryl alkyl asymmetric ether linkage at 1225.6 cm^{-1}. All of these peaks of itraconazole are also present in the spectra of solid dispersion indicating no chemical interaction between drug and milk components.

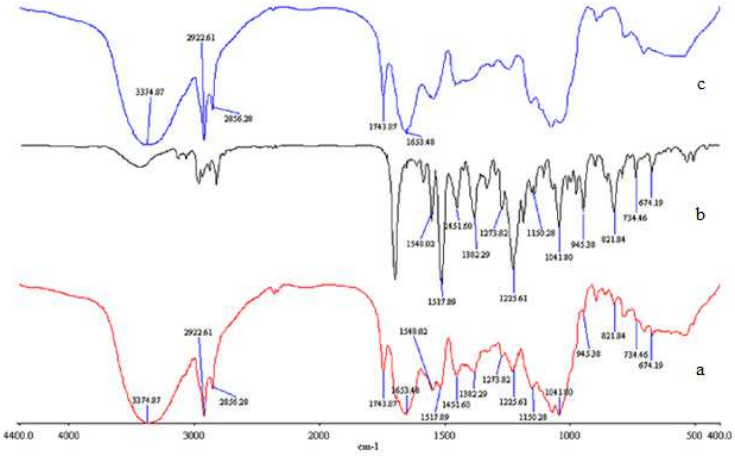

Fig. (1)FTIR spectra of (a) Solid dispersion (MSD-1), (b) Itraconazole and (c) Milk

Figure 2 shows the X-ray diffraction pattern of itraconazole, skimmed milk powder and solid dispersions (MSD-1, MSD-3 and MSD-5). The diffraction pattern of itraconazole is typical of crystalline substances with its characteristic peaks appearing at 8.83^o, 10.83^o, 14.53^o, 17.65^o, 18.09^o, 19.48^o, 20.45^o, 21.28^o, 22.50^o, 23.59^o, 25.39^o, 31.64^o (2θ). No sharp peak was observed in diffraction pattern of skimmed milk which is indicative of amorphous character. The diffraction plots of various batches of itraconazole milk solid dispersion showed disappearance of peaks of itraconazole, which appeared at 8.83^o, 21.28^o, 31.64^o (2*θ*). Further, it can be observed that there was marked decrease in the intensity and area of the characteristic peaks of itraconazole, with the solid dispersion containing higher amount of itraconazole showing peaks of higher intensity.

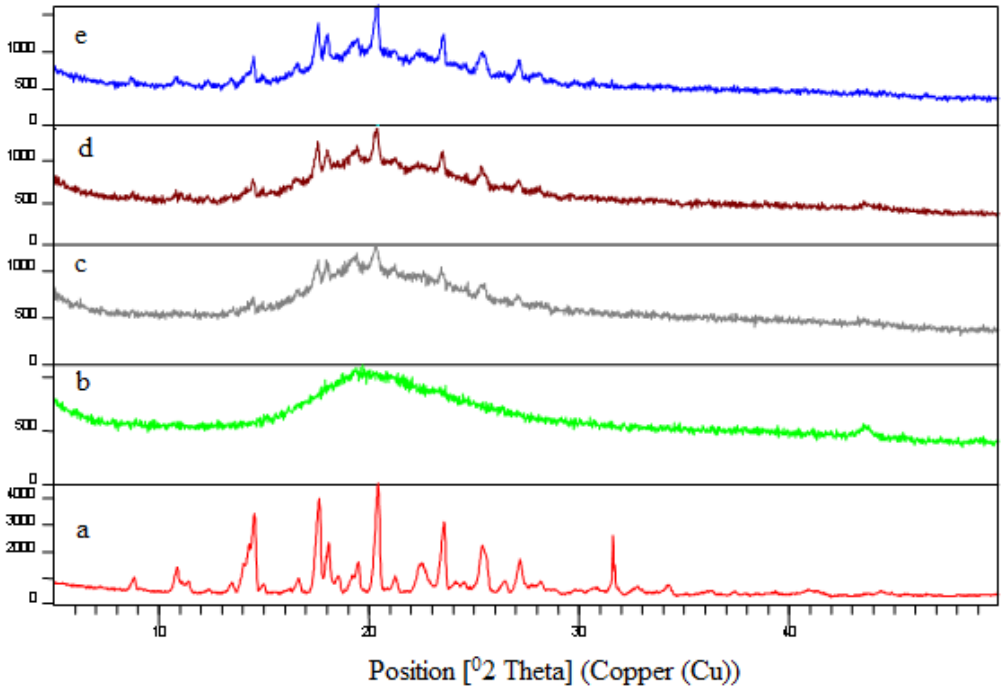

Fig. (2)X-Ray diffraction pattern of (a) Itraconazole, (b) Milk, (c) MSD-1, (d) MSD-3 and (e) MSD-5

In order to have better comparison, relative degree of crystallinity (RDC) was computed. RDC was determined by comparing some representative peak heights/peak area in diffraction pattern of samples with those of a reference. RDC was calculated using the following equation [26, 27]. –

$$\text{RDC} = \frac{A_{samp}}{A_{ref}} \qquad (1)$$

Where, Asamp is the peak area of samples at a specific 2θ, Aref – is the peak area of drug with highest intensity at that value of 2θ. Table 2 compares the relative degree of crystallinity (RDC) of different batches of solid dispersion. The values Relative Degree of Crystallinity appeared decreasing with increase in amount of skimmed milk.

Table 2.P-XRD interpretations of various batches of itraconazole-milk solid dispersion

°2θ	ITZ	*MSD-1*		*MSD-3*		MSD-5	
	Peak area	Peak area	RDC	Peak area	RDC	Peak area	RDC
14.53	563.10	29.36	0.05	52.17	0.092	71.99	0.127
17.65	906.31	144.20	0.16	170.05	0.18	237.74	0.26
23.59	525.59	47.80	0.09	48.60	0.092	197.36	0.375
25.39	413.14	29.08	0.07	34.02	0.082	142.91	0.345

(ITZ-itraconazole)

The DSC thermograms of skimmed milk, itraconazole, physical mixture of itraconazole and skimmed milk (PM MSD 1) and various batches of itraconazole and skimmed milk (MSD 1, MSD-3 and MSD-5) are illustrated in Fig. 3. The thermal curve of itraconazole showed a sharp endothermic peak at 169.99^{o}C with the heat of fusion of 92.66 J/g, while broad endothermic peaks at 115.81^{o}C and 180.82^{o}C characterize milk. Both characteristics peaks of itraconazole (drug melting) and milk were clearly distinguishable in binary physical mixture (PM MSD-1). The DSC thermogram of physical mixture of skimmed milk and itraconazole showed a sharp endothermic peak at 167.44^{o}C with heat of fusion of 25 J/g, showing the weak interaction between itraconazole and milk, as there is shift of 2.55^{o}C. The DSC curves of Milk and itraconazole solid dispersion of batches MSD-1, MSD-3 and MSD-5 showed the endothermic peak contributed by itraconazole at 164.77^o, 165.26^o, 166.26^o C with heat of fusion of 1.417, 4.749, 11.46 J/g respectively. Thus, there was shift in the endothermic transition and decrease in heat of

fusion with highest change appeared in MSD-1. In all binary solid dispersions, fusion endotherm of itraconazole was markedly reduced in intensity. Therefore, it can be inferred from the DSC that an interaction had taken place between milk and itraconazole. The findings of DSC study corroborate with the X-RD observations.

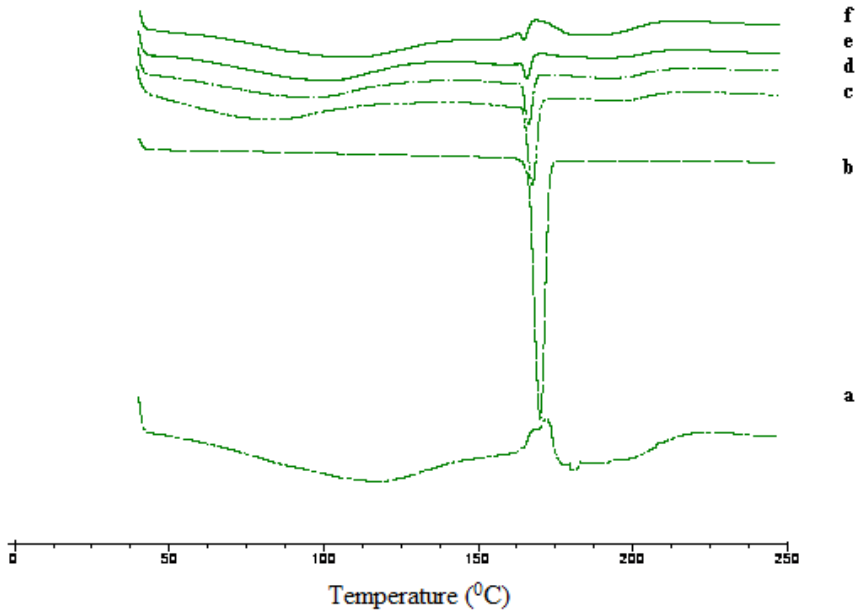

Fig. (3)DSC thermogram of (a) Milk, (b) Itraconazole (c) PM MSD-1 (d)MSD-5 (e) MSD-3 and (f) MSD-1

Figure 4 displays the scanning electron micrographs of plain drug, physical mixture of itraconazole and milk and solid dispersion of itraconazole with milk. The photomicrograph revealed the needle shaped crystals of itraconazole. Similar structure of itraconazole was also visible in physical mixture of itraconazole and milk, while the solid dispersion showed amorphous matrix with few tiny crystals, indicating the decrease in crystallinity of itraconazole in solid dispersion.

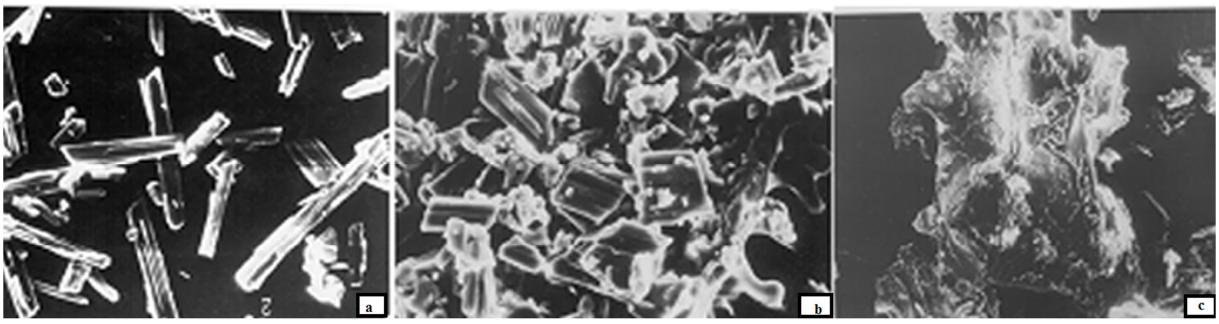

Fig. (4) Scanning Electron Micrograph of (a) Itraconazole, (b) Physical mixture (PM MSD-1) and (c)Binary solid dispersion (MSD-1)

Solubility study of drug and solid dispersions was conducted in SGF (pH 1.2). It is well evident from the results (Table 1), as the proportion of milk in solid dispersion was increased, a corresponding increase in solubility was observed. The enhancement factor was also calculated. The solubility was enhanced over 3.54 fold for MSD-1, whereas in case of physical mixture over 1.57-fold increase in solubility was observed. For the batch having least milk proportion (MSD-5), over 2.12-fold enhancement in solubility was estimated.

Earlier, it has been reported that milk proteins have surfactant properties. It is surmised that casein micelles entrap water insoluble drug into its hydrophobic region and reduce surface tension between drug and gastric media when taken orally, thereby, augmenting dissolution rate [24, 17] . Therefore, in order to affirm surface active properties of

milk proteins, surface tension measurement was performed using stalagmometer. Table 3 represents the surface tension values of itraconazole, physical mixture of itraconazole and skimmed milk and solid dispersions of drug with skimmed milk. Surface tension measurements were performed for pure drug, skimmed milk solid dispersions and physical mixture in SGF (pH 1.2). The surface tension values for the MSDs were significantly low compared with the values of surface tension that were registered for the drug (P value < 0.01). The surface tension of physical mixture was significantly lower (P value< 0.01), although higher than the values obtained for MSD. From these observations it can be speculated that the reduction of surface tension values of PM and MSD was ascribed to the surface active properties of milk proteins. One-way ANOVA test was performed followed by dunnett't test.

Table3.Surface tension values of drug, physical mixture and solid dispersions

Samples	Surface tension	Mean*(X)	SD	SEM
	66.78			
Drug	65.12	65.42	1.229	0.709
	64.38			
	62.29			
MSD-5	62.11	61.973	0.4082	0.2325
	61.52			
	60.20			
MSD-4	59.99	60.396	0.5329	0.3077
	61			
	57.60			
MSD 3	59.64	59.076	1.291	0.7452
	59.99			
	58.18			
MSD-2	57.87	58.233	0.3927	0.2267
	58.65			
	57			
MSD-1	56.56	56.813	0.2274	0.1313
	56.88			
	59.67			
PM(MSD-1)	60.67	61.56	2.459	1.420
	64.34			

Dissolution profiles of itraconazole, physical mixture and solid dispersions are presented in Fig. 5. The prepared binary formulations exhibited a considerable improvement in the dissolution rate of itraconazole. The order of dissolution rate in the binary solid dispersions and physical mixture was found to be MSD-1 > MSD-2 > MSD-3 > MSD-4 >MSD-5 > PM-MSD-1 > Drug. For comparative analysis, % Dissolution Efficiency at 20 min ($\%DE_{20min}$) and Relative Dissolution Rate at 5 min (RDR_{5min}) was computed. Dissolution efficiency is the area under dissolution curve up to the time 't' expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time. It can be calculated by following equation [28].

$$DE\ (\%) = \frac{\int_0^t y \times dt}{y100 \times t} \times 100 \qquad (2)$$

Where y is the % drug dissolved at time 't'

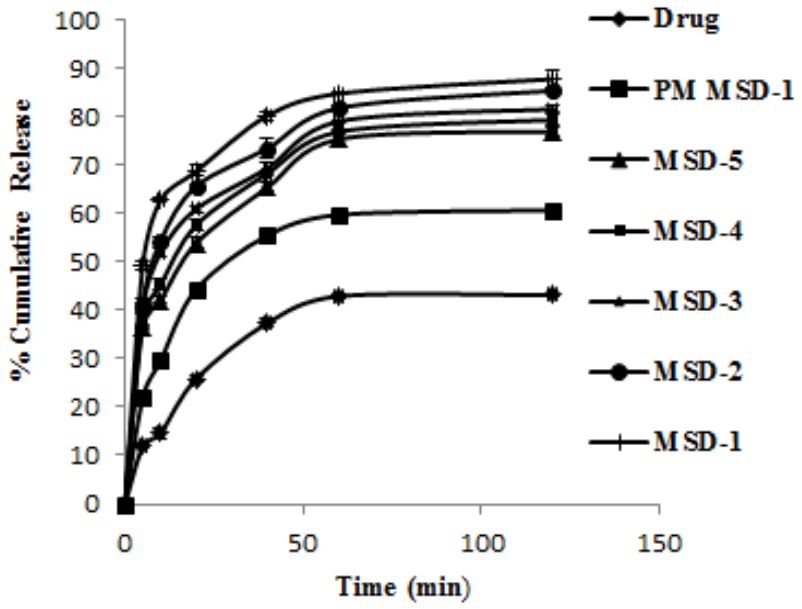

Fig.(5)Release profile of drug, physical mixture and binary solid dispersions

As shown in Table 4, the value of % DE_{20min} for pure drug was enhanced from miniscule (14.9%) to the highest (53.42%) for MSD-1. For physical mixture (PM MSD-1), value of % DE_{20min} was increased up to 1.8 fold. There was a proportional increase in % DE with increase in skimmed milk content. Analogous to % DE, RDR_{5min} value reached maximum to 4.16 in solid dispersion (MSD-1) while physical mixture attained RDR_{5min} of 1.84. On comparative analysis of different batches of itraconazole milk solid dispersion for RDR_{5min}, MSD-1 was found to provide fastest dissolution in first 5 min while in case of other formulations, no significant difference in RDR_{5min} was apparent. Solid dispersions showed increase in their dissolution rate corresponding to decrease in their crystallinity as shown in DSC and X-RD data. In addition to this, this observation of enhanced dissolution rate is concordant with surface tension data. Thus, possible underlying mechanisms directing increase in solubility and dissolution rate include: Reduction in crystallinity of itraconazole, decrease in particle size and surfactant action of amino acids and proteins.

Table 4Dissolution parameters of itraconazole, solid dispersions and physical mixtures

Formulation Code	Dissolution parameters		
	DP_{5min}	% Dissolution Efficiency	RDr_{5min}
Drug	11.85	14.9	-
MSD-1	49.35	53.02	4.16
MSD-2	41.22	47.05	3.47
MSD-3	40.36	44.76	3.40
MSD-4	36.55	40.92	3.21
MSD-5	21.87	38.37	3.08
PM (MSD-1)	21.87	27.66	1.84

Table 5 Release kinetic parameters of solid dispersions

Formulation Code	Mathematical model for release kinetics									
	Zero order		First order		Higuchi		Hixoncrowell		Koressmeyerpeppas	
	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2	Slope	r^2
MSD-1	0.4873	0.4627	-0.864	0.7281	4.1563	0.7465	-0.0152	0.6394	0.8215	0.6815
MSD-2	0.5088	0.5329	-0.006	0.7730	7.2496	0.8092	-0.0149	0.6954	0.827	0.7125
MSD-3	0.4876	0.5403	-0.005	0.7527	6.9212	0.8142	-0.0135	0.6852	0.8171	0.7119
MSD-4	0.4928	0.5723	-0.005	0.7578	6.9087	0.7001	-0.0132	0.7001	0.8715	0.7277
MSD-5	0.4882	0.5924	-0.004	0.7565	6.783	0.8554	-0.012	0.7068	0.8163	0.7009

Table 5 displays the regression parameters obtained after fitting various release kinetic models to the *in vitro* dissolution data. The order of fitness for various models investigated for all binary solid dispersion ranked as Higuchi > first-order > Hixson-Crowell cube root law > Korsemeyer–Peppas ≫ zero-order. The best fit to Higuchi kinetic model indicates that the diffusion was the predominant mechanism of drug release.

CONCLUSION

Based on the present study, it can be concluded that skimmed milk was found to be a suitable carrier for preparation of solid dispersion of itraconazole by lyophilization technique. Our study proved that solid dispersion with skimmed milk remarkably improved the solubility and dissolution rate of itraconazole. However, solubility and dissolution improvement of itraconazole was also observed with PM but lesser than the solid dispersion. Results of differential scanning calorimetry, powder X-ray diffractometry, scanning electron microscopy, surface tension measurement study were congruous with the results obtained from solubility and dissolution study. The fast release of itraconazole through solid dispersion was likely attributed to transition from crystalline to amorphous state and to surfactant action of skimmed milk. Thus, objective of the work was achieved using milk as a carrier for formulation of solid dispersion of itraconazole by lyophilization technique.

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