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Formulation and Evaluation of Stable Solid Dispersion of Anti-Protozoal Drug

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ABSTRACT

The solubility behaviour of drug is one of most challenging aspect in formulation development. Thus a greater understanding of dissolution & absorption behaviour of drug with low aqueous solubility is required to successfully formulate them into more soluble and hence bioavailable drug product. The current investigation includes formulating solid dispersions of Tinidazole, an antiprotozoal and antiamoebic drug which is characterized by poor solubility and rapid absorption (BCS-2). Thus an attempt was made to prepare stable solid dispersion by improving the solubility and dissolution rate of tinidazole using a mixture of polymers viz: hydroxypropylmethyl cellulose (HPMC), Polaxomer-F-188 (POL), Polyvinyl pyrrolidone (PVP), Polyethylene glycol (PEG) and polymeric surfactants in different proportions as carrier. Solid dispersions of tinidazole were prepared by spray drying technique, melting technique and as physical mixture to conduct a comparative evaluation of the methods that could yield the most stable formulation and then were evaluated for number of analytical parameters. Solid dispersions containing Drug: PEG 6000: Poloxamer 188 as carrier in 1:0.3:0.2 ratios by melting method showed the best result. POL and PEG combination revealed a synergistic effect, on solubility, dissolution and stability of crystalline drug and both crystallization inhibitor (PEG) and wetting agent (poloxamer) in solid dispersion can improve solubility and release profile of tinidazole. The study demonstrates high potential of Melting method for obtaining large surface area and amorphicity of drug using hydrophilic carrier. The solid dispersions were compressed into Fast Dissolving Tablets by incorporating appropriate superdisintegrants.

Keywords: Tinidazole, Solid dispersion, dissolution enhancement, HPMC 100LV, Poloxamer 188, Poly ethylene glycol (PEG), Crossprovidone and Sodium starch glycolate.

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INTRODUCTION

Tinidazole is a nitroimidazole derivative, antiparasitic drug used against protozaon infection. It is used as tissue amoebicides for both intestinal and extra intestinal amoebiasis. It has broad spectrum cidal activity against protozoa including Giardia Lamblia many anaerobic bacteria such as fragilis, fusobacterium, clostridium perfrigens, cldifficile, helicobacter pylori.

But as it is a BCS Class-II drug [1], the dissolution from its dosage forms is too low and is a rate limiting step in absorption of drug. Poor aqueous solubility [2] and poor dissolution rate poses difficulties in achieving predictable and reproducible in vivo/in vitro correlations and also bioavailability related problems. Further it also undergoes extensive first pass effect leading to low and variable bioavailability. Thus improvement in the extent and rate of dissolution is highly desirable for such compounds, as this can lead to an increased and more reproducible oral bioavailability and subsequently to clinically relevant dose reduction and more reliable therapy.

Different techniques like micronization, salt formation, use of surfactants, liposomes, nanoparticles, etc. are available for enhancement of dissolution of poorly soluble drugs, but all of these have certain limitations. Micronization have limited control over important characteristics with regards to fine particle such as size, shape, morphology, surface properties, electrostatic charge, poor wettability and flow properties. All poorly water-soluble drugs [3] are not suitable for improving their solubility by salt formation. Potential disadvantages of salt forms include high reactivity with atmospheric carbon dioxide and water resulting in precipitation of poorly water-soluble drug, epigastric distress due to high alkalinity. Use of surfactants to improve dissolution rate poses problems, such as patient compliance and commercialization. Similarly liposomes and nanoparticles also enhance dissolution rate of drugs but they have disadvantage of poor drug loading and so high cost.

Hence an attempt will be made to improve the solubility of tinidazole by solid dispersion [4-7] technique and the prepared solid dispersions will be formulated in the form of fast disintegrating tablets using various superdisintegrants to improve the solubility, bioavailability and patient compliance and clinical efficacy of tinidazole [8-10].

MATERIALS AND METHODS

Tinidazole was received as a Gift Sample by Ajanta Pharma, Mumbai. HPMC K100LV, PVP K30, PEG 6000, Poloxamer were all given as gift sample by Svizera Pharmatech, Mumbai. Croscarmellose Sodium, Sodium Starch Glycolate, Crossprovidone were obtained as a gift sample from Loba chemic Pvt. Ltd and were of analytical grade.

EXPERIMENTAL:

Preparation of solid dispersion:

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Solid dispersions were formulated by three diferent method, viz: melting technique, physical mixtures and spray drying technique to have a comparative estimation on the solid dispersion produced with best solubility profile. These are elaborated below:

Melting Technique: In melting method, the carriers such as, PEG 6000 [11, 12] and PVP K 30 [12] and Poloxamer F 188 [11] were weighed accurately. The carrier was first melted in the china dish at about 60^{o}C and the drug was dispersed in the molten mixture with constant stirring. The dispersion was poured and cooled immediately. The solid dispersions obtained from this method were tacky enough. The resultant solid dispersion was passed through 44 mesh sieve and stored in desiccators.

Physical Mixture: Physical mixtures were prepared by mixing the calculated amounts of Tinidazole and carriers in a glass mortar and then it was triturated. The resultant solid dispersion was passed through 44 mesh sieve and stored in desiccators until used for further study.

Spray Drying Technique [15]: Tinidazole, HPMC K 100 LV [11, 13], PVP K 30, PEG 6000 and Poloxamer F 188 were weighed accurately in appropriate ratios and dissolved in sufficient amount of solvent. The above solution was fed into spray dryer to evaporate the organic solvent.

The ratios, contents and corresponding batch numbers are enlisted below in Table 1 for further clarity.

Table 1: Content and Composition of solid dispersions:

Sr. No	Batch	Composition	Ratio %(w/w)	Spray drying	Melting tech.	Physical mixture
1	F1	Tinidazole : HPMC100LV	1 : 0.5	F1-S	---	F1-P
2	F2	Tinidazole : Poloxamer188	1 : 0.5	F2-S	F2-M	F2-P
3	F3	Tinidazole : PVP	1 : 0.5	F3-S	F3-M	F3-P
4	F4	Tinidazole : PEG6000	1 : 0.5	F4-S	F4-M	F4-P
5	F5	Tinidazole : HPMC100LV : Poloxamer188	1 : 0.25 : 0.25	F5-S	---	F5-P
6	F6	Tinidazole : PVP : Poloxamer188	1 : 0.25 : 0.25	F6-S	F6-M	F6-P
7	F7	Tinidazole : PEG6000 : Poloxamer188	1 : 0.25 : 0.25	F7-S	F7-M	F7-P
8	F8	Tinidazole : PEG6000 : Poloxamer188	1 : 0.2 : 0.3	F8-S	F8-M	--
9	F9	Tinidazole : PEG6000 : Poloxamer188	1 : 0.3 : 0.2	F9-S	F9-M	F9-P
10	F10	Tinidazole : PEG6000 : Poloxamer188	1 : 0.1 : 0.4	F10-S	F10-M	--
11	F11	Tinidazole : PEG6000 : Poloxamer188	1 : 0.4 : 0.1	F11-S	F11-M	--

ISSN: 0975-8585

Evaluation of solid dispersions:

Drug content:

The physical mixture and solid dispersion equivalent to 20mg of Tinidazole were taken and dissolved separately in 20ml of 0.1 N HCL (pH 1.2). The solutions were filtered using 0.45 µ m membrane filter and were further diluted and assayed by UV spectrophotometer at 276.8 nm. The actual drug content was calculated using by following equation. The data for drug content is provided in Table 2.

$$\%\text{Drug content} = \frac{\text{Actual Tinidazole content in weight quantity of solid dispersion}}{\text{Theoretical amount of Tinidazole solid dispersion}} \times 100$$

Table 2: Drug Content:

Sr.No.	Batch	Spray drying		Melting technique		Physical mixture	
		Code	% Drug Contents	Code	% Drug Contents	Code	% Drug Contents
1	**F1**	**F1-S**	89.03	**---**	---	**F1-P**	76.12
2	**F2**	**F2-S**	90.40	**F2-M**	90.13	**F2-P**	86.32
3	**F3**	**F3-S**	88.36	**F3-M**	87.27	**F3-P**	74.08
4	**F4**	**F4-S**	92.44	**F4-M**	91.59	**F4-P**	88.86
5	**F5**	**F5-S**	98.02	**---**	---	**F5-P**	94.49
6	**F6**	**F6-S**	97.62	**F6-M**	96.43	**F6-P**	90.40
7	**F7**	**F7-S**	99.38	**F7-M**	98.43	**F7-P**	98.02
8	**F8**	**F8-S**	99.72	**F8-M**	98.99	**---**	**---**
9	**F9**	**F9-S**	100.85	**F9-M**	101.3	**F9-P**	**98.53**
10	**F10**	**F10-S**	99.72	**F10-M**	98.02	**---**	**---**
11	**F11**		96.43	**F11-M**	95.11	**---**	**---**

Invitro drug release:

Dissolution was carried out in rotating basket using 0.1N HCl as dissolution medium at 37ºC ± 2ºC. During dissolution study 5 ml aliquot was withdrawn at different time intervals of 2, 4, 6, 8 ---20 minute and same was replaced with equal volume of fresh medium. The withdrawn samples were filtered through Whatman filter paper no. 42 and absorbance was measured at 276.8 nm for 0.1 N HCL (pH 1.2). Dissolution data is given below at 10 and 20 minutes for various formulations (F1-F11) prepared by different methods such as melting technique, physical mixture and spray drying. The results are as depicted in Table 3.

Fourier Transform Infrared (FTIR) Spectroscopy [15]

FTIR spectra were recorded on samples prepared in potassium bromide (KBr) disks using a Shimadzu Corporation, (Koyto, Japan) Model - 8400S. Samples were prepared in KBr disks by means of a hydrostatic press at 6-8 tons pressure. The scanning range was 500 to 4000 cm -1. Fig 1-5 depicts the FTIR spectra of Tinidazole and its combination with polymers and excipients.

ISSN: 0975-8585

Table 3: Dissolution data at 10 and 20 mins:

Formulations	Spray Drying		Melting method		Physical Mixture	
	DD 10	DD20	DD10	DD20	DD10	DD20
F1	36.78±0.03	77.83±0.06	----	----	30.26±0.09	57.72±0.15
F2	32.86±0.03	82.16±0.05	39.23±0.12	87.7±0.11	31.08±0.04	64.47±0.14
F3	23.93±0.01	63.31±0.15	32.14±0.02	74.66±0.12	14.16±0.05	49.33±0.07
F4	39.93±0.03	82.97±0.05	45.53±0.16	94.34±0.08	29.77±0.07	71.9±0.02
F5	34.44±0.15	89.19±0.09	----	----	24.19±0.09	75.03±0.02
F6	32.82±0.11	74.42±0.07	41.73±0.01	85.87±0.12	31.9±0.09	67.89±0.09
F7	43.77±0.08	94.19±0.11	46.77±0.09	97.29±0.06	38.99±0.04	78.67±0.05
F8	43.81±0.12	90.02±0.07	----	-----	46.27±0.02	96.09±0.02
F9	----	----	----	-----	44.11±0.07	97.89±0.14
F10	39.07±0.05	94.92±0.01	50.68±0.09	98.81±0.08	----	----
F11	42.45±0.13	88.65±0.01	48.23±0.09	94.16±0.06	-----	----

Figure 1: FTIR of Tinidazole

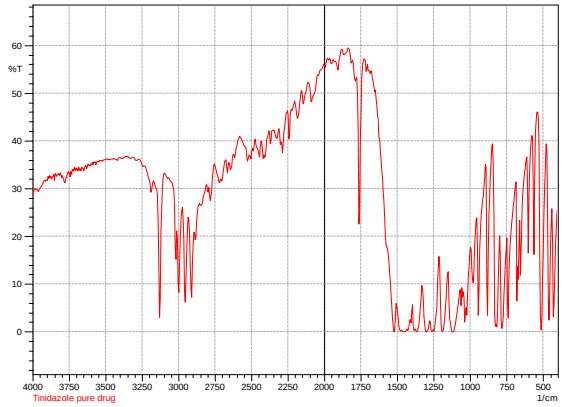


Figure 2: FT-IR Tini, PEG and Polo

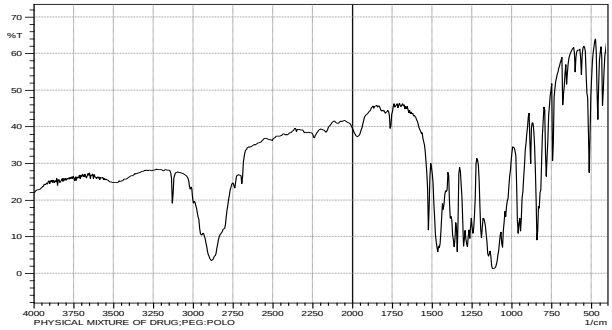


Figure 3: FT-IR Tini, PVP and HPMC

ISSN: 0975-8585

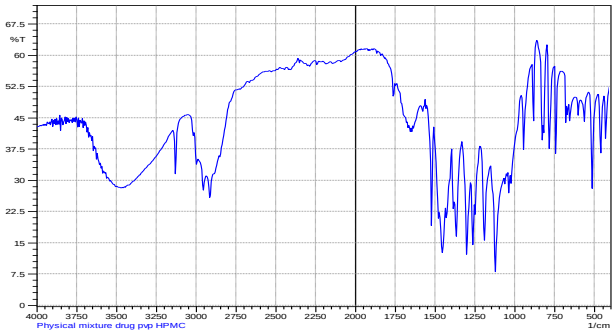


Fig 4: FT-IR Tini, SSG and Crosspro

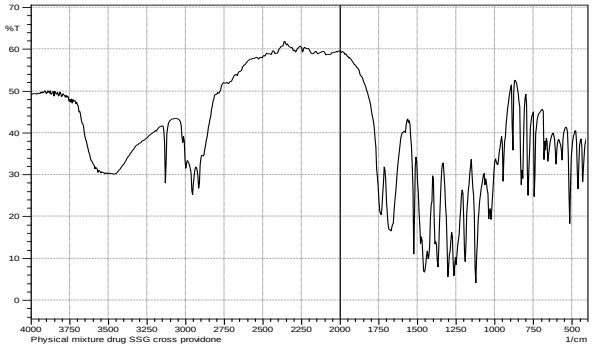
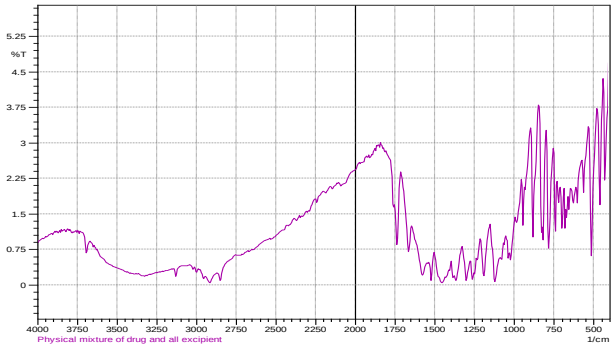


Figure 5: FT-IR of Tini and all the excipient



Differential Scanning Calorimetry (DSC) [15]**:**

The DSC study was carried out using Model No. METTLER DSC 30S by using crucible Al 40µL, at10^{0}C /min heating rate, under nitrogen environment. The temperature range used was 20 ^{0}C - 240^{0}C. DSC spectra are shown in Figure 6 and 7.

ISSN: 0975-8585

Figure 6: DSC Spectra of Tinidazole

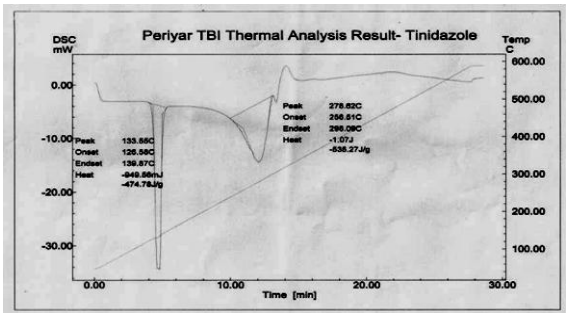
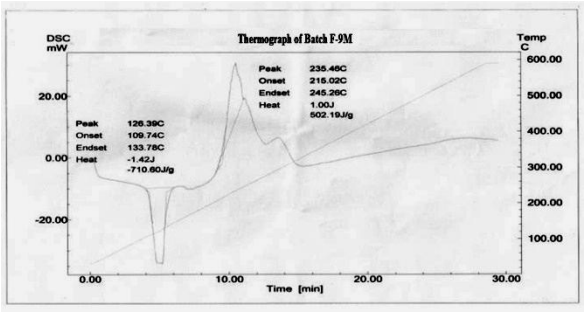


Figure 7: DSC Spectra formulation F -9M



X-Ray Diffraction (XRD) [15]**:**

The study was carried out using X-ray powder diffraction system, Model No. XPERT-PRO Diffract meter System, at, A.I.S.S.M.S College of pharmacy, Puneby using copper target, a voltage of 45 Kv and a current of 40 m A. The scanning was done over 2θ range of 5º to 80º. Figure 8 -10 depicts XRD of Tinidazole, and the optimized batches.

Figure 8: XRD spectra of pure drug Tinidazole

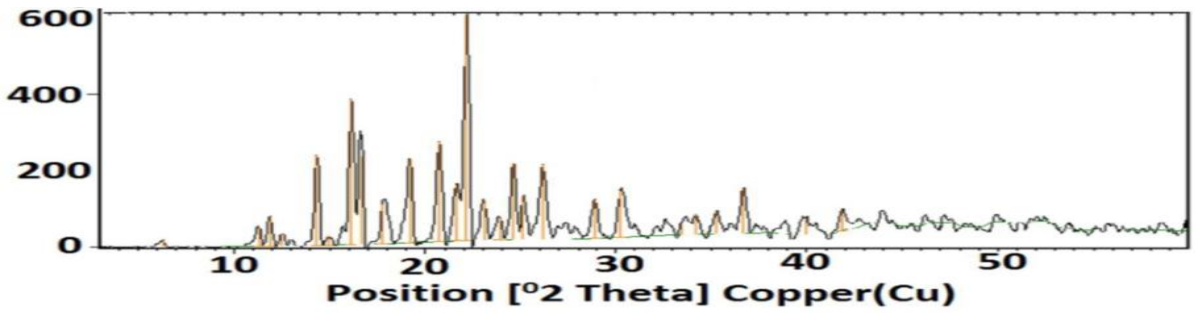


Figure 9: XRD spectra of Batch F 9-S

ISSN: 0975-8585

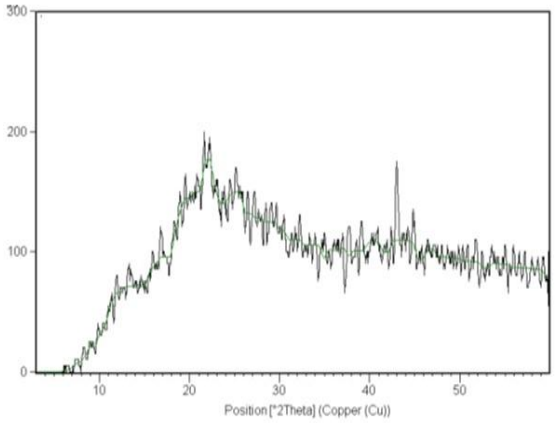
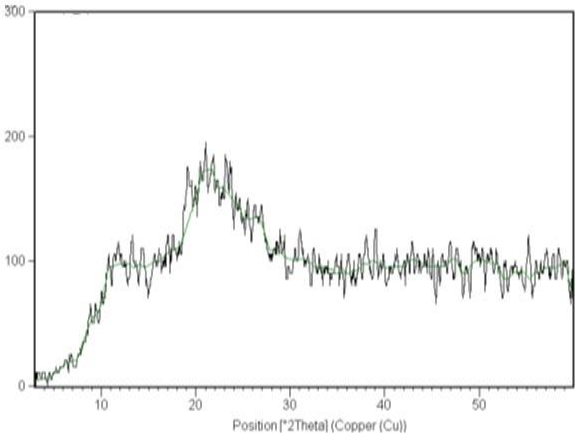


Figure 10: XRD spectra of Batch F 9-M



SEM:

Pure drug Tinidazole and optimized solid dispersion formulation (F-9S), (F-9M) and (F-9P) were coated with platinum and visualized under Analytical Scanning Electron Microscope (SEM), using Model No. JSM-6380A., at Diya Laboratory, Mumbai The SEM Photomicrographs showing surface morphology are shown in the Figures 11 - 14 below.

Figure 11: SEM of Tinidazole

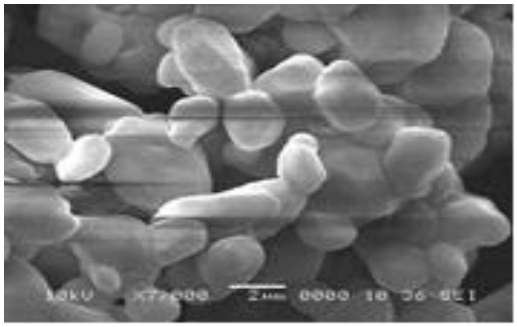


Figure 12: SEM of S.D. (F9-M)

ISSN: 0975-8585

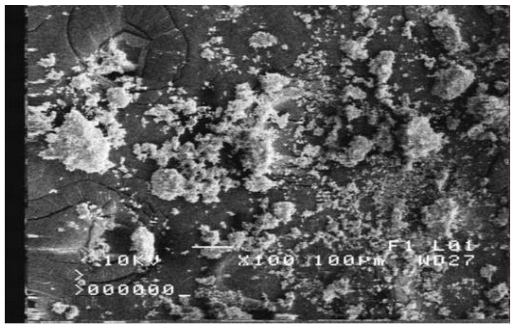


Figure 13: SEM of S.D. (F9-S)

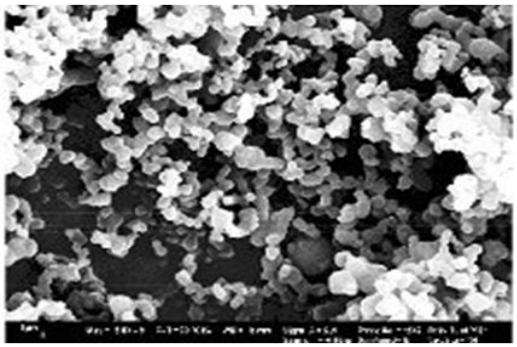
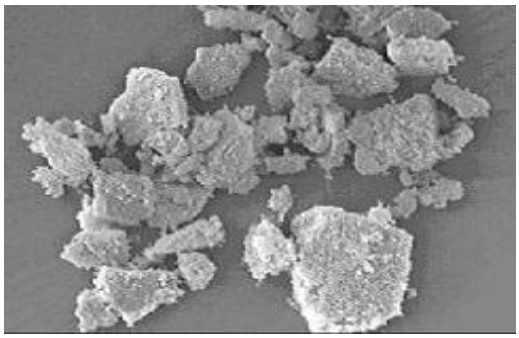


Figure 14: Physical mixture. (F9-P)



Formulation of fast disintegrating tablets:

Tablets containing equivalent of 300 mg of Tinidazole were compressed with Avicel PH 101, Crospovidone, Sodium starch glycolate, Mannitol. Prepared tablets were evaluated for hardness (Tablet Tester Model No. C – WWTDH 500N Campbell Electronics, Mumbai.), friability (Roche Friabilator), weight variation, and drug content. In vitro dissolution studies of F9-M and commercial tablets of Tinidazole were also carried out.

RESULTS AND DISCUSSION

TNZ: HPMC K 100 LV dispersions (trial -01) were prepared in the ratio of 1:0.5 by spray drying and physical mixture. The increased dissolution was attributed to fast diffusion of drug through highly swelled hydrophilic polymer. The release was not sufficient as depicted in Table 3. As seen in trial-02, the conjugates prepared by melting technique gave the drug release 87.16 % in 20 min and solid dispersions by spray drying gave drug release 82.16 % in 20 min. and physical mixing yielded a drug release of 64.47 % at the end of 20 minutes. This trial, consisting of TNZ: Poloxamer F 188 in ratio of 1:0.5, gave more drug release when compared to previous trial. Enhancement of DRs in SDs or PMs is caused by two mechanism: either by micellar solubilization or by reducing the activity coefficient of the drug by reducing the hydrophobic interaction (surface lowering effect to medium resulting in wettability of hydrophobic) or by both processes. The presence of amorphous form of drug in SDs as compared with PMs in which drug was present in crystalline form resulted in greater drug release as solid dispersions. The improved dissolution rates in trial -03 may be attributed to the improved wetting of tinidazole in the presence of PVP K-30 probably due to formation of intermolecular hydrogen bonding between the carbonyl group of PVP K-30 and the hydrogen atom in the NH2 group. Enhancement of DRs is caused by the inhibition of crystallization of drugs because of antiplasticizing effect of PVP. But the drug release was found to be less as compare to previous trial.

The PEG 6000 dispersions in trial-04, which were prepared by melting technique, gave the drug release 94.34% in 20 min while in the conjugates prepared by spray drying, drug release was found to be 82.97% at the end of 20 min. and the conjugate prepared by physical mixing gave the drug release only 71.90 % at the end of 20 minutes. This trial gave more drug release when compared to previous all trial. The enhanced dissolution rates may be probably due to an increased wetability (attributed to its hydrophilic oxyethylene groups) and dispensability of drug coupled with a higher viscosity which hinder the precipitation of the drug following dissolution of the carrier. In trial- F5, F6 and F7, Effect of polymers in combination were studied for improving the drug release profile. By formulating ternary batches. Batch no. F-7 which was prepared by melting technique by use of drug: PEG 6000: Poloxamer F 188 in the ratio of 1:0.25:0.25 respectively shown the good result in terms of both i.e. drug release as well as drug content as compare to all other (drug release: 97.29±0.06% and drug content : 98.43%). Mechanism of higher dissolution rate in ternary solid dispersion might be the synergistic effect of polymer and surfactant that is, whenever dissolution media enter in polymeric matrix of solid dispersion a high concentration of surfactant in near vicinity of drug, which is already transformed in amorphous form from crystalline form due to solid dispersion with polymer, enhances local dissolution of drug and then dissolved drug releases from polymer matrix. The synergistic effect of Poloxamer F 188 can be attributed to the possibility that the surfactant can increase the chain mobility of PEG 6000 with the result that drug release is facilitated. For optimizing and selecting the most appropriate formulation, batches F-8, F-9, F-10 and F-11 were formulated by a combination of Poloxamer 188 and PEG 6000 in different ratios. Batch no. F-9 M which was prepared by melting technique by use of Drug: PEG 6000: Poloxamer F 188 in the ratio of 1:0.3:0.2 depicted good results with a drug release of 98.97±0.02% in 16 minutes (as shown in Fig 17) and drug content of 101.3%. This proves that synergistic effect of

ISSN: 0975-8585

Poloxamer 188 and PEG 6000 was found to be higher in the ratio of 0.3:0.2. Thus by the drug content and dissolution profile, it can be stated that dispersions prepared by melting method showed better dissolution profile than dispersions prepared by spray drying and physical mixture technique.

Figure 15: Dissolution profile of optimized fast dissolving tablet of F9-M

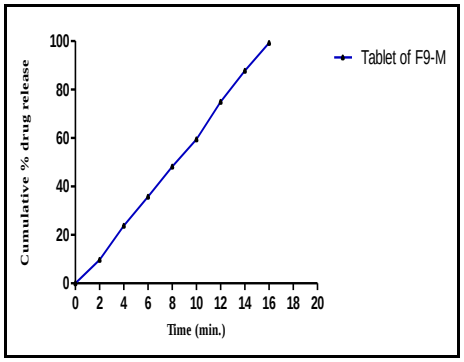


Fig 16: Dissolution profile of marketed formulation of Tinidazole

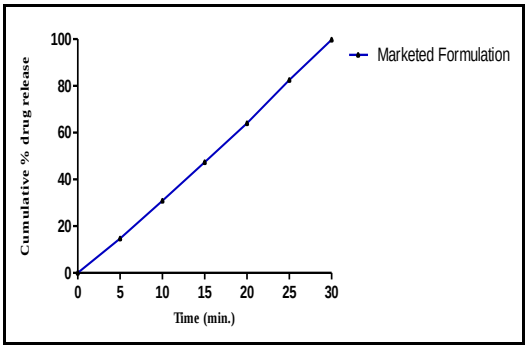
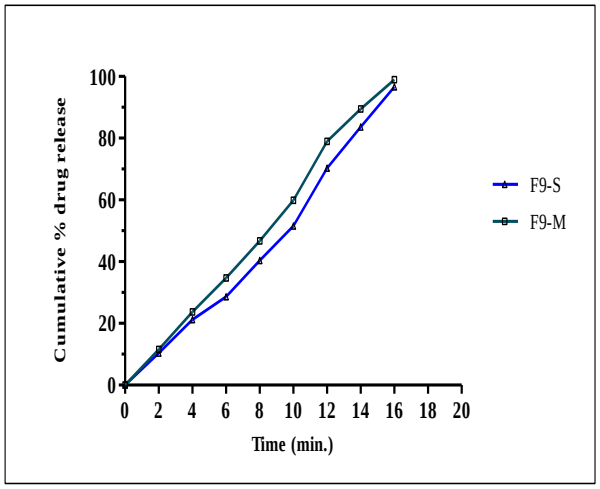


Fig 17: Dissolution profile of 9-S and F9-M



This may be due to grinding; uniform distribution of drug in the polymer crust at molecular level in a highly dispersed state via intensive mixing and compact packing of drug in melted polymer and polymeric surfactant.

The *in vitro* drug release of the polymers and polymeric combination is as shown in an ascending manner below:

PVP-K30 < HPMC K 100 LV <Poloxamer F 188<PEG-6000 <PEG6000: Poloxamer F 188 (0.3:0.2**)**

FT-IR:

FT-IR spectrum of pure TNZ was shown in Fig. 1. The characteristic peak of TNZ spectrums areas follows. 1741 due to N-O stretching, 2956.72 due to aliphatic C-H stretching and 3219 due to aromatic stretching. There was no change in the intensity of principal peak of drug found. Hence, the FTIR results suggested that the drug and polymers were compatible.

Differential Scanning Colorimetry (DSC):

Differential scanning of pure drug Tinidazole and optimized formulation F -9M are shown in Fig. no. 6 and 7. The DTA diagram of pure TNZ shows sharp endothermic peak at 133.55 ^{0}C. The DSC diagram of optimized batch F -9M gave the broad endothermic peak of TNZ at 126.39^{0}C with reduced Peak height. Reduction in the thermal glass transition temperature and the broadness of the endotherm suggest the reduction in the crystallinity of the drug. This Tg at lower value suggesting a possible plasticizing effect for the surfactant. This suggests that the drug was molecularly dispersed in the carriers.

X – Ray Diffraction (XRD)

Tinidazole showed 26 peaks at different points. This proved crystalline structure of drug. XRD of batch (F9-S) shown the reduction in crystallinity of drug as observed in XRD of pure drug but small crystallinity might be present as it gave small peak. But no such sharp peaks were observed in XRD of batch (F9-M) at 0 month and even after period of 3 month. These results indicated that the crystallanity of tinidazole was almost absent in this batch (F9-M) and was found to be most stable.

Scanning Electron Microscopy (SEM):

Surface Photomicrographs of pure drug Tinidazole and optimized solid dispersion formulations are shown in Figure no. 11 to 14 indicates almost uniform surface characteristics of solid dispersion.

Preparation of Fast Disintegrating Tablet from S.D. of TNZ

The tablets were prepared by using the selected optimized formulation and formulated by direct compression technique. Crosspovidone (CP) is used in the final formulation as a superdisintegrant [14]. Cross providone is basically densely cross-linked homopolymers of N-vinyl 2-pyrrolidones. Their porous particle morphology enables them to rapidly absorb liquids into the tablet by capillary action and to generate rapid volume expansion and hydrostatic pressures that result in disintegration. Overall Crosspovidone showed comparatively faster disintegration times than Croscarmellose sodium (CCS) and Sodium starch glycolate (SSG).

Tablets formulated with CCS and SSG appeared to disintegrate much more slowly into more or less uniform coarser particles. Tablets containing Crosscarmellose sodium and Sodium starch glycolate seemed to swell immediately. This was in accordance with earlier findings where tablets prepared with Crosscarmellose sodium and Sodium starch glycolate showed tremendous swelling before disintegration. The disintegrating action of CP was owing to its high capillary activity and rapid swelling in all direction in presence of any physiological fluid. This leads to the rapid development of high internal stresses, which causes the tablet to disintegrate.

Table 4:

Time(min)	Tablet of F9-M
0	0
2	9.74±0.05
4	23.82±0.09
6	35.83±0.01
8	48.24±0.04
10	59.49±0.11
12	74.92±0.06
14	87.81±0.01
16	99.27±0.08
18	--
20	--

Table 5:

Time (min)	Marketed Formulation
0	0
5	14.7±0.07
10	30.87±0.03
15	47.37±0.09
20	64.01±0.13
25	82.52±0.05

30	99.8±0.11
35	--
40	--

(* Represents mean ± S.D.) (n=3)

For further improving drug release profile and other parameter, trials were carried out by using combination of two Superdisintegrant i.e. cross providone and sodium starch glycolate in different ratios. Finally it was found that formulation batch containing cross providone and sodium starch glycolate gave good results in the ratio of 3:1. The disintegration time was seen to be 27 seconds which facilitated faster dispersion in the mouth. And the drug release was found to be 99.27±0.08% in 16 minutes as shown in Table 4. This can be attributed to the extent of water uptake and consequently the strong swelling power of this disintegrants causing sufficient hydrodynamic pressure to induce complete disintegration. This can be correlated to tablet pore size distribution due to synergistic effect of two superdisintegrants which makes the larger pores in the network of skeleton.

CONCLUSION

The solubility and dissolution rate of Tinidazole from solid dispersion i.e. batch F-1 and F-11 was increasing significantly than that of pure drug. Solid dispersion prepared by melting technique showed faster drug release than the solid dispersion prepared by spray drying and physical mixture. Thus *in vitro* drug release increased in the following order as per the technique of preparation was concerned:

Physical mixture < spray drying technique <melting technique

The general trend indicated that there was increase in dissolution rate for solid dispersion in the following order:

PVP-K30 < HPMC K 100 LV < Poloxamer F 188 < PEG-6000 < PEG-6000: Poloxamer F 188

Among all the formulation batches, F-9M containing TNZ:PEG-6000: Poloxamer F188 (1:0.3:0.2) gave good drug content and drug release and hence was selected as an optimized batch. Fast dissolving tablets of solid dispersion containing cross providone and sodium starch glycolate in ratio of (3:1) found to give good results (99.27 % within 16 minute) which was much better than the marketed formulation of Tinidazole (99.8 at 30 minutes). The stability studies revealed no significant changes in hardness, friability, disintegration time and *in vitro* dissolution time after three months study.

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