Influence of Crystal Habit on Trimethoprim Suspension Formulation

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Purpose. The role of crystal habit in influencing the physical stability and pharmacokinetics of trimethoprim suspensions was examined.
Methods. Different habits of trimethoprim (TMP) were obtained by recrystallizing the commercial sample (PD) utilizing solvent-change precipitation method. Four distinct habits (microscopic observation) belonging to the same polymorphic state (DSC studies) were selected for studies. Preformulation and formulation studies were carried out on suspension dosage forms containing these crystals. The freshly prepared suspensions were also evaluated for their pharmacokinetic behaviour on healthy human volunteers using a cross over study.
Results. Variation of crystallization conditions produces different habits of TMP. Among the different crystal habits exhibiting same polymorphic state, the most anisometric crystal showed best physical stability in terms of sedimentation volume and redispersibility. However, habit did not significantly affect the extent of TMP excreted in urine.
Conclusions. Modification of surface morphology without significantly altering the polymorphic state can be utilized for improving physical stability of TMP suspensions. However, the pharmacokinetic profile remains unaltered.

KEY WORDS: crystal habit; trimethoprim suspension; physical stability; pharmacokinetics.

INTRODUCTION

A crystalline solid is characterized by its internal (polymorphic) and external (habit) structures. An interference with the crystallizing molecules to the different faces of a growing crystal may affect its shape without changing its internal structure (1,2).

Although, the influence of crystal shape on compression was first reported by Jaffe and Foss in 1959 (3), who found that "substances belonging to the cubic crystal system presented no difficulty for direct compression," only a few researchers have so far investigated this seemingly trivial crystal property, and that too in solid dosage forms (4–7). While the influence of polymorphism on stability and bioavailability of drugs has been amply studied, the role of crystal habit in suspensions has not been investigated in isolation.

An equidimensional crystal habit has been found to improve flow and compaction of ibuprofen granules as compared to elongated needle-like crystals (8). Recently, Byrn *et al.* have suggested the importance of crystal morphology of polymorphs in evaluating the performance or manufacturing reproducibility of dosage forms (9). Hence, it is hypothesized that the influence of habit would be more pronounced in suspensions because of availability of more space for reorientation of particles during storage.

The aim of the present investigation is to obtain different crystal habits (belonging to the same polymorphic state) of trimethoprim and evaluate their influence on suspension stability and bioavailability.

MATERIALS AND METHODS

Materials

Triemthoprim I.P. (Burroughs Wellcome, Bombay, India); methanol, propylene glycol, glycerol, PEG 200 and 400, acetone (E. Merck, India); n-propanol, iso-propanol, N-N-dimethylformamide (BDH); ethanol B.P. (Bengal Chemicals, India); HPMC (Loba Chem, India). All other chemicals were of analytical grade.

Methods

Purity of the commercial sample (PD) of TMP and prepared crystals was determined by using $\epsilon = 7.4 \times 10^3$ (0.4% NaOH) at 288 nm (10) on Beckman 34 spectrophotometer.

Preparation, Selection, and Determination of Physico-Chemical Properties

Different crystal habits of TMP were obtained by adding a saturated solution of the drug in co-solvent into water. The speed of stirring was adjusted to give a vortex at the top surface of liquid. The co-solvents used were methanol, ethanol, n-propanol, iso-propanol, propylene glycol, glycerol, PEG 200, 400, acetone and N-N-dimethylformamide (DMF). Process variables studied were ratio of co-solvent:water (1:5, 1:10, 1:20, 1:50 and 1:100) and the rate of cooling. Qualitatively different rates of cooling were obtained by adding the co-solvent at room temperature and 70°C to crystallizing solvent (water) maintained at room temperature and 70°C. Then the crystallization was left to occur at room temperature and 0°C for each case. Various crystal habits (microscopic examination) were isolated by filtration and vacuum drying (5 mm Hg, at 50 ± 2°C).

Crystals of four different habits belonging to the same polymorphic state as that of PD were evaluated for their shape parameters (length and width). Photographs were taken under Jenamed-2-histology Carl Zeiss microscope.

The polymorphic state (ΔH) of PD and selected crystals was determined by DSC analysis employing a heating rate of 10°C/min over a temperature range of 30–250°C on Mettler TA 3000 instrument.

Bulk density was determined by tapping to a constant volume. Zeta potential (Zp) was measured using an assembled microelectrophoresis unit and was calculated using the formula, $Zp\ (mV) = 150 \times V/E \times 1000$, where, V is the electrophoretic mobility (cm/sec), E is the potential gradient (Volts/cm) and 150 is a constant for aqueous medium.

Dissolution studies (160 mg, 100/200 # sieve fraction) were performed in distilled water, at 100 rpm using USP XXI

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dissolution test apparatus II. The calculations for amount of drug dissolved over 60 minutes were done on the basis of the amount of drug initially loaded into the dissolution vessel.

Suspension Formulation and Stability Testing

The suspension formulations consisted of TMP 100/200 # sieve (0.160g), PEG 400 (0.04 ml), HPMC 2% w/v solution (6.70 ml) and distilled water (Qs 10.0 ml). 10 ml portion of each suspension in stoppered test tube was evaluated for chemical and physical stability [sedimentation volume (F), ease of redispersibility (RD), and zeta potential (Zp)] at 45°C over 60 days.

'F' was calculated as the ratio of height of the sediment to the original height of the suspension. 'RD' was determined using an assembled mechanical unit fitted with an electrical motor, timer, digital counter and side arm for holding the test tube. The test tube was inverted (180°) from an upright position (5 seconds), held for inversion of the sediment (3 seconds) and again reverted to upright position along the same path (5 seconds). This was counted as one turn. The process was repeated till all the sediment was removed from the bottom of the test tube. Data collected was represented as 'number of turns to achieve homogeneity'. 'Zp' of the suspensions was determined in the same way as for crystals.

Evaluation of Bioavailability

Urinary excretion data was obtained by administering freshly prepared suspensions of PD and investigational crystals (160 mg TMP) to healthy male volunteers (23 ± 1 year age; 55 ± 5 Kg, weight). The subjects were fasted overnight and for four hours after drug administration. Each group consisting of 5 volunteers were subjected to cross-over studies with wash out period of one week. Written consent was obtained from all the volunteers for this study.

The urine samples were analyzed for the free drug according to the method reported by Kaplan *et al.* (11), using Hitachi 650-10S fluorescence spectrophotometer. Activation and detection wavelengths were 275 and 345 nm, respectively and the slit opening was set at 5/3.

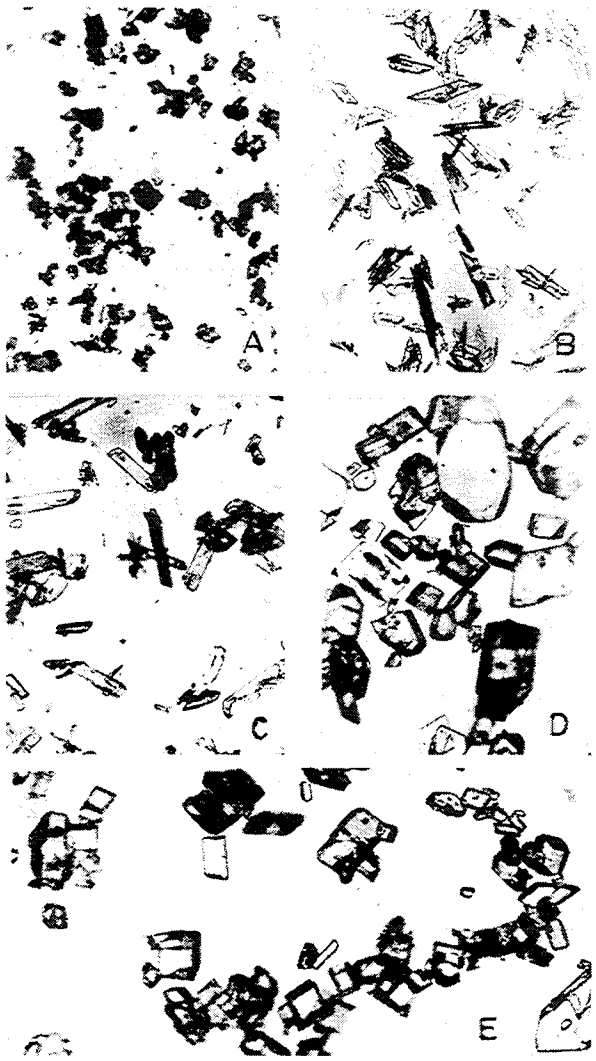

Fig. 1. Photomicrographs of PD and investigational crystals (mag. 200 ×). A, PD; B, Crystal I; C, Crystal II; D, Crystal III; E, Crystal IV.

RESULTS

Habit Modification and Physico-Chemical Properties

The crystallization conditions for obtaining investigational TMP crystals are summarized in Table I. The photomicrographs (Fig. 1A-1E) and ΔH_f values (Table I) indicate that while the crystals exhibit different habits, they belong to the same polymorphic state. The purity of the prepared crystals ranged between 99.62 and 102.79 as compared to 100.00 ± 0.18 of PD. Thus, it is concluded that the method of preparing crystals did not result in drug degradation.

Table I. Crystallization Conditions and Physicochemical Properties of PD and Investigational Crystals of TMP

Crystal no.	Cosolvent	Crystallization conditions co-solvent: water			Crystal habit	ΔH_f (Kcal/mole)	Zp (mV)	Bulk density (gm/cc)	Length; breadth (μm)
		Ratio	Temp.	Cooling					
I	DMF	1:10	RT-RT	RT	Thin rods	8.73 ± 0.9	−46.31	0.138 ± 0.017	130.12; 17.46
II	PEG200	1:5	RT-RT	RT	Thick rods	9.03 ± 0.06	−27.67	0.214 ± 0.003	110.60; 22.54
III	DMF	1:10	70-70	RT	Polyhedra	9.14 ± 0.11	−35.74	0.513 ± 0.005	129.13; 116.95
IV	DMF	1:20	RT-70	RT	Cubes	9.20 ± 0.18	−18.06	0.368 ± 0.004	52.67; 44.09
PD	—	—	—	—	Irregulars	9.56 ± 0.24	−27.51	0.406 ± 0.012	80.43; 41.40

Note: DMF: dimethyl formamide; PEG: poly ethylene glycol; RT: room temperature.

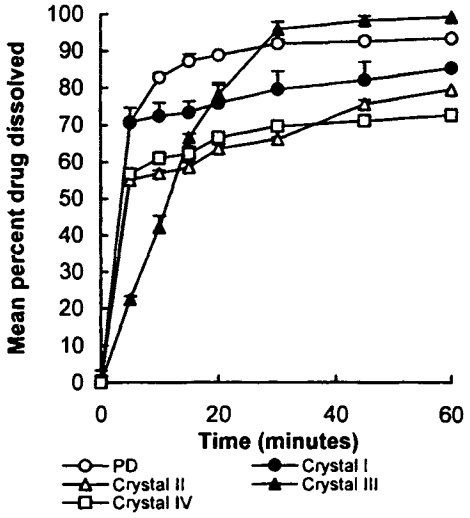

Fig. 2. Dissolution profile of TMP investigational crystals and PD in distilled water.

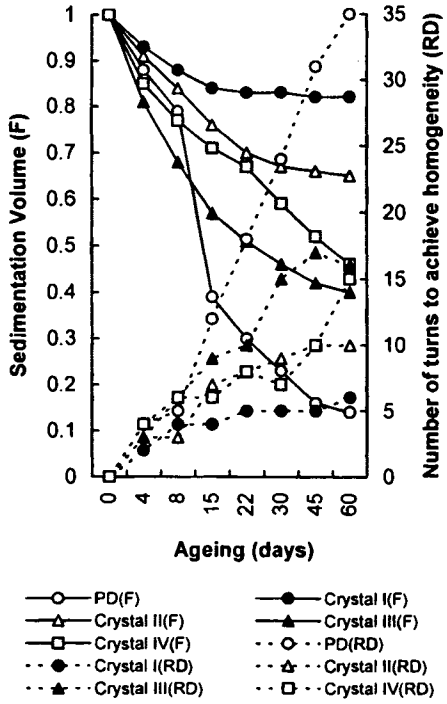

Fig. 3. Sedimentation (F) and redispersibility (RD) behavior of PD and investigational crystal suspensions of TMP upon aging at 45°C.

Crystals I, II and IV exhibited lower bulk density than PD. Whereas, crystal III had 26% higher bulk density than PD. The bulk density and shape parameters are summarized in Table I. Dissolution profile of all the investigational crystals in distilled water was less than that of PD ($P < 0.05$), as shown in Fig. 2.

Stability of Suspensions

All the formulations containing crystals of different habits were stable in suspension formulation as evidenced by drug loss of less than 1.2% during ageing. F, RD and Zp behaviour of these suspensions during ageing is summarized in Table II. The equilibrium F value follows the order: crystal I > II > IV > III > PD, that is 5.8, 4.6, 3.2 and 2.8 times that of PD. Figure 3 shows that the suspensions exhibiting higher F value were free from caking and were easily redispersible.

Bioavailability of Suspensions

The rate of excretion of unchanged TMP in urine is depicted in Fig. 4. The pharmacokinetic parameters reveal that TMP is rapidly absorbed from the GIT after oral administration of the suspensions. Excretion rates ranged from 3.71 mg/hr (crystal IV) to 5.04 mg/hr (PD) and extent of excretion from 35.11% (crystal III) to 51.15% (PD). Anova ($P < 0.05$) did not reveal any difference between the products for these two parameters.

DISCUSSION

Crystallization studies employing various alcohols (methanol to polyethylene glycol 400), acetone and dimethylformamide exhibited different habits depending upon the processing variables, like ratio of co-solvent to crystallizing solvent, temperature of co-solvent and/or crystallizing solvent and cooling condition. All these factors are expected to change the supersaturation level of TMP thus leading to modifications in the rate of crystal growth at different crystal faces. It was found that

Table II. Sedimentation Volume (F), Redispersibility (RD), and Zeta Potential (Zp) Behavior of PD and Investigational Crystal Suspensions of TMP upon Storage at 45°C

Aging (days)	PD			Crystal I			Crystal II			Crystal III			Crystal IV		
	F	RD	Zp	F	RD	Zp	F	RD	Zp	F	RD	Zp	F	RD	Zp
0	1.00	—	48.52	1.00	—	62.09	1.00	—	43.91	1.00	—	46.85	1.00	—	32.57
4	0.88	4	45.47	0.93	2	59.68	0.91	3	50.73	0.81	3	49.02	0.85	4	40.49
8	0.79	5	48.60	0.88	4	55.19	0.84	3	54.22	0.66	6	45.68	0.77	6	42.70
15	0.36	12	33.78	0.84	4	49.42	0.76	7	48.66	0.57	9	38.35	0.71	6	38.92
22	0.30	C18	20.94	0.83	5	53.16	0.70	8	43.19	0.51	10	31.70	0.67	8	41.60
30	0.23	C24	23.01	0.82	5	47.77	0.67	9	40.34	0.46	C15	34.38	0.59	7	37.05
45	0.16	C31	15.12	0.82	5	50.50	0.65	10	45.45	0.42	C17	30.77	0.52	10	41.73
60	0.14	C35	17.04	0.82	6	48.94	0.65	10	39.26	0.40	C16	26.67	0.46	C15	30.27

Note: C: caking.

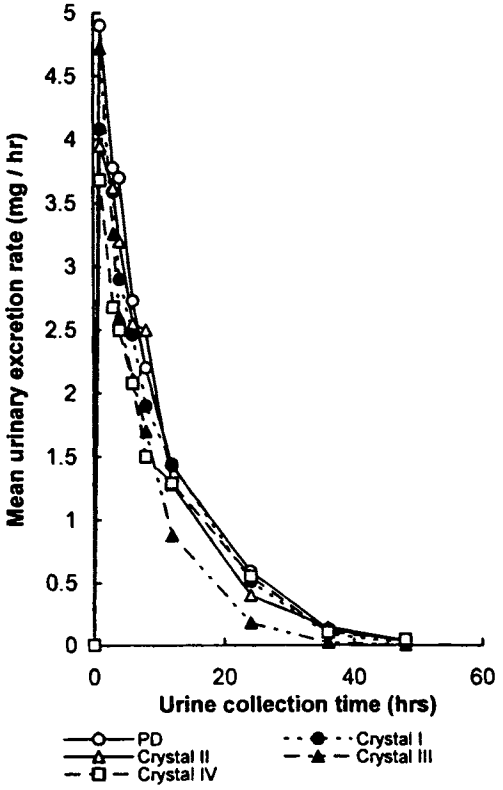

Fig. 4. Urinary excretion rate of free TMP following oral administration of PD and investigational crystal suspensions.

the higher solubility of TMP in a co-solvent required a lower ratio of co-solvent to crystallizing solvent for producing a well defined morphology. Also, a decrease in initial supersaturation due to elevated temperature of co-solvent or crystallizing solvent eliminated the chances of formation of rod-like crystals. This is evident from Table I. Variation in cooling condition did not affect the crystal morphology, probably due to spontaneous precipitation and rapid growth of TMP crystals. Thus, Carstensen's view point that rapid cooling usually produces needle-shaped crystals (12) could not be confirmed in the present investigation.

The crystals selected for investigation (Fig. 1A-1E) exhibit different habits. Their ΔH_f (a measure of crystal lattice energy) summarized in Table I do not differ significantly, indicating that they belong to the same polymorphic state. The macromeritic properties (100/200 # sieve fraction) show that crystal III has 26% higher bulk density than PD (Table I). This crystal was multi-faced and had the lowest shape factor (length/width). It is probably due to such a shape that the crystals of this system underwent orientation during packing so as to achieve the lowest volume among the other habits. Crystal I (rods) having the highest shape factor did not allow easy orientation during packing and hence, resulted in highest bulk volume. Thus, the anisometric particles, having a high elongation ratio seem to build up open packing with high porosity.

It is evident from Fig. 2 that crystals with asymmetric shape exhibit low rate and extent of dissolution than PD. Crystal I, although, shows high dissolution in the early phase, probably undergoes changes in shape during later phase, thus altering its dissolution rate. Crystal II, having a shape factor less than crystal I exhibits an intermediate dissolution. It has earlier been hypothesised that crystals of symmetric shape (spherical and cubical) dissolve faster due to equal exposure of all the surfaces (13). The dissolution rate increased in the order PD > I > II = IV (Anova, $P < 0.05$). The rapid dissolution of crystal III during later phase compensates for its slow dissolution during the early phase to such an extent that its overall dissolution rate does not differ from others. The size factor (length × width) of crystal III was almost five times that of PD. Hence, its lowest dissolution rate during the initial phase seems to be due to its size factor. However, its most symmetrical morphology exposed the maximum surface for solvent attack, essentially maintaining the same shape due to equal dissolution from all faces during the later phase, thus resulting in highest extent of dissolution. PD crystals apparently rank second in the extent of dissolution despite irregular shape, possibly due to surface roughness/imperfections. Microscopic observation of crystal IV shows massive aggregation. It is noteworthy that crystal IV exhibits the lowest Zp value. Hence, despite being cuboidal shaped and expected to show better dissolution, it exhibited the least release. In fact, excluding PD, Zp of the particles (Table I) seems to directly influence dissolution of TMP crystals. Hence, the role of particle-particle interaction in modifying the release of TMP from crystals cannot be ruled out as suggested for erythromycin and its hydrates (14). These results suggest that although crystal habit plays a major role in modifying the dissolution characteristics, the contribution of surface charge and imperfections cannot be neglected.

The decline in sedimentation volume (Fig. 3) upon storage is apparently exponential. The ultimate volume can be ranked as crystal I > II > IV > III > PD, that is 5.8, 4.6, 3.2 and 2.8 times that of PD. This can be explained on the basis of asymmetricity of the crystals. The highest shape factor of crystal I showed highest F value due to formation of porous pack structure. This resulted in the highest sedimentation volume followed by crystal II. An end-to-face rather than end-to-end framework may be hypothesized to be operative during formation of the sediment as this will result in a decrease in the free energy of the system. Also, such a structure will be less susceptible to the overhead pressure of settling particles. Thus, despite having high zeta potential, the anisometric particles tend to induce 'self-flocculation' which provides a scaffold-like, porous pack structure. Although, PD suspension should have ranked third in the order, it showed the least F value due to irregular-shaped particles which could undergo 'close fit' re-orientation upon storage. The high F value of crystal IV suspension as compared to III can be correlated with their respective bulk densities. Also, crystal IV shows aggregation (Fig. 1). Due to this, crystal IV was less susceptible to overhead sediment pressure as compared to crystal III. The redispersibility pattern (Fig. 3) substantiates the view-point that suspensions maintaining higher sedimentation volume are less prone to caking.

Zp of the suspensions varied from 32.57 (crystal IV) to 62.09 (crystal I) mV (Table II). A comparison of the Zp of the crystals in suspensions (Table II) with the initial Zp of particles (Table I) shows that the addition of HPMC resulted in gain in negative surface charge of about 11 (crystal III) to 21 (PD) mV. A similar phenomenon is reported for nitrofurantoin upon addition of Carbopol 943 (15). An analysis of the Zp during storage indicates that the suspensions undergoing rapid decline

in Zp are more prone to caking. In fact, an inverse correlation (log RD = -0.025 Zp + 1.901; r = -0.9213; n = 33, $P < 0.001$) between redispersibility and zeta potential and a direct correlation (F = 0.019 Zp − 0.133; r = 0.9456; n = 33, excluding controls; $P < 0.001$) between sedimentation volume and zeta potential suggests that with decrease in surface charge, the physical stability of TMP suspensions decreases during storage.

The rate of urinary excretion of intact TMP following oral administration of freshly prepared suspensions is compared in Fig. 4. Crystal IV suspension apparently exhibits the least while PD and crystal III exhibit the highest absorption rate. Crystal I and II suspensions exhibit intermediate rates of absorption. These profiles follow the trend as observed in dissolution studies. Although, crystal III and IV differed with respect to absorption and distribution rate constants, the C_{max}, T_{max} and extent of drug excretion did not differ significantly in any of the formulations. This seems to be due to the insignificant inter-crystalline variation in ΔH_f (16). Also, because TMP is inherently rapidly absorbed, the small inter-crystalline variations in ΔH_f does not seem to influence the pharmacokinetic parameters. A similar reason has been suggested for diflunisal (17). However, studies on slowly absorbed drugs are advocated to validate this view-point.

The results indicate that selection of a proper crystal habit of a drug may be advantageously utilized to generate a floc structure strong enough to withstand the overhead pressure of the sediment. Such a system would make "self-flocculation" possible for formulating suspensions with enhanced physical stability without using flocculating agents. For rapidly absorbable drugs such an approach will not adversely affect the pharmacokinetics.

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