RESEARCH PAPER

Accepted 23 March 2000
Revised 7 March 2000
Received 30 August 1999
Indian J. Pharm. Sci., 2000, 62 (4) 267-272

Enhanced Suspension Stability: Choice of Crystal Habit

A.K. TIWARY* AND G.M. PANPALIA[1]
Department of Pharmaceutical Sciences and Drug Research,
Punjabi University, Patiala - 147 002
[1]Department of Pharmaceutical Sciences, Birla Institute of Technology,
Mesra, Ranchi - 835 215

It is well established that the polymorphic state of a solid can modify physicochemical properties and suspension stability of drugs. However, not much attention has been paid towards crystal habit, the other seemingly trivial property of a crystalline solid. This study reveals that different habits of sulphamethoxazole belonging to the same polymorphic state can be obtained by varying the crystallization conditions, normally associated with preparation of different polymorphs. This implies that the same polymorphic form can exist in different habits. The selected habits were found to differ significantly with respect to shape parameters, bulk density and dissolution rate. The suspension formulations containing these habits exhibited significantly different physical stability than that formulated with the parent drug material. However, due to insignificant difference in ΔH_f value of the crystals and inherent nature to be rapidly absorbed, the K_e, T_{max} and extent of free as well as acetylated sulphamethoxazole excreted were not significantly altered.

Crystal structure of a solid comprises of its internal and external structures. The internal structure, commonly referred to as the polymorphic state, is the molecular arrangement within the crystal lattice. Habit describes the shape or outer appearance of a crystal. The habit of a crystal may be altered due to interference with the uniform approach of crystallizing molecules to the different faces of a crystal, resulting in anhedral (irregular) or euhedral (regular) crystals[1,2]. Hence, the environment of a growing crystal may affect its external shape without changing the internal structure.

Polymorphism is known to influence solubility and dissolution rates that directly affect absorption and bioavailability of drugs[3,4]. However, a change in habit may not necessarily be associated with a change in the polymorphic state. Crystal habit can be easily altered by changing the solvent and/or crystallization conditions. Hence, the same drug may be made available to the formulator in different habits by different bulk drug manufacturers. Literature reveals the importance of crystal habit in influencing the flowability and compressibility of dry powders during formulation of solid dosage forms[5-9]. An equidimensional crystal habit has been reported to improve flow and compaction of ibuprofen granules as compared to needle-shaped crystals[10]. Characterization of crystal morphology has also been suggested to be important in assessing the reproducibility of dosage forms[11]. It is noteworthy that in most studies, the role of crystal habit has not been delineated from the polymorphic effect. Hence, in order to clearly bring out the role of crystal habit, it seems imperative to keep the influence of polymorphism to the minimum.

The influence of crystal habit is envisaged to be more pronounced in suspensions due to availability of more space for re-orientation of particles during settling. Selection of a stable habit assumes more importance in suspensions because of the presence of solid-liquid interactions that often result in Ostwald ripening. Recently,

*For Correspondence
E mail : tiwary@pbi.ernet.in

TABLE 1 : CRYSTALLIZATION CONDITIONS AND PHYSICOCHEMICAL PROPERTIES OF PD AND INVESTIGATIONAL CRYSTALS OF SMZ

Crystal No.	Cosolvent	Crystallization Conditions Co-solvent: Water Ratio	Temp.(°)	Cooling	Crystal Habit	ΔH_f (Kcal/mole)	Zp (mV)	Bulk Density (g/cc)	Length; Breadth (μm)
I	DMF	1:10	RT-70	4°	Thick rods	5.79±0.10	-62.45±1.76	0.22±0.02	95.70; 16.60
II	Methanol	1:20	RT-50	4°	Thin rods	5.82±0.17	-53.91±1.68	0.18±0.014	180.80; 16.60
III	PEG 400	1:50	RT-70	4°	Cuboids	5.83±0.04	-69.04±1.55	0.28±0.005	81.90; 73.00
IV	PEG 200	1:20	RT-70	4°	Thin flakes	5.85±0.24	-71.76±1.87	0.22±0.013	146.08; 104.03
PD	-	-	-	-	Irregulars	7.47±0.06	-76.38±1.92	0.40±0.008	59.56; 45.89

DMF : Dimethyl Formamide; PEF : Poly ethylene glycol; RT : Room Temperature.

a rod-shaped habit has been reported to significantly improve the physical stability of trimethoprim suspension[12]. The present work aims at obtaining different habits of sulphamethoxazole (SMZ) belonging to the same polymorphic state and evaluating their influence on suspension stability and bioavailability.

MATERIALS AND METHODS

Sulphamethoxazole USP XXI was a gift sample from SOL, Hyderabad, India. Methanol, propylene glycol, glycerol, PEG 200, 400 and acetone were purchased from E. Merck, India. n-Propanol, iso-propanol, n-butanol and N-N-dimethylformamide were procured from BDH, India. Ethanol B.P. and HPMC were products of Bengal Chemicals and Loba Chem, respectively. All other chemicals were of analytical grade. Purity of the parent drug sample (PD) of SMZ and crystals selected for final investigation was determined by using E value of 673 (1 %, 1 cm) in 0.1 N NaOH at 257 nm[13].

Preparation, selection and determination of physico-chemical properties:

Different crystal habits of SMZ were obtained by adding a saturated solution of the drug (in various co-solvents) into water at a constant rate of stirring. Appearance of a vortex provided complete mixing. The co-solvents used for obtaining investigational crystals were methanol, polyethylene glycol (PEG) 200 and 400 and dimethylformamide (DMF). Other co-solvents tried in this study included, ethanol, n-propanol, iso-propanol, propylene glycol, glycerol and acetone. Initially, studies with different co-solvent:water ratios (1:5, 1:10, 1:20, 1:50 and 1:100) at room temperature were conducted using each co- solvent in order to select the ratio(s) that gave well-defined habit. After identifying the ratio(s) for each co-solvent, the influence of different temperatures of water (25, 50 and 70°) was investigated. Furthermore, the influence of two cooling rates was studied by leaving the crystallization to occur at room temperature and 4°. All crystals having distinctly different habits and least contamination with other habits (microscopic examination) were filtered and vacuum dried (5 mmHg, at 50±2°). The crystallization conditions for obtaining investigational crystals are summarized in Table 1.

Crystals of four different habits exhibiting insignificant difference from PD with respect to ΔH_f (t-test, $P<0.05$) were selected from the various crystals obtained and evaluated for their shape parameters (length and width). Photo graphs were taken under a Jenamed-2-histology Carl Zeiss microscope. The ΔH_f of PD and selected crystals was determined by DSC (heating rate of 10°/min over a temperature range of 30-250°) using a Mettler TA 3000 instrument.

Bulk density was determined by tapping to constant volume. Zeta potential (Zp) was measured using an indigenously developed microelectrophoresis unit [Zp (mV) = 150 x V/E x 1000, where, V is the electrophoretic mobility (cm/sec), E is the potential gradient (Volts/cm) and 150 is a constant for aqueous medium]. At least forty particles were counted for each case, taking 'polarity reversal' into consideration.

Dissolution studies (800 mg, 100/200 # sieve fraction) were performed in 0.1 N hydrochloric acid at 100 rpm using USP XXI dissolution test apparatus II. The amount of drug dissolved till 45 min. was analysed spectrophotometrically after dilution with 0.1 N sodium hydroxide solution as for purity determination of PD.

Formulation and stability testing of suspensions:

Suspension formulation of PD and selected crystals consisted of (100/200 # sieve fraction) SMZ (0.80 g), PEG 400 (0.25 ml), HPMC 2% w/v aqueous solution (5.0 ml) distilled water (Qs 10.0 ml). The suspension was subjected to evaluation of chemical and physical stability [sedimentation volume (F), ease of redispersibility (RD), zeta potential (Zp)] at 45° over 60 days.

'F' was calculated as the ratio of height of the sediment to the original height for all the suspensions and compared by t-test ($P<0.05$). '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 s), held for inversion of the sediment (3 s) and again reverted to upright position along the same path (5 s). 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:

Freshly prepared suspensions of PD and investigational crystals (800 mg SMZ) were administered to healthy male volunteers (age: 23±1 y and weight: 55±5 Kg). The subjects were fasted overnight and were given fixed amount of water till 4 h after drug administration. After 4 h, they were fed with a standard vegetarian diet. Each group consisting of 5 volunteers were subjected to crossover urinary excretion studies with wash out period of one week. The volunteers were asked to note the volume of entire urine voided at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, 36 and 48 h. A portion (10 ml) was collected in a test tube and analysed by Bratton and Marshall method[14] for both free and total SMZ excreted. The free SMZ was estimated by adding trichloroacetic acid (2 ml of 15% w/v solution) to diluted urine sample (1 ml) followed by addition of 4 N HCl (0.5 ml). The mixture was cooled for 15 min in a freezer and then sodium nitrite (2 ml of 0.1% w/v solution) was added. This mixture was again cooled for 15 min, brought to room temperature and ammonium sulphamate (2 ml of 0.5% w/v solution) added. After shaking the mixture for 5 min, N-(1-naphthyl) ethylenediamine dihydrochloride (2 ml of 0.1% w/v solution) was added and absorbance of the pink colour developed was read at 545 nm. For estimation of total SMZ, the diluted urine sample was first hydrolysed by heating for 30 min in the presence of 6 N HCl (2ml), cooled and then subjected to further processing as for free SMZ. The pharma-cokinetic parameters were compared by t-test ($p<0.05$). An informed consent was obtained from all the volunteers.

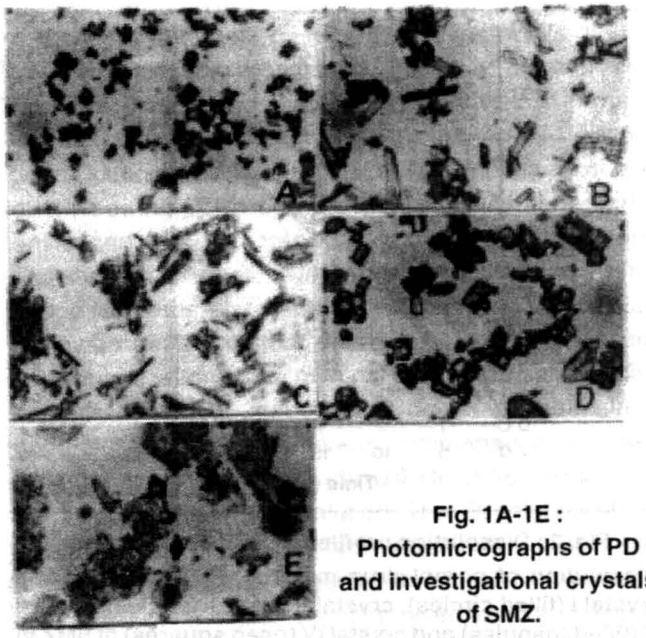

Fig. 1A-1E : Photomicrographs of PD and investigational crystals of SMZ.

Magnification is 200 x for A-PD., B-Crystal I., C-Crystal II., D-Crystal III and E-Crystal IV

RESULTS

The photomicrographs (Fig. 1A-1E) and the ΔH_f values Table 1 indicate that while the crystals exhibit different habits, they belong to the same polymorphic state. The purity of all crystals was observed to be between 99.86 and 100.92 as compared to 100.00±0.16 of PD. This indicates that the method of crystallization did not result in drug degradation. Table 1 shows that all the investigational crystals exhibited lower bulk density than PD, with the minimum being exhibited by crystal II (44% of PD). Extent of dissolution of only crystal I was less than PD Fig. 2.

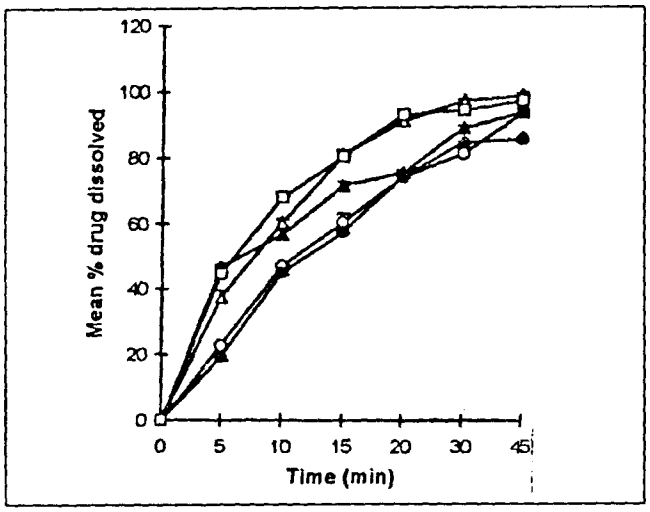

Fig. 2 : Dissolution profiles of SMZ preparations

Dissolution of parent drug material, PD (open circles), crystal I (filled circles), crystal II (open triangles), crystal III (filled triangles) and crystal IV (open squares) of SMZ in 0.1 HCl

The drug was stable in all habits in the presence of formulation additives as evidenced by less than 1.3% of drug loss during ageing. As evident from Fig. 3, the equilibrium F value follows the order, crystal II>I>IV>III>PD ($p<0.05$). Suspensions exhibiting higher F value were less prone to caking and were easily redispersible.

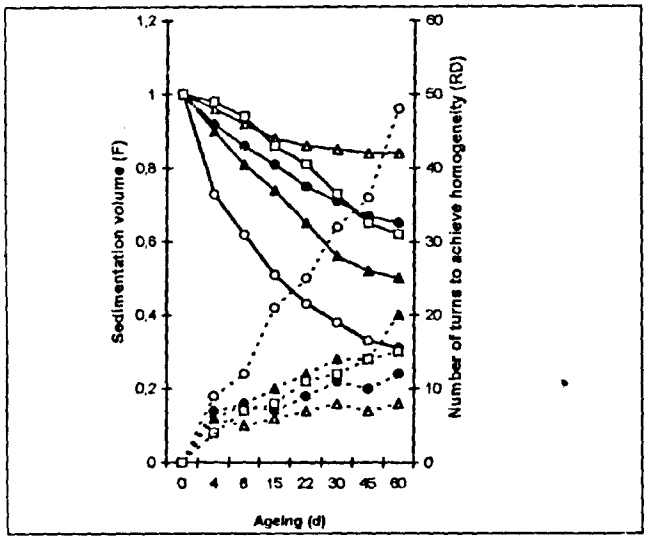

Fig. 3 : Physical stability of SMZ suspensions

Suspensions have been formulated with parent drug material (open circles) or crystal I (filled circles) or crystal II (open triangles) or crystal III (filled triangles) or crystal IV (open squares) and stored at 45°. Solid lines represent sedimentation volume and broken lines represent redispersibility

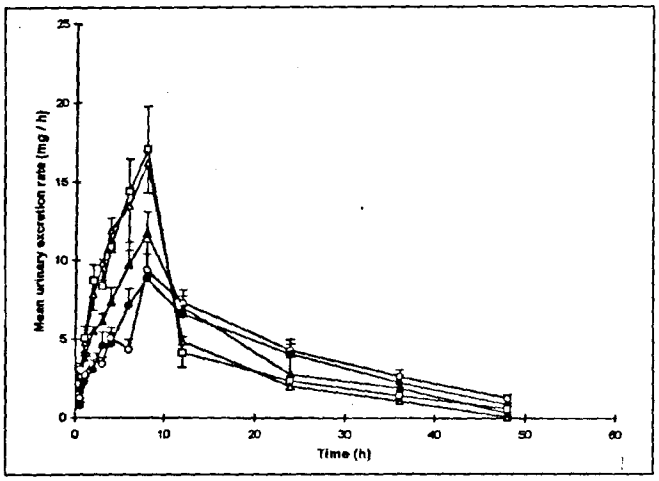

Fig. 4 : Urinary Excretion rates of various crystal suspensions of SMZ.

Healthy human volunteers were administered suspensions of parent drug material (open circles) or crystal I (filled circles) or crystal II (open triangles) or crystal III (filled triangles) or crystal IV (open squares) after an overnight fast. Urine samples were collected and drug content was analysed by Bratton and Marshall method

Urinary excretion rate of free SMZ following oral administration of freshly prepared suspensions is shown in Fig. 4. Suspension containing crystal I and IV exhibited the minimum and maximum values, respectively for K_a and C_{max}. However, K_e, T_{max} and percent free drug excreted from any product were insignificantly different ($P<0.05$).

DISCUSSION

The selection of investigational crystals was based on the observation that the crystals exhibited well-defined habit, differing from each other but belonged to the same polymorphic state. This was necessary for delineating the role of crystal habit on the performance of SMZ suspensions. In addition, the selected habits had to be free from contamination with other habits that are usually produced due to delayed crystallization of some amount of drug. No generalization could be made regarding the conditions leading to contamination of a particular crystal habit by other habits. Therefore, only four crystals were selected for further studies out of the numerous crystals obtained after initial crystallization trials.

Different crystal habits were obtained from the same co-solvent depending on the crystallization conditions.

This is due to the change is supper saturation of the drug during crystallization under different conditions that ultimately leads to different growth at adjacent crystal faces[1]. Viscous solvents produced more symmetric crystals as compared to fluid solvents. This seems to be because of formation of small nuclei and slow, uniform deposition of crystallizing molecules at all the faces due to high viscosity of the co-solvent. Co-solvents having high affinity for SMZ required higher co-solvent:crystallizing solvent ratio and higher temperature of crystallizing solvent for obtaining a well-defined habit Table 1. It is noteworthy, that crystallization occurred immediately when crystallizing solvent (water) was at 4° as against 5 min when it was at room temperature. However, at any cooling rate, crystallization was delayed when the co-solvent was added to water at higher temperature. Crystallization was also delayed when the co-solvent possessed more affinity for SMZ. It seems that the two extreme rates of cooling influenced the onset of nuclei formation by altering the super saturation level of the drug. Although, extreme cooling at lower co-solvent:crystallizing solvent ratios produced elongated structures, they were not needles. Thus, Carstensen's contention[15] that rapid cooling produces needles could not be confirmed in the present study.

The crystals selected for investigation exhibit different habits Fig. 1A-1E. Their ΔH_f (a measure of crystal lattice energy) does not differ significantly, indicating that they belong to the same polymorphic state Table 1. Also all the four crystals exhibited a single sharp endotherm, indicating their monotropic nature without any solvent entrapment. Table 1 shows that all the crystals (100/200 # sieve) exhibited lower bulk density than PD. As compared to PD, the lowest (44%) and highest (70%) bulk density among the crystals was exhibited by crystal II and III, respectively. This indicates that the symmetric crystals having a high elongation ratio arrange loosely as compared to irregulars.

SMZ is practically insoluble in water. Due to its amphoteric property it exhibits greater solubility at lower pH values[16]. The USP specifies that not less than 50% of the labelled amount of SMZ be dissolved from sulphamethoxazole tablets USP in 20 min using dilute hydrochloric acid as the dissolution medium. These facts imply that the *in vivo* performance of SMZ is dissolution rate limited. Also, choice of dissolution medium seems to be critical. Hence, the present study employed 0.1 N HCl as the dissolution medium. The rate of dissolution Fig. 2 follows the order, crystal IV = II > PD = I ($p<0.05$). It is important to note that the dissolution profile of crystal III did not differ significantly from that of other crystals. The same trend is exhibited for extent of drug release. The results indicate that the early rapid dissolution of cuboidal-shaped crystal III is balanced by subsequent slowing down of the process. It has been earlier envisaged that cubic and spherical particles dissolve equally from all sides, whereas other habits change their shape factor thereby altering the dissolution rate[17]. Thus, it may be postulated that during later stages of dissolution, shape alterations of most asymmetrical and thin crystals (II and IV, respectively) helped in maintaining good dissolution rate. Whereas, maintenance of essentially the same shape by cuboidal structure, made crystal III to show lower dissolution. The dissolution of PD despite having half the surface factor (1/L x 1/B) is better than crystal I. This suggests that for thick crystals, a higher degree of asymmetry is required to achieve higher dissolution. However, the role of surface imperfections of PD in increasing the solvent-particle interaction cannot be ruled out.

The decrease in equilibrium 'F' can be ranked as, crystal II>I>IV>III>PD ($p<0.05$) that is 2.7, 2.1, 2.0 and 1.6 time that of PD, respectively Fig. 3. The increased F value of crystal suspensions can be attributed to their asymmetric shape. Crystal II with high elongation ratio tend to build up open packing of high porosity and exhibits the highest sedimentation volume. An end-to-face rather than end-to-end framework may be hypothesised to be operative during settling of crystal I, as this will result in a decrease in the free energy of the system. Also, such a structure will be less susceptible to overhead pressure of settling particles. Crystal IV ranks third, possibly due to formation of a 'card-house' structure, susceptible to crumbling unlike that produced by rods. PD (as isometric as crystal III), being irregular-shaped can undergo 'close-fit' orientation in such-a-way so as to yield less porous sediment during the settling process. Thus, the effect of polydispersity on packing seems to diminish with increase in anisometricity of crystals.

It is evident from Fig. 3 that the suspensions exhibiting a high F value were easily redispersible. An inverse correlation ($\log RD = -0.1164\ Zp + 1.8934$; $r = -0.9475$; $n = 33$, $P<0.05$) between redispersibility and zeta potential and a direct correlation ($F = 0.0087\ Zp + 0.0705$; $r = 0.9468$; $n = 33$, excluding controls; $P<0.05$) between sedimentation volume and zeta potential suggests that maintenance of surface change during storage is essential

for physical stability of suspensions. The dispersion of SMZ in HPMC resulted in gain in ZP, ranging from 16.05 mV (crystal III) to 45.71 mV (crystal II) during storage. A similar phenomenon is reported for nitrofurantoin[18]. The gain in ZP follows the order, crystal II>I>IV>III>PD. This correlates very well with the improvement in physical stability in all respects. Comparison of zeta potential with change in sedimentation volume during ageing shows that crystal II maintained a high sedimentation volume due to retention of the charged sheath around the particles. The rapid drop in ZP of PD suspension, due to formation of a compact sediment, resulted in squeezing out the hydration layer with associated particle discharge. Therefore, PD suspension exhibited caking.

DSC analysis of PD and investigational crystals of aged suspensions (filtration, repeated washing with water and drying) did not reveal any evidence of formation of a semihydrate. This indicates that HPMC also retards the formation of semihydrate as has been suggested for methylecellulose, povidone and sucrose[19].

Hence, from the results of these studies it may be concluded that crystal habit plays a major role in the physical stability of suspensions. However, the role of surface imperfections and particle discharge cannot be ruled out during the sedimentation process.

The urinary excretion rate of free SMZ upon oral administration of freshly prepared suspensions follows the order, crystal II = IV>III>I = PD ($p < 0.05$). It is interesting to note that the extent of SMZ dissolved was also higher from crystal II and IV. Hence, the absorption of SMZ seems to be dissolution rate limited when formulated in suspensions with different habits. Crystal IV did not differ significantly from crystal II but differed from crystal I or PD with respect to K_a and C_{max}.However, an insignificant difference was obtained between all the suspensions with respect to K_e T_{max} and extent of free and acetylated drug excreted ($P<0.05$). An insignificant difference in the ratio of acetylated:free drug at C_{max} suggests that alteration in crystal habit did not change the metabolic behaviour of SMZ. These findings seem to be due to little inter-crystalline variation in ΔH_f[20]. Also, because SMZ 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[21].

The results of this investigation reveal significant improvement in physical stability of SMZ suspensions due to habit modification of the parent drug particles. Such an approach may be utilized for generating a 'scaffold-like', three-dimensional structure capable of withstanding the overhead pressure of settling particles without using flocculating agents. This formulation design will not adversely affect the bioavailability of rapidly absorbable drugs. However, studies on drugs exhibiting slow or site-specific absorption are advocated to generalise the findings.

REFERENCES

1. Haleblian, J.K., **J. Pharm. Sci.,** 1975, 64, 1269.
2. Takubo, H., Kume, S. and Koizumi, M., **J. Cryst. Growth,** 1984, 67, 217.
3. Moustafa, M.A., Kahlil, S.A., Ebian, A.R. and Motawi, M.M. **J. Pharm. Pharmacol.,** 1972, 24, 921.
4. Khalafallah, M. , Khalil, S.A. and Moustafa, M.A., **J. Pharm. Sci.,** 1974, 63, 861
5. Jaffe, J. and Foss, N.E., **J. Am. Pharm. Assoc. Sci. ed.,** 1959, XLVIII, 26.
6. Shell, J.W., **J. Pharm. Sci.,** 1963, 52, 100.
7. Alpar, O., Hersey, J.A. and Shotton, E., **J. Pharm. Pharmacol.,** 1970, 22, S, 1S.
8. Shotton, E. and Obiorah, B.A., **J. Pharm. Pharmacol.,** 1973, 25S, 37.
9. Summers, M.P., Enever, R.P. and Carless, J.E., **J. Pharm. Pharmacol.,** 1976, 28, 89.
10. Gordon, R.E. and Amin, S.I., **European Patent Appl.,** 1984, EP 120 587.
11. Byrn, S., Pfeiffer, R., Ganey, M., Hoiberg, C. and Poochikian, G., **Pharm. Res.,** 1995, 12, 945.
12. Tiwary, A.K. and Panpalia, G.M., **Pharm. Res.,** 1999, 16, 261.
13. Moffat, A.C., Eds., In: Clarke's Isolation and Identification of Drugs, 2nd Edn, Pharmaceutical Press, London, 1986, 988.
14. Bratton, A.C. and Marshall, E.K., **J. Biol. Chem.,** 1939, 128, 537.
15. Carstensen, J.T., Eds., In: Theory of Pharmaceutical Systems: Heterogeneous System, Vol. II, Academic Press, New York, 1973, 262.
16. Dahlan, R., McDonald, C. and Sunderland, V.B., **J. Pharm. Pharmacol.,** 1987, 39, 246.
17. Shami, F.G., Dudzinski, J.R., Jr., In: Jackman, L., Lieberman, H.A., and Kanig, J.L., Eds., The Theory and Practice of Industrial Pharmacy, 2nd Edn, K.M. Varghese Co., Bombay, 1976, 18.
18. Gallardo, V., Delgado, A.V., Parera, A. and Salcedo, J.S., **J. Pharm. Pharmacol.,** 1990, 42, 225.
19. Graf, E., Beyer, C. and Abdallah, O., **Pharm. Ind.,** 1982, 44, 1071.
20. Simmons, D.L., Ranz, R.L., and Gyanchandani, N.D., **Can. J. Pharm. Sci.,** 1973, 8, 125.
21. Dresse, A., Gegard, M.A., and Lays, A., **Pharm. Acta Helv.,** 1978, 53, 177.