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Crystal modification of dipyridamole using different solvents and crystallization conditions

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Abstract

Dipyridamole crystals having different types of habits, improved dissolution rate were prepared by recrystallization from selected solvents, such as acetonitrile, benzene and methanol (Method I); crystals have also been made by solvent change using methanolic solution of dipyridamole in the presence of 2% solutions of Tween-80, Povidone K_{30} and polyethylene glycol (PEG) 4000 (Method II). Scanning electron microscopy, X-ray powder diffractometry, IR spectrometry and differential scanning calorimetry were used to investigate the physicochemical characteristics of the crystals. The comparative dissolution behavior of the newly developed crystals and that of the untreated dipyridamole were also studied. It was found that the newly developed crystals were different from each other with respect to physical properties but are chemically identical. The crystals, obtained (Method I) from benzene and acetonitrile, produced needle shaped crystals and that obtained from methanol produced rectangular shaped crystals. But the crystals obtained (Method II) with the methanolic solution of the drug in the presence of Tween-80, Povidone K_{30} and PEG-4000 produced smooth needle shaped crystals. X-ray diffraction spectra and differential scanning calorimetry study of the newly developed crystals, clearly indicate that dipyridamole exist in different crystal modification. The dissolution rate of newly developed crystals was found to be greater than the pure drug dipyridamole. Stability studies at 40 °C (75% RH) for 1 month for the modified crystals as well as the pure drug did show some changes in the XRD and DSC but not in IR studies.

Keywords: Dipyridamole; Recrystallization; Physicochemical characterization

1. Introduction

Different physiological and formulation factors are responsible for the bioavailability of drug from the dosage form. One of the most important physical factors, which affect the bioavailability and therapeutic efficacy of drug, is the existence of active ingredients in various crystal forms having different internal structure and physical properties (Kapoor et al., 1998). The different crystal form of a drug have different physicochemical characteristics, namely crystal shape, crystal size, melting point, density, flow properties solubility pattern, dissolution characteristics and XRD pattern, though they are chemically identical. A physical form having improved dissolution rate and solubility is useful for improving the bioavailability of a drug (Burt and Mitchell, 1980; Watanable et al., 1982). The crystal habit is an important variable in pharmaceutical manufacturing, where some factors, such as the polarity of crystallization solvent and the presence of impurities in the solvent, affect crystallization (Chow et al., 1985; Femi-Oyewo and Spring, 1994; Garekani et al., 2000). Among them, solvent strongly affects the habit of crystalline materials; however, the role-played by solvent interactions in enhancing or inhibiting crystal growth is still not completely understood (Lahra and Leiserowitz, 2001). The drug dipyridamole used herein is practically insoluble in water. Its main use in therapy as antiplatelet aggregating and peripheral vasodilating effect is well known. But the water insolubility and the poor bioavailability are the limitations of its effective use clinically. Keeping this in view, crystal modification of dipyridamole has been undertaken to improve dissolution and bioavailability. Dipyridamole is a derivative of 1,3,5,7-tetra azanaphthalene and used mainly for cardiovascular diseases for the above-mentioned purposes. It has been recrystallized

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from selected solvents and solvent system. The newly developed dipyridamole crystals were characterized by some physicochemical approaches.

2. Materials and methods

2.1. Materials

Dipyridamole was obtained as generous gift from German Remedies (Mumbai, India). The solvents used for the present work were acetone, benzene, methanol, obtained from Ranbaxy Chemical Laboratories (S.A.S. Nagar, India) and Tween-80, Povidone K_{30} and polyethylene glycol (PEG) 4000 were obtained from SDS Chemical Limited (Boisar, India).

2.2. Preparation of dipyridamole crystals

Two different methods used in this study to observe the effect of solvents on the development of crystal habits in the changed environment are given below.

2.2.1. Method I

One gram of dipyridamole was dissolved separately in 50 ml of selected solvents in a conical flask. The solution was heated at the boiling point of the respective solvents and filtered, concentrated and the solution was left at room temperature (28–30 °C) until the solvent was completely evaporated. The crystals were further dried under vacuum at room temperature and stored in appropriate airtight container for further use.

2.2.2. Method II

One gram of dipyridamole was dissolved in 40 ml of methanol in a conical flask and the solution was heated and filtered. The resultant solution was concentrated at 60 °C and then cooled down at room temperature (28–30 °C). The clear solution, thus obtained, was rapidly added to equal volume of cold water (5 °C) containing 2% solution of Tween-80, PVP K_{30} and PEG-4000, separately under agitation by means of a glass rod and then left for 1 h at 10–15 °C. The crystals were then recovered by filtration under vacuum using a sintered glass funnel. They were then kept in airtight container for further use.

2.3. Stability studies

One month's accelerated stability test was carried out for each sample after preparation, when the crystals were kept in humidity chambers (75% RH) and at a temperature 40 °C and the physicochemical changes of the crystals as observed are compared with that of the drug dipyridamole under identical conditions. The results are summarized in Figs. 9 (XRD) and 10 (DSC), respectively.

2.4. Scanning electron microscopy

Electron micrograph of crystals was obtained using a scanning electron microscope (JEOL JSM—5200) operating between 5 and 24 kV. The specimens were mounted on a metal stub (with double side adhesive tape) and coated under vacuum with gold in an argon atmosphere prior to observation.

2.5. X-ray powder diffraction

The cavity of the metal sample holder of X-ray diffractometer was filled with ground sample powder and then smoothed out with a spatula. X-ray diffraction pattern of dipyridamole crystals were obtained using the X-ray diffractometer (Rich Seifert Model 3000P) at 30 kV, 30 mA over a range of 10–100 2θ, using Cu Kα radiation wavelength 1.5405 Å.

2.6. Infrared spectroscopy

The spectra were recorded on an IR spectrophotometer (PERKIN-ELMER USA MODEL—248), after respective samples were mixed with dried KBr powder and compressed to a 12 mm disc by a hydraulic press at 10 tonnes compression for 30 s.

2.7. Thermal analysis

Differential scanning calorimetry (DSC) of the samples, 10 mg, was carried out using a thermal analysis system (METTLER TA 4000 System). Calibration with standard was undertaken prior to subjecting the samples, which were heated at 10 °C/min in an aluminum pan under a nitrogen atmosphere and a similar empty pan was used as the reference. The instrument automatically calculated onsets of melting points and enthalpy of fusion.

2.8. Dissolution studies

Dipyridamole and its crystals, 25 mg in each case were accurately weighed and dissolution profile of the drug was determined in a USP Type II Dissolution test apparatus at 37 °C, with basket (100 mesh) with a stirring speed of 50 rpm. The dissolution medium was 600 ml of phosphate buffer pH 4.0, I.P. (Indian Pharmacopoeia). Samples were withdrawn from the dissolution vessels at selected time intervals and analyzed for dipyridamole content at 285 nm on a UV spectrophotometer (BECKMAN-UM-64). The results are shown as the graphical plots in Figs. 7 and 8, respectively.

3. Results and discussion

3.1. Morphology of crystals

Fig. 1 shows the scanning electron micrographs (SEM) of untreated and recrystallized dipyridamole from different solvents under solvent evaporation method (Method I). It is clear from the figure that the untreated dipyridamole is having small irregular needle shaped crystals (Fig. 1d), whereas the crystals obtained from acetonitrile is needle shaped (Fig. 1c) and that from benzene is rod shaped (Fig. 1b). Recrystallization of dipyridamole from methanol solution with the same method produced

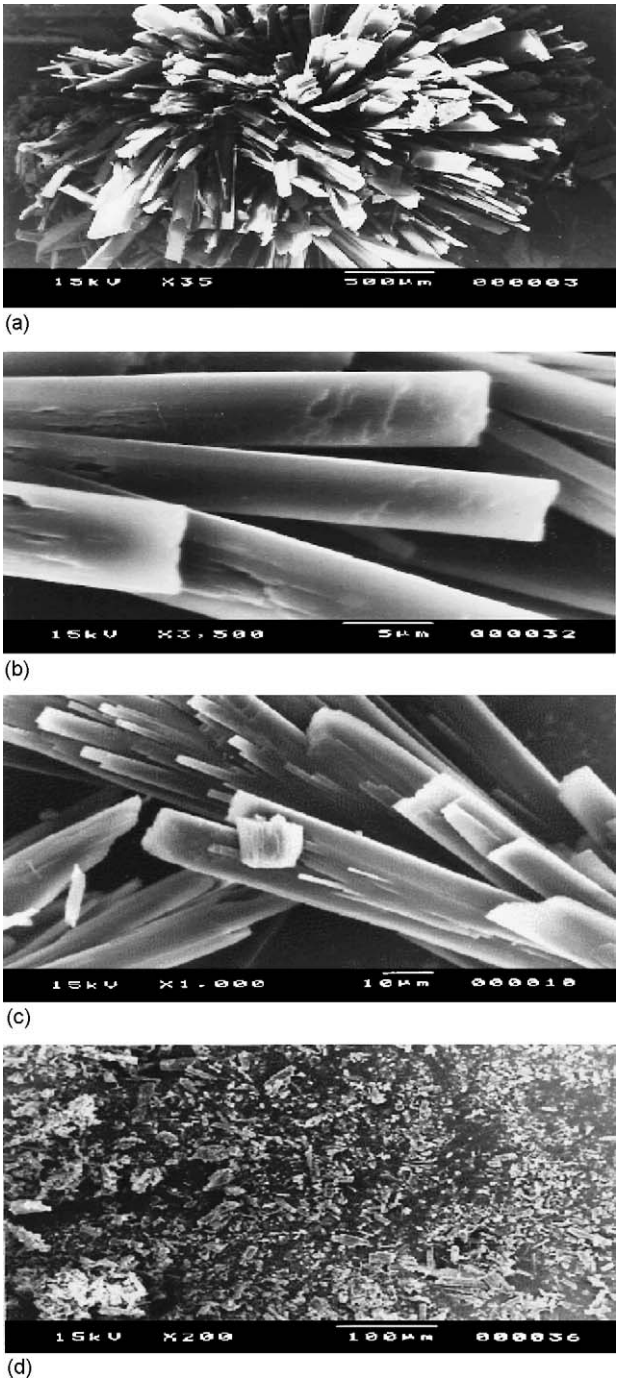

Fig. 1. Scanning electron micrographs of dipyridamole recrystallized from (a) methanol, (b) benzene, (c) acetonitrile and (d) untreated dipyridamole.

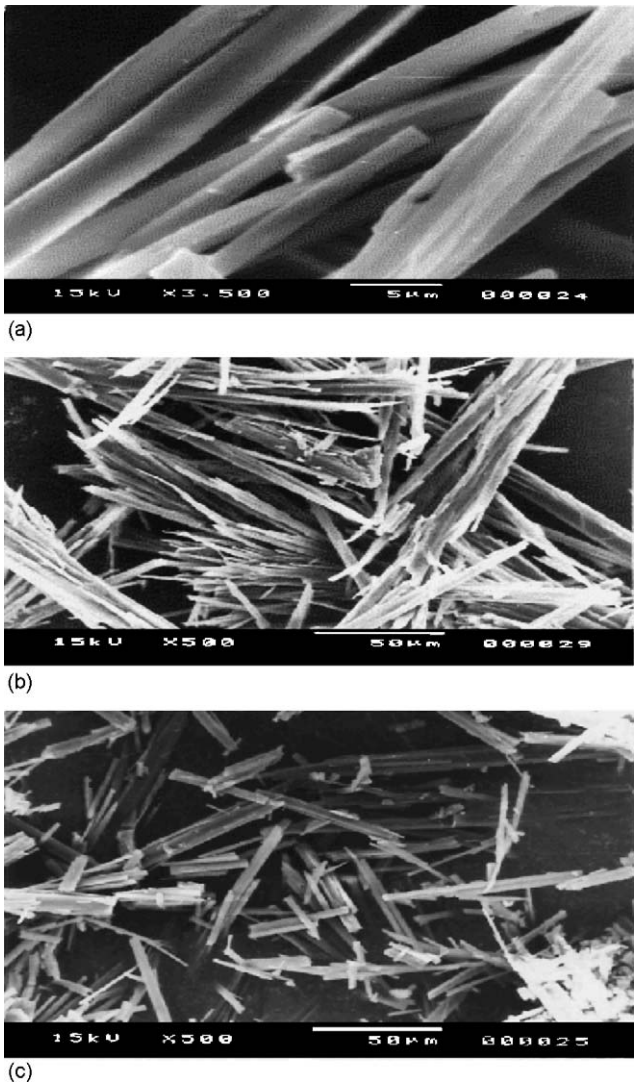

Fig. 2. Scanning electron micrographs of dipyridamole recrystallized from methanol with 2% solutions of (a) Tween-80 (SCT); (b) PEG-4000 (SCPEG); (c) $PVPK_{30}$ (SCPVP).

rectangular needle shaped crystals (Fig. 1a), while using solvent change method (Method II), the shape of crystals changes to fine needles (Fig. 2a–c). The results also showed that the size of crystals produced from Methods I and II are somewhat different from the size of untreated dipyridamole and follows the order, i.e. Method I > Method II (compare the magnification of the SEM in Figs. 1 and 2). Therefore, it can be concluded that cooling rate decreases the crystal size due to incomplete growth of large number of small crystals (Garekani et al., 1999).

3.2. X-ray diffraction

To obtain information on the physicochemical characteristics of the prepared crystals, X-ray powder diffraction measurements were conducted.

XRD spectra for all crystals are presented in Figs. 3 and 4. In the powder diffractogram sharp peak at diffraction angle (2θ) 30.04, 20.74, 20.81, 12.33, 17.45, 10.25, and 20.93 were obtained in case of drug dipyridamole and the modified crystals obtained from methanol, benzene, acetonitrile, Tween-80, PEG-400, PVP K_{30}, respectively. The presences of these sharp peaks are clearly evident in the comparative diffractogram presented in Figs. 3 and 4 and the data recorded therein. From the data recorded, it is clearly evident that there is significant difference in the entire diffraction pattern or *d*-spacing values between treated and untreated dipyridamole samples. The intensity of the peak in methanol is the highest than that of all other modified crystals reported herein. This is probably due to higher crystal

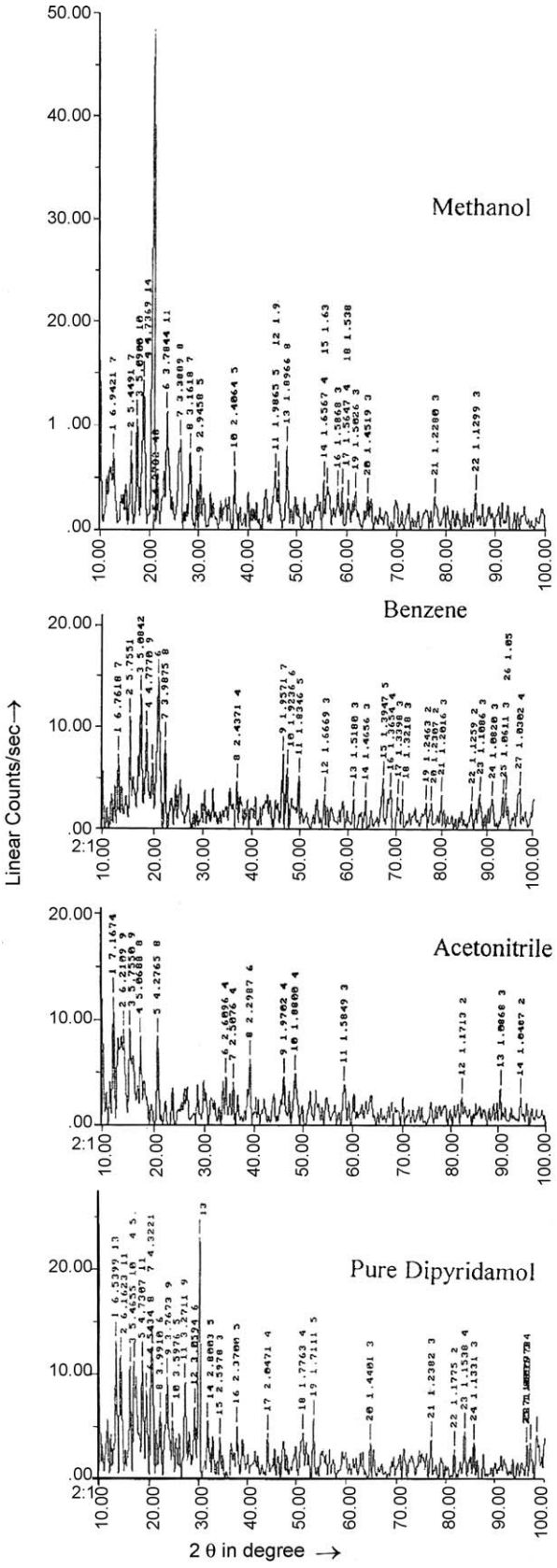

Fig. 3. X-ray powder diffraction pattern of pure dipyridamole and dipyridamole recrystallized from methanol; benzene; acetonitrile.

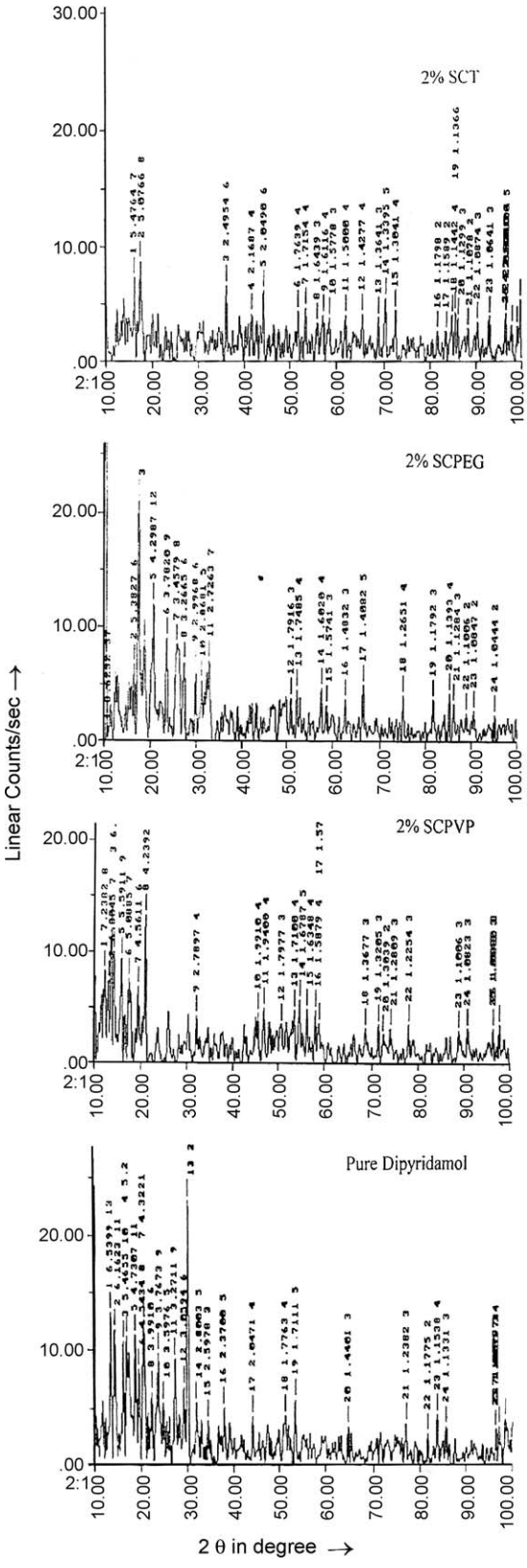

Fig. 4. X-ray powder diffraction pattern of pure dipyridamole and dipyridamole recrystallized from methanol with 2% solutions of Tween-80 (SCT); PEG-4000 (SCPEG); PVP K_{30} (SCPVP).

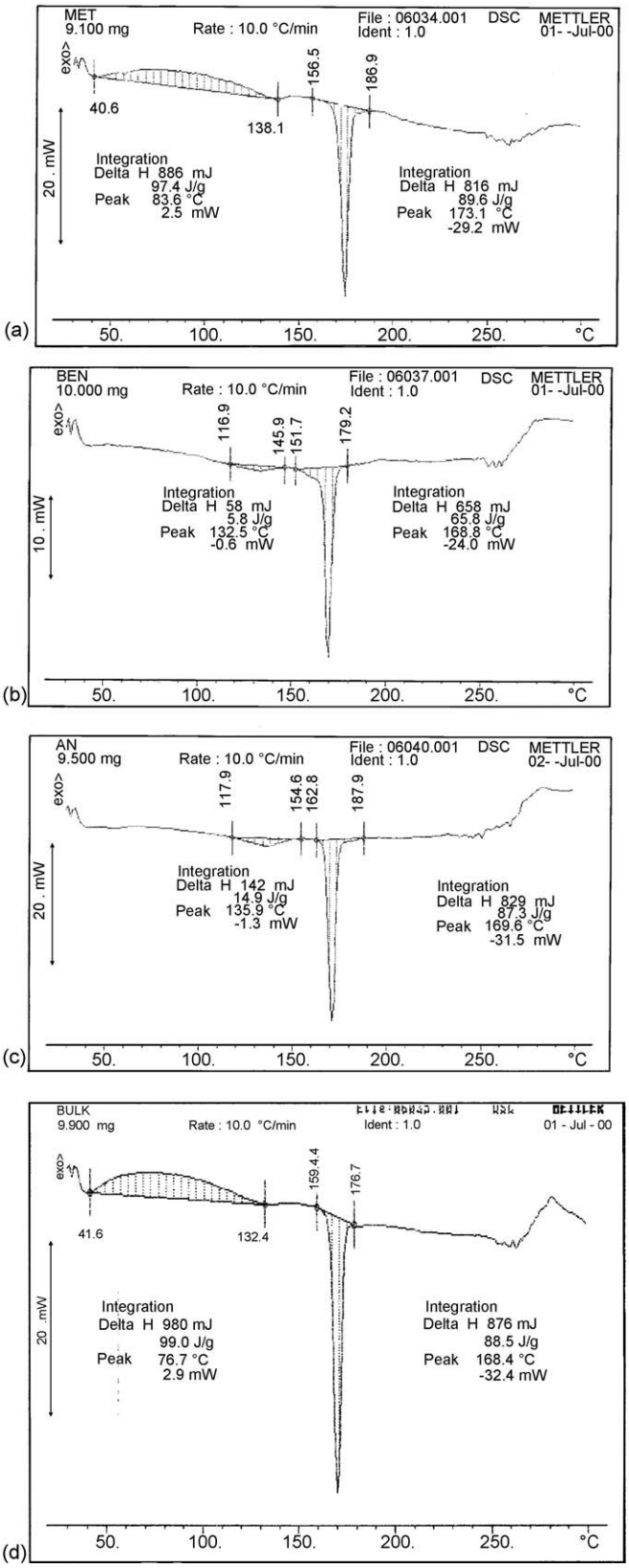

Fig. 5. Differential scanning calorimetric thermographs of dipyridamole recrystalized from (a) methanol; (b) benzene; (c) acetonitrile; (d) untreated dipyridamole.

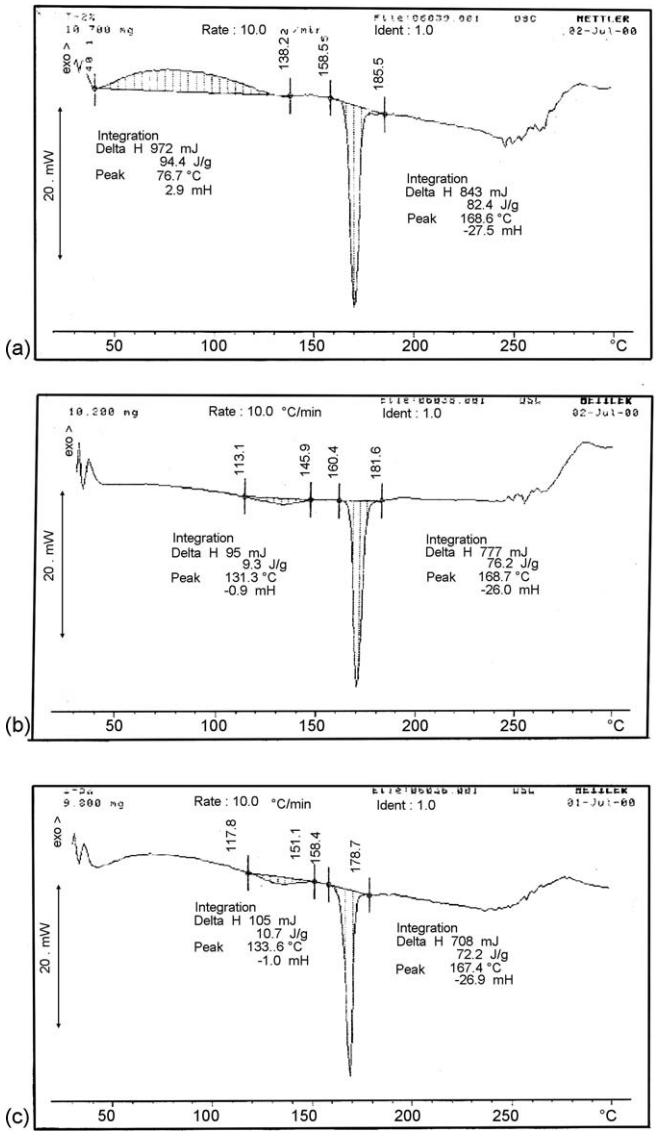

Fig. 6. Differential Scanning Calorimetric thermographs of dipyridamole recrystalized from methanol with 2% solution of (a) Tween-80(SCT); (b) PEG-4000 (SCPEG); (c) PVP K_{30} (SCPVP).

perfection in this condition of crystallization (Nokhodchi et al., 2003).

3.3. Infrared spectroscopy

The spectra of all modified crystals were identical and the main absorption bands of dipyridamole appeared in all of the spectra. This indicates that there were no difference between the internal structure and conformations of these samples, because these were not associated with changes at molecular level.

3.4. Thermal analysis

The DSC data for drug dipyridamole (untreated) and the modified crystals are shown in Figs. 5 and 6. It should be noted that the DSC thermo grams (Figs. 5 and 6) of all modified crystals showed only slight variation. However, the modified crystal

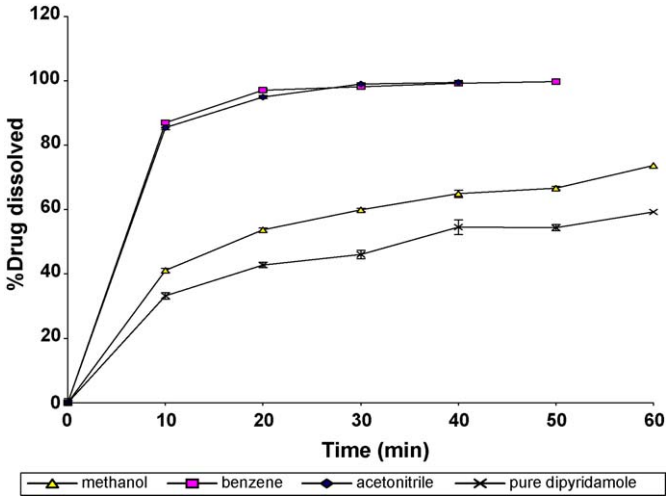

Fig. 7. Dissolution profile of pure Dipyridamole and modified crystals obtained using various solvents in phosphate buffer pH 4.0. (I.P). (a) Methanol; (b) benzene; (c) acetonitrile; (d) untreated dipyridamole.

obtained from methanol shows significant changes due to high crystal perfection.

The DSC curve of crystals from SCT (2%, v/v) and methanol shows broad exothermic peaks and very slight but insignificant variation in transition temperature and a little difference (not significant) in enthalpy of fusion. This may be due to oxidation or phase transformation. Crystals obtained by using acetonitrile, benzene, SCPVP (2%, w/v) and SCPEG (2%, w/v) show a weak endothermic peak and there is no significant variation in transition temperature, but significant difference in enthalpy of fusion is observed in case of acetonitrile, SCPEG (2%, w/v) and SCPVP (2%, w/v) while compared with the thermo gram obtained in case of benzene. The appearance of weak endothermic peaks in this case may be due to solvation of the crystals (Gordon and Chow, 1992).

Results from IR spectroscopy, X-ray diffraction analysis and DSC taken together led to the conclusion that only habit modifications were observed during recrystallization of dipyridamole under various conditions of the crystallization.

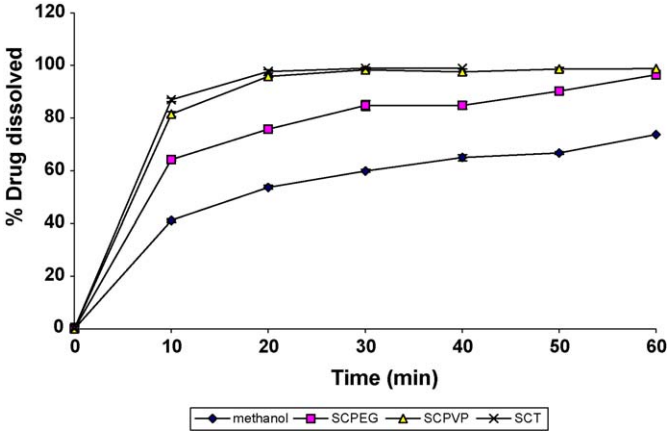

Fig. 8. Dissolution profile of modified crystals of dipyridamole from methanol and also from methanol with 2% solutions of PEG-4000, PVP $K_{30,}$ and Tween-80 in phosphate buffer pH 4.0. (I.P). (a) Methanol; (b) Tween-80 (SCT); (c) PEG-4000 (SCPEG); (d) PVP K_{30} (SCPVP).

3.5. Dissolution studies

The dissolution profile of dipyridamole and its modified crystals from different solvents are shown in Figs. 7 and 8, respectively.

Recrystallization of the parent drug from various solvents, given earlier (Method I), resulted in the increase of the dissolution rate of different modified crystals than dipyridamole. Especially, crystals obtained from benzene and acetonitrile, show higher dissolution rate than untreated dipyridamole because of the better crystallinity of the modified crystals in these cases. Crystals obtained using only methanol show lower dissolution rate than other crystals obtained (Method II). However, it is evident that after the addition of Tween-80 and other polymer solution, the dissolution rates were increased. This may be due to

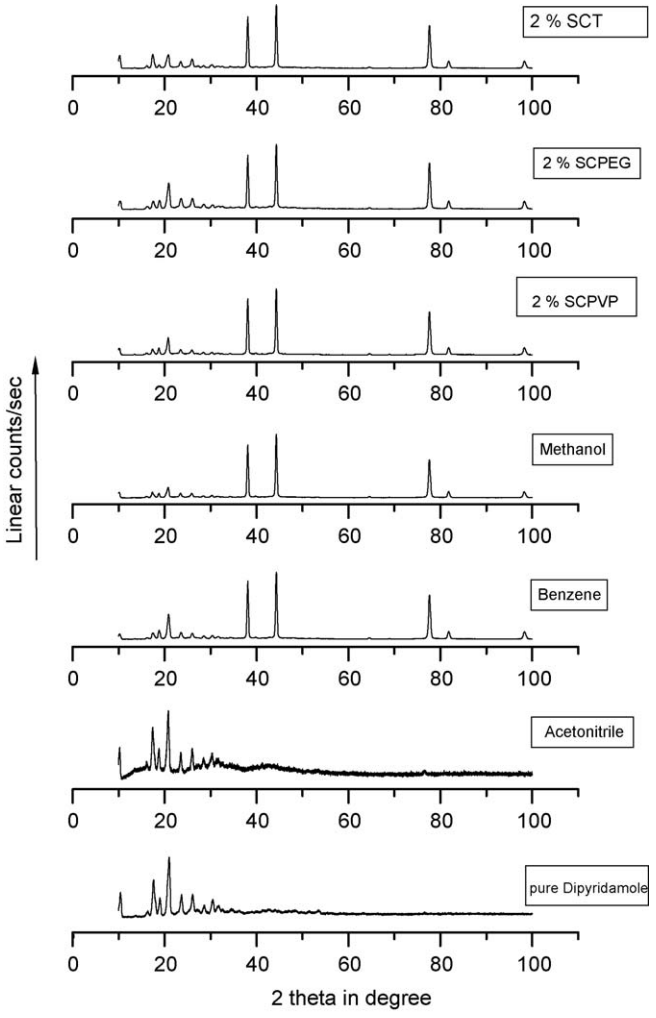

Fig. 9. Comparative X-ray powder diffraction pattern of pure dipyridamole and dipyridamole recrystallized from acetonitrile; benzene; methanol and dipyridamole recrystallized from methanol with 2% solutions of PEG-4000 (SCPEG), $PVPK_{30}$ (SCPVP), Tween-80 (SCT) and kept at elevated temperature (40 °C) and 75% RH for one month.

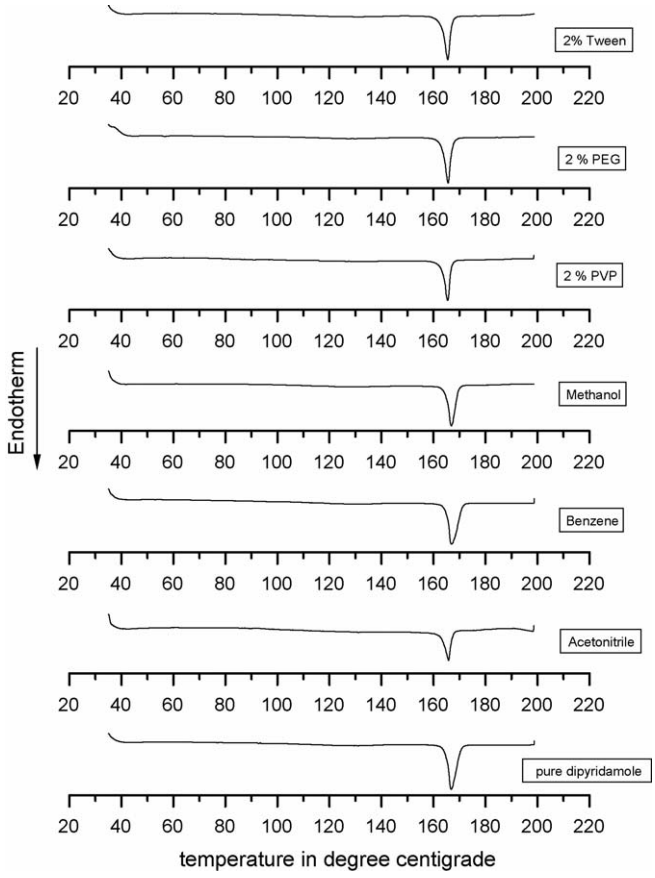

Fig. 10. Comparative Differential scanning calorimetric thermographs of pure dipyridamole and dipyridamole recrystallized from acetonitrile; benzene; methanol and dipyridamole recrystallized from methanol with 2% solutions of PEG-4000 (SCPEG), $PVPK_{30}$ (SCPVP), Tween-80 (SCT) and kept under elevated temperature (40 °C) and 75% RH for one month.

the adsorption of surfactant and polymers on the crystal surface (Majumdar et al., 1992).

3.6. Stability studies

The results obtained in the stability test showed slight changes in XRD, DSC data for all samples under investigation. XRD spectra for all the crystals kept at the elevated temperature (40 °C) are presented in Fig. 9. In the powder diffractogram of dipyridamole and the modified crystals, sharp peak at diffraction angle (2θ), 20.98, 20.79 and 44.25, respectively were obtained incase of drug dipyridamole and crystals from acetonitrile and benzene. But in the case of crystals obtained from methanol (Method I) and other crystals obtained (Method II), all of them showed the sharp peak at diffraction angle (2θ), 44.5. These clearly indicate that under the circumstance all retain the same state. However, there is significant difference in the *d*-spacing values between the freshly prepared crystals and the crystals obtained after storing at elevated temperature including pure drug. This is probably due to the existence of different crystal habits in the crystalline materials at elevated temperature. The DSC data for drug dipyridamole (untreated) and the modified crystals kept at elevated temperature are shown in Fig. 10. It is clearly evident from the DSC thermo grams for all the samples including pure dipyridamole under investigation that the modified crystals (Methods I and II) showed slight change in the value of enthalpy and the heat of fusion. However, the DSC curve of crystals from SCT (2%, v/v) and PEG (2%, w/v), very weak exothermic peaks were seen in a position significantly different from the samples, studied under ambient conditions, leading to significant variation in transition temperature and in enthalpy of fusion. This may probably be due to oxidation or phase transformation under such stress condition. But it is very much interesting to note that none of the samples studied under such stress condition did show any change in the IR spectrum confirming the presence of its chemical identity.

4. Conclusion

In conclusion, it can be said that the crystallization conditions and the medium used have major effect on dipyridamole crystals habit modification under ambient conditions. The crystals showed significant changes in the shape, size, melting points, dissolution rate, XRD patterns and DSC curves. This suggests that the newly developed crystals of dipyridamole under ambient conditions exist in different crystalline modification facilitating significantly improved dissolution rate as compared to dipyridamole. There are enough references (Dalton et al., 2001; el-Yazigi and Sawchuk, 1985) available in the literature wherein it has been proved that in vitro dissolution data are good predictor of in vivo performance in reality. Therefore, it can be safely concluded that the improvement obtained in the present study in the modified crystals will give better bioavailability and better therapeutic activity clinically. But the stability study undertaken at 40 °C and a relative humidity of 75% shows some physical changes probably due to some phase transitions but retaining the chemical identity. The effect of such changes in reality needs to be explored in actual situations, if any.

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