

Enhancement of Dissolution rate of Rifabutin by preparation of Microcrystals using Solvent Change Method

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Abstract: Rifabutin (RFB) is a potent semi-synthetic spiropiperidyl-rifamycin derivative with high permeability and low solubility. The aim of the present study was preparation of rifabutin microcrystals by solvent change method to enhance its solubility and dissolution rate. The microcrystals were screened for solubility, dissolution rate, morphology, stability, wettability and crystallinity. At the end of 10 min, more than 50 % of the drug was released from the microcrystals; however only 23 % of the drug was released from the control. Results of solubility, X-RD and DSC studies supports to the observations of dissolution studies. Stability and wettability of the crystals was found to be improved.

Key Words: Rifabutin (RFB), solvent change method, poloxamer, PEG.

Introduction :

A common observation throughout the pharmaceutical industry is that as the potency and specificity of new drug candidates are improving, the poor aqueous solubility is often becoming a problem. This may result in poor bioavailability of the active pharmaceutical ingredients. Given the increasing number of compounds emerging from discovery programs having poor aqueous solubility and/or dissolution, pharmaceutical scientists are constantly seeking new formulation approaches in order to obtain an adequate oral bioavailability.¹ A lot of research has been going on during the last two decades to developing adequate drug delivery systems for challenging drug candidates which belong to the classes II and IV of the biopharmaceutical classification system (BCS) and it gave birth to the techniques used to solve solubility problems associated with poorly soluble drugs.^{2,3}

Poor water solubility of the new chemical entity is caused by hydrophobicity, i.e., inability to form hydrogen bonds with water and/ or by high lattice energy. One market analysis report estimated that worldwide sales of about \$37 billion of drugs were insoluble or poorly soluble.⁴ The conversion of an aqueous insoluble drug to a salt form, pH adjustment and the use of cosolvents are typically initial steps in the formulation strategy taken to dissolve the drug. If the log P is very high, conventional approaches such as salts, pH or cosolvents may not be sufficient to formulate the drug. For such molecules an emulsion or lipidic system may be feasible, if the melting point is low. Alternatively, inclusion complexation may also be explored. The molar ratio of inclusion complexing agent to drug ratio is generally 1:1 or 2:1.

The dose of a drug is therefore limited by the allowable high molecular weight inclusion complexing agent used in the formulation. Micronization of drugs is one of the ways to enhance the solubility of the drugs.⁵⁻⁷ Generally micronization is carried out by jet-milling or top down methods which provide limited opportunity for the control of important product characteristics such as size shape morphology, surface properties and electrostatic charge.^{5,8}

The aim of the present study was to prepare microcrystals of rifabutin (RFB), a poorly water soluble drug by solvent change approach to enhance its solubility and dissolution rate. RFB is a potent semi-synthetic spiropiperidyl-rifamycin derived from rifamycin-S having high permeability and low solubility.⁹ During crystal precipitation new surfaces are formed with high surface charges. Surfactants used in the processing stabilize these newly formed surfaces. The obtained crystals were screened for solubility, dissolution rate, stability, wettability, morphology and crystallinity.

Experimental:

Materials

Rifabutin (RFB) was supplied as a gift sample by Lupin Ltd, (Aurangabad, India). Poloxamers (Grade 407 and 188) were supplied by Glenmark Pharma (Mumbai, India). Polyethylene glycol (PEG, grade 6000 and 4000) were procured from Alembic (Vadodara, India). Methanol and hydrochloric acid (HCL) were of A.R. grade (S. D. Fine, Mumbai, India).

Preparation of Microcrystals

Microcrystals of RFB were prepared by solvent change method. Briefly, a fixed amount RFB (1.5 g) was dissolved in 5 ml of methanol. This organic phase was added at room temperature, under constant mechanical stirring (2000 rpm) (Remi Stirrer, Mumbai), to 100 ml of 0.2 % w/v aqueous solution of surfactants. Stirring was continued for 30 min. Microcrystals were collected after filtration, washed with deionized water and dried at room temperature.

Drug Content

A weighed quantity of the samples was dispersed in 10 ml of 0.01 N HCL. It was sonicated for 10 min and the samples were centrifuged at 2000 rpm for 10 min. The supernatant was diluted with suitable quantity of 0.01 N HCL and analyzed by UV-Visible Spectrophotometer (Shimadzu UV-1700, Japan) at 281nm. It was carried out in triplicate.

Solubility Studies

Solubility studies were carried out in water in triplicate according to the method described by Higuchi and Connors. Saturation solubility measurements were assayed through ultraviolet absorbance determination at 281 nm using UV-Visible Spectrophotometer (Shimadzu UV-1700, Japan). To 10 ml of the deionized water excess quantity of samples (50 mg) were added. Apparent solubility study was performed by standardized shake flask method at 37 ± 0.5 °C. After shaking for 48 hrs, the samples were filtered through 0.2 μ membrane filters and the filtrate was analyzed for drug content.

Dissolution Studies

Dissolution studies were carried out under sink conditions in 0.01 N HCL (USP dissolution media) according to the paddle method using a LabIndia Disso 2000 apparatus (Mumbai, India). Stirring speed employed was 100 rpm, and the temperature was maintained at 37 ± 0.5 °C. Quantification of the samples was carried out spectrophotometrically at 281 nm (UV-

VIS Spectrophotometer, Shimadzu UV-1700, Japan). All samples were analyzed in triplicate.

Scanning Electron Microscopy (SEM)

Morphological characteristics of the drug and samples were analyzed using JSM-6400 scanning electron microscope (JEOL, Tokyo, Japan). Samples were fixed on aluminum stubs with conductive double sided adhesive tape and coated with gold by sputter coater at 50mA for 50s.

X-Ray Diffraction

XRD patterns of the drug and samples were recorded by using Philips Analytical X-Ray diffractometer (Model: PW3710, Holland) with tube copper anode over the interval 5 to 60° of 2 θ . The operation data were as follows: generator tension (voltage) 40 kV, generator current 30mA and scanning speed 0.02 °/min.

Differential Scanning Calorimetry (DSC)

DSC curves were obtained by a TA Instruments, (USA, Model: SDT 2960) equipped with a thermal analysis automatic program. Aliquots of each sample were placed in an aluminium pan. Conventional DSC measurements were performed by heating the samples from 30 to 350 °C at a rate of 10 °C/ min.

Stability Studies

For stability analysis, known amount (n = 3) of the sample was kept in a capped glass vials at 40 ± 2 °C and $75 \pm 5\%$ RH for 3 months in environmental test chamber (HMG INDIA, Mumbai). After 30, 60 and 90 days, the samples were taken out and analyzed for the drug content.

Wettability

A weighed quantity (1 g) of the untreated drug and crystals were placed on a sintered glass disk forming the bottom of glass tube on which methylene blue crystals were placed. The whole device was brought into contact with water. The time taken for the capillary rising of water to the surface so as to dissolve methylene blue crystals was noted.

Table 1: The drug content and solubility of the pure drug and microcrystals

Name	Batch ID	Drug Content*	Solubility*
RFB	R	-	0.129 \pm 0.002
RFB/WS	R1	99.97 \pm 0.05	0.146 \pm 0.007
RFB/PEG 4000	R2	98.45 \pm 0.05	0.326 \pm 0.015
RFB/PEG 6000	R3	98.84 \pm 0.17	0.347 \pm 0.011
RFB/Poloxamer 407	R4	98.73 \pm 0.08	0.389 \pm 0.012
RFB/Poloxamer 188	R5	98.92 \pm 0.05	0.353 \pm 0.009

WS: without surfactant, * : mean \pm S.D.

Moisture Uptake Study

A weighted quantity of crystals (10 mg) were placed in crucibles at accelerated condition of temperature & humidity, 40 ± 2 °C & 75 ± 5 % RH respectively (environmental test chamber, HMG INDIA, Mumbai). The changes in weight of samples were determined.

Results and Discussion:

Preparation of Microcrystals

Commercial RFB used in the study was characterized with low solubility. Microcrystals of RFB were prepared by solvent change method. Addition of organic phase to the aqueous phase results in precipitation of drug particles. The manufacturing of a microcrystals implies the creation of additional surface area and hence interface. As the Gibbs free energy change, associated with the formation of additional interface is positive, the microcrystals formed are thermodynamically unstable and will tend to minimize their total energy by agglomeration. Kinetically, the process of agglomeration depends on its activation energy. This activation energy can be influenced by adding stabilizers to the system. A first requirement for a stabilizing system is that it provides wetting of the hydrophobic surfaces of the drug particles.^{5,10} Surfactants used in the preparation of microcrystals stabilized these particles and avoided its growth. The solvent change method for preparation of microcrystals was found to be efficient.

Drug Content, Solubility and Dissolution Studies

The drug content was found to be good and uniform among the different batches of the prepared samples and ranged from 98.45 to 98.92 % (Table 1). As water is a universal solvent, apparent solubility studies were carried out in deionized water. In solubility studies of the samples, the crystals prepared using poloxamer 407 have showed highest solubility of the drug in water (0.389 ± 0.012 mg/ml) as compared with the untreated drug (0.129 mg/ml) (Table 1).

The dissolution profiles of the prepared crystals and the control (raw drug material) are illustrated in Figure 1. Those samples prepared with surfactants showed the faster dissolution rate, with approximately more than 50% of the drug being released within 10 min compared to 43 % for the samples prepared without surfactant and 23 % for the control. At the end of 50 min, more than 90 % of the drug was released from all crystals except for the crystals prepared without surfactants and the control. This effect can be explained by an increased specific surface area which is hydrophilized due to the adsorbed hydrophilic polymers. The fact that the microcrystals prepared by solvent change approach exhibited a faster dissolution rate than the control shows that the solvent change method itself was responsible for increased dissolution. Surfactants applied in the process have been shown to reduce the aggregation tendencies of particles

compared to milling. The mechanism for how this process yields enhanced dissolution properties is believed to be a microenvironment surfactant effect whereby surfactant dissolution creates a local surfactant concentration in the boundary layer surrounding the drug particles, providing a lower energy pathway for drug dissolution.¹¹ This process of microcrystal preparation is believed to be particularly effective at enhancing the rate of drug dissolution because the drug particles are maintained in direct contact with the surfactants particles during drug dissolution.

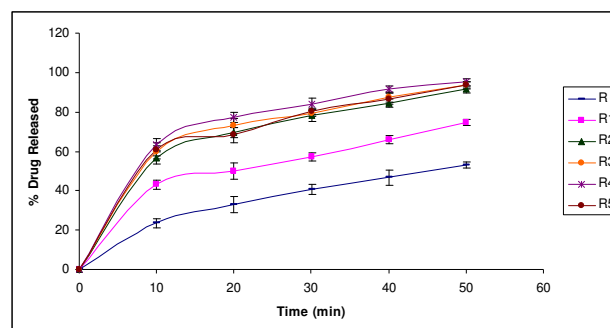


Figure 1: In-vitro drug release profile with error bar

(n = 3; mean) of (R) the drug and its crystals prepared (R1) without surfactant and with (R2) PEG 4000, (R3) PEG 6000, (R4) Poloxamer 407, (R5) Poloxamer 188

SEM, X-RD and DSC Studies

The particle morphologies before and after comminution were not simply related. Figure 2a and 2b shows the particles before and after comminution without using surfactants, indicating very less change in the particle morphology. However Figure 2c-f shows the particles after comminution with various surfactants were morphologically quite different from the starting material. Therefore, the addition of surfactants aids in breaking and stabilization of the larger particles.

The diffractogram of the samples after treatment attested that no modifications occurred after comminution. All samples were found to contain amorphous material, as shown by their X-ray diffraction patterns (Figure 2a and b) which contained no peaks indicative of amorphous material and were similar to that of the starting raw material. The amorphous solid state has the advantage of increased solubility and therefore faster dissolution rate compared to crystalline material.¹¹

Thermal behavior of pure drug and crystals are shown in Figure 4. The DSC curve showed that RFB appeared a sharp endothermic peak at about 122.45 °C corresponding to its melting. However, the crystals prepared with PEG 4000 and poloxamer 188 shows shift of endothermic peak towards lower temperature at 94.22 and 105.96 °C respectively. Shift of the endothermic peak

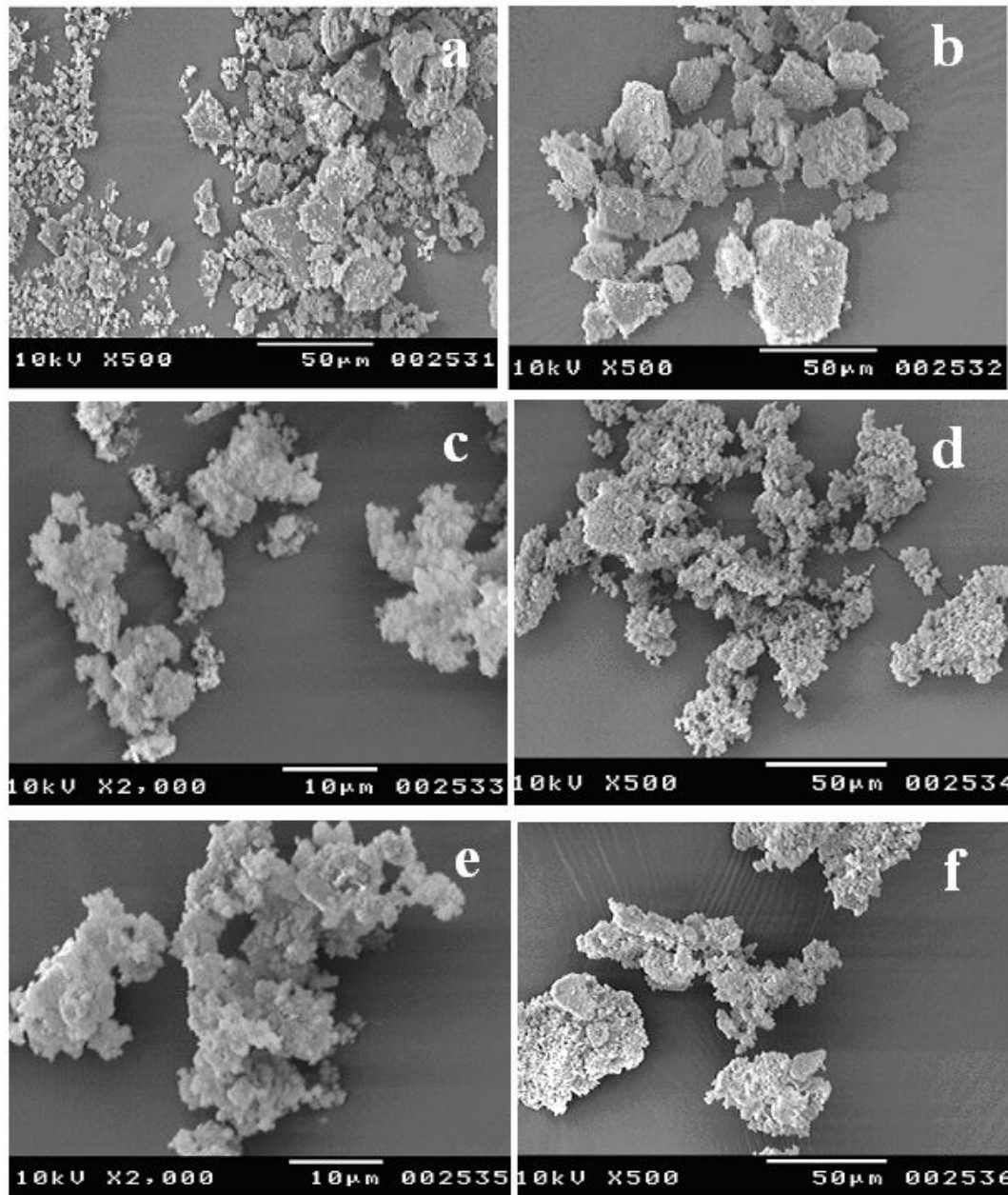


Figure 2: SEM photomicrographs of (a) the drug and its crystals prepared (b) without surfactant and with (c) PEG 4000, (d) PEG 6000, (e) Poloxamer 407, (f) Poloxamer 188.

towards lower temperature dictates decreased melting point of the drug in the formed crystals. This decreased melting point accounts for increased solubility of the drugs.¹² In contrast, the crystals prepared with PEG 6000 and poloxamer 407 did not show any peak. Usually this may be caused by a variation of the different crystal habit or to a reduction in particle size. From the DSC analysis of the crystals, positive influence of the hydrophilic polymer on the solid state of the drug was attested.

Wettability, Moisture Uptake and Stability Studies

The results of the accelerated stability studies indicated that crystals did not show physical changes during the study period and the drug content was found to be more than 98 %. The drug content for the R1, R2, R3, R4 and R5 crystals were found to be (n=3; mean \pm S.D.), after 30 days: 99.41 ± 0.07 , 99.56 ± 0.03 , 99.49 ± 0.07 , 99.78 ± 0.04 and 99.57 ± 0.03 , after 60 days: 98.87 ± 0.12 , 99.08 ± 0.06 , 99.18 ± 0.09 , 99.06 ± 0.05 and 98.97 ± 0.10 , after 90 days: 98.48 ± 0.09 ,

98.67 ± 0.06, 98.39 ± 0.14, 98.79 ± 0.08 and 98.43 ± 0.07 respectively, dictates that the samples are stable at accelerated conditions of temperature and humidity.

The powder bed hydrophilicity test was performed to confirm the wettability of the crystals. For R1, R2, R3, R4 and R5 samples, the methylene blue crystals were dissolved after 52, 39, 41, 37 and 39 min respectively, whereas even after 55 min the methylene blue crystals did not get dissolved for the untreated drug, indicating an augmented wetting property of the drug in the prepared crystals.

Use of hydrophilic surfactants may result in formation of hygroscopic particles. The moisture uptake study was performed to examine the hygroscopic nature of the prepared crystals. No change in the weight of the crystals was noticed, denoting non-hygroscopic nature of the crystals.

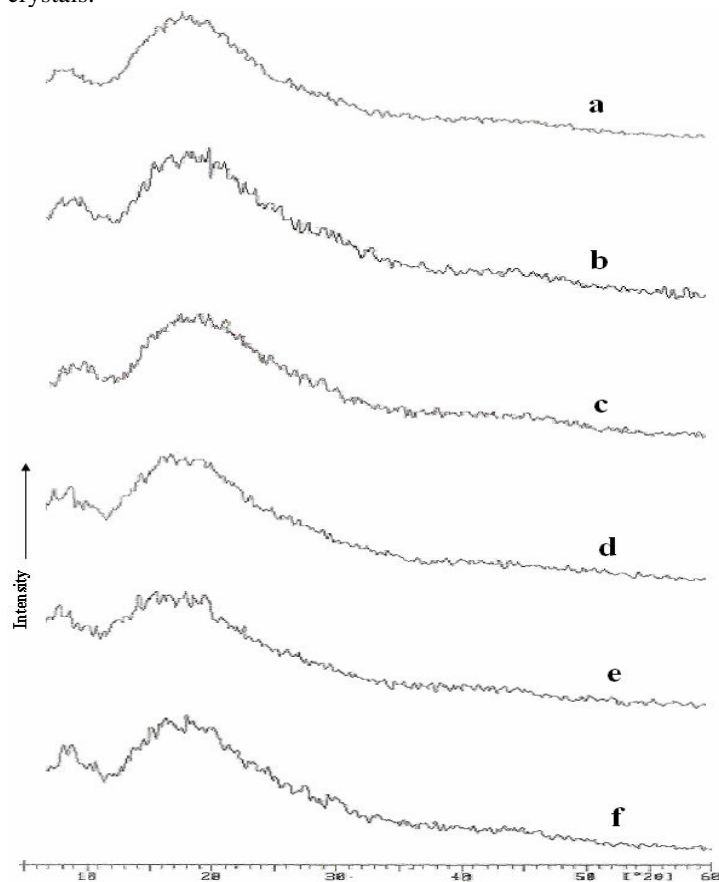


Figure 3: XRD pattern of (a) the drug and its crystals prepared (b) without surfactant and with (c) PEG 4000, (d) PEG 6000, (e) Poloxamer 407, (f) Poloxamer 188.

Conclusion:

From the above discussion it has been concluded that solubility and dissolution rate of the prepared crystals was observed to be increased. This indicated that both the solvent change method and the inclusion of the hydrophilic surfactant contributed to enhanced dissolution rates. The improved particles stability and

wetting conferred by the presence of the hydrophilic surfactants.

Acknowledgements:

We are thankful to Lupin Ltd, Aurangabad for providing gift sample of RFB. We are grateful towards Glenmark Pharma, Mumbai and Alembic, Vadodara for providing excipients and Shivaji University, Kolhapur for getting facilities to perform XRD and DSC.

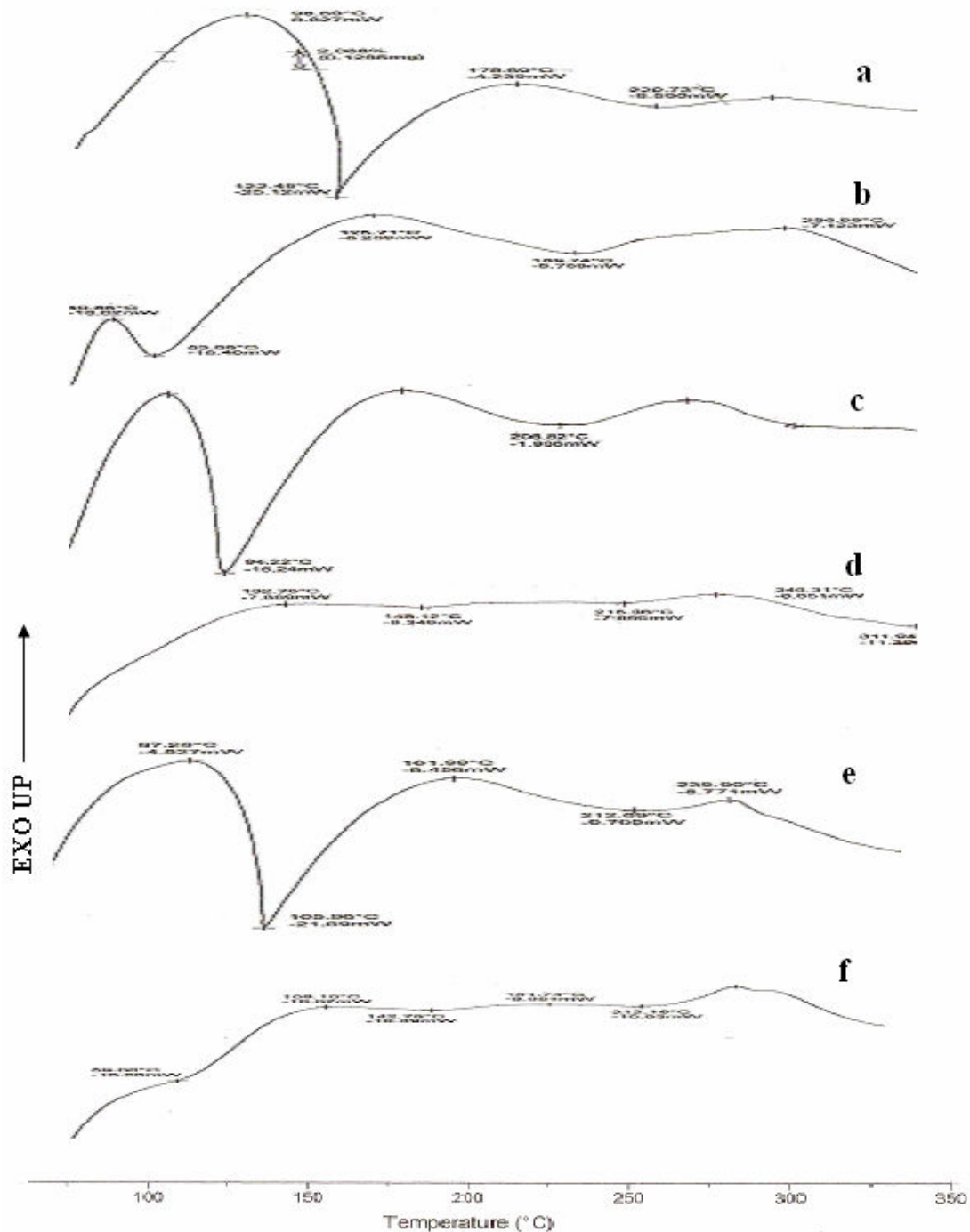


Figure 4: DSC thermo-grams of (a) the drug and its crystals prepared (b) without surfactant and with (c) PEG 4000, (d) PEG 6000, (e) Poloxamer 407, (f) Poloxamer 188.

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