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Research Article

Studies on microcrystals for Improved dissolution of piroxicam

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ABSTRACT

Improved bioavailability is an added advantage for most of the poorly soluble drugs in water. In recent years research work is concentrated on various methods to improve the solubility characteristics of poorly soluble drugs and crystallization phenomenon is one among them. The solubility problem can be solved by changing the crystal habit of drug, which improves the solubility and dissolution. Crystallization is also a purification process to remove the impurities from pharmaceutical products by, recrystallization technique. So, in the present investigation an attempt has been made to improve the solubility characteristics of Piroxicam (NSAID's) using crystallization method by solvent evaporation technique. To increase the therapeutic efficiency and quality of existing marketed dosage formulations. In this method, crystallization takes places mainly due to the removal of solvent by evaporation and reprecipitation in water in which drug is insoluble. The biphasic layer formed due to water immiscible solvent and is evaporated by maintaining the temperature above to corresponding boiling point of solvents. In our work three immiscible solvents, chloroform, ethyl acetate and dichloromethane used and microcrystals prepared under slow and turbulent stirring conditions. The precipitated crystals were filtered using whatmann paper and dried at 60° C for 1 hour. The formulated crystals of Piroxicam were subjected to various physico-chemical parameters like size distribution, shape and drug, solvent interactions with DSC, IR, XRD etc., and found to be smaller in size than pure and crystalline in nature with free from any interactions. For all the samples *in-vitro* drug release parameters studied U.S.P. XXIII dissolution rate test apparatus (Electrolab) employing paddle stirrer for one hour. In 900 ml phosphate buffer (pH 7.4) and compared with pure drug samples. The microcrystals produced with 30% v/v chloroform and 30% v/v ethyl acetate as solvents prepared under turbulent stirring conditions precipitated in water as crystallization media found to be best formulations for improved drug dissolution when compared to the pure drug for Piroxicam respectively. The results thus conclusively proved that the method of precipitation by solvent evaporation technique can be used to produce microcrystals of poorly soluble non-steroidal anti-inflammatory drugs.

Keywords: Piroxicam, Microcrystals, Chloroform, Ethyl Acetate, Dichloromethane.

INTRODUCTION

Crystallization is the spontaneous arrangement of the particles into a repetitive orderly array, i.e., regular geometric patterns. Crystallization is a phenomenon in which solid particles formed by solidification under favorable conditions of a chemical element or a compound, whose boundary surfaces are planes symmetrically arranged at definite angles to one another in a definite geometric form. In the matter, particles are present randomly due to thermal agitation. In gases the disorderliness is highest and in liquids it is moderate. The liquids can solidify into crystalline forms, whenever attraction forces between particles are strong enough to overcome the disorderliness. Crystallization can proceed directly from vapor of a substance. Examples are solid camphor from camphor vapor, solid iodine from iodine vapor. Such a process is known as sublimation. Crystals are commonly obtained from liquid state. Example is salt from brine. Crystallization deals with the later type, i.e., from solution to solid state. Crystallization differs from precipitation in that the product is deposited from a supersaturated solution. Precipitation occurs when solutions of materials react chemically to form a product, which is sparingly soluble in the liquid and therefore deposits out. The polymorphic changes will have a

definite influence on the solubility and thereby bioavailability of a particular compound due to structural differences resulting from different arrangements of molecules in the solid state[1,2]. More than 40% of active substances during formulation development by the pharmaceutical industry are poorly water soluble. Poor water solubility, which is associated with poor dissolution characteristics. Dissolution rate in the gastro-intestinal tract is the rate limiting factor for the absorption of these drugs, and so they suffer from poor oral bioavailability. For BCS class II-drugs, the dissolution rate is the limiting factor for the drug absorption rate. An enhancement in the dissolution rate of these drugs can increase the blood-levels to a clinically suitable level. Several techniques are commonly used to improve dissolution and bioavailability of poorly water-soluble drugs[3,4]. There are several methods of crystallization reported in the literature survey; aspirin microcrystal's by spherical crystallization, Sulfaguanidine microcrystal by microprecipitation technique, Paracetamol microcrystals by solvent change method. The present work, is focused on the influence of polymorphism phenomenon on the solubility profile of meloxicam which belongs to sulfoanilide group, a non-steroidal anti inflammatory drug[5,6].

MATERIAL AND METHODS

Piroxicam was a gift sample from Bal Pharma. Pvt. Ltd., Mumbai. Chloroform, ethyl acetate and dichloromethane and other chemicals used were of analytical grade.

Preparation of microcrystals

Solvent evaporation technique has been used in the present study to prepare microcrystals. In this method, crystallization takes place mainly due to the removal of solvent by evaporation and precipitation in water in which drug is insoluble. The basic procedure employed for preparing microcrystals of drugs consists of the following steps. Preparation of drug solution in different water immiscible solvents i.e., chloroform, ethyl acetate and dichloromethane in 10 ml, 20 ml and 30 ml, respectively with 1g. of drug. The above prepared drug solution was added drop-wise to water i.e., 10%, 20% and 30% v/v in a 250 ml. beaker with continuous stirring (slow and turbulent). The biphasic layer formed due to water immiscible solvent is evaporated by maintaining the temperature corresponding to their boiling point. Microprecipitation takes place due to the evaporation of water immiscible solvents and reprecipitation of drug in water. The precipitated crystals were filtered using Whatmann filter paper and dried at 60° C for 1 hour[7,8].

Characterization

Size and Size Distribution: There are many methods used for the determination of particle size of pharmaceutical solids. As the crystals obtained in the present work were small and belong to sub-sieve range in their size, microscopic method was used to determine their size, projection microscope Sipcon SP585/SP585A was used to measure the diameter of not less than 400 particles from each batch. From the basic size data, cumulative percent under size and their corresponding probit values were computed. Probit analysis refers to the analysis of quantal response data, which is using the probit transformation. The data has been graphed as cumulative percent undersize in probits versus diameter in microns on logarithmic scale. From these log probability graphs, geometric mean length diameter (dg) and geometric standard deviation were obtained. The former is equal to median or 50% diameter, and latter, which defines the slope equal to the diameter at 84.1% undersize divided by the medium diameter or the median diameter divided by the diameter at 15.9% undersize[9,10].

Mean Volume Surface Diameter (d_{vs}): The values of mean volume surface diameter (d_{vs}) was computed by using Hatch-Chotes equation, as the sample were found to obey log normal distribution[11,12]. The equation used to calculated the mean volume surface diameter (d_{vs}).

Determination of Specific Surface Area (S_w): It is defined as the surface area per unit weight. This was determined by using the formula:

$$S_w = \frac{6}{\rho \times d_{vs}}$$

Where, d_{vs} = mean volume surface diameter in microns.

ρ = crystal density in gm/cc.

Density of the crystal samples: The density of various batches of microcrystals were determined in water. The density of these samples are less than the water. The drug particles were found to float on the surface when added to water. In order to find the up thrust (apparent loss of weight) sample is completely immersed in water, a metal piece sufficiently heavy to sink the solid has been used to find out the density of samples[13].

Photomicrographs: The shape of micro precipitated crystals produced in different solvents by solvent evaporation technique in different magnifications (10x, 20x and 30x) was analyzed by using Nickon research microscope HFX-DX. This was carried out in the Department of Botany at Gulbarga University, Gulbarga.

Infrared Spectral Analysis: Infrared spectroscopy is one of the most powerful analytical techniques when it comes to the determination of presence of various functional groups involved in making up the molecule. It provides very well accountable spectral data regarding any changes in the functional group characteristics of a drug molecule occurring while in the processing of a formulation. Infrared spectra original drug and microcrystals prepared from different solvents were obtained by KBr pellet method using Perkin Elmer FTIR series model 1615 spectrometer in order to rule out drug solvent interaction occurring during the crystallization process. To assess the purity of microcrystals in different solvent systems, their infrared spectra were taken and compared with those of that are obtained by original drug sample, by pellet KBR method using FTIR 1615 Perkin Elmer due to lack of facility in the college, the samples were analyzed at Sipra Lab, Hyderabad.

X-ray Powder Diffraction: The X-ray diffraction studies are based on the scattering of X-ray by crystals. X-ray diffraction studies are generally used for investigating the internal structures, size of crystallites and crystallinity. Crystalline materials in powder form exhibit highly characteristic X-ray diffraction pattern in which the positions and relative intensity of peak are well-defined and reproducible. Powder X-ray diffraction is both rapid and relatively simple method for the detection of change in form. The amorphous materials do not show any pattern and is unique to each polymorphic form. XRD 6000 of Shimadzu, Japan was used to obtain X-ray powder diffraction patterns for original sample microcrystals in different solvents. This work was also carried out at Common Facility Centre, Shivaji University, Kolhapur due to lack of facilities in our institution.

Differential Scanning Calorimetry Analysis (DSC): Thermagravimetry is a technique in which a change in weight of a substance is recorded as a function of temperature or time. In DSC a test sample and an inert reference material (alumina) undergo a controlled heating program. If the sample undergoes any physical or chemical change than the difference ΔT will occur between the sample and reference material. The melting characteristics as well as the incidence of any interaction between and different solvents during recrystallization by solvent evaporation method were determined by carrying out differential scanning calorimetry on the respective crystalline samples i.e., original drug and microcrystals. DSC analysis were carried on Pyris-6 Perkin Elmer thermal analyzer at a heating rate of 5° C / min. in the range of 50-200°C in the nitrogen atmosphere. This was also carried out at Common Facility Centre, Shivaji University, Kolhapur due to lack of facilities in our institution.

***In vitro* Dissolution Studies**

Dissolution of drug microcrystals were studied using U.S.P. XXIII dissolution rate test apparatus (Electrolab) employing paddle stirrer. The in-vitro drug dissolution studies were carried out for 60 minutes In 900 ml phosphate buffer (pH 7.4) at 37^0 C. The stirrer speed was fixed at 50 rpm throughout the dissolution period. A quantity of 100 mg of drug microcrysals was taken in muslin bag and tied to the paddle. The samples were withdrawn at intervals of 10 minutes and analyzed for drug content at 348 nm using shimadzu UV-visible spectrophotometer[14].

RESULTS

The average diameter value of Piroxicam original crystals found to be 33.42 microns, whereas its microcrystals prepared in 30% v/v of Dichloromethane in turbulent stirring conditions were found to be 27.17 microns. The surface area (S_w) of Piroxicam original crystals was found to be 2.96 x 10^3 gm/cm², whereas the microcrystals prepared in 30% v/v of Dichloromethane in turbulent stirring conditions were found to be 5.02 x 10^3 gm/cm². Photomicrographs of microcrystals of drug produced by solvent evaporation method showed reduced size in shape when compared to pure drug crystals. The density of original drug was found to be 0.230 gm/cc, and microcrystals was found to be 0.228 gm/cc. The infrared spectra of microcrystals of drug with different solvents was almost similar to that of spectra of pure drug indicating that there is no interaction between drug and solvent. The X-ray diffractograms of pure drug, and microcrystals of Piroxicam in Dichloromethane solvent. Revealed that maximum intensity of the peak of pure drug found to be 453.6900 and number of peaks 43, whereas for form of the maximum intensity of peak found to be 707.56 and total number of peaks 58. This clearly indicates the purity of dosage form. Differential Scanning Calorimetry (DSC) studies of original drug of Piroxicam showed a sharp endothermic peak with highest peak area at a melting point of 205.34°C, and microcrystals of Piroxicam produced in Dichloromethane solvent has exhibited endothermic peaks with comparatively reduced areas at lower melting point of 204.96°C. The crystals, produced in Dichloromethane solvent system gave lower area of endothermic peak. So it can be concluded from the results of DSC that the solvents used for recrystallization have a marked effect on the melting characteristics of the crystals. In-vitro drug dissolution of Piroxicam microcrystals with 30% Dichloromethane showed promising results in 60 min. It showed 97.51% drug release in turbulent stirring condition. Among

several experiments conducted with different solvents and varied stirring conditions, the microcrystals produced with 30% v/v Dichloromethane as solvent prepared under turbulent stirring condition precipitated in water as crystallization media found to be best formulation for improved drug dissolution when compared to the pure drug. The results thus conclusively proved that the method of precipitation by solvent evaporation technique can be used to produce microcrystals of poorly soluble non-steroidal anti-inflammatory drugs, which can be formulated into quick acting dosage forms for acute arthritic conditions and musculo-skeletal disorders.

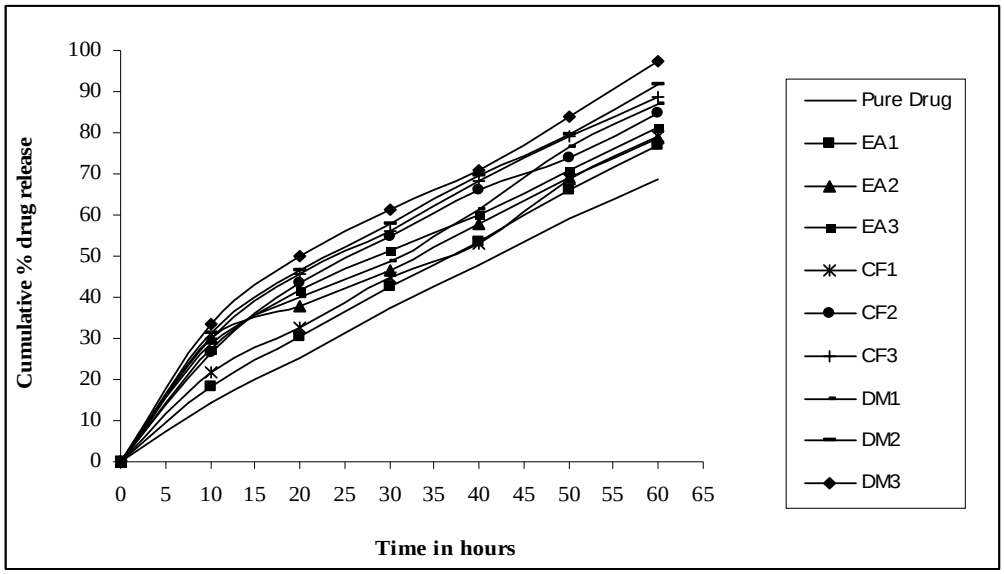
Table 1: Statistical Significance data of Prepared Piroxicam microcrystals

Sr. No.	Formulation	dg	6g	dav	SD	CV%	ρ	dvs	SW
1	Pure Drug	17.78	2.23	33.42	20.83	62.32	0.230	88.10	2.96×10^3 gm/cm^2
2	Piroxicam microcrystals	13.48	2.08	27.17	14.02	51.60	0.228	52.33	5.02×10^3 gm/cm^2

dg = Geometric mean diameter in microns. 6g = Geometric standard deviation in microns.
dav = Averagediameter in microns. SD = Standard Deviation.
CV = Coefficient variance in percentage. ρ = Density in gm/cc.
dvs = Mean volume surface diameter in micron. SW = Surface area by weight.

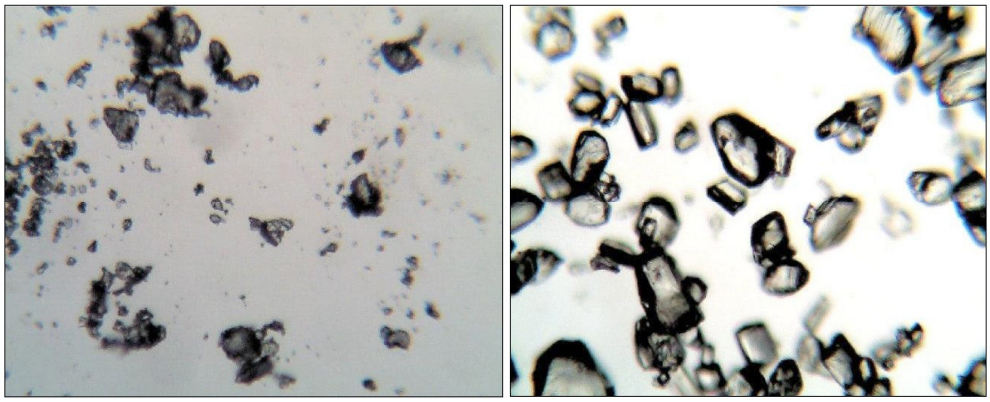
Table 2: *In vitro* Dissolution of Piroxicam in pure form and Microcrystals prepared using Chloroform, Ethyl Acetate and Dichloromethane Solvent at Turbulent stirring condition in pH 7.4 Phosphate buffer

Time (min)	Pure Drug	Cumulative % drug release								
		EA_1	EA_2	EA_3	CF_1	CF_2	CF_3	DM_1	DM_2	DM_3
10	14.55	18.39	30.00	27.33	21.69	26.45	29.91	28.79	31.10	33.56
20	25.15	30.26	37.80	41.59	32.76	43.56	45.72	39.79	46.65	49.87
30	37.33	42.61	46.37	51.11	44.58	54.79	56.23	48.86	57.88	61.43
40	47.85	53.43	57.62	59.88	52.99	65.88	68.38	61.43	69.35	70.98
50	59.22	65.88	68.93	70.75	68.71	73.97	79.17	76.58	79.67	83.75
60	68.48	76.76	78.86	81.23	79.17	84.77	88.89	87.14	91.77	97.51



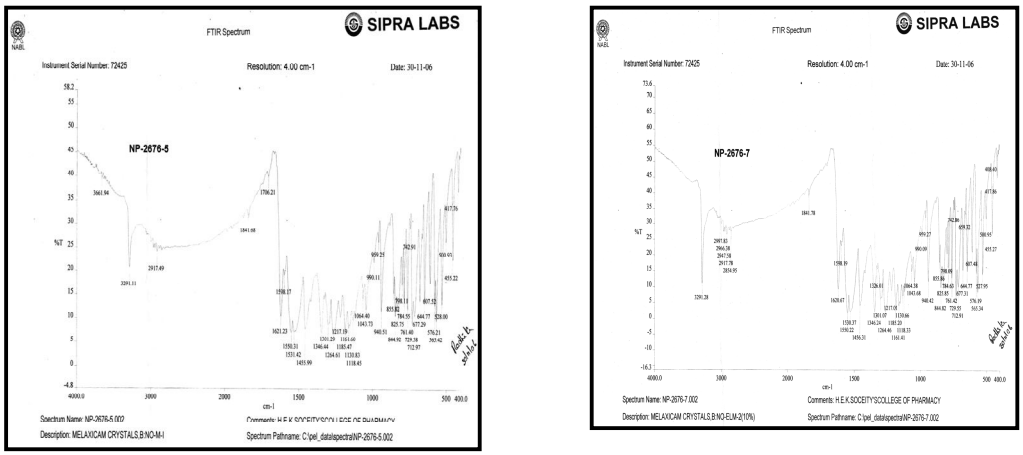
CF_1-Piroxicam microcrystals with 10% v/v chloroform EA_1-Piroxicam microcrystals with 10% v/v Ethyl acetate
CF_2-Piroxicam microcrystals with 20% v/v chloroform EA_2-Piroxicam microcrystals with 20% v/v Ethyl acetate
CF_3-Piroxicam microcrystals with 30% v/v chloroform EA_3-Piroxicam microcrystals with 30% v/v Ethyl acetate
DM_1-Piroxicam microcrystals with 10% v/v Dichloromethane
DM_2-Piroxicam microcrystals with 20% v/v Dichloromethane
DM_3.Piroxicam microcrystals with 30% v/v Dichloromethane

Fig. 1: *In vitro* Dissolution of Piroxicam in pure form and Microcrystals prepared using Chloroform, Ethyl Acetate and Dichloromethane Solvent at Turbulent stirring condition in pH 7.4 Phosphate buffer



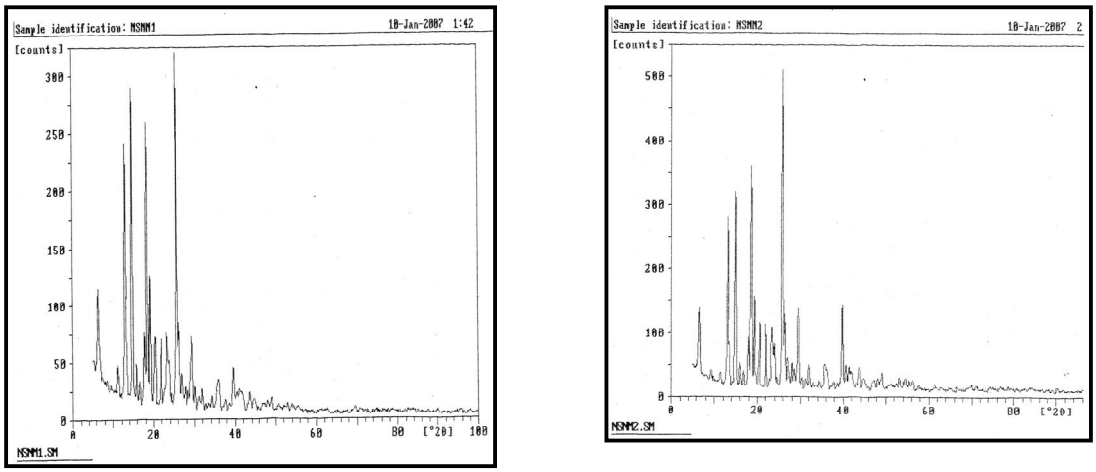
Pure drug **Microcrystals prepared**

Fig. 2: Photomicrograph of Piroxicam



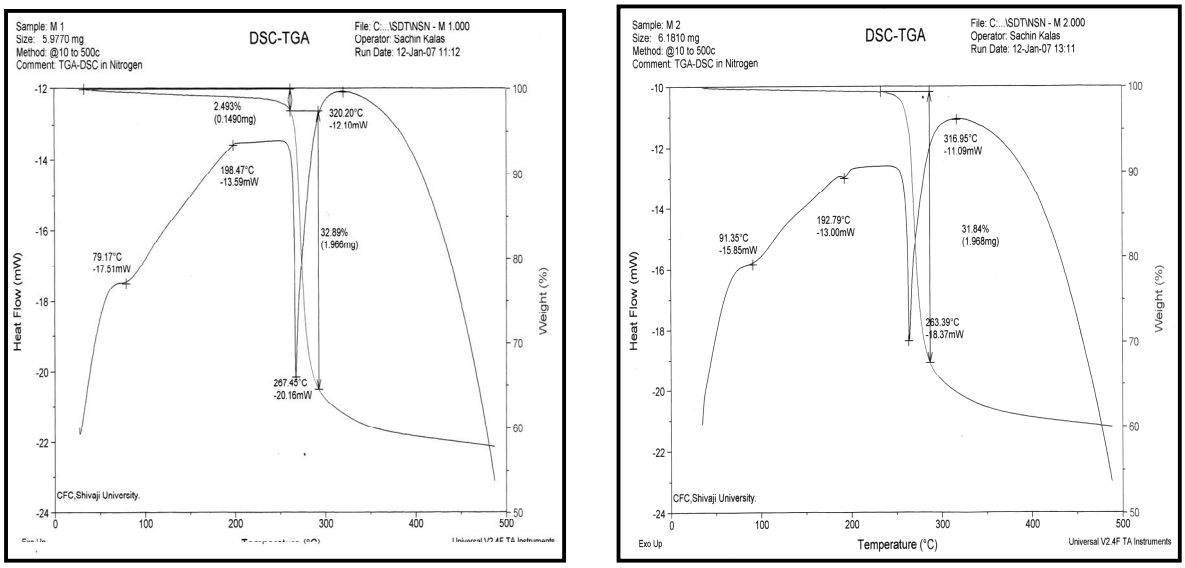
Pure drug **Microcrystals prepared**

Fig. 3: FTIR Spectrum of Piroxicam



Pure drug **Microcrystals prepared**

Fig. 4: X-Ray diffraction analysis of Piroxicam



Pure drug **Microcrystals prepared**

Fig. 5: Differential Scanning Calorimetry of Piroxicam

DISCUSSION

Among several experiments conducted with different solvents and varied stirring conditions, the microcrystals produced with 30% v/v Dichloromethane as solvent prepared under turbulent stirring condition precipitated in water as crystallization media found to be best formulation for improved drug dissolution when compared to the pure drug. Thus the results proved that the method of precipitation by solvent evaporation technique can be used to produce microcrystals of poorly soluble non-steroidal anti-inflammatory drugs especially drugs, which can be formulated into quick acting dosage form for acute arthritic condition and musculo-skeletal disorders.

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