Original Article

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Influence of Solvents on the Crystal Habit and Properties of Rofecoxib

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ABSTRACT
In the present investigation of a total of four rofecoxib crystals were prepared successfully. Most pharmaceutical powders have crystals and used in the design of dosage forms, crystalline materials are often employed, but in some cases they are not recommended. Many drugs can exist in more than one crystalline form. Though the polymorphs are chemically identical, they exhibit different physicochemical properties, viz., melting point, solubility etc. These physicochemical properties further affect the biological properties of drug molecules. Suitable analytical methods (UV spectroscopy) were established and validated in water and hydrochloric acid (pH, 1.2) solutions. The melting point and FT-IR were used as characterization tools. The pharmaceutical importance was derived using solubility and dissolution studies. The rofecoxib crystals were prepared successfully using low temperature time cooling for longer period (4 to 5 days) in closed containers. The crystal morphology exhibited differences, when observed under magnifying lens. The melting point of rofecoxib crystals obtained from methanol, isopropanol, ethyl acetate, and acetone were different indicating the prima facie evidence for differences in crystal habit or polymorphism. These are characterized, though it was not conclusive.
Key words: *Rofecoxib, Crystal habit, aqueous solubility, Dissolution rate, FT IR, Crystal's preparation.*

INTRODUCTION

Most pharmaceutical powders have crystals in the range of 0.5-300 µm in diameter. In the design of dosage forms, crystalline materials are often employed but in some cases they are not recommended. Many drugs can exist in more than one crystalline form, i.e., the molecules exhibit different space lattice arrangements in the crystal. There are a variety of reasons for such changes in the crystal [1]. It largely depends on the how the crystallization of the drug is conducted, the nature of solvent(s) used, the conditions such as temperature, pressure, cooling rate, agitation, use of the cosolvents, presence of other solutes and ions. Such information regarding the industrial processing of bulk drugs is a closely guarded secret by the manufacturers.

Though the polymorphs are chemically identical, they exhibit different physicochemical properties, viz., melting point, solubility etc. Crystal size and habit variables may conceivably affect various measurements. These physicochemical properties further affect the biological properties of drug molecules. Roficoxib exists in polymorphic state and shown to influence significantly the bioavailability of drugs. Metastable polymorph and amorphous Roficoxib have better bioavailability compared to its stable polymorph. The influence of crystal habit is predicted to be more obvious in suspensions due to availability of more space for re-orientation of particles during settling. Selection of stable habit assumes more importance in suspensions because of the presence of solid-liquid interactions that often result in Ostwald ripening. Chemicals that are capable of forming hydrogen bonding can exhibit polymorphism[2]. Nearly all the organic compounds having long chains also exhibit polymorphism. Aqueous solubility of drugs is important for bio absorption and drug action. In case of slightly soluble drugs, polymorphism will significantly alter the biological properties. Since dissolution is an important prerequisite for drug absorption in most of acidic drugs, the polymorphism influences the drug absorption to a great extent.

MATERIALS & METHODS

Rofecoxib: Ami Trading Company Ahmedabad, Acetone, Ethanol, Methanol, Isopropyl Alcohol, Ethyl Acetate, S.D.Fine Chemicals Limited, Mumbai.

PREPARATION OF CRYSTALS

Several studies on the preparation of polymorphs had included exaggerated stress conditions such as rapid cooling, low temperature crystallization, rapid evaporation, freeze drying etc. The saturated solutions were prepared and the normal room temperature cooling (no stress conditions) did not yield crystals. Simple evaporation of solvents was observed. Further the residue was poor in crystallinity [3]. The present study employed sudden cooling technique by keeping the saturated solutions of drug in the solvents in deep freezer at around 4 to 5˚C. The crystals from the solution were separated and dried at room temperature. The surface moisture was removed by storing the sample over calcium chloride desiccators for 1 to 2 weeks. The influence of solvents on the crystal habit can be highlighted. Solvents having wide range of polarity were selected for the preparation of crystals.

CHARACTERIZATION OF CRYSTALS

Melting Points: The melting points of the crystals were carried in open capillaries by using electrical melting point apparatus [4].

Fourier Transformed Infrared (FT-IR) Spectroscopy: The sample powder was dispersed in KBr powder and analysed [5]. FT-IR spectra were obtained by powder diffuse reflectance on a FT-Infrared spectrophotometer type FT-IR 1600 Perkin-Elmer.

PHYSICOCHEMICAL PROPERTIES

Solubility Studies

Rofecoxib*:* The solubility of rofecoxib crystals was studied in distilled water. About ten mg of crystals were added to 10 ml of distilled water in glass ampoules and the ampoules were sealed. This amount was sufficient to obtain saturated solution. These ampoules were shaken for 8 hours at 25^0C by keeping in a constant temperature shaker bath[6]. The ampoules were then broken and solutions were filtered with the help of Whattman filter paper. The absorbance of the solution was measured at $\lambda_{max,}$ 262 nm. This method was repeated for three times.

ROFECOXIB

Preparation of Rofecoxib Crystals

Among the solvents used for crystallization, only five solvents gave encouraging results. The other solvents such as hexane, water and chloroform, did not give crystals, may be due to poor solubility. Though several methods were used for their preparation, only shock cooling at low temperature (4 to 6˚C) gave encouraging results. During the cooling, the containers were always closed with stoppers. Occasional agitation was used to verify the crystals [7]. This has enhanced the formation of crystals. The nuclei were separated and acted as seeds for further crystal growth. Time of about 2 to 3 days was required for obtaining good quantity of crystals. About three times the crystals were prepared and obtained reproducibility.

General Morphology of Rofecoxib Crystals

The crystals of rofecoxib obtained from different solvents were observed by magnifying lens and microscopic method. The visual observations made convincing results about the differences in the crystalline of all the above crystals characterization [8]. The general determinations were given in Table 1.

RESULTS & DISCUSSION

Melting Points of Rofecoxib Crystals

Melting points of the rofecoxib crystals were determined using open capillaries method. The data were recorded as shown in Table 2.

Though the commercial sample is amorphous, it has high melting point. In other words, the crystals of low melting points are an encouraging observation. Crystal obtained from isopropanol has lowest melting point among the crystals. Reproducible results were obtained though the melting points were monitored over a period of 45 days. In other words, the polymorphic transformations during the storage were not observed [9].

FT-IR Spectroscopic Analysis

Solid samples of drugs must be used, since polymorphs of a compound may have identical spectra in solution. This technique can be used for both quantitative and qualitative identification.

Rofecoxib Crystal Analysis:

The FT-IR spectra were obtained for the crystals of rofecoxib from different solvents for commercial sample, methanol, isopropanol, acetone and ethyl acetate, respectively. The commercial sample was used for comparison[10]. The spectral analysis was done in two parts, first is for the identification of drug using characteristic bands and second is for identification of polymorphs (or crystal

habit)commercial & literature bands as shown in Table 3 & characteristic polymorphic (crystal habit) changes of rofecoxib crystals as shown in Table 4.

Solubility Behavior of Rofecoxib Crystals

Solubility data in water have been obtained for different crystals of rofecoxib after shaking for eight hours at 25ºC. Crystals of rofecoxib obtained in acetone and isopropanol were shown to have aqueous solubility to the same extent. On the other hand, crystals obtained in ethyl acetate and methanol were shown to have aqueous solubility to the same extent, but reduced when compared to that of acetone and isopropanol. Commercial sample showed the highest aqueous solubility than any crystals. It is understood, as it was amorphous in nature. In general, crystals have low aqueous solubility compared to amorphous as show in Table 5..

Dissolution Behavior of Rofecoxib Crystals

For the dissolution studies, water was selected as a dissolution medium. There was no dissolution of drug due to low aqueous solubility. Therefore, several alternative media were studied and finally hydrochloric acid (pH, 1.2) solution was selected. Dissolution studies were conducted using Dissolution Apparatus Type I.

In this study, crystals were filled in the hard gelatin capsule and added to the dissolution medium [11]. The dissolution rate-time data are reported in Tables 5 and 6 for the different crystals. The dissolution-time profile of rofecoxib crystals was given in Figure 6. The trend of dissolution rate is different from the solubility data as because of using hydrochloric acid (pH, 1.2) solution, instead of distilled water as in the solubility. The order of the dissolution of the crystals after 60 minutes is isopropanol > ethyl acetate> acetone > methanol > commercial sample > ethanol.

INTERRELATIONSHIPS OF PHYSICOCHEMICAL PROPERTIES OF CRYSTALS

Rofecoxib: Melting Point Vs Aqueous Solubility

The melting points and aqueous solubility data were abstracted and compiled in Table 8 for ready reference[12]. The data were plotted in Figure 7.

Though commercial sample showed high melting point, its aqueous solubility was also high. It is difficult to explain this phenomenon. Among crystals also, a similar behavior was observed.

Rofecoxib: Aqueous Solubility Vs Percent Dissolution

The aqueous solubility data of rofecoxib crystals and percent dissolution at 60 minutes time were abstracted and compiled in Table 9 as a ready reference. The data were plotted in Figure 8.

A perusal to Figure 4 indicated that the percent of dissolution of rofecoxib commercial sample in hydrochloric acid (pH, 1.2) solution was low, though its aqueous solubility was high [13]. For crystals obtained from ethyl acetate, methanol, ethanol and acetone, the aqueous solubility is decreased; percent dissolution of rofecoxib was increased. It is difficult to explain such a behavior. The hydrochloric acid (pH, 1.2) medium has antagonistic effect on the aqueous medium. One possible reason for this behavior was the dissociation constant. This can be ruled out, because rofecoxib did not have any functional groups that exhibit dissociation constant. Hence, correlation may not be relevant.

Rofecoxib: Melting Point Vs Percent Dissolved

The melting point of rofecoxib crystals and percent dissolution at 60 minutes data were abstracted and compiled in Table 7 as a ready reference. The data were also plotted in Figure 9.

A perusal to Figure 5 indicated that the lower the melting point, the higher the dissolution of crystals in hydrochloric acid (pH, 1.2) solution. This behavior was observed in case of commercial sample also.

Table 1: General observations of rofecoxib crystals obtained from different solvents

Sl. No.	Solvent of crystallization	Description of crystals
1	Ethyl acetate	Small size, irregular shaped, platy type.
2	Methanol	Long needles with finger like crystal growth in a few cases.
3	Acetone	Small size, irregular shaped, platy type.
4	Ethanol	Needle shaped, length was shorter compared to crystals obtained from methanol and longer than the crystals obtained from isopropanol.
5	Isopropanol	Needle shaped, shorter in length and sticky.

Table 2: Melting points of rofecoxib crystals

Sl. No.	Solvent of crystallization	Melting point, ˚C
1	Ethyl acetate	183
2	Methanol	180
3	Acetone	185
4	Ethanol	184
5	Isopropanol	178
6	Commercial Sample	203

Table 3: Comparison of characteristic bands between literature and commercial sample

Characteristic bands	Literature values, cm^{-1}	Observed in this study, cm^{-1}
C-H Stretching-aromatic	3030 (s)	3018
C-H Stretching-alkane	2962-2853 (s)	2929
C-C Multiple bond stretching-aromatic	~ 1660 (s)	1646
Cyclic, α, β unsaturated lactone	1760-1740 (s)	1747
S=O Stretching vibrations-sulfones	1160-1140 (s)	1149
Intermolecular hydrogen bonding	3550-3450 (s)	3461

Note:'s' means strong values.

Table 4: IR spectra for characteristic polymorphic (crystal habit) changes of rofecoxib crystals.

Sl. No.	Solvent from which crystals were obtained	Characteristic changes, bands, cm^{-1}	Intensity of bands	Inference
1	Commercial Sample	3461 3018, 2929, 495 1595	Broad Sharp Sharp	Polymorph
2	Methanol	3018 495 1089, 1035, 960 1594	Not sharp No band Low intensity Disappeared	Polymorph
3	Isopropanol	2929 3436 495 1089, 1035, 960 661, 617, 592	Not sharp High No band Low Disappeared	Polymorph
4	Acetone	1089, 1035, 960	Low intensity	Same as commercial sample
5	Ethyl acetate	3018, 2929 1089, 1035, 960 1594	Not sharp Low Disappeared	Polymorph

Table 5: Solubility data of crystals of rofecoxib obtained from different solvents.

Sl. No.	Solvent of crystallization	Solubility, mg/ml Mean ± S.D*
1.	Acetone	0.0033 ± 0.0005
2.	Isopropanol	0.003299 ± 0.0003
3.	Ethyl acetate	0.002543 ± 0.0001
4.	Methanol	0.002468 ± 0.0005
5.	Ethanol	0.002938 ± 0.0003
6.	Commercial sample	0.003575 ± 0.0004

* Each reading is an average of three determinations.

Table 6 : Dissolution rate-time data of crystals of rofecoxib obtained from different solvents.

Time min.	Percent rofecoxib dissolved, Mean ± SD*		
	Acetone	Isopropanol	Ethyl Acetate
20	34.24 ± 1.1002	40.56 ± 0.8808	39.32 ± 2.3243
40	38.37 ± 2.9815	49.75 ± 4.2528	43.29 ± 1.3661
60	41.66 ± 1.8228	71.62 ± 2.5647	53.13 ± 3.5786
80	44.89 ± 0.7775	77.15 ± 1.4911	60.11 ± 4.5347
100	52.18 ± 0.1026	82.93 ± 1.2543	65.49 ± 3.2280
120	57.26 ± 0.9779	87.14 ± 1.8879	75.81 ± 5.6042

* Each reading is an average of three determination

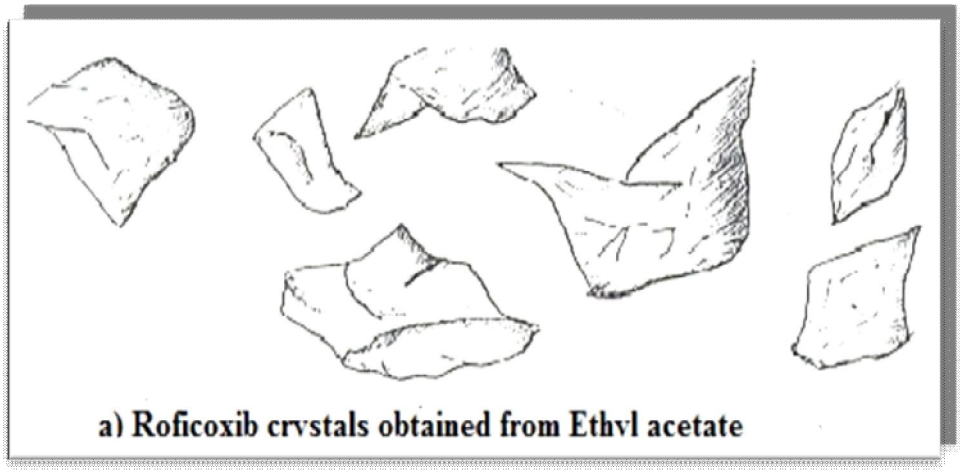
Table 7: Dissolution rate-time data of crystals of rofecoxib obtained from different solvents.

Percent rofecoxib dissolved, Mean ± SD*		
Methanol	Ethanol	Commercial Sample
28.29 ± 1.4568	31.16 ± 4.3611	31.17 ± 0.8929
35.59 ± 1.1829	35.55 ± 2.4472	36.45 ± 1.0372
40.66 ± 0.8282	38.92 ± 1.7658	39.59 ± 0.8577
42.73 ± 1.4828	43.29 ± 1.8536	43.70 ± 0.5823
44.97 ± 0.4375	48.27 ± 1.3319	48.95 ± 1.3216
51.47 ± 1.6095	53.65 ± 2.1261	52.92 ± 1.0401

*Each reading is an average of three determinations.

Table 8: Co-relation between percent drugs dissolved, solubility and melting point of rofecoxib crystals obtained from different solvents.

Sl. No.	Solvent of crystallization	Melting point, ˚C.	Aqueous solubility, mg/ml	Percent Dissolution at 60 minutes.
1	Ethyl acetate	183	0.002543	53.13
2	Methanol	180	0.002468	40.66
3	Acetone	185	0.003333	41.66
4	Ethanol	184	0.002938	38.92
5	Isopropanol	178	0.003299	71.62
6	Commercial sample	203	0.003575	39.59



a) Roficoxib crystals obtained from Ethyl acetate

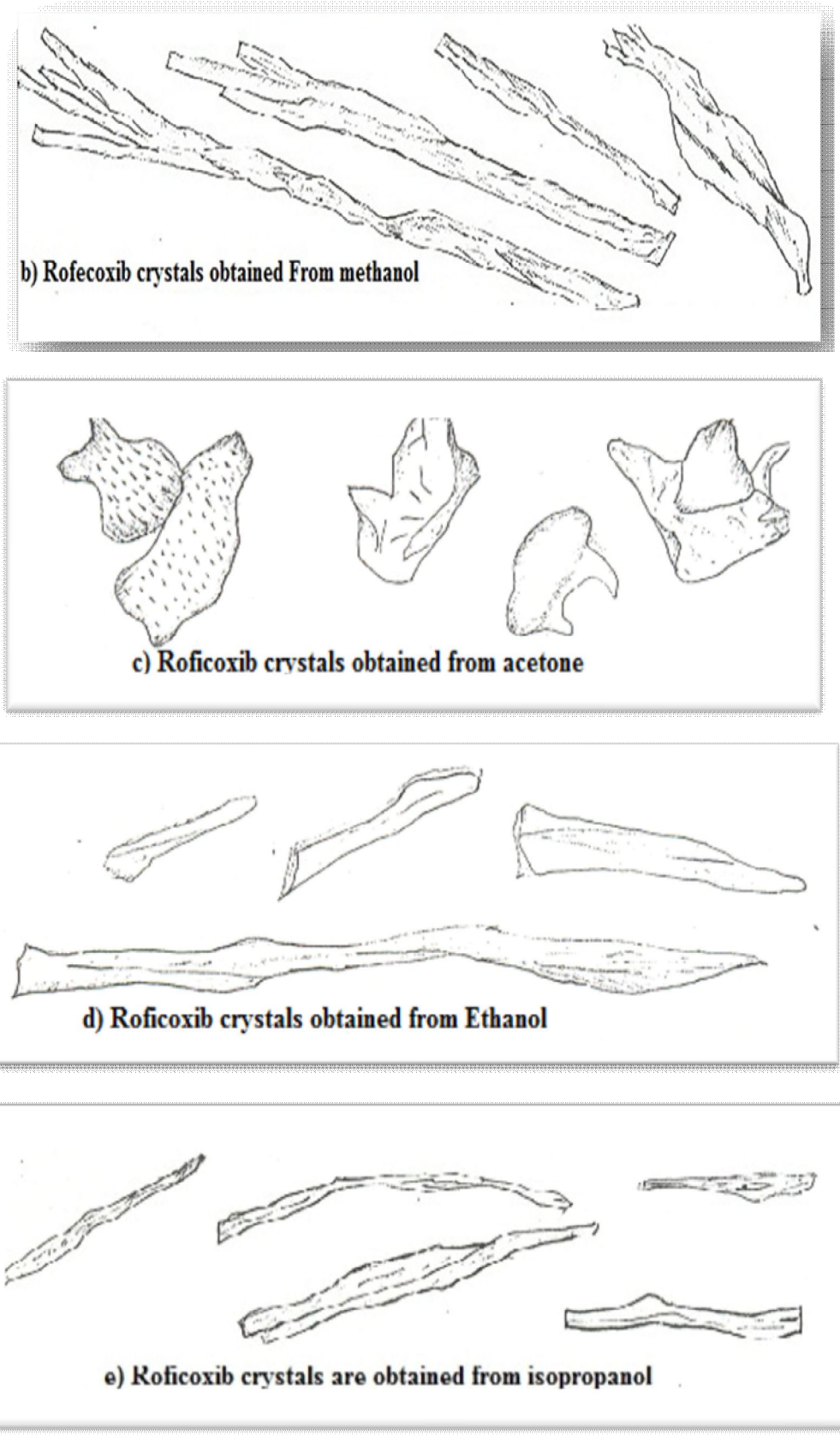


Fig. 1 Binocular observation roficoxib crystals

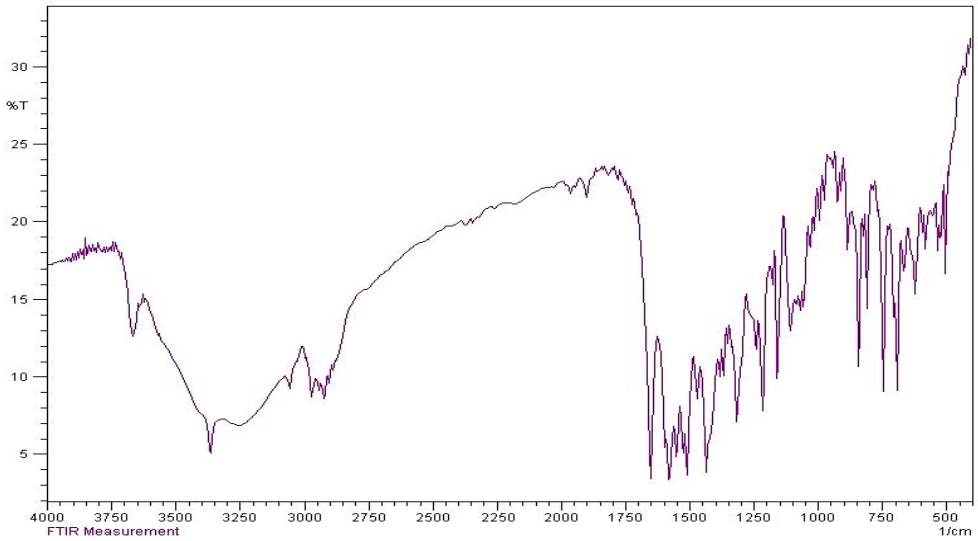


Fig: 2 FT IR Spectra of Rofexcoxib Crytals obtained from ethanol

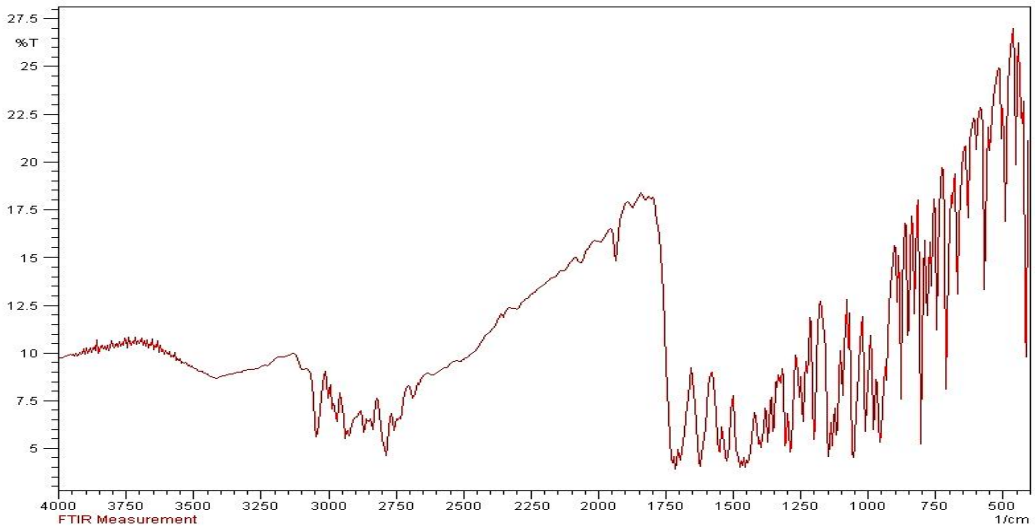


Fig: 3 FT IR Spectra of Rofexcoxib Crytals obtained from Isopropanol

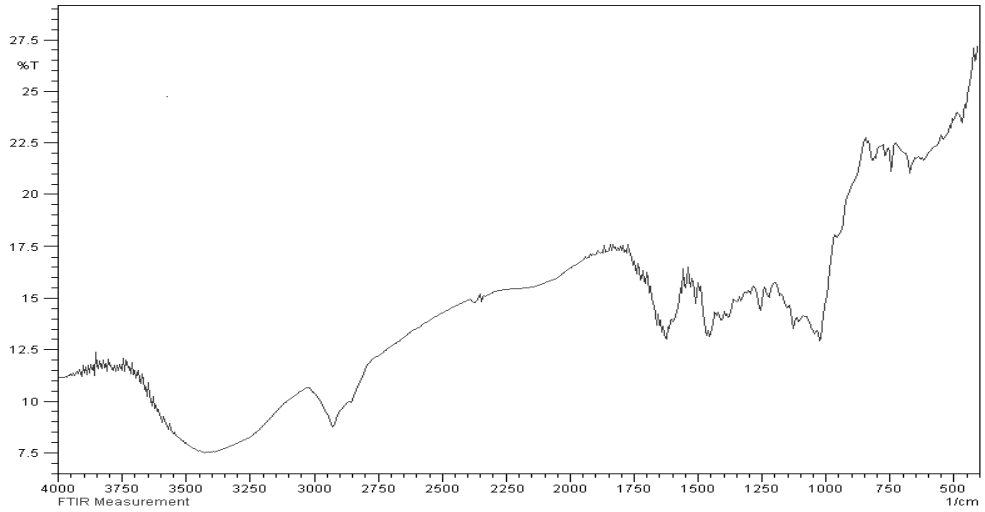


Fig: 4 FT IR Spectra of Rofexcoxib Crytals obtained from Ethyl acetate

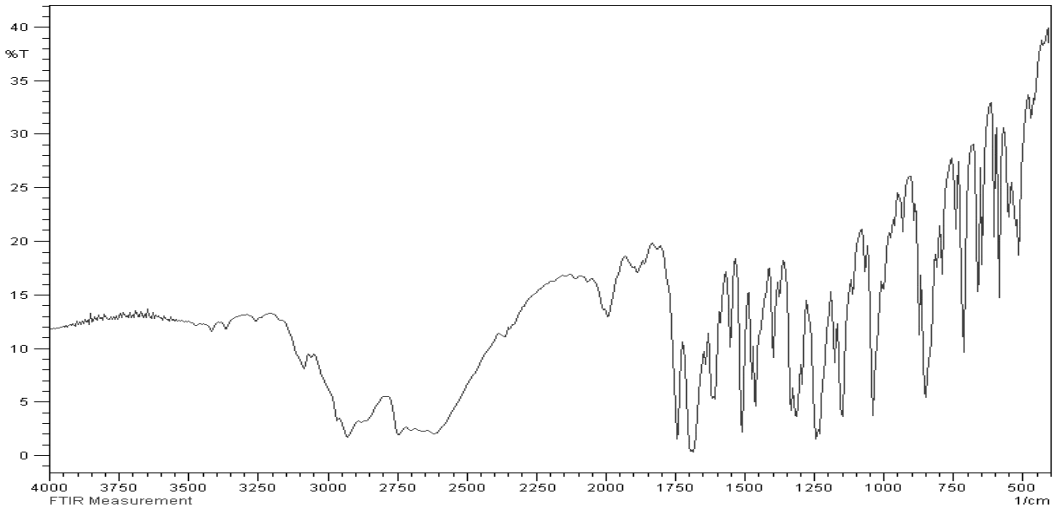
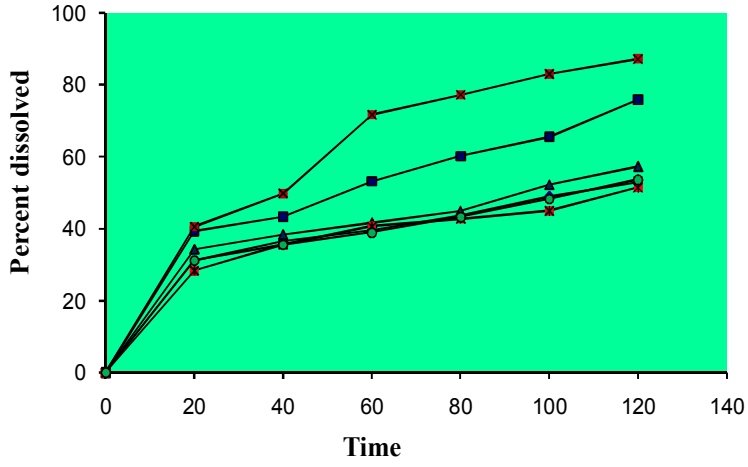


Fig: 5 FTIR Spectra of Rofecoxib Crystals obtained from Acetone



◆ Pure drug rofecoxib, ■ Ethyl acetate crystals, ▲ Acetone crystals
● Ethanol crystals, ✖ Methanol crystals, × Isopropanol crystals

Fig 6: Dissolution profile of the drug rofecoxib and its crystals obtain from different solvents

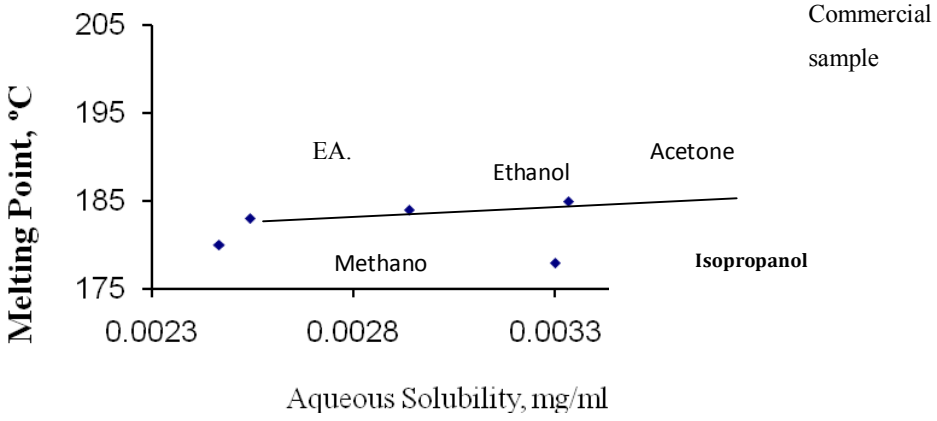


Fig 7: Co-relation between melting point Vs aqueous solubility of rofecoxib crystals

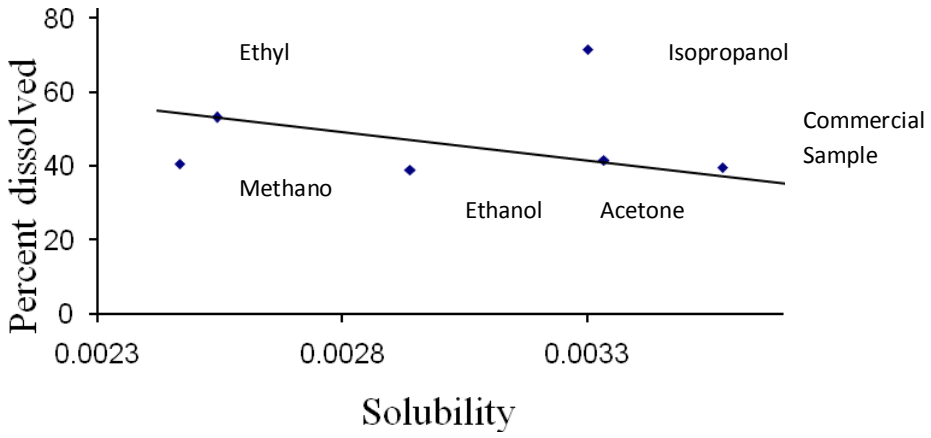


Fig 8: Co-relation between percent dissolution Vs aqueous solubility of rofecoxib crystals.

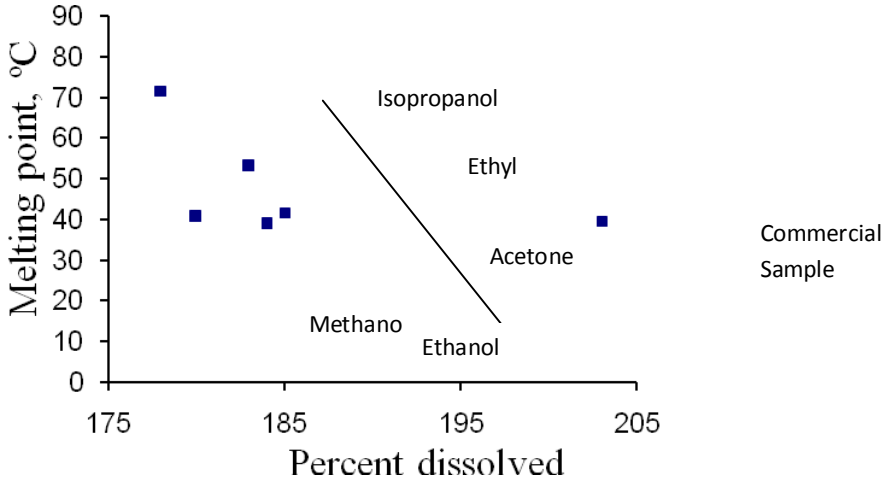


Fig 9: Co-relation between melting point and percent dissolved of rofecoxib crystals.

CONCLUSIONS

The rofecoxib crystals were prepared successfully using low temperature time cooling for longer period (2 to 3 days) in closed containers. The crystal morphology exhibited differences, when observed under magnifying lens. The melting point of rofecoxib crystals obtained from methanol, isopropanol, ethyl acetate, and acetone were different indicating the *prima facie* evidence for differences in crystal habit or polymorphism. The IR spectra confirm that three types of crystals of rofecoxib obtained from methanol, isopropanol and ethyl acetate were different polymorphs or crystal habits. All crystals showed lower aqueous solubility compared to commercial sample, amorphous. The order of dissolution of rofecoxib crystals after 60 minutes was isopropanol > ethyl acetate > acetone > methanol > commercial sample > ethanol. In case of inter-relationships, an important conclusion was that the lower the melting point of crystals of the rofecoxib, the higher the dissolution of rofecoxib in hydrochloric acid (pH, 1.2) solution, though lower aqueous solubility was observed.

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