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Research Article

Evaluation of crystal forms of Nateglinide

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ABSTRACT

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. The different crystal form of a drug have different physico-chemical characteristics namely crystal shape, crystal size, melting point, density, flow properties solubility pattern, dissolution characteristics and XRD pattern, though they are chemically identical. 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. 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. The drug nateglinide used here is practically insoluble in water. But the water insolubility and the less bioavailability are the limitations of its effective use clinically. Keeping this in view crystal modification of nateglinide has been undertaken to improve solubility, dissolution and bioavailability. New crystals prepared by two different methods using solvents like Benzene, Ethanol and Acetone in this study to observe the effect of solvents on the development of crystal habits in the changed environment, and prepared crystals were characterized by some physico chemical techniques like melting point, UV and I.R spectroscopy, solubility and invivo dissolution studies, scanning electron microscopy, X-ray powder diffraction and DSC analysis.

Keywords: Crystal; DSC analysis; Nateglinide; X-ray powder diffraction

INTRODUCTION

Active pharmaceutical ingredients (APIs) are frequently delivered to the patient in the solid-state as part of an approved dosage form (e.g., tablets, capsules, etc.). Solids provide a convenient, compact and generally stable format to store an API or a drug product. Understanding and controlling the solid-state chemistry of APIs, both as pure drug substances and in formulated products, is therefore an important aspect of the drug development process. APIs can exist in a variety of distinct solid forms, including crystals, solvates, hydrates, salts, co-crystals and amorphous solids. Each form displays unique physicochemical properties that can profoundly influence the bioavailability, manufacturability purification, stability and other performance characteristics of the drug (S.R. Byrn et al., 1999). Hence, it is critical to understand the relationship between the particular solid form of a compound and its functional properties. The choice and design of pharmaceutical solid forms can be critically important to successful drug development.

Solid form discovery and design depends on the nature of the molecule of interest and type of physical property challenges faced in its development. The preferred solid form is generally the thermodynamically most stable crystalline form of the compound (S.R. Byrn et al., 1999; H. Brittain., 1999). However, the stable crystal form of the parent compound may exhibit inadequate solubility or dissolution rate resulting in poor oral absorption, particularly for water-insoluble compounds. In this case, alternative solid forms may be investigated. For ionizable compounds, preparation of salt forms using pharmaceutically acceptable acids and bases is a common strategy to improve bioavailability (S.R. Byrn et al., 1999; S.M. Berge et al., 1977; P.L. Gould., 1986). Like the parent compound, pharmaceutical salts may exist in several polymorphic, solvated and/or hydrated forms.

Many pharmaceutical solids can exist in different physical forms. Polymorphism is often characterized as the ability of a drug substance to exist as two or more crystalline phases that have different arrangements and/or conformations of the molecules in the crystal lattice[1].

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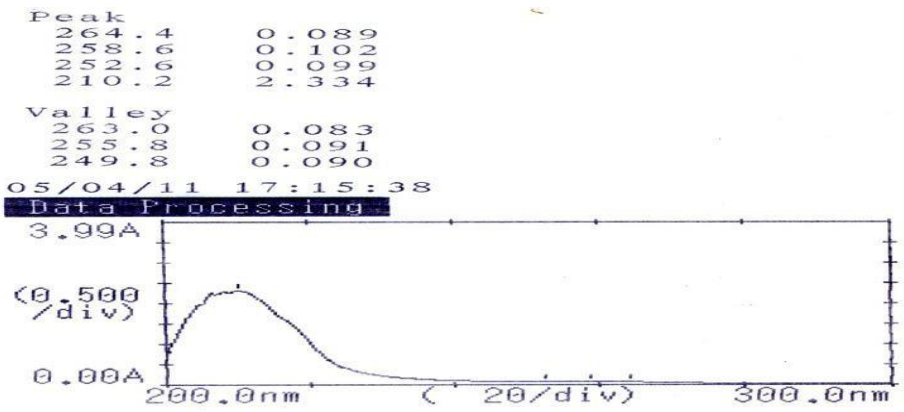

Figure 1: Common UV spectra for Nateglinide and its new crystal forms obtained from different solvents/ solvent systems

Table 1: Comparative melting points data of Nateglinide with its new crystal forms obtained from different solvents/solvent systems (I) and after kept at elevated temperature (II)

S.NO	Solvents/solvent systems used	Melting point (°C) (I) (± S.D)	Melting point(°C) (II) (± S.D)
1	Acetone	130 ± 1	131 ± 2
2	Benzene	128 ± 2	129 ± 1
3	Ethanol	126 ± 2	127 ± 1
4	SCPVP (2%)	104 ± 1	105 ± 2
5	SCPEG (2%)	129 ± 1	130 ± 1
6	SCT-80 (2%)	130 ± 2	131 ± 1
7	Nateglinide	139 ± 1	139 ± 1

Pharmaceutical polymorphic solids of the same chemical compound differ in internal solid-state structure and, therefore, possess different chemical and physical properties, including packing, thermodynamic, spectroscopic, kinetic, interfacial, and mechanical properties. These properties can have a direct impact upon drug substance processability, drug product manufacturability, and drug product quality/performance, such as stability, dissolution, and bioavailability (D.J.W. Grant., 1999).

MATERIALS AND METHODS

Materials used

Drug : Nateglinide

Solvents : Benzene, Acetone and Ethanol.

Polymers: Polyethylene glycol-4000 (PEG-4000), Polyvinyl pyrrolidone (PVP K_{30}) and Tween-80 (T-80)

Nateglinide was obtained as generous gift from Alembic chemical works Co Limited (Baroda, 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).

Preparation of Nateglinide 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.

Method I

1 gm of Nateglinide 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 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.

Method II

1 gm of Nateglinide was dissolved in 40 ml of ethanol in a conical flask and the solution was heated and filtered. The resultant solution was concentrated on a water bath at 78^{o}C and then the clear solution thus obtained was rapidly added to equal volume of cold water (5^{o} 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 1hr at room temperature. The crystals were then recovered by filtration under vacuum using a sintered glass funnel. They were then kept in airtight container for further use.

Physico Chemical Characterization of the New Crystal Forms of Nateglinide

The newly developed crystal forms of Nateglinide were characterized by using different physico chemical techniques.

Melting point determination, Spectroscopic Analysis (Ultra-violet Spectroscopy (UV) , Infra-red Spectroscopy (IR)), Solubility and Dissolution rate studies, X-ray

powder diffraction (XRPD) study, Differential Scanning Calorimetry (DSC) and Accelerated stability studies.

***In vivo* studies**

In vivo studies were performed using a cross-over technique in 12 male wistar albino rats. The weights of wistar rats ranged from 200-300gms.The male wistar albino rats will be divided into 4 groups. In that each group containing 3 animals. The animals were fasted overnight until the end of the experiment but allow water ad libitum. Group 1-4 will administer with nateglinide and it crystal obtained from benzene, ethanol and SCPVP. A dose equivalent to 2 mg/kg of all samples dispersed in 1 ml phosphate buffer P^H 6.8 was administered orally to each of the animals. Each sample was administered orally. Blood was taken prior to administration and at 0.5.1.0, 1.5, 2, 4, 8 and 24 hrs after dosing. Plasma was separated by centrifugation (3000 rpm for 10min) and stored in a freezer at -20^{o}C unit analyzed. All the studies were carried out under ambient conditions.

High performance liquid chromatographic (HPLC) analysis

Waters HPLC systems were used for the analysis of all samples and the below mentioned conditions were kept as constant for entire study.

Stationary phase: Phenomenex GEMINI C_{18} (250 x 4.6 mm i.d, 5μ)

Mobile Phase: Di Sodium Hydrogen phosphate (pH 2.0): Acetonitrile

Mobile phase ratio: 50:50 % v/v

Flow rate: 0.7 ml/min

Sample volume: 50μl using Rheodyne 7725i injector

Detection: 210 nm using dual wavelength absorbance detector

Data station: Class-VP 6.01 data station

The mobile phase was filtered through a 0.22 μL membrane and degassed using ultrasonicator. The experiments were carried out at room temperature of about 20^{0}C.

Preparation of plasma samples

At the time of analysis, the samples were removed from the deep freezer and kept in the room temperature and allowed to thaw. A volume of 0.5 ml of sample was pipetted into 2.0 ml centrifuge tube with this 500 μl of internal standard solution (50.0 μg/ml) and 1.0 ml of precipitating agent (Acetonitrile) was added. The resulting solution was Vortexed for 5 minutes and centrifuged at 4000 rpm for 10 min. Supernatants from the above solutions were separated and used for the analysis.

RESULTS AND DISCUSSION

UV Analysis

A 0.001% (W/V) of sample solution in methanol was used to scan the sample by using UV-visible spectrophotometer (Schimadzu 160-UV-visible spectrophotometer). The trace is showing in Fig.1.

Melting point

From the melting point determination study it was found that the melting points of all the new crystals stored under ambient condition and kept at elevated temperature (40^o C) were considerably varying with parent drug (Nateglinide) and the results are shown in Table 1.

Infrared Spectroscopy

The spectra of all modified crystals stored under ambient condition and kept at elevated temperature (40^o C) were identical and the main absorption bands of Nateglinide appeared in all of the spectra (Fig.2a, 2b, 2c & 2d). 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.

X-ray Powder Diffraction

To obtain information on the physico-chemical characteristics of the prepared crystals, X-Ray powder diffraction measurements were conducted.

XRD spectra for all crystals are presented in Fig.4. In the powder diffractogram sharp peak at diffraction angle (2 θ) 19.75, 13.95, 14.10, 18.80, 18.95, 18.85, and 13.90 were obtained in case of drug Nateglinide and the modified crystals obtained from acetone, benzene, ethanol, SCPVP (2%w/v), SCPEG (2%w/v) and SCT (2%w/v) respectively. The presences of these sharp peaks are clearly evident in the diffractogram presented in Fig.3 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 Nateglinide samples (Table 2). The intensity of the peak in parent drug is the highest than that of all other modified crystals reported herein. This is probably due to higher crystal perfection (A. Nokhodchi et al., 2003).

Thermal Analysis

The DSC data for drug Nateglinide (untreated) and the modified crystals are shown in Fig.4. The DSC thermographs of all modified crystals showed significant variation in transition temperature, enthalpy of fusion and in melting point (Table 3). It should be noted that the all samples shows broad exothermic peaks and slight but insignificant variation in. This may be due to oxidation or phase transformation. Crystals obtained by using ethanol, SCPVP (2%w/v) and SCPEG (2%w/v) show a weak endothermic peaks and there is no significant variation in transition temperature but significant

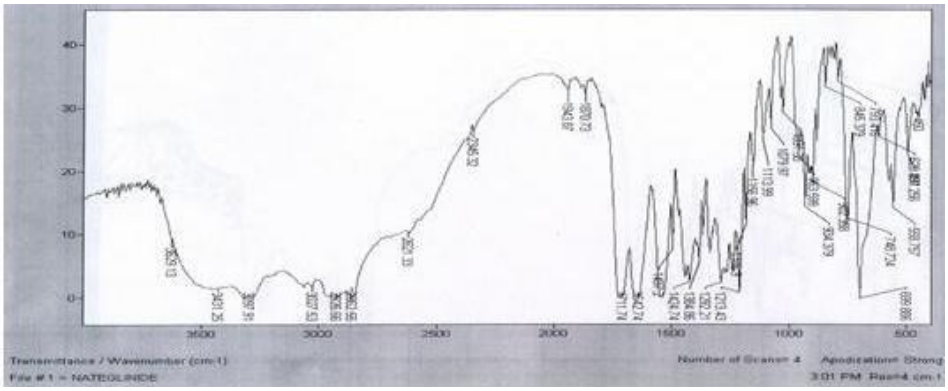

Figure 2a: IR Spectra of parent drug (Nateglinide)

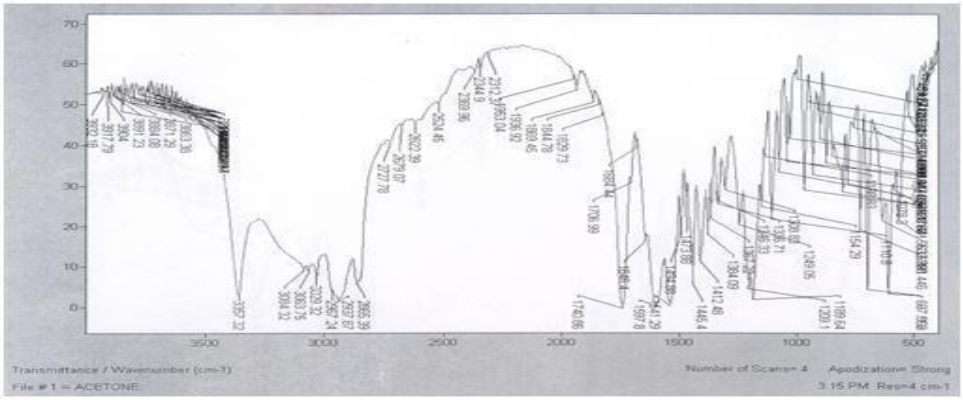

Figure 2b: IR Spectra of drug Nateglinide re-crystallized from Acetone

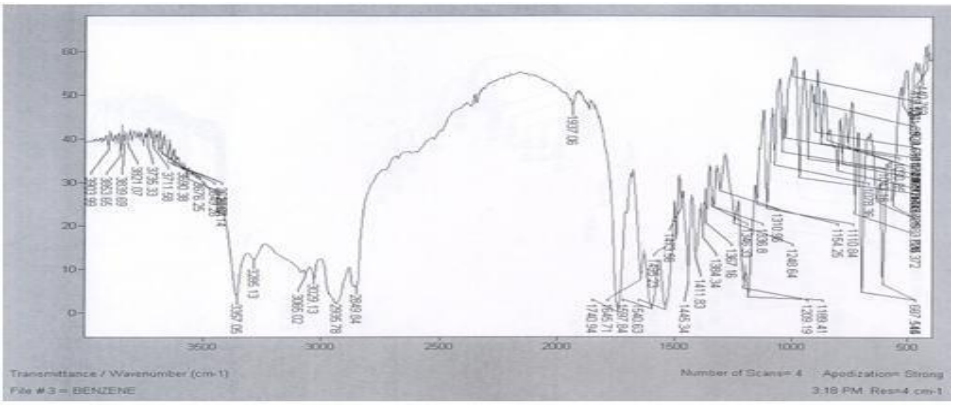

Figure 2c: IR Spectra of drug Nateglinide re-crystallized from Benzene

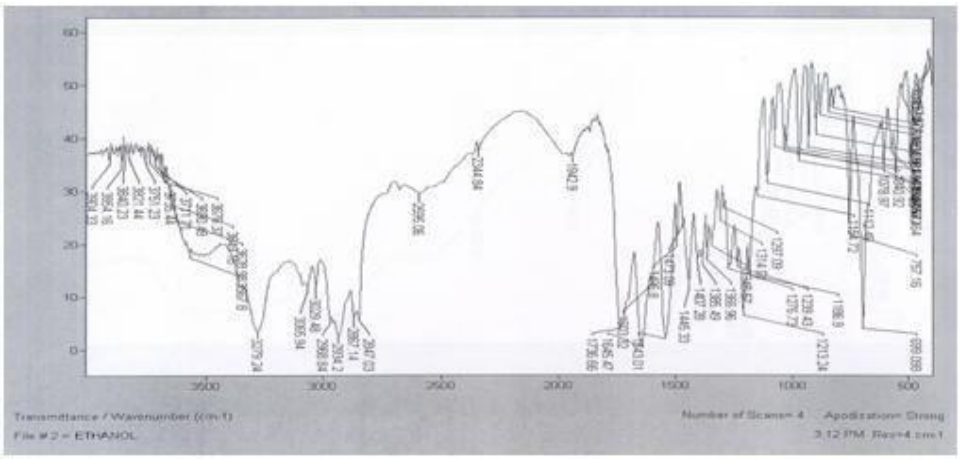

Figure 2d: I.R. Spectra of drug Nateglinide re-crystallized from Ethanol

Table 2: Comparative X-ray powder diffraction pattern data of Nateglinide with its new crystal forms obtained from different solvents/solvent systems

S.NO	Solvents/solvent systems used	d-value	Intensity (cps)	2θ	Peak position
1	Acetone	6.3429	664	13.950	4
2	Benzene	6.2757	716	14.100	4
3	Ethanol	4.716	434	18.800	9
4	SCPVP (2%)	4.6791	714	18.950	8
5	SCPEG (2%)	4.7037	667	18.850	9
6	SCT-80 (2%)	6.3656	844	13.900	6
7	Nateglinide	4.4913	1468	19.750	8

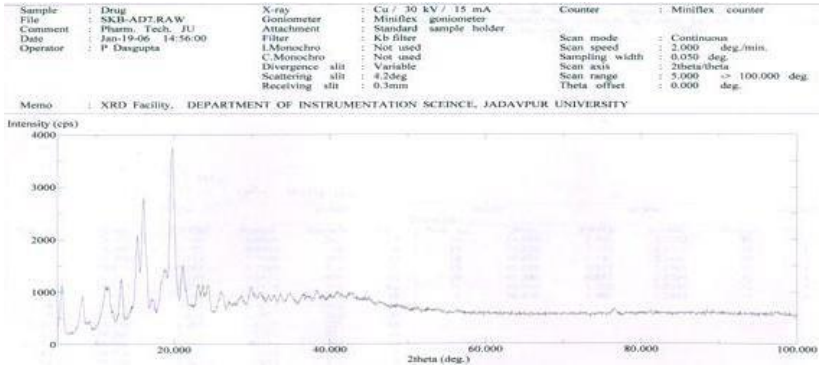

Figure 3a1: X-ray powder diffraction pattern of parent drug (Nateglinide)

Figure 3a2: X-ray powder diffraction peaks of parent drug (Nateglinide)

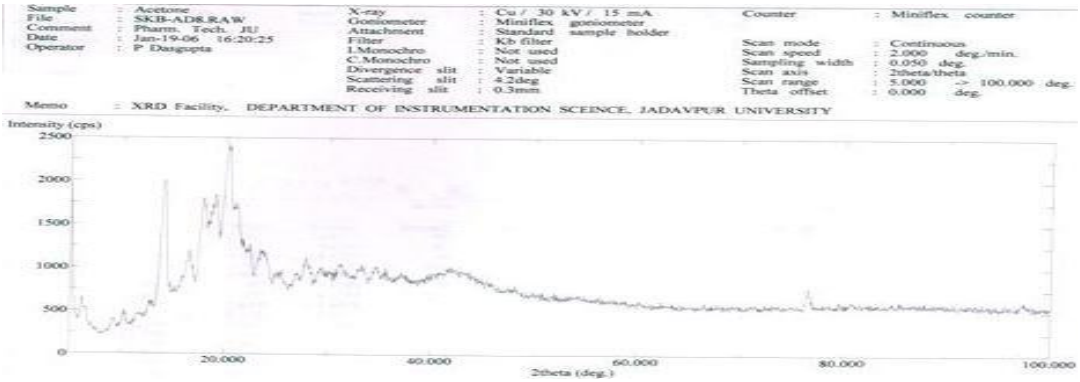

Figure 3b1: X-ray powder diffraction pattern of drug Nateglinide recrystallized from Acetone

difference in enthalpy of fusion is observed. The appearance of weak endothermic peaks in this case may be due to solvation of the crystals (Gordon et al., 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 Nateglinide under various conditions of the crystallization.

Solubility Studies

In the solubility studies it was found that the drug crystals obtained from benzene, acetone, ethanol, SCPVP (2%), SCT (2%) showed slight increase in solubility over the parent drug. In the case of SCPVP (2%) shows higher solubility than other crystals. This may be due to adsorption on the crystal surface and also due to the reduction of size of the crystals by the super cooling process (Table 4).

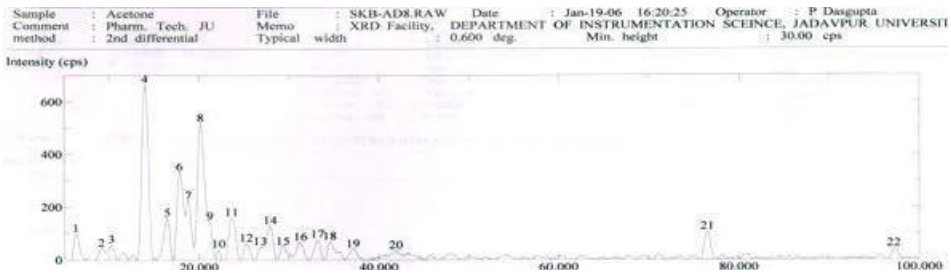

Figure 3b2: X-ray powder diffraction peaks of drug Nateglinide re-crystallized from Acetone

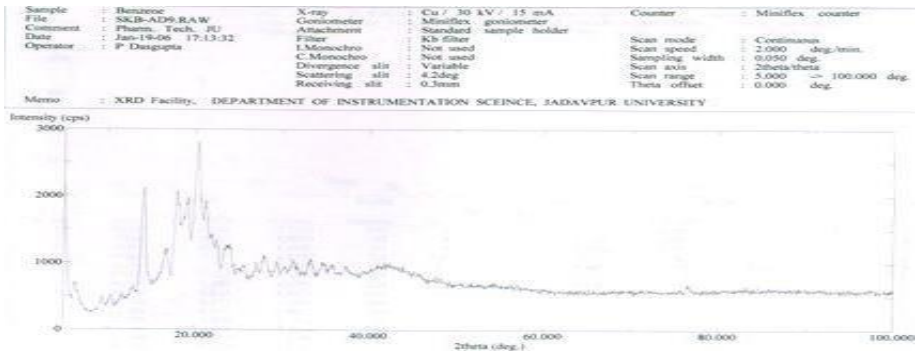

Figure 3c1: X-ray powder diffraction pattern of drug Nateglinide recrystallized from Benzene

Figure 3c2: X-ray powder diffraction peaks of drug Nateglinide re-crystallized from Benzene

Figure 3d1: X-ray powder diffraction pattern of drug Nateglinide recrystallized from Ethanol

Figure 3d2: X-ray powder diffraction peaks of drug Nateglinide re-crystallized from Ethanol

Table 3: Comparative Differential scanning calorimetric thermograph of Nateglinide with its new crystal forms obtained from different solvents/solvent systems

S. NO	Solvents/solvent systems used	Heat of fusion (ΔH) J/g		Peak (°C)	
		Endothermic	Exothermic	Endothermic	Exothermic
1	Acetone	67.6	628.1	130.1	245.2
2	Benzene	79.6	------	128.3	240
3	Ethanol	38.4 86.4 28.1	------	126.6 53.4 101.2	240.7
4	SCPVP (2%)	45.3 130.8	941.5	104.6 52.2	241.5
5	SCPEG (2%)	44.0 22.3 125.9	885.7	129.1 103.6 49.9	237.0
6	SCT-80 (2%)	114.0	------	130.1	240
7	Nateglinide	103.4	737.0	139.1	237.3

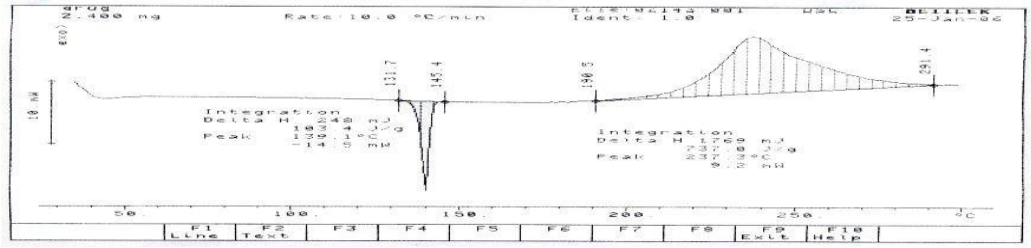

Figure 4a: Differential scanning calorimetric thermograph of parent drug (Nateglinide)

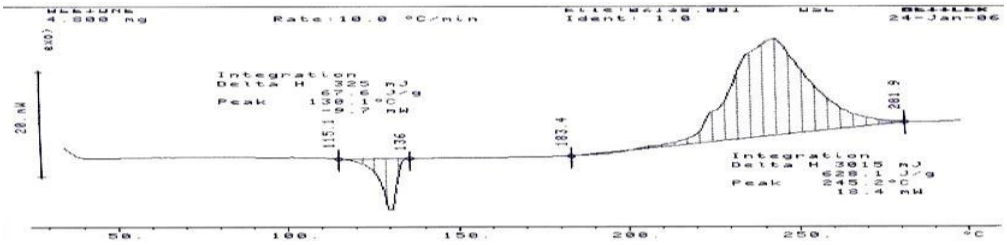

Figure 4b: Differential scanning calorimetric thermograph of drug Nateglinide re-crystallized from Acetone

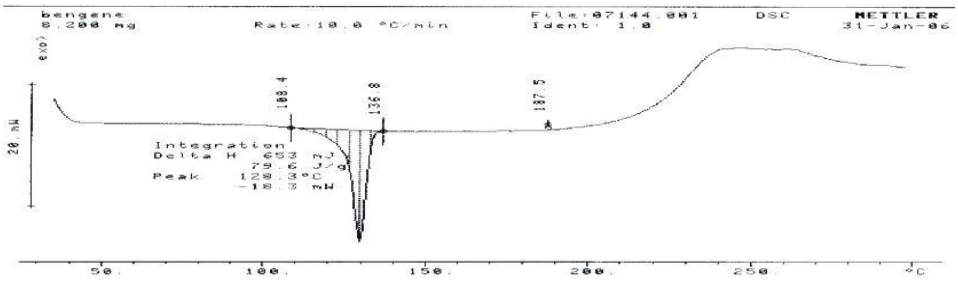

Figure 4c: Differential scanning calorimetric thermograph of drug Nateglinide re-crystallized from Benzene

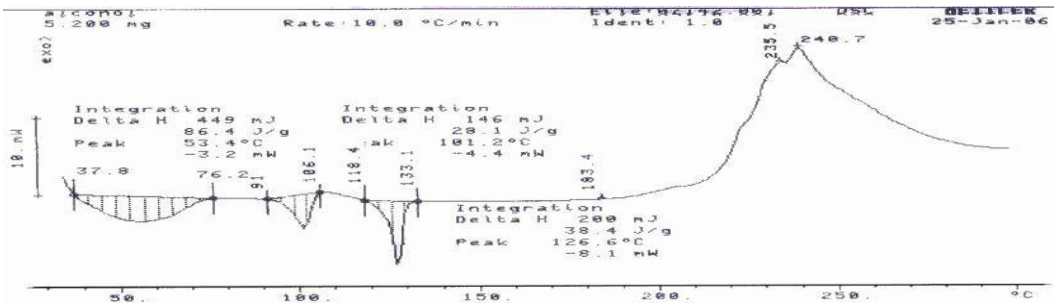

Figure 4d: Differential scanning calorimetric thermograph of drug Nateglinide re-crystallized from Ethanol

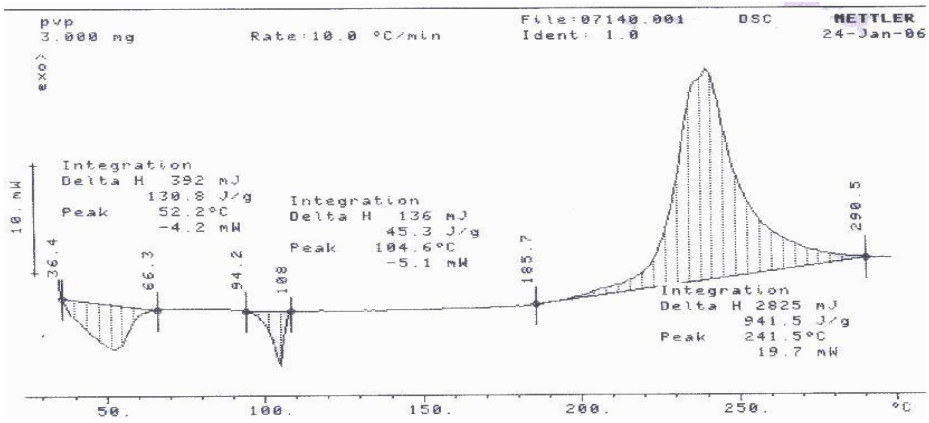

Figure 4e: Differential scanning calorimetric thermograph of nateglinide re-crystallized from ethanol with 2% solution of PVP (SCPVP)

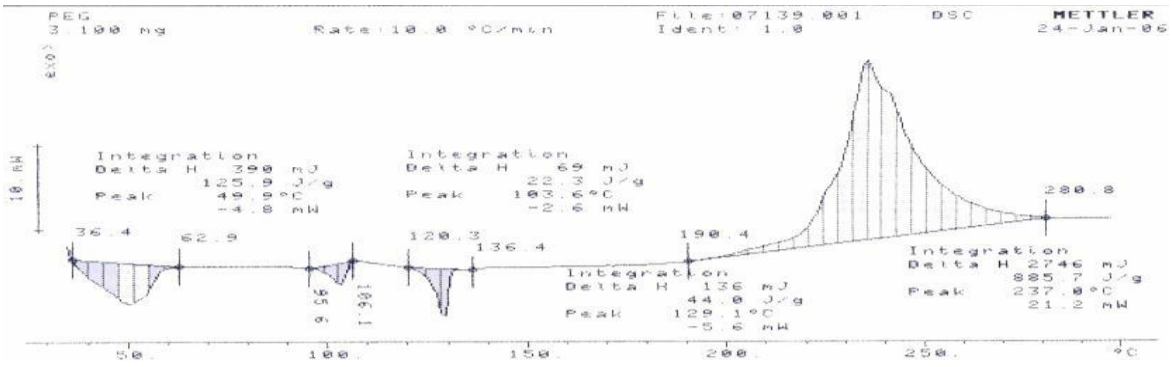

Figure 4f: Differential scanning calorimetric thermograph of nateglinide re-crystallized from ethanol with 2% solution of PEG (SCPEG).

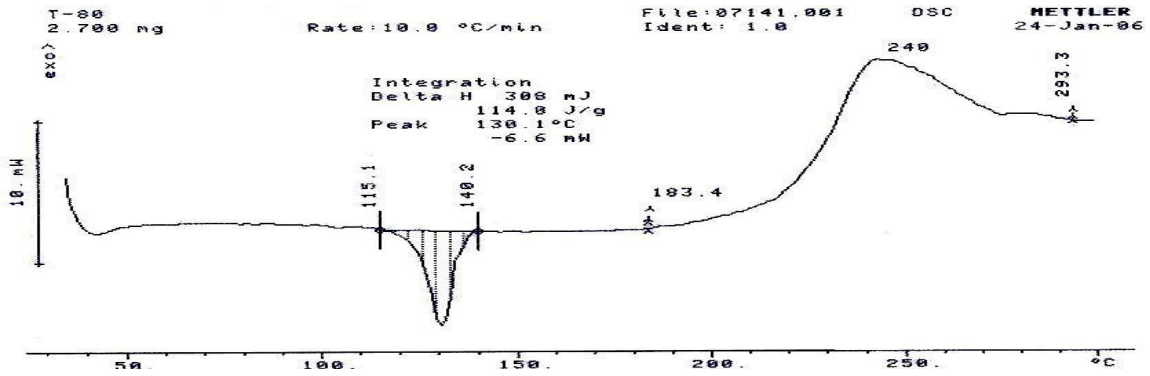

Figure 4g: Differential scanning calorimetric thermograph of nateglinide re-crystallized from ethanol with 2% solution of Tween-80 (SCT).

Table 4: Comparative solubility studies of the parent drug (Nateglinide) with its new crystal forms obtained from different solvents/solvent systems in phosphate buffer PH 6.8 at 37± 0.5 0C

Solvents/solvent systems	Solubility (mg/ml) (± S.D)
Benzene	0.5655± 0.0021
Acetone	0.5566± 0.0028
Ethanol	0.5144± 0.0018
SCPVP (2%w/v)	0.5772± 0.0024
SCPEG (2%w/v)	0.5570± 0.0012
SCT(2%w/v)	0.556± 0.0032
Nateglinide	0.4942±0.0025

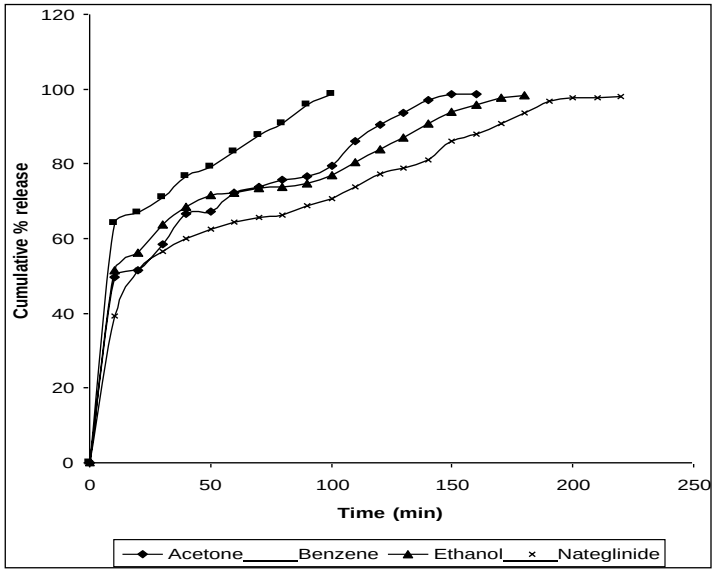

Figure 5: Dissolution profiles of nateglinide recrystallized from: (a) acetone; (b) benzene;(c) ethanol; (d) nateglinide

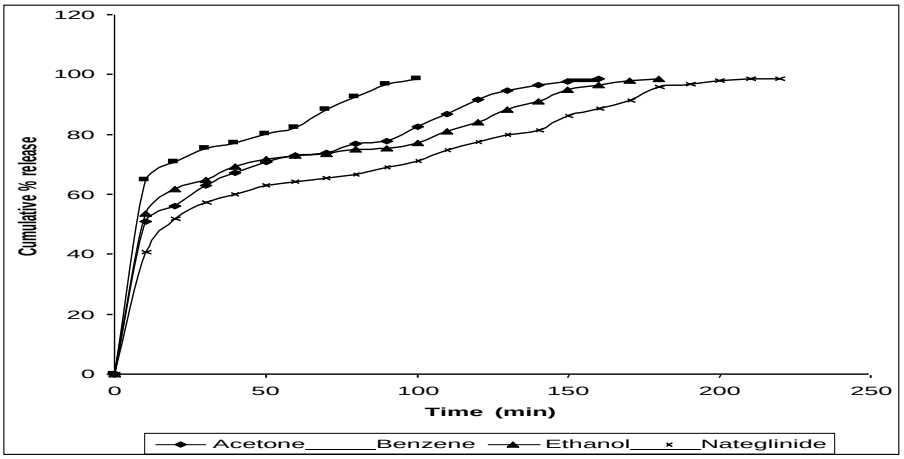

Figure 6: Dissolution profiles of nateglinide recrystallized from: (a) acetone; (b) benzene ; (c) ethanol; (d) nateglinide and kept at elevated temperature (40oC), 75% RH for one month

Table 5: Plasma concentration profiles of Nateglinide and its crystal forms obtained from benzene, ethanol and PVP

TIME	DRUG(µg/ml)	BENZENE(µg/ml)	ETHANOL(µg/ml)	SCPVP(µg/ml)
0	0	0	0	0
0.5	0.359 ± 0.027	0.452 ± 0.032	0.632 ± 0.027	0.624± 0.026
1	0.478 ± 0.016	0.828 ± 0.046	0.741 ± 0.016	0.957± 0.032
1.5	0.662 ± 0.023	0.847 ± 0.031	0.780 ± 0.028	0.936± 0.029
2	0.704 ± 0.026	0.808 ± 0.013	0.622 ± 0.023	1.008± 0.017
4	0.624 ± 0.015	0.658 ± 0.024	0.459 ± 0.015	0.732± 0.038
8	0.424 ± 0.039	0.560 ± 0.021	0.333 ± 0.033	0.613± 0.023
24	0.348 ± 0.012	0.208 ± 0.036	0.250 ± 0.022	0.355± 0.019

Dissolution Studies

The dissolution profile of Nateglinide and its modified crystals from different solvents was stored under ambient condition and kept at elevated temperature (40^{o} C) are shown in Figs.5.

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 Nateglinide. Especially, crystals obtained from benzene and SCPVP (2%w/v) show higher dissolution rate than untreated Nateglinide because of the better crystallinity of the modified crystals in these cases. Crystals obtained using only ethanol show lower dissolution rate than other crystals obtained (Method I). 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 the adsorption of

Table 6: Pharmacokinetic parameters of Nateglinide and its crystal forms obtained from benzene, ethanol and SCPVP

	DRUG	BENZENE	ETHANOL	SCPVP
C_{max}(µg/ml)	0.704 ± 0.036	0.847± 0.025	0.780 ± 0.042	1.008 ± 0.062
t_{max} (h)	1.00 ± 0.15	1.30 ± 0.18	1.30 ± 0.09	2.00 ± 0.12
AUC_{0-24} (µg h/ml)	10.26 ± 1.71	11.435 ± 1.53	7.833 ± 1.46	12.984 ± 1.67
K_e (h^{-1})	0.025313 ± 0.0021	0.060619 ± 0.0042	0.042783 ± 0.0013	0.032039 ± 0.0021
$t_{½}$ (h)	27.38 ± 0.42	11.4345 ± 0.28	16.20 ± 0.36	22.95 ± 0.89
$AUC_{0-\infty}$ (µg h/ml)	24.053 ± 2.13	14.822 ± 1.89	13.687 ± 1.65	24.742 ± 1.42

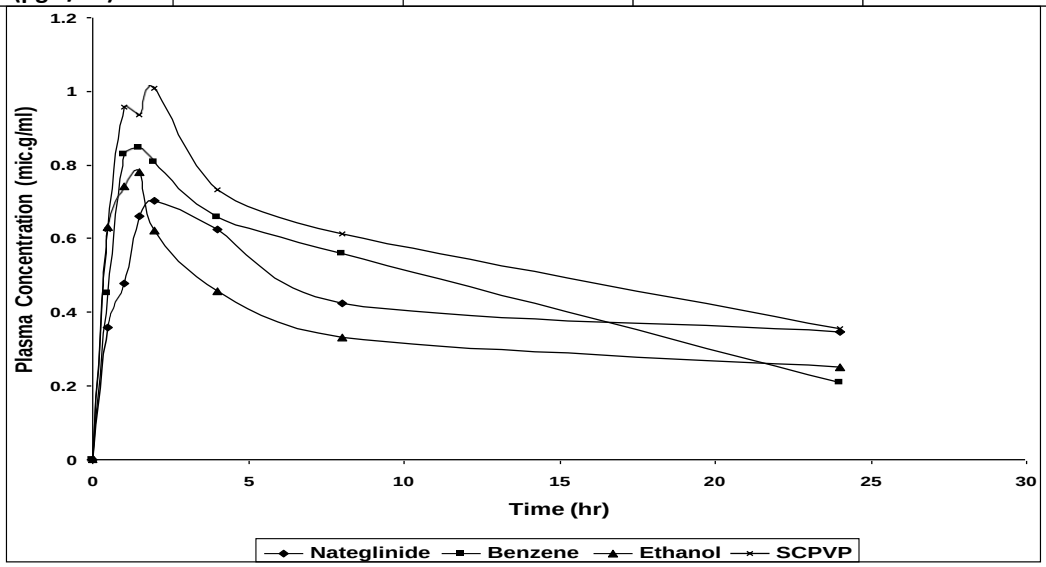

Figure 7: Plasma concentration profiles of Nateglinide and its crystal forms obtained from benzene, ethanol and PVP

surfactant and polymers on the crystal surface (Majumdar et.al 1998).

Accelerated stability studies

The results obtained in the stability test showed slight changes in dissolution rate and melting point for all samples under investigation. 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 (Fig.6).

Pharmacokinetic studies

The mean plasma concentration-time profiles after oral administration of Nateglinide, crystals obtained from benzene, ethanol and from ethanol with 2% solution of PVP. Compared to Nateglinide alone, the crystal forms tend to show higher plasma concentrations. The pharmacokinetic parameters of Nateglinide and the three crystal forms were evaluated and it was found that there were some significant differences in the C_{max}, T_{max}, AUC_{0-24} and K_e. In order to determine the relationship between the dissolution rate and the bioavailability, the dissolution profiles of nateglinide and its crystals were measured and the results with the exception that the dissolution rate of crystals from benzene and from SCPVP (2%w/v) showed higher dissolution rate followed by crystals from ethanol and Nateglinide. The order of dissolution rate matched that of the plasma concentration profile (Fig.7). The results of the *in vivo* study indicate that the higher plasma profile of Nateglinide obtained by the formation of new crystal habit with benzene and ethanol with 2%solution of PVP (SCPVP) mainly due to increased dissolution rate of the new crystal forms (Table 5 & 6).

SUMMARY AND CONCLUSION

In summary, it can be said that the crystallization conditions and the medium used have major effect on Nateglinide crystals habit modification under ambient conditions. The crystals showed significant changes in the shape, size, melting points, dissolution rate, XRD patterns, DSC curves, and slight changes in solubility. This suggests that the newly developed crystals of Nateglinide under ambient conditions exist in different crystalline modification facilitating significantly improved dissolution rate as compared to Nateglinide. But the stability study undertaken at 40° C and a relative humidity of 75% shows some physical changes like melting point and slight changes in dissolution rate probably due to some phase transitions but retaining the chemical identity. The effect of such changes in reality need be explored in actual situations if any. The order of dissolution rate matched that of the plasma concentration profile. The results of the *in vivo* studies indicate that the higher plasma profile of Nateglinide obtained by the formation of new crystal habit with benzene and ethanol with 2%solution of PVP (SCPVP)

mainly due to increased dissolution rate of the new crystal forms. Therefore, it can be safely concluded that the improvement in the dissolution rate would result in an improved bioavailability because the dissolution rate is the rate controlling step in drug absorption following the oral administration of a drug.

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