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Polymorphic Study of L-Arginine Salt of Ragaglitazar (DRF-2725)†

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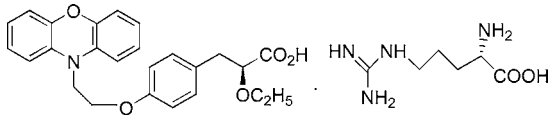
Abstract:

In the manufacturing process, during our attempts to get a suitable polymorph by reacting the aqueous solution of L-arginine with 3-[4-[2-(phenoxazin-10-yl) ethoxy]phenyl]-2-ethoxypropanoic acid (ragaglitazar) in different solvents, we have encountered various polymorphic modifications. These forms were characterized using differential scanning calorimetry (DSC), thermogravimetric analysis TGA, and powder X-ray diffraction (PXRD) techniques. The polymorphic forms of arginine salt of ragaglitazar (I) defined below is known to lower sugar level, lower total cholesterol (TC); increase high-density lipoprotein (HDL) and decrease low-density lipoprotein (LDL), which have a beneficial effect on type-II diabetes, coronary heart disease, and atherosclerosis.

Introduction

Molecules which activate PPAR (peroxisome proliferator-activated receptor)-γ are found to improve the blood glucose levels in type-II diabetes by an insulin-sensitizing mechanism.[1] On the other hand, the fibrates reduce triglycerides and increase HDL levels via activation of PPAR-α.[2]



(I)

During our intense search for molecules that activate both PPAR-α and PPAR-γ, arginine salt of ragaglitazar, was discovered.[3] This phenoxazine analogue of phenyl propanoic acid showed dual PPAR-α and -γ activity. It is chemically and pharmacologically different from presently marketed PPAR agonists such as rosiglitazone,[4] pioglitazone,[5] and troglitazone.[6] It is expected to be among the first to reach the market from a new generation of dual-acting sensitizers.[7]

The latest trend that has, of late, crept into the pharmaceutical industry is the studies on polymorphism in drugs and the difference in the potency of different polymorphic forms of a given drug.[8] Polymorphic forms would include different physical forms, crystalline and noncrystalline (amorphous) forms. This has especially become very interesting after observing that many antibiotics, antibacterials, tranquilizers, etc., exhibit polymorphism and that some or one of the polymorphic forms of a given drug exhibits superior bioavailability and consequently shows much higher activity compared to other polymorphs.[8] Ranitidine, sertraline, frentizole are some of the important examples of pharmaceuticals which exhibit polymorphism. Polymorphism in drugs is a topic of current interest and is evident from the host of patents being granted. To cite a few, U.S. 5,700,820[9] discloses six polymorphic forms of troglitazone, U.S. 5,248,699[10] discusses about five polymorphic forms of sertraline hydrochloride, while European Patent, EP 014590[11] describes four polymorphic forms of frentizole. European Patents, EP 490648[12] and EP 022527[13] also deal with the subject of polymorphism in drugs.

Experimental Section

The thermal properties of the polymorphs were characterized on a differential scanning calorimeter (Shimadzu

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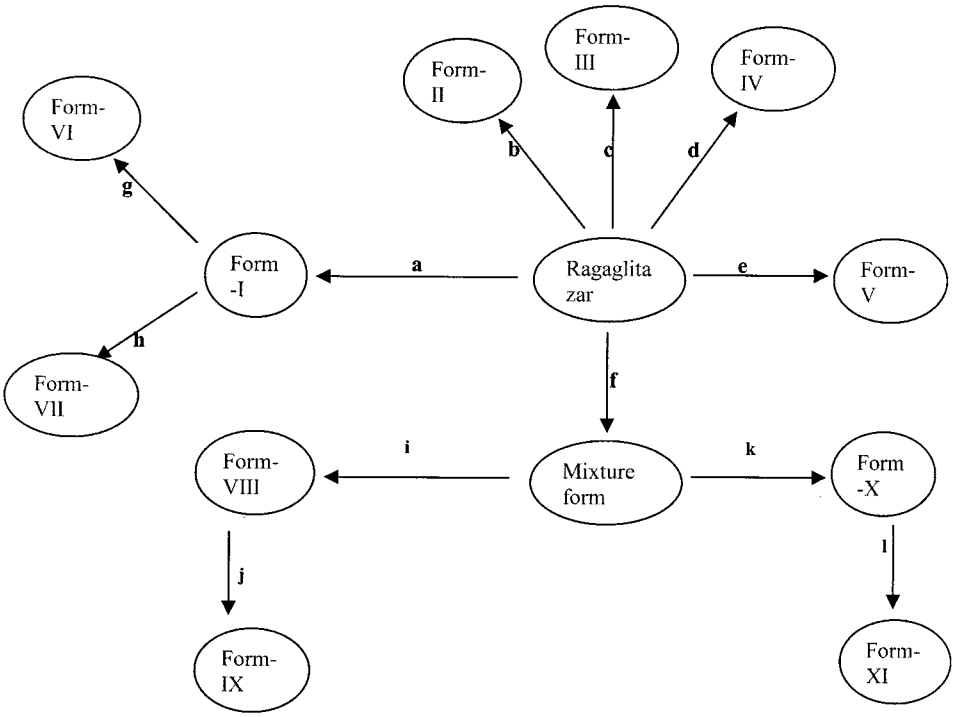
Table 1. **DSC and PXRD data of the polymorphic forms of L-arginine salt of ragaglitazar**

form	DSC peak temperature (°C)[a,b]	2θ values (in deg) from PXRD data
I	181↓	8.18, 12.40, 16.66, 18.80, 19.44, 22.32, 22.84, 23.10, 23.50, 24.72, 29.84
II	131↓, 166↓170↑ and 179↓	6.78, 11.5, 12.08, 16.44, 19.34, 22.30, 22.72, 24.40, 26.66
III	100↓, 164↓ 168↑, 182↓	6.80, 12.10, 15.84, 17.02, 19.40, 22.32, 22.68, 24.38, 26.36
IV	150↓, 165↓, 172↑ 186↓	6.78,12.66, 15.96, 16.54, 19.34, 22.78, 24.42, 26.70, 31.70
V	120↓, 165↓, 173↓, 174↑, 186↓	6.76, 12.10, 15.96, 17.00, 18.50, 19.40, 22.38, 22.44, 24.44, 26.30
VI	78↓ 158↑ 179↓, 184↓	no diffraction peaks due to its amorphous nature
VII	133↑, 177↓, 184↓	no diffraction peaks due to its amorphous nature
VIII	153↓, 158↑, 178↓	4.16, 11.02, 15.94, 19.50, 20.22, 22.22, 27.38
IX	177↓	8.20, 12.42, 16.66, 18.80, 19.44, 22.30, 23.08, 27.38, 28.48, 29.84
X	163↑,185↓	no diffraction peaks due to its amorphous nature
XI	185↓	7.38, 7.56, 11.90, 12.32, 14.80, 16.40, 19.58, 20.48, 22.34, 22.90, 23.54
mix	181↓, 185↓	8.16, 12.40, 16.64, 18.78, 19.42, 22.34, 22.80, 23.08, 29.84

[a] ↓ denotes endotherm in DSC. [b] ↑ denotes exotherm in DSC.

Scheme 1. **Graphical presentation of various forms**



a	L-arginine/ethanol/water
b	L-arginine/acetone/water
c	L-arginine/1, 4 dioxane/water
d	L-arginine/DMSO/water
e	L-arginine/DMF/water
f	L-arginine/Isopropanol/water
g	Dissolve in water and freeze drying
h	Dissolve in methanol and evaporation
i	1, 4 Dioxane, Reflux
j	Isopropanol, Reflux
k	Heat to 185°C and cool to room temperature
l	Heat to 175°C and cool to room temperature

DSC-50) and thermogravimetric analyzer (Shimadzu TA-50). The thermograms were recorded under nitrogen atmosphere at a heating rate of 5 °C/min. X-ray powder diffraction patterns were recorded on a Rigaku D/Max-2200 model diffractometer equipped with horizontal goniometer in $\theta/2\theta$ geometry. Copper Kα (λ = 1.5418 Å) radiation was used, and the sample was scanned between 3 and 45° 2θ. The differential scanning calorimetry (DSC) and powder X-ray diffraction (PXRD) data for these forms are summarized in the Table 1.

Preparation of Form I. To a solution of ragaglitazar (1 g) in ethanol (25 mL) heated to 45−50 °C was added L-arginine (0.415 g) dissolved in water (1.2 mL) slowly with constant stirring. After the completion of the addition, the reaction mixture was refluxed for 10−12 h. The reaction mixture was cooled to 45−50 °C. The white crystalline precipitate formed was filtered and dried under vacuum (1 mmHg) at 60−65 °C for 10−12 h to yield Form I of L-arginine salt of ragaglitazar (1.15 g).

Preparation of Form II. To a solution of ragaglitazar (1 g) in acetone (25 mL) was added L-arginine (0.415 g) dissolved in water (1.2 mL) slowly with constant stirring. The reaction mixture was stirred at room temperature for 24 h. The white crystalline precipitate formed was filtered and dried under vacuum at 40−45 °C for 6−8 h to yield Form II of L-arginine salt of ragaglitazar (1.29 g).

Preparation of Form III. To a solution of ragaglitazar (1 g) in 1,4-dioxane (25 mL) was added L-arginine (0.415 g) dissolved in water (1.2 mL) slowly with constant stirring. The reaction mixture was stirred at room temperature for 24 h. The white crystalline precipitate formed was separated and dried under vacuum at 40−45 °C for 6−8 h to yield Form III of L-arginine salt of ragaglitazar (1.25 g).

Preparation of Form IV. To a solution of ragaglitazar (1 g) in DMSO (10 mL) was added L-arginine (0.415 g) dissolved in water (1.2 mL) slowly with constant stirring. The reaction mixture was stirred at room temperature for 24 h. The white crystalline precipitate formed was separated and dried under vacuum at 40−45 °C for 6−8 h to yield Form IV of L-arginine salt of ragaglitazar (1.3 g).

Preparation of Form V. To a solution of ragaglitazar (1 g) in DMF (10 mL) was added L-arginine (0.415 g) dissolved in water (1.2 mL) slowly with constant stirring. The reaction mixture was stirred at room temperature for 24 h. The white crystalline precipitate formed was separated and dried under vacuum at 40−45 °C for 6−8 h to yield Form V of L-arginine salt of ragaglitazar (1.17 g).

Preparation of Form VI. Polymorphic Form I of L-arginine salt of ragaglitazar (1 g) was dissolved in water (10 mL) and freeze-dried to yield Form VI of L-arginine salt of ragaglitazar, as an amorphous white powder (0.95 g).

Preparation of Form VII. Polymorphic Form I of L-arginine salt of ragaglitazar (1 g) was dissolved in methanol (25 mL) and evaporated under vacuum to yield Form VII of L-arginine salt of ragaglitazar, as an amorphous white powder (0.9 g) which has the characteristics given earlier.

Preparation of Form VIII. A mixture of two forms of L-arginine salt of ragaglitazar (1 g) was refluxed in 1,4-dioxane (10 mL), filtered, and dried under vacuum to yield Form VIII of L-arginine salt of ragaglitazar which has the characteristics given earlier.

Preparation of Form IX. Polymorphic Form VIII of L-arginine salt of ragaglitazar (1 g) was refluxed in 2-propanol (10 mL), filtered and dried under vacuum to yield Form IX of L-arginine salt of ragaglitazar.

Preparation of Form X. A polymorphic mixture form of L-arginine salt of ragaglitazar was heated to 185 °C and cooled to room temperature to yield Form X of the L-arginine salt of ragaglitazar.

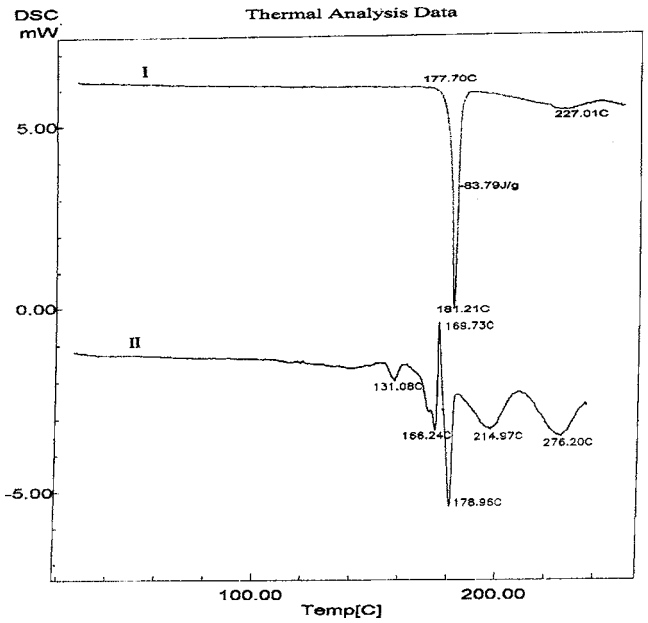


Figure 1. **Overlayed DSC thermograms of Forms I and II.**

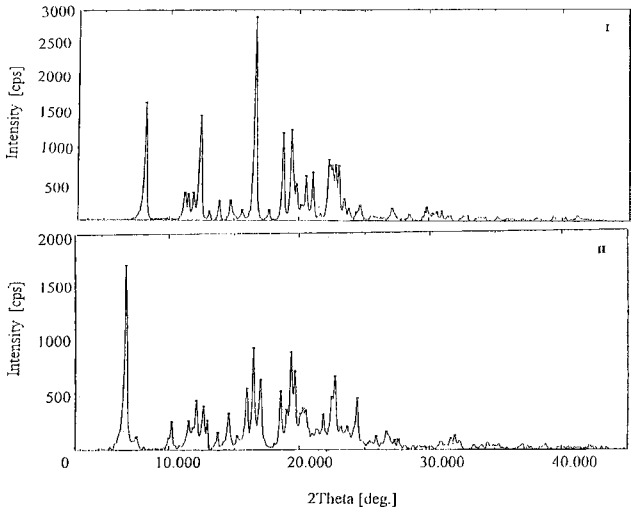


Figure 2. **Powder XRD pattern of Forms I and II.**

Preparation of Form XI. Polymorphic Form X of L-arginine salt of ragaglitazar was heated to 175 °C and cooled to room temperature to yield Form XI of the L-arginine salt of ragaglitazar which has the characteristics given earlier.

Preparation of Mixture Form. To a solution of ragaglitazar (1 g) in 2-propanol (25 mL) was added L-arginine (0.415 g) dissolved in water (1.2 mL) slowly with constant stirring. The reaction mixture was stirred at room temperature for 24−40 h. The white crystalline powder formed was filtered and dried at 30−35 °C for 4−8 h to yield the mixture form of the L-arginine salt of ragaglitazar (1.05 g).

Results and Discussion

During our attempts to get a suitable polymorph by reacting the aqueous solution of L-arginine with ragaglitazar

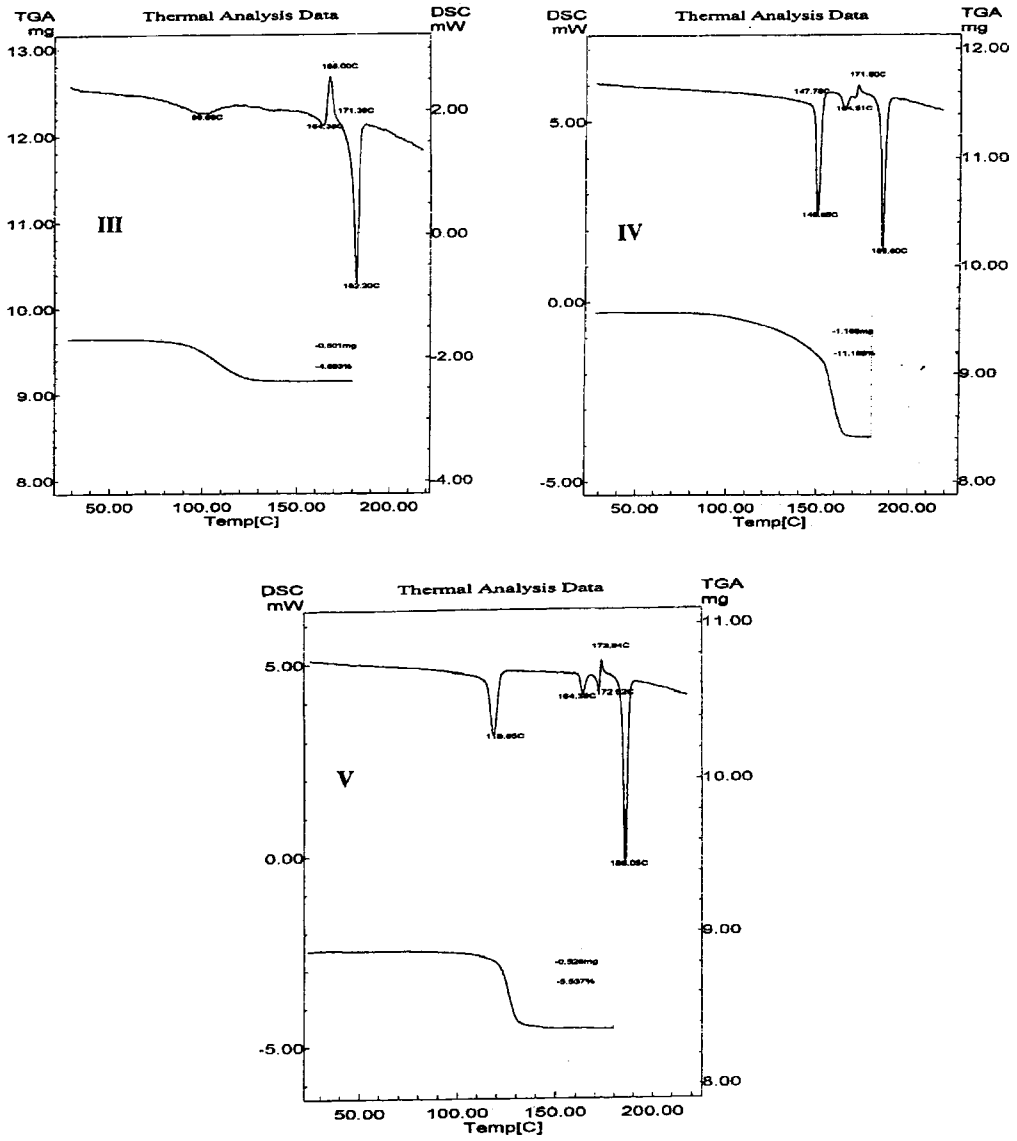


Figure 3. **Overlayed DSC and TG thermograms of Forms III, IV, and V.**

in different solvents, we have encountered various polymorphic modifications. All these polymorphic forms were proved to be identical in solution as evident from NMR, UV, and mass spectral data. These forms were characterized using techniques such as DSC, thermogravimetric analysis (TGA), and PXRD. These solid-state techniques revealed the difference among these forms. Among these forms, six were solvent free, three were solvates, and three were amorphous in nature.

The forms presented in the following sections are the outcome of the standard inhouse strategy followed for polymorph screening applied to new molecular entities. The processes for producing these forms are well established and patented.[14] During these studies no attempts have been made to study the effect of seeding the supersaturated solution of ragaglitazar·Arg with different forms.

The formation of various morphs and their inter-relationships are depicted in Scheme 1.

The thermoanalytical data of the various forms are depicted in Figures 1, 3, 5, 7, 9. The powder XRD data are shown in Figures 2, 4, 6, 8, 10.

Form I has been obtained from ethanol with acceptable solid-state properties for an API. The form was a free-flowing crystalline solid with a characteristic melting endotherm at 181 °C in DSC (Figure 1). This form can also be obtained from methanol, 2-propanol, and acetonitrile.

Though Form I could be obtained by the method referred above, many a time a material with following characteristics was obtained. The PXRD would match with those of Form I. On the other hand, the DSC thermogram will have an additional endotherm at 185 °C in addition to the characteristic melting endotherm at 181 °C. This would probably indicate that the material on hand is a mixture of Form I and an amorphous form which melts at 185 °C and which does not contribute to X-ray diffraction. This impure form is henceforth referred as mixture form.

During our attempts to obtain a stable polymorph with better stability and free-flowing, particle size, and surface

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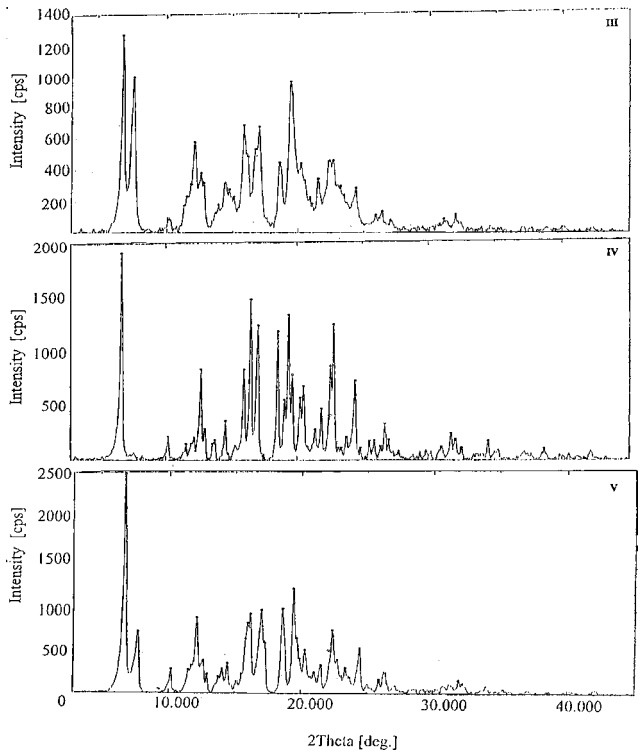

Figure 4. **Powder XRD pattern of Forms III, IV, and V.**

properties, we have encountered the following novel polymorphic forms.

The Form II obtained from acetone is also crystalline in nature as evidenced by PXRD data. DSC thermogram (Figure 1) displays two endotherms at 131 and 166 °C followed by an exothermic peak at 170 °C in addition to its melting endotherm at 179 °C.

Form III is obtained from 1,4-dioxane. DSC thermogram (Figure 3) displays a small endotherm at 100 °C, an exotherm at 168 °C followed by a melting endotherm at 182 °C. The initial endotherm at 100 °C could be due to the loss of solvent. The exotherm at 168 °C could be due to crystallization of desolvated phase, and this crystallized material melts at 182 °C. TG data show a weight loss of 4.7% in the temperature range of 70−120 °C, corresponding to the first endotherm in DSC. This weight loss corresponds to 0.3 mol of 1,4-dioxane. Powder X-ray diffractogram (Figure 4) shows the sample to be crystalline in nature as evidenced by diffraction peaks. However, the presence of significant amount of amorphous substance is indicated by the large background noise in the diffractogram.

Form IV was obtained from dimethylsulphoxide (DMSO). DSC thermogram (Figure 3) shows a major endotherm at 150 °C, a minor endotherm at 165 °C, followed by an exotherm at 172 °C followed by a melting endotherm at 186 °C. In the temperature range of the first endotherm (120−170 °C), a weight loss of 11.2 % in the TG analysis corresponds to 1 mol of dimethylsulphoxide in the lattice. This material is crystalline in nature as seen in PXRD (Figure 4). Good-quality single crystals of this pseudomorph, suitable

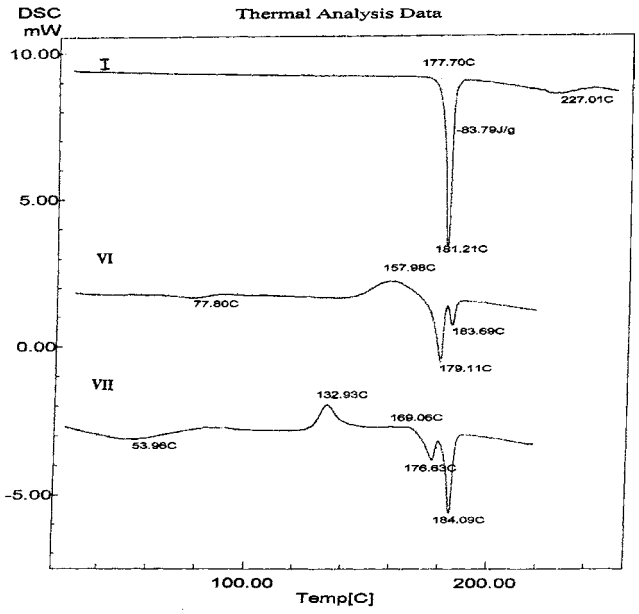

Figure 5. **Overlayed DSC thermograms of Forms I, VI, and VII.**

for X-ray diffraction, have been obtained, and the X-ray structure has been solved.[15]

Form V was obtained from dimethylformamide (DMF). DSC thermogram (Figure 3) shows a major endotherm at 120 °C, two minor endotherms at 165 and 172 °C, an exotherm at 174 °C followed by a melting endotherm at 186 °C. In the temperature range of the first endotherm (110−140 °C), a weight loss of 5.54 % in the TG analysis corresponds to 0.5 mol of dimethylformamide in the lattice. This material is crystalline in nature as indicated by PXRD data (Figure 4).

Form VI has been prepared by freeze-drying the aqueous solution of Form I of the ragaglitazar·Arg. The DSC thermogram (Figure 5) showed an endotherm at 78 °C, which could be due to the presence of traces of water. The exotherm at 158 °C could be attributed to crystallization of amorphous material. PXRD data (Figure 6) also confirm the amorphous nature of the form. On further heating, two endotherms at 179 and 184 °C are observed.

It is interesting to note that these two endotherms match reasonably well with those of the mixture form.

Form VII was made by quick stripping of methanol from the methanolic solution of Form I of ragaglitazar·Arg. This form also turned out to be amorphous (Figure 6) with a thermal profile (Figure 5) of broad endotherm at 54 °C, exotherm at 133 °C, followed by two endotherms at 177 and 185 °C. Unfortunately, this form was also not acceptable for further use due to its fluffy and less dense nature.

At this juncture of failure to realize a better polymorph, attempts were made to convert the mixture form to the Form I by following methods. Here again, all the following

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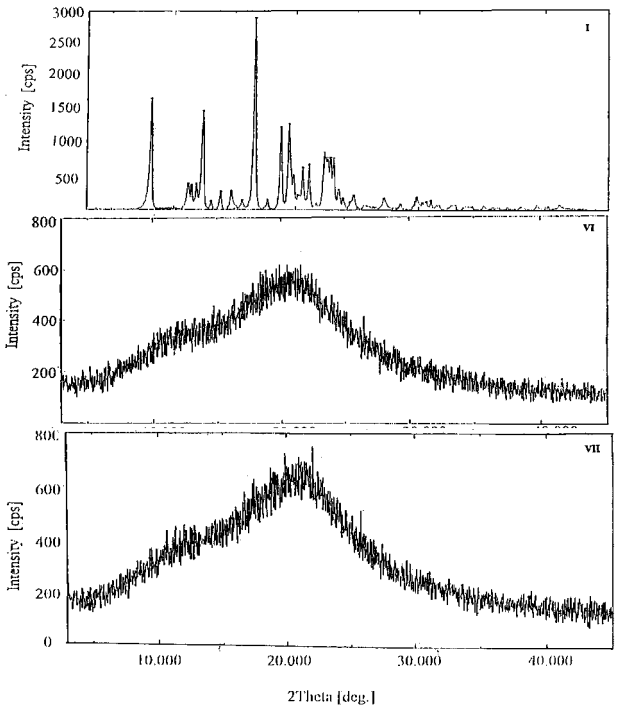

Figure 6. **Powder XRD pattern of Forms I, VI, and VII.**

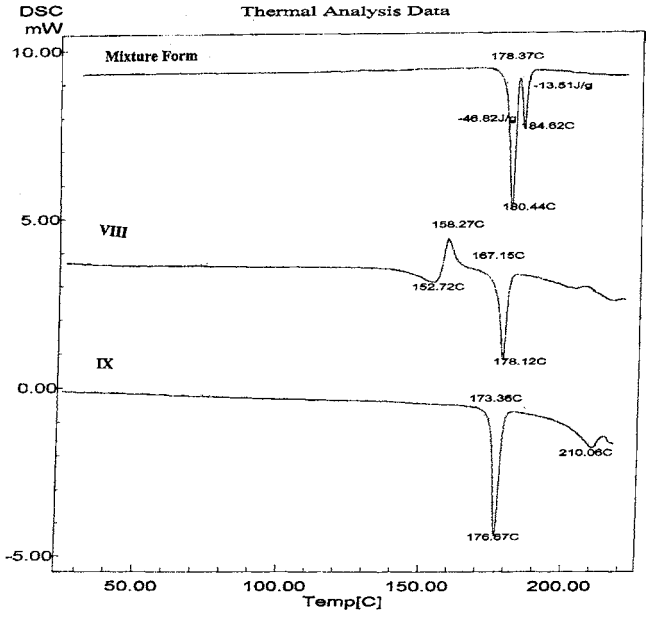

Figure 7. **Overlayed DSC thermograms of mixture form and Forms VIII and IX.**

attempts lead to new polymorphic forms instead of the desired Form I.

Refluxing the mixture form in 1,4-dioxane lead to Form VIII, which shows a minor endotherm at 153 °C followed by an exotherm at 158 °C in addition to the melting endotherm at 178 °C (Figure 7). It is crystalline in nature (Figure 8).

With a fond hope of getting Form I, Form VIII was refluxed in 2-propanol only to yield Form IX. It shows a melting endotherm at 177 °C (Figure 7). The PXRD data (Figure 8) of this form closely resembles with those of Form I. However the intensity of the peak at about 30° (2θ) in the

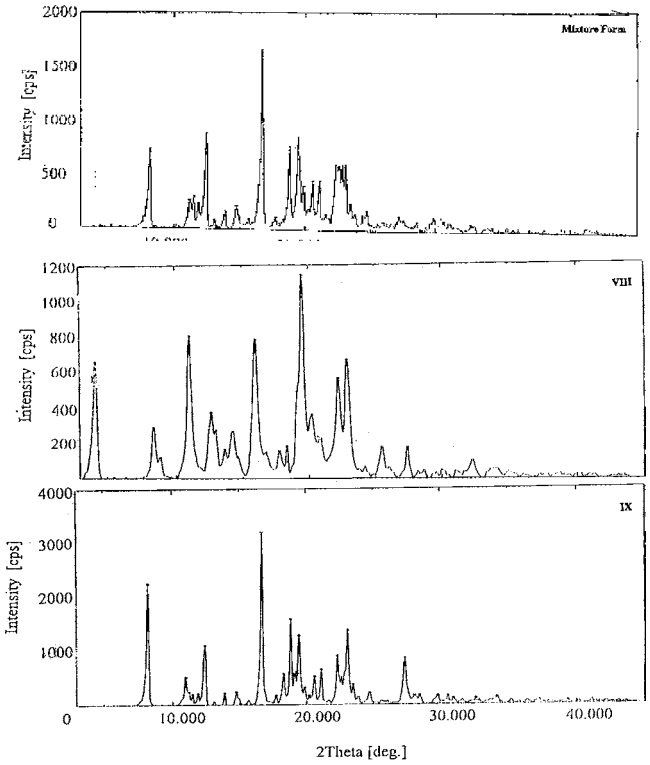

Figure 8. **Powder XRD pattern of mixture form and Forms VIII and IX.**

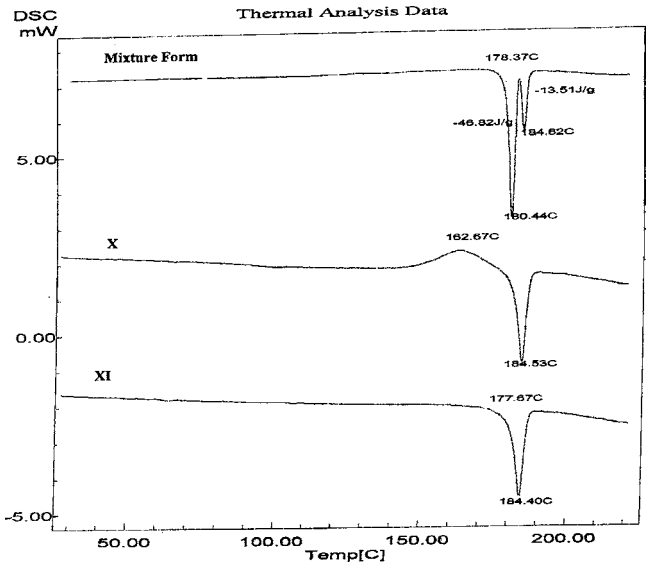

Figure 9. **Overlayed DSC thermograms of mixture form and Forms X and XI.**

XRD data is significant in this form when compared to Form I. Further, careful examination of IR data (Figure 11) reveals that this form displays a peak at 1581 cm^{-1}, while Form I has a peak at 1709 cm^{-1}. These characteristic differences qualify this form to be different from Form I.

Form X is obtained by heating the mixture form in solid state to 185 °C and cooling to room temperature. The XRD data shows that this form is amorphous in nature (Figure 10). The DSC thermogram displays a broad exotherm at 163 °C followed by a melting endotherm at 185 °C (Figure 9). The initial exotherm can be rationalized in terms of

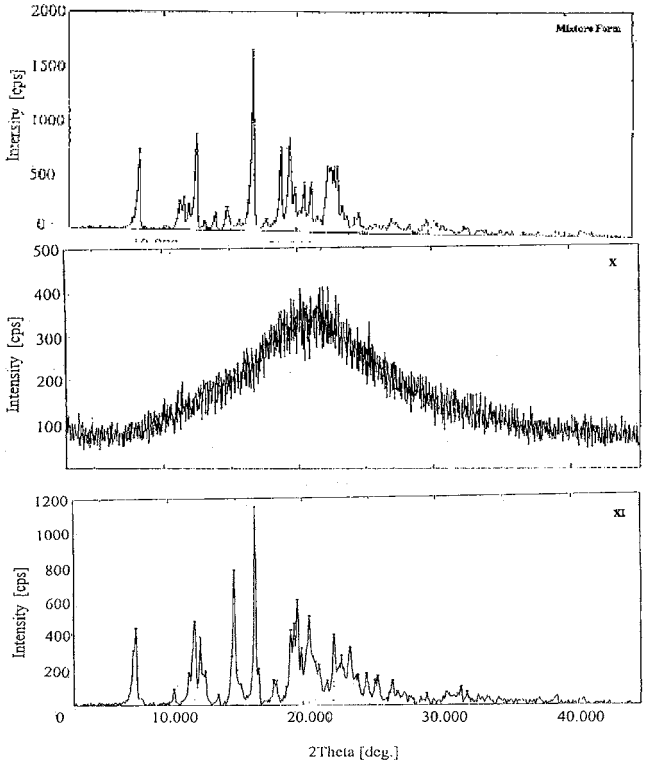

Figure 10. **Powder XRD pattern of mixture form and Forms X and XI.**

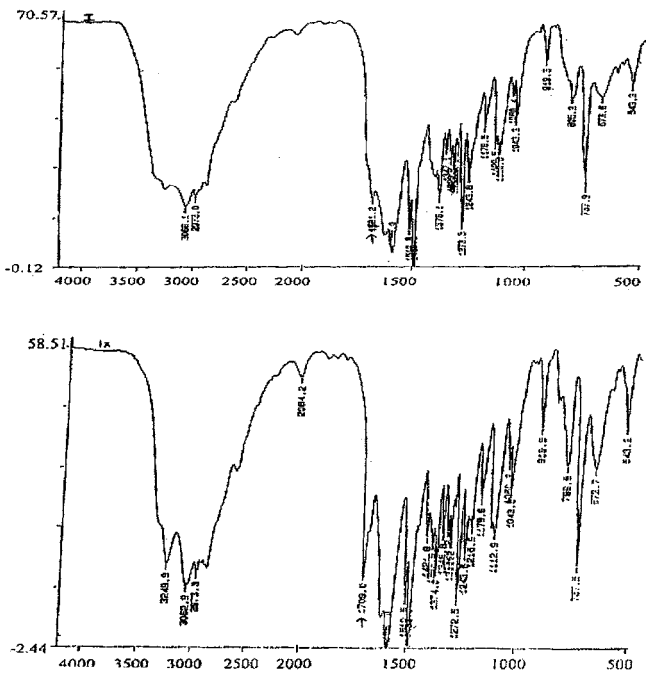

Figure 11. **IR Patterns of Forms I and IX.**

crystallization of the amorphous material followed by the melting of the crystallized material. To test this hypothesis, this form was heated to ~175°C (i.e. the point of completion of the initial exotherm of Form X). As anticipated, this thermal treatment yields a crystalline Form XI, which melts at 184 °C (Figure 9). This crystalline form also turned out to be different from that of Form I (Figure 10).

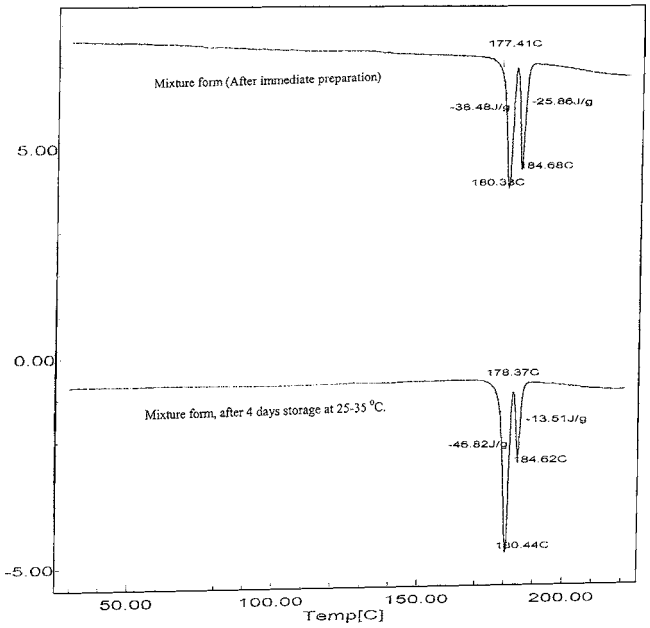

Figure 12. **Overlayed DSC thermograms of mixture form collected at day 0,4 at room temperature.**

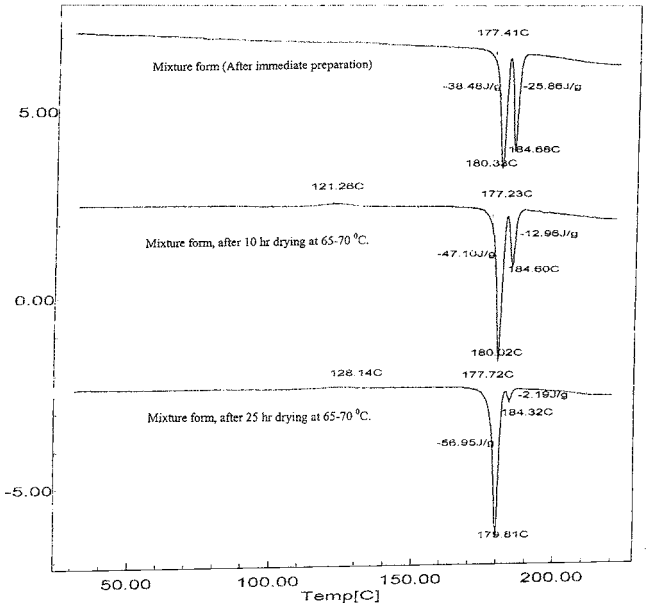

Figure 13. **Overlayed DSC thermograms of mixture form collected 0, 10 and 25 h at 65−70 °C under vacuum.**

As all the rational attempts described above have failed, the only option open was to critically examine the sequence of events in the process of preparing Form I. During this exercise, certain interesting observations were made. DSC data of the mixture form collected on day 0 and 4 at room temperature are compared (Figure 12). The data clearly indicate that the second endotherm at 185 °C disappears with time, rather slowly, at ambient conditions. Perhaps the rate of disappearance could be hastened by drying the salt at a suitably higher temperature range, preferably under vacuum. Interestingly, when the wet ragaglitazar·Arg was dried at 65−75 °C under vacuum (1 mmHg), the second endotherm at 185 °C wa reduced drastically over a period of 25 h (Figure 13). These facts clearly indicate that the undesirable

form characterized by the second endotherm at 185 °C is an unstable polymorph, which disappears with time and temperature. It is worth mentioning that the vanishing peak at 185 °C does not reappear on standing at ambient conditions. Hence, it is reasonable to conclude that this unstable (and undesirable) form has a monotropic relationship with the desirable Form I.

Conclusions

Twelve polymorphs of arginine salt of ragaglitazar were prepared by different methods. Among them, six were solvent free, three were solvates, and three were amorphous. A robust process was designed to obtain the desired stable polymorph (Form I) with acceptable quality. All the forms encountered were characterized by DSC, TG, and PXRD.

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