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(54) Title: ORAL COMPOSITIONS CONTAINING TACROLIMUS IN AMORPHOUS FORM

(57) Abstract: Fast release or modified release oral compositions containing as the active principle Tacrolimus and/or derivatives thereof in amorphous form, and: a) a "solubilising agent" of the active principle suitable to maintain it in the amorphous form over time, b) at least one "diluting agent" having the function to stabilize the active principle in the amorphous form, c) at least one agent being "modulator" of the dissolution rate of the active principle from the pharmaceutical form. These compositions in form of paste, granules, powder, solution, suspension can be formulated in hard or soft gelatine capsules or can be formulated in other pharmaceutical forms for oral use. These compositions are characterized in that all the relative operations of preparation are carried out in the absence of water and/or organic solvents.

ORAL COMPOSITIONS CONTAINING TACROLIMUS IN AMORPHOUS FORM

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Field of the invention

The present invention relates to fast or controlled-release oral formulations containing as active principle Tacrolimus or its derivatives in amorphous form.

Background of the invention

Tacrolimus (also known as **FK-506**) is an immunosuppressant agent that acts through inhibition of T cells activation, binding to protein 12 and forming a complex that blocks the activity of serine/threonine phosphatases.

Tacrolimus is a macrolide isolated from a fermentation broth of *Streptomyces tsukubaensis* and whose main use is to reduce the activity of the patient's immune system after allogeneic organ transplant and therefore the risk of organ rejection. Its activity is similar to cyclosporin, but is more potent in equivalent doses. As cyclosporin, it has a wide range of adverse interactions, including that with grapefruit juice that increases its concentration in plasma. It is also used in a topical preparation in the treatment of severe atopic dermatitis ("eczema"), severe refractory uveitis after bone marrow transplants, and the skin condition vitiligo.

It is marketed by Astellas with the tradename Prograf®. It was approved by *FDA* (US Food and Drug Administration) in 1994 for liver transplants and its use has been extended to include kidney, heart, small bowel, pancreas, lung, trachea, skin, cornea, bone marrow, and limb transplants.

In Europe Prograf (Astellas) was authorised for the first time in France on August 21, 1995.

Nevertheless the molecule has low bioavailability by the oral route (no more than 15-20% of the administrated dose) mainly due to its poor solubility in water (3.6 µg/ml at 25°C), therefore several patents disclose methods to improve its bioavailability.

For example US Patent No. 5,260,301 describes the preparation of Tacrolimus solution in ethanol.

In US2006177500 the preparation of solid dispersions are described with high dissolution rates and increased bioavailability due to the presence of a surfactant with function both of a carrier or vehicling agent for Tacrolimus and of dissolution

enhancer in aqueous medium. The surfactant has a value of hydrophilic lipophilc balance (HLB) higher than or equal to 7 and it is solid at room temperature. This surfactant is selected from sodium Lauryl Sulfate (HLB=40), or poloxamers with HLB values higher than or equal to 7, esters of fatty acids and sucrose with HLB values between 7 and 18. In the mentioned invention Tacrolimus and the surfactant are preferably present in ratios from 1:0.1 to 1:100, more preferably from 1:3 to 1:50.

The solid dispersion in all cases is obtained by dispersing Tacrolimus and solid surfactant in an organic solvent followed by removing the solvent under vacuum and obtaining the powder by spray drying or fluid bed granulation.

WO2006/062334 describes the preparation of micro-emulsions for oral use containing completely dissolved Tacrolimus, a surfactant, a co-surfactant, an oil and an organic acid with improved stability and high bioavailability.

Hard or soft gelatine capsules are preferably filled with these microemulsions.

It is known that Tacrolimus in amorphous state has improved physical characteristics with respect to the crystalline form, namely higher dissolution rate and superior bioavailability that in rat is 7.5 times higher than that of the crystalline form (Improvement by solid dispersion of the bioavailability of KRN633, a selective inhibitor of VEGF receptor-2 tyrosine kinase, and identification of its potential therapeutic window", Kazuhide Nakamura et al, *Mol Cancer Ther.* **2006**, *5*, 80-88, 2006 American Association for Cancer Research Naoki Matsunaga).

Processes to obtain oral pharmaceutical forms containing Tacrolimus in amorphous state with improved solubility and dissolution rates have been disclosed.

WO20066083486 describes a method to obtain Tacrolimus in amorphous state and the preparation of tablets containing Tacrolimus in amorphous state. In this document the conversion of crystaline Tacrolimus into the amorphous form is carried out by dissolving the active principle in an organic solvent such as acetone, ethyl acetate, methanol, ethanol, acetonitrile and other organic solvents and mixtures thereof followed by evaporation of the solvents,

WO2006083130 describes oral solid formulations containing Tacrolimus in amorphous form obtained by dissolving the active principle in an organic solvent

and mixing the solution thus obtained with a dispersion or solution in an organic solvent of cyclodextrins and an organic acid followed by final removal of solvents by spray drying.

The dispersions described in this application can be formulated as powders, granules, tablets or charged into hard or soft gelatine capsules or as coated formulations.

In conclusion also for these two prior art documents the use of organic solvents is necessary not only to manufacture the target formulations but also to obtain Tacrolimus in amorphous form.

The use of organic solvent in production processes implies issues related to their toxicity and, in several cases, also high in inflammability, moreover further dangers are connected to the removal of these solvents generally carried out via evaporation.

Therefore there is a need to overcome the aforesaid drawbacks.

SUMMARY OF THE INVENTION

The applicant has now found a process to prepare fast or delayed-release oral pharmaceutical compositions containing Tacrolimus in amorphous form comprising:

a) a "solubilizing agent" of the active principle suitable to maintain it in the amorphous form over an extended period of time

b) at least one "diluting agent" having the function to stabilize the active principle in amorphous form

c) at least one agent "modulating" the rate of dissolution of the active principle from the pharmaceutical form.

The compositions of the present invention can be formulated in the form of hard or soft gelatine capsules, in the form of powder, granules, paste, solution or suspension which can be splitted up into said hard or soft gelatine capsules, or can be formulated in other pharmaceutical forms for oral administration.

The above compositions are characterised in that all phases of their preparation are carried out in the absence of water and/or organic solvents.

Therefore, the present invention further relates a process for preparation of pharmaceutically acceptable capsules according to the present invention without

using water and/or organic solvents. Moreover the method of preparation described does not comprise the use of surfactants and organic acids.

In particular, the process of the present invention comprises the following steps:

A) Melting the lipid component formed by the solubilizing agent and by the diluting agent(s) and by the modulating agents at a temperature comprised between 40°C e 80°C, and dispersing Tacrolimus in the molten mass under mild stirring until complete dissolution of the active principle

B) Dispersing the molten lipidic mass coming from step (A) in the technological adjuvants at room temperature until said mass is completely adsorbed and homogenised,

C) sizing the mixture prepared in step (B) by sieving.

D) Charging the mixture into hard gelatine capsules

For the preparation of hard or soft gelatine capsules containing a paste, a solution or a suspension, step A) can be followed immediately by step D).

DESCRIPTION OF THE FIGURES

Figure 1 represents the graphic of the dissolution profile of Tacrolimus in an aqueous medium, reporting in ordinates the % by weight of released Tacrolimus and in abscissae the time in minutes, from hard gelatine filled with oral compositions in the form of granules prepared as described in example 1 (without modulating agent) in examples 2-4 with the modulating agent Gelucire 39/01 and compared with the commercial form PROGRAF 5 mg.

Figure 2 represents the graphic of the dissolution release profile of Tacrolimus in an aqueous medium, reporting in ordinates the % by weight of released Tacrolimus and in abscissae the time in minutes, from hard gelatine capsules filled with oral compositions in the form of a granulate prepared as described in Example 1 (without modulating agent) and in Examples 5-7 in the presence of modulating agent Precirol ATO5 as described in Examples 5-7 and compared with the corresponding release of the commercial form PROGRAF 5 mg.

Figure 3 represents dissolution profile of Tacrolimus in an aqueous medium, reporting in ordinates the % by weight of released Tacrolimus and in abscissae the time in minutes, from hard gelatine capsules filled with the oral compositions in the form of a paste prepared as described in Example 8, in the absence of a

modulating agent, and with the modulating agent Gelucire 39/01 as described in the Examples 9-11 and compared with the commercial form PROGRAF 5 mg.

Figure 4 represents the graphic of the dissolution profile in an aqueous medium of Tacrolimus, reporting in ordinates the % by weight of released Tacrolimus and in abscissae the time in minutes, from capsules of hard gelatine filled with the oral compositions in the form of a paste prepared as described in Example 8 in the absence of modulating agent and with modulating agent Precirol ATO5 as described in Examples 12-14 and compared with the corresponding release of the commercial form PROGRAF 5 mg.

Figure 5 represents the graphic of the release profile of dissolution of Tacrolimus in an aqueous medium reporting in ordinates the % by weight of released Tacrolimus and in abscissae the time in minutes from hard capsule of gelatine filled with the oral composition in the form of a paste prepared as described in Example 15 in the absence of modulating agent Precirol ATO5 as described in the Examples 16-18 and compared with the corresponding release of the commercial form PROGRAF 5 mg.

Figure 6 represents the graphic of the dissolution profile of Tacrolimus in an aqueous medium, reporting in ordinates the % by weight of released Tacrolimus and in abscissae the time expressed in minutes, from hard gelatine capsule filled with the oral composition in the form of a paste prepared as described in Example 15 in the absence of modulating agent and with the modulating agent Gelucire 39/01 as described in examples 19-21 and compared with the commercial form PROGRAF 5 mg.

Figure 7 represents DSC analysis of crystalline Tacrolimus.

Figure 8 represents DSC analysis of Tacrolimus in oral solid composition in granular form charged into a hard gelatine capsule as described in example 3 and compared with DSC analysis of Tacrolimus in crystalline form.

Figure 9 represents DSC analysis of Tacrolimus in oral solid composition in granular form charged into a hard gelatine capsule as described in example 4 and compared with DSC analysis of Tacrolimus in crystalline form.

Figure 10 reports the DSC analysis of Tacrolimus in the oral composition in the form of a paste contained in the hard gelatine capsule as described in example 9 and compared with DSC analysis of Tacrolimus in crystalline form.

Figure 11 represents DSC analysis of Tacrolimus in oral solid composition in paste form contained in a soft gelatine capsule as described in Example 16 and compared with DSC analysis of Tacrolimus in crystalline form.

Figure 12 represents DSC analysis of Tacrolimus in oral solid composition in paste form charged into a soft gelatine capsule as described in Example 12 and compared with DSC analysis of Tacrolimus in crystalline form.

Figure 13 represents DSC analysis of a 1:1 (weight/weight) mixture of crystalline Tacrolimus and mannitol.

DETAILED DESCRIPTION OF THE INVENTION

The terms "active principle" or "active substance" in the oral compositions according to the present invention indicate Tacrolimus or its derivatives (such as (monohydrated Tacrolimus present in the final product in the amorphous state).

The terms "solubilizing agent" indicate a substance soluble in liquids and liquid at room temperature such as diethylenglycol monoethylether (as, for example, Transcutol HP, Trascutol P), polyethylenglycols (with average molecular weight preferably between 1000 and 20000 Da) and macroglycerides of liquid fatty acids (as for example Labrasol , a mixture of PEG-8 with capric o caprylic acid). All these substances are reported in pharmacopoeia and are considered non-toxic for oral use.

The term "diluting agent" indicates a substance with amphiphilic properties characterised by high HLB value: higher than 10, such as mixtures of macroglycerides of semi-solid C12-C18 fatty acids preferably by HLB value between 13 and 20, as for example Gelucire 44/14: mixtures of lauroyl esters of PEG32 with HLB= 14, Gelucire 50/13: mixtures of palmitostearyl esters of PEG 32 with HLB = 13 and therefore high capability to act as diluting agent for lypophilic substances.

The diluting agent imparts stabilising properties to the active principle Tacrolimus in the final dosage form while maintaining the same in the amorphous form.

With terms agent "modulating" the rate of dissolution of the active principle, for the purpose of the present invention, components are meant characterised by a low HLB value lower than 5, preferably between 1 and 2, such as mixtures of semi-solid esters of fatty C12-C18 acids with glycerol such as Gelucire 39/01 (with HLB=1), Precirol ATO5 that is a mixture of palmitin and stearin (triglycerides of palmitic and stearic acids), with HLB=2.

According to the present invention Tacrolimus is preferably present in the formulation in weight ratio with respect to the lipidic mass (consisting of the solubilizing, the diluting and the modulating agent(s) between1:1000 and 1:1, most preferably between 1:100 and 1:10. For the scope of this invention the solubilizing, the diluting and the modulating agent(s) are used respectively in weight ratios ranging from 1:1:1 to 1:0.05:0.05, more preferably from 1:0.6:0.6 to 1:0.4:0.4.

The production process may encompass the use of other excipients with technological functions preferably selected from dibasic calcium phosphate (for example Fujicalin), mannitol (for example Pearlitol 160C), lactose, pre-gelatinised maize starch, calcium silicate (for example Zeopharm 600), microcrystalline cellulose or mixtures thereof, antioxidants (vitamin E acetate and derivatives thereof, ascorbyl palmitate, sodium metabisulfite), lubricants ed antiadherents (for examples: talc, magnesium stearate, glycerol behenate).

The first step or step (A) of the production process is carried out by heating the solubilizing agent, the diluent(s), the modulating agent(s) at a temperature preferably between 50 and 65°C and dispersing Tacrolimus in the lipidic molten mass and stirring gently until the active principle is completely dissolved.

In step (B) the molten lipidic mass prepared in step (A), is dispersed in the mixture of technological adjuvants at room temperature, adding optionally lubricants, antiadherents or other excipients having technological uses, until obtaining complete absorption and homogenisation.

In step (C) the mixture prepared in step (B) is sized on a sieve of appropriate mesh.

In step (D) the sized mixture obtained in step (C) is splitted up into capsules using normal filling machines capable to fill capsules with either powders or paste according to the desired formulation to be obtained.

In the case of preparation of soft gelatine capsules, the apparatus employed is the standard machinery used for preparation of these pharmaceutical forms.

The following examples are reported of the preparation of some of the oral formulations according to the present invention for illustrative, but not limitative purpose.

The compositions reported hereunder contain, the same amount of "solvent" component, whereas overall decreasing amounts of diluting agent(s) and corresponding increasing amounts of the modulating agent(s) according to schemes reported in table 1-3.

Table1.

Hard gelatine capsules containing a granulated solid

Weight ratio: "solubilizing" component: diluent(s): modulating component(s) in the formulation

Formulation	"solubilizer"	"diluent"	"modulator"
F807RS 30(*)	1 (Transcutol HP)	1 (Gelucire 44/14)	0
F807RS32	1 (Transcutol HP)	0.75 (Gelucire 44/14)	0.25 (Gelucire 39/01)
F807RS33	1 (Transcutol HP)	0.5 (Gelucire 44/14)	0.5 (Gelucire 39/01)
F807RS34	1 (Transcutol HP)	0.25 (Gelucire 44/14)	0.75 (Gelucire 39/01)
F807RS36	1 (Transcutol HP)	0.75 (Gelucire 44/14)	0.25 (Precirol ATO5)
F807RS37	1 (Transcutol HP)	0.5 (Gelucire 44/14)	0.5 (Precirol ATO5)
F807RS38	1 (Transcutol HP)	0.25 (Gelucire 44/14)	0.75 (Precirol ATO5)

(*) comparative formulation without modulator

Table 2.

Hard gelatine capsules containing a paste

Weight ratio: "solubilizing" component: diluent(s): modulating component(s) in the formulation

Formulation	"solubilizer"	"diluent"	"modulator"
F807RS31(*)	1 (Transcutol HP)	3.5 (Gelucire 44/14)	0
F807RS35	1 (Transcutol HP)	2.625 (Gelucire 44/14)	0.875 (Gelucire 39/01)
F807RS40	1 (Transcutol HP)	1.75 (Gelucire 44/14)	1.75 (Gelucire 39/01)
F807RS41	1 (Transcutol HP)	0.875 (Gelucire 44/14)	2.625 (Gelucire 39/01)

F807RS46	1(Transcutol HP)	2.625 (Gelucire 44/14)	0.875 (Precirol ATO5)
F807RS47	1 (Transcutol HP)	1.75 (Gelucire 44/14)	1.75 (Precirol ATO5)
F807RS48	1 (Transcutol HP)	0.875 (Gelucire 44/14)	2.625 (Precirol ATO5)

(*) comparative formulation without modulator

Table 3.

Soft gelatine capsules

Weight ratio: "solubilizing" component: "diluent(s)": modulating component(s) in the formulation

Formulation	"solubilizer"	"diluent"	"modulator"
F807RS43(*)	1 (Transcutol HP)	3.5 (Gelucire 44/14)	0
F807RS39	1 (Transcutol HP)	2.625 (Gelucire 44/14)	0.875 (Gelucire 39/01)
F807RS44	1 (Transcutol HP)	1.75 (Gelucire 44/14)	1.75 (Gelucire 39/01)
F807RS45	1 (Transcutol HP)	0.875 (Gelucire 44/14)	2.625 (Gelucire 39/01)
F807RS49	1 (Transcutol HP)	2.625 (Gelucire 44/14)	0.875 (Precirol ATO5)
F807RS50	1 (Transcutol HP)	1.75 (Gelucire 44/14)	1.75 (Precirol ATO5)
F807RS51	1 (Transcutol HP)	0.875 (Gelucire 44/14)	2.625 (Precirol ATO5)

(*)comparative formulation without modulator

Example 1 (batch F807RS30) comparative formulation without modulator *(Hard gelatine capsules containing a granulated solid)*

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	25.55 mg
3. Gelucire 44/14	25.34 mg
4. Dibasic Calcium phosphate	67.20 mg
5. Mannitol	12.60 mg
6. Talc	4.20 mg
Total content in 1 capsule	**140.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**190.00 mg**

Production method

The production is carried out in four phases:

Phase I: melting of the lipid mass and dissolution of the active principle

Phase II: dispersion of the molten mass in the mixture of technological adjuvants

Phase III: sizing

Phase IV: splitting up into capsules

Description of process:

Phase I

All lipid components and the solubilising agent (Transcutol) are placed in a becker mantained in a thermostated container at 65°C.

The mixture is gently stirred until complete fusion of all the lipid components.

After melting, while stirring, monohydrated Tacrolimus is added.

The mixture is left under stirring until complete solubilisation of the active principle.

Phase II

The molten lipidic mass, containing the dissolved monohydrate Tacrolimus is dispersed in a second becker containing the mixture of solid diluents (manintol, basic calcium phosphate and talc) by manual stirring. The manual stirring is continued until complete dispersion of the lipidic mass.

Phase III

Once cooling is completed, the mixture obtained in Phase III is sized with a 500 micron sieve.

Phase IV

The sieve mixture is splitted up into hard gelatine capsules.

Example 2 (batch F807RS32)

(Hard gelatine capsules containing a granulated solid)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	25.55 mg
3. Gelucire 44/14	19.00 mg
4. Gelucire 39/01	6.34 mg
5. Dibasic calcium phosphate	67.20 mg
6. Mannitol	12.60 mg

7. Talc	4.20 mg
Total content in single capsule	**140.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**190.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 1

(lipids used in Phase I: excipients: 2, 3 and 4)

(technological adjuvants used in Phase III: excipients 5 and 6)

(antiadherent in phase IV: excipient 7)

Example 3 (batch F807RS33)

(Hard gelatine capsules containing a granulated solid)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	25.55 mg
3. Gelucire 44/14	12.67 mg
4. Gelucire 39/01	12.67 mg
5. Dibasic calcium phosphate	67.20 mg
6. Mannitol	12.60 mg
7. Talc	4.20 mg
Total content in 1 capsule	**140.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**190.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 1.

(lipids used in Phase I: excipients 2, 3, and 4)

(technological adjuvants used in Phase III: excipients 5 and 6)

(antiadherent in phase IV: excipient 7)

Example 4 (batch F807RS34)

(Hard gelatine capsules containing a granulated solid)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg

(Tacrolimus content	5.00 mg)
2. Transcutol HP	25.55 mg
3. Gelucire 44/14	6.34 mg
4. Gelucire 39/01	19.00 mg
5. Dibasic calcium phosphate	67.20 mg
6. Mannitol	12.60 mg
7. Talc	4.20 mg
Total content in 1 capsule	**140.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**190.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 1

(lipids used in Phase I: excipients 2, 3 and 4)

(technological adjuvants used in Phase III: excipients 5 and 6)

(antiadherent in phase IV: excipient 7)

Example 5 (batch F807RS36)

(Hard gelatine capsules containing a granulated solid)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	25.55 mg
3. Gelucire 44/14	19.00 mg
4. Precirol ATO5	6.34 mg
5. Dibasic calcium phosphate	67.20 mg
6. Mannitol	12.60 mg
7. Talc	4.20 mg
Total content in 1 capsule	**140.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**190.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 1.

(lipids used in Phase I: excipients 2, 3 and 4)

(technological adjuvants used in Phase III: excipients 5 and 6)

(antiadherent in phase IV: excipient 7)

Example 6 (batch F807RS37)

(Hard gelatine capsules containing a granulated solid)

Composition of the capsule content expressed in weight is as follows:

1. Monohydrated tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2.Transcutol HP	25.55 mg
3. Gelucire 44/14	12.67 mg
4. Precirol ATO5	12.67 mg
5. Dibasic calcium phosphate	67.20 mg
6. Mannitol	12.60 mg
7. Talc	4.20 mg
Total content in 1 capsule	**140.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**190.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 1

lipids used in Phase I: excipients 2, 3, and 4)

(technological adjuvants used in Phase III: excipients 5 and 6)

(antiadherent in phase IV: excipient 7)

Example 7 (batch F807RS38)

(Hard gelatine capsules containing a granulated solid)

Composition of the capsule content expressed in weight is as follows:

1.Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	25.55 mg
3. Gelucire 44/14	6.34 mg
4. Precirol ATO5	19.00 mg
5. Dibasic calcium phosphate	67.20 mg
6. Mannitol	12.60 mg
7. Talc	4.20 mg

Total content in 1 capsule	**140.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**190.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 1.

(lipids used in Phase I: excipients 2, 3 and 4)

(technological adjuvants used in Phase III: excipients 5 and 6)

(antiadherent in phase IV: excipient 7)

Example 8 (batch F807RS31) - comparative formulation without modulator *(Hard gelatine capsules containing a paste)*

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	43.30 mg
3. Gelucire 44/14	151.59 mg
Total content in 1 capsule	**200.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**250.00 mg**

Production method

The production is carried out in two phases:

Phase I: melting of the lipid mass and dissolution of the active principle

Phase II: filling of the capsule

Description of the process:

Phase I

All the lipid components are placed in a beaker maintained in a thermostated container at 65°C.

The whole is gently mixed until complete fusion of all the lipids. Once the fusion is completed monohydrated Tacrolimus is added. The mixture is left under stirring until complete solubilisation of the active ingredient.

Phase II

The molten mass is splitted up into hard gelatine capsules using a pipette Eppendorf (200 mg per capsule).

Example 9 (batch F807RS35)

(Hard gelatine capsules containing a paste)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	43.30 mg
3. Gelucire 44/14	113.67 mg
4. Gelucire 39/01	37.92 mg
Total content in 1 capsule	**200.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**250.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 8

Example 10 (batch F807RS40)

(Hard gelatine capsules containing a paste)

Composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2.Transcutol HP	43.30 mg
3. Gelucire 44/14	75.80 mg
4. Gelucire 39/01	75.80 mg
Total content in 1 capsule	**200.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**250.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 8

Example 11 (batch F807RS41)

(Hard gelatine capsules containing a paste)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	43.30 mg

3. Gelucire 44/14	37.92 mg
4. Gelucire 39/01	113.67 mg
Total content in 1 capsule	**200.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**250.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 8.

Example 12 (batch F807RS46)

(Hard gelatine capsules containing a paste)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	43.30 mg
3. Gelucire 44/14	113.67 mg
4. Precirol ATO5	37.92 mg
Total content in 1 capsule	**200.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**250.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 8.

Example 13 (batch F807RS47)

(Hard gelatine capsules containing a paste)

Composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2.Transcutol HP	43.30 mg
3.Gelucire 44/14	75.80 mg
4.Precirol ATO5	75.80 mg
Total content in 1 capsule	**200.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**250.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 8.

Example 14 (batch F807RS48)

(Hard gelatine capsules containing a paste)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	43.30 mg
3. Gelucire 44/14	37.92 mg
4. Precirol ATO5	113.67 mg
Total content in 1 capsule	**200.00 mg**
Capsule (size 3)	**50.00 mg**
Total	**250.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 8.

Example 15 (batch F807RS43) - Comparative formulation without modulator *(soft gelatine capsules containing a paste)*

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	43.30 mg
3. Gelucire 44/14	151.59 mg
Total content in 1 capsule	**200.00 mg**
Soft gelatine capsule (size 3)	**50.00 mg**
Total	**250.00 mg**

Production method

The production is carried out in two phases:

Phase I: melting of the lipid mass and dissolution of the active principle

Phase II: filling of the capsule

Description of the process:

Phase I

All lipid components and a solubilising agent (Transcutol) are mixed in a beaker maintained at 65°C in a thermostated container under gentle stirring until a completely molten mass is obtained, then Tacrolimus monohydrate is added and stirring is continued until complete dissolution.

Phase II

The molten mass is splitted up into gelatine capsules

Example 16 (batch F807RS39)

(Soft gelatine capsules)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2 Transcutol HP	43.30 mg
3. Gelucire 44/14	113.67 mg
4. Precirol ATO5	37.92 mg
Total content in 1 capsule	**200.00 mg**
Soft gelatine capsule	**80.00 mg**
Total	**280.00 mg**

The operating conditions for manufacturing the capsules were conducted as described in Example 15

Example 17 (batch F807RS44)

(Soft gelatine capsules)

The composition of the capsule content expressed in weight is as follows:

1.Tacrolimus monohydrate	5.11 mg
(Tacrolimus base	5.00 mg)
2.Transcutol HP	43.30 mg
3. Gelucire 44/14	75.80 mg
4. Precirol ATO5	75.80 mg
Total content in 1 capsule	**200.00 mg**
Soft gelatine capsule	**30.00 mg**
Total	**230.00 mg**

The operating conditions for manufacturing the capsules were the same as those described in Example 15.

Example 18 (batch F807RS45)

(Soft gelatine capsules)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	113.67 mg
3. Gelucire 44/14	43.30 mg
4. Precirol ATO5	37.92 mg
Total content in 1 capsule	**200.00 mg**
Soft gelatine capsule	**80.00 mg**
Total	**280.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 15.

Example 19 (batch F807RS49)

(Soft gelatine capsules)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	43.30 mg
3. Gelucire 44/14	113.67 mg
4. Gelucire 39/01	37.92 mg
Total content in 1 capsule	**200.00 mg**
Soft gelatine capsule	**80.00 mg**
Total	**280.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 15.

Example 20 (batch F807RS50)

(Soft gelatine capsules)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	43.30 mg

3. Gelucire 44/14	75.80 mg
4. Gelucire 39/01	75.80 mg
Total content in 1 capsule	**200.00 mg**
Soft gelatine capsule	**80.00 mg**
Total	**280.00 mg**

The operating conditions for manufacturing the capsules were conducted as described in Example 15.

Example 21 (batch F807RS51)

(Soft gelatine capsules)

The composition of the capsule content expressed in weight is as follows:

1. Monohydrated Tacrolimus	5.11 mg
(Tacrolimus content	5.00 mg)
2. Transcutol HP	113.67 mg
3. Gelucire 44/14	43.30 mg
4. Gelucire 39/01	37.92 mg
Total content in 1 capsule	**200.00 mg**
Soft gelatine capsule	**80.00 mg**
Total	**280.00 mg**

The operating conditions for manufacturing the capsules were similar to those described in Example 15.

Capsules Dissolution studies

Aim of the following dissolution studies is to test the *in vitro* release rates of tacrolimus capsule to evaluate the influence of the modulating components in the formulations.

Three comparative formulations were prepared (batches 30, 31 and 43) without modulating agent to determine the influence of these agents.

Experimental conditions:

apparatus 2 (paddle)

medium: hydroxypropylcellulose (MW 100.00) 0.05% in purified water at pH 4.5 with phosphoric acid

temperature: 37°C (+/- 0.5°C)

volume of medium:900 ml

rotating speed: 50 rpm

volume of the sample: 5 ml, with initial volume restore

used of sinkers

The analyte was quantified by HPLC .

Results:

Table 4.

Hard gelatine capsules containing a granulated solid

	Release Tacrolimus (%)/Time				
Formulation	15'	30'	60'	90'	120'
F807RS 30(*) (ex.1)	72	73	80.3	83.6	78,9
F807RS32 (ex.2)	56.9	65.9	71.4	73.9	75.34
F807RS33 (ex.3)	55.2	63	67.7	70.3	73.5
F807RS34 (ex.4)	35.6	44.7	50	54.6	53.2
F807RS36 (ex.5)	60.3	70.1	74.1	78.3	78.9
F807RS37(ex.6)	42.7	51.7	59.2	65.7	64.5
F807RS38(ex.7)	26.9	35.7	44.3	50.3	51.9
Prograf 5 mg	42.7	50.2	68.2	72.4	74.3

(*) comparative formulation without modulating agent

(**) Commercial drug product

Table 5.

Hard gelatine capsules containing a paste

	Release Tacrolimus (%)/Time				
Formulation	15'	30'	60'	90'	120'
F807RS31(*) (ex.8)	93.7	102.9	101.8	103.8	104.1
F807RS35 (es.9)	78.1	86.4	83.4	85.3	86.1
F807RS40 (ex.10)	58.2	65.4	73.8	75.9	78.6
F807RS41 (ex.11)	35.1	46.5	51.7	53.8	54.9
F807RS46 (ex.12)	75.3	82.7	80.9	84.6	87.0
F807RS47 (ex.13)	55.9	66.4	71.8	70.1	71.2
F807RS48 (ex.14)	32.6	43.8	50.6	48.3	51.6
Prograf 5 mg	42.7	50.2	68.2	72.4	74.3

(*) comparative formulation without modulating agent

(**) Commercial drug product

Table 6.

Soft gelatine capsule

	Release Tacrolimus (%)/Time				
Formulation	15'	30'	60'	90'	120'
F807RS43(*) (ex.15)	79.2	88.1	98.2	101.1	102.6
F807RS39 (ex.16)	77.9	90.8	95.3	100	87.4
F807RS44 (ex.17)	61.7	71.2	78.5	91.6	95.5
F807RS45 (ex.18)	38.3	50.0	61.3	69.8	74.2
F807RS49 (ex.19)	76.8	88.5	93.1	89.9	91.4
F807RS50 (ex.20)	39.6	44.6	70.2	73.3	80.5
F807RS51 (ex.21)	32.5	44.4	57.1	62.2	69.1
Prograf 5 mg	42.7	50.2	68.2	72.4	74.3

(*) comparative formulation without modulating agent

(**) Commercial drug product

The results evidence that the formulations according to the present invention allow to modulate the release of the active principle from the capsule. These results were compared with those obtained by using the same methodology with the commercial product Prograf (capsules containing 5 mg of Tacrolimus) the results reported in the above tables are also represented in the diagrams reported hereunder in figures 1-6.

Differential Scanning Calorimetry diagrams

The content of the capsules prepared according to the method described in examples 2, 3, 4, 9 and 16 was analysed by Differential Scansion Calorimetry (DSC),

Experimental Conditions:

sample weight: 3.00- 5.00 mg

scanning rate: 10.0 °C/min

start temperature: 40°C

final temperature: 300°C.

In all depicted thermograms the scanning of the active principle only in the crystalline form (displaying a peak at about 120°C) is superimposed,

The endothermic peak displayed in some formulations of capsules, at about 170°C, is due to the transition state of mannitol, when present in the formulations.

No peak that can be referred to Tacrolimus appears in any of the thermograms relative to content of capsules in all formulations according to the present inventions, demonstrating that the active principle is contained in amorphous form in the final formulation.

5

CLAIMS

1. Fast- or controlled-release oral compositions containing as the active principle Tacrolimus in amorphous form comprising:

a) a "solubilizing agent" of the active principle able to maintain it in the amorphous form over an extended the time period

b) at least one "diluting agent" having function to stabilize the active principle in amorphous form

c) at least one agent "modulating" the dissolution rate of the active principle from the pharmaceutical form

2.The compositions according to claim 1, characterised in that they are in the form of powder, granules, paste, solution or suspension.

3. The compositions according to claim 2, characterised in that they can be charged into hard or soft gelatine capsules

4. The compositions according to claim 2, characterised in that they are in the form of a paste which is charged into soft gelatine capsules

5. The compositions according to any of claims 1-5, characterised in that the solubilizing agent is a substance solubile in lipophilic media at room temperature selected from the group consisting of: diethyleneglycol monoethylether, polyethylenglycol with average molecular weight comprised between 1000 e 20000 Da and liquid fatty acid macroglycerides.

6. The compositions according to any of claims 1-5, characterised in that the "diluting agent" is a substance with amphiphilic properties characterised by a HLB value higher than 10.

7. The compositions according to claim 6, characterised in that said substance is a mixture of macroglycerides of semisolid fatty acids C12-C18 with HLB comprised between 13 and 20.

8. The compositions according to any of claims 1-7, characterized in that the modulating agent is a substance with HLB <5.

9. The compositions according to claim 8, characterized in that the HLB is comprised between 1 and 2 and that the substance is selected from the group consisting of mixtures of C12-C18 semi-solid esters of fatty acids with glycerol.

10. The compositions according to any of claims 1-9, characterized in that Tacrolimus is present in the formulation in weight ratio with respect to the lipidic component consisting of the solubilizing agent(s), the diluting agent(s) and the modulating agent(s) between 1:1000 and 1:1.

11. The compositions according to claim 10, characterised in that such weight ratio is comprised between 1:100 and 1:10.

12. The compositions according to any of claims 1-11, characterized in that the solubilizing agent(s), the diluting agent(s) and the modulating agent(s) are used in weight ratios between 1:1:1 and 1:0.05:0.05,

13. The compositions according to claim 12, wherein this ratio is comprised between 1:0.6:0.6 and 1:0.4:0.4.

14. Process for preparing compositions in the form of a granulated solid or powder to be charged into the hard gelatine capsules according to claim 3 comprising the follollowing steps:

A) Melting a mixture of the solubilizing agent, the diluent(s), the modulating agent(s) at a temperature between 40 and 80°C, dispersing Tacrolimus in the lipidic molten mass and stirring gently until the active principle is completely dissolved;

B) Dispersing the molten lipidic mass in the technological adjuvants at room temperature until said mass is completely adsorbed and homogenised;

C) Sizing the mixture prepared in step (B) on a sieve of appropriate mesh;

D) Charging the sized mixture obtained in step (C) into capsules using normal filling machines capable to fill capsules with powders or paste according to the desired form to be obtained

15. The process for preparing the compositions in form of a paste, solution or suspension splitted up into hard gelatine capsules according to claim 3 comprising the following steps:

A') Melting a mixture of the solubilizing agent, the diluent(s), the modulating agent(s) at a temperature preferably between 40 and 80°C and adding Tacrolimus to the lipidic molten mass, stirring gently until the active principle is completely dissolved;

B') Filling the molten mass into the hard gelatine capsules.

16. Process for preparing the compositions in form of a paste, solution or suspension charged into soft gelatine capsules according to claim 4 comprising the following steps:

A'') Melting a mixture of the solubilizing agent, the diluent(s), the modulating agent(s) at a temperature between 40 and 80°C and adding Tacrolimus to the lipidic molten mass stirring gently until the active principle is completely dissolved;

B") charging the molten mass obtained from step A") into soft gelatine capsules.

17. The process according to any of the claims 14-16, wherein the step (A), (A') or (A") are carried out at temperatures between 50 and 65°C.

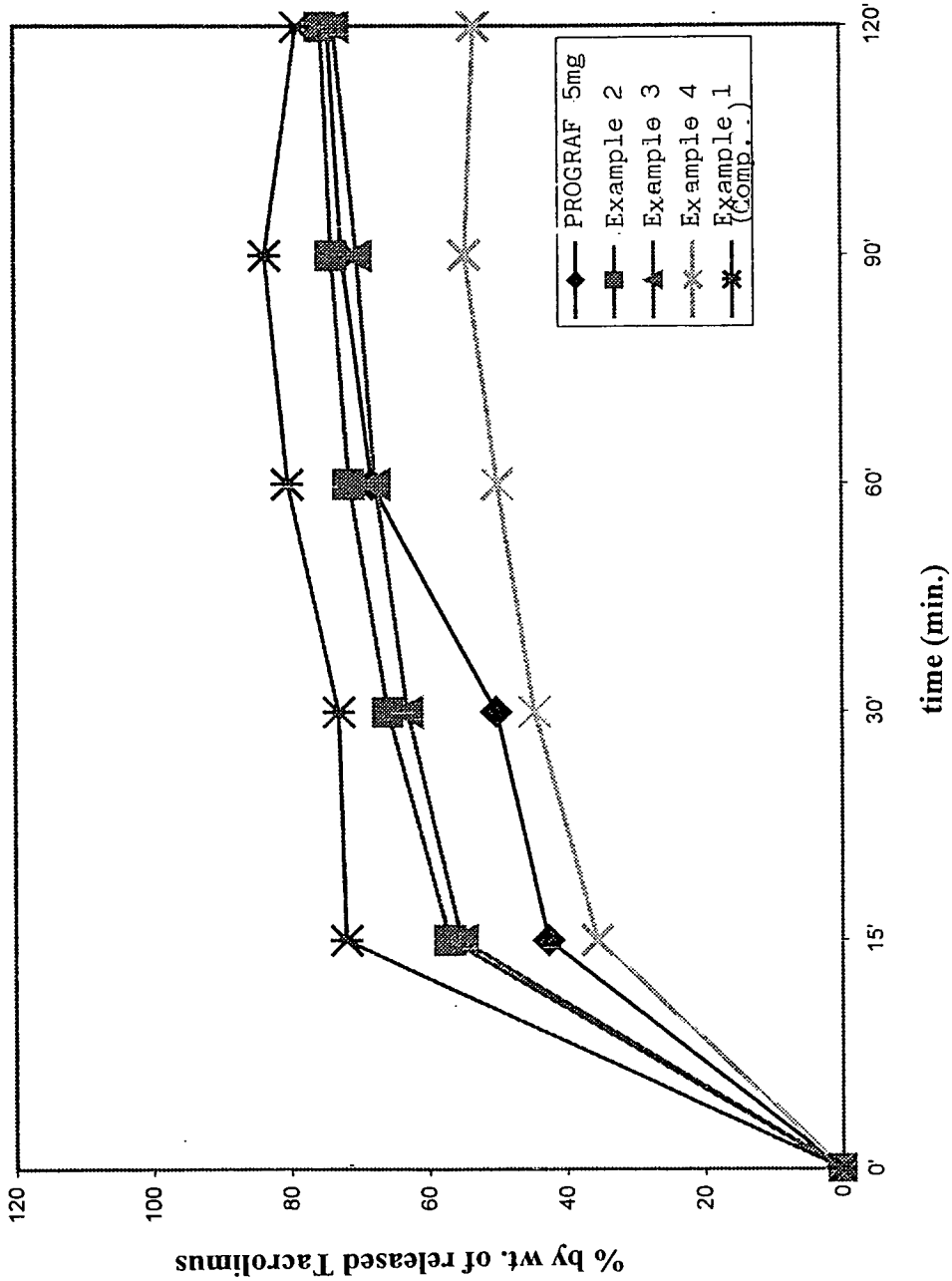

% by wt. of released Tacrolimus
120
100
80
60
40
20
0
0'
15'
30'
60'
90'
120'
time (min.)
PROGRAF 5mg
Example 2
Example 3
Example 4
Example 1 (Comp.)

Figure 1

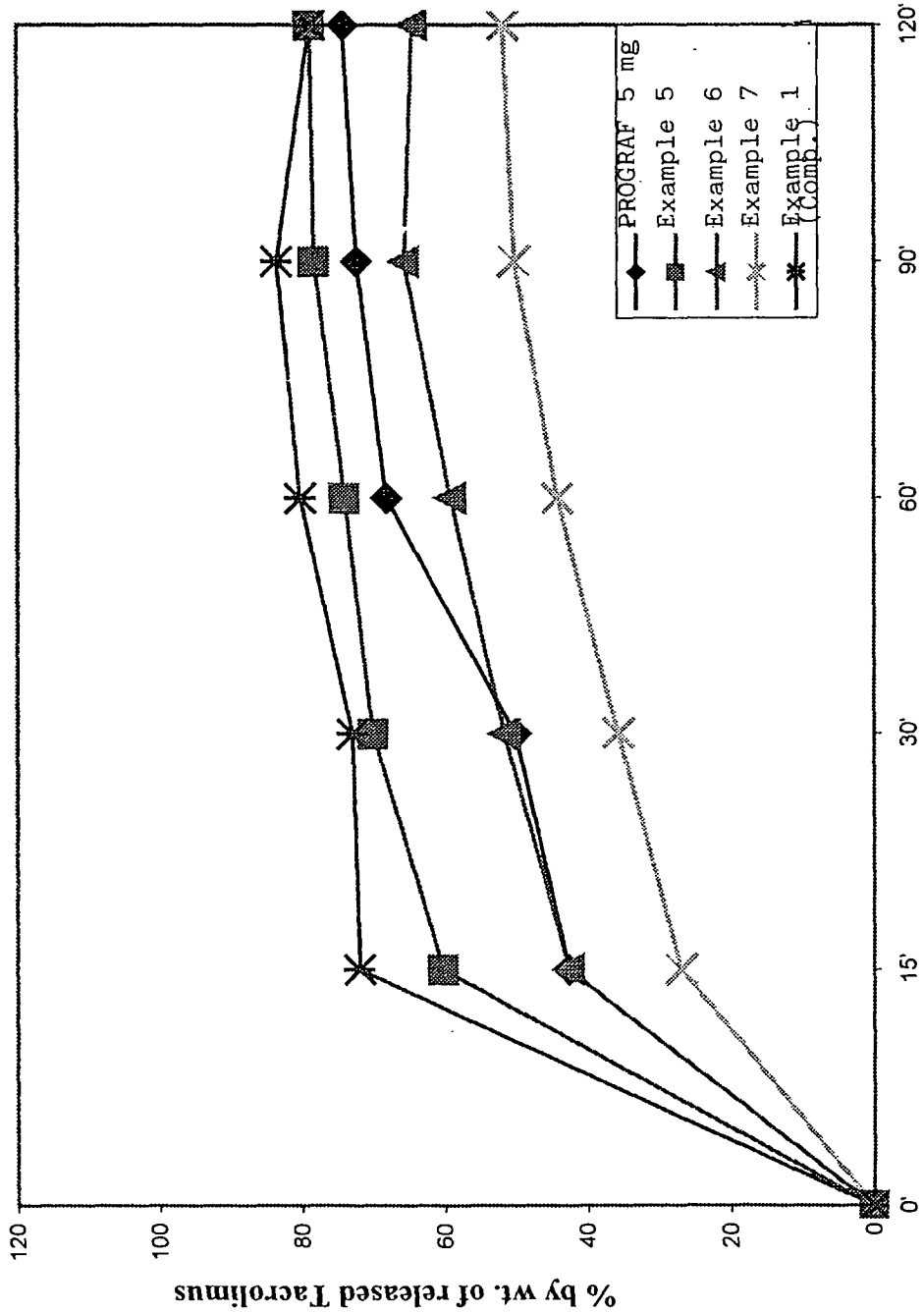

% by wt. of released Tacrolimus
0
20
40
60
80
100
120
0'
15'
30'
60'
90'
120'
time (min.)
PROGRAF 5 mg
Example 5
Example 6
Example 7
Example 1 (Comp.)

Figure 2

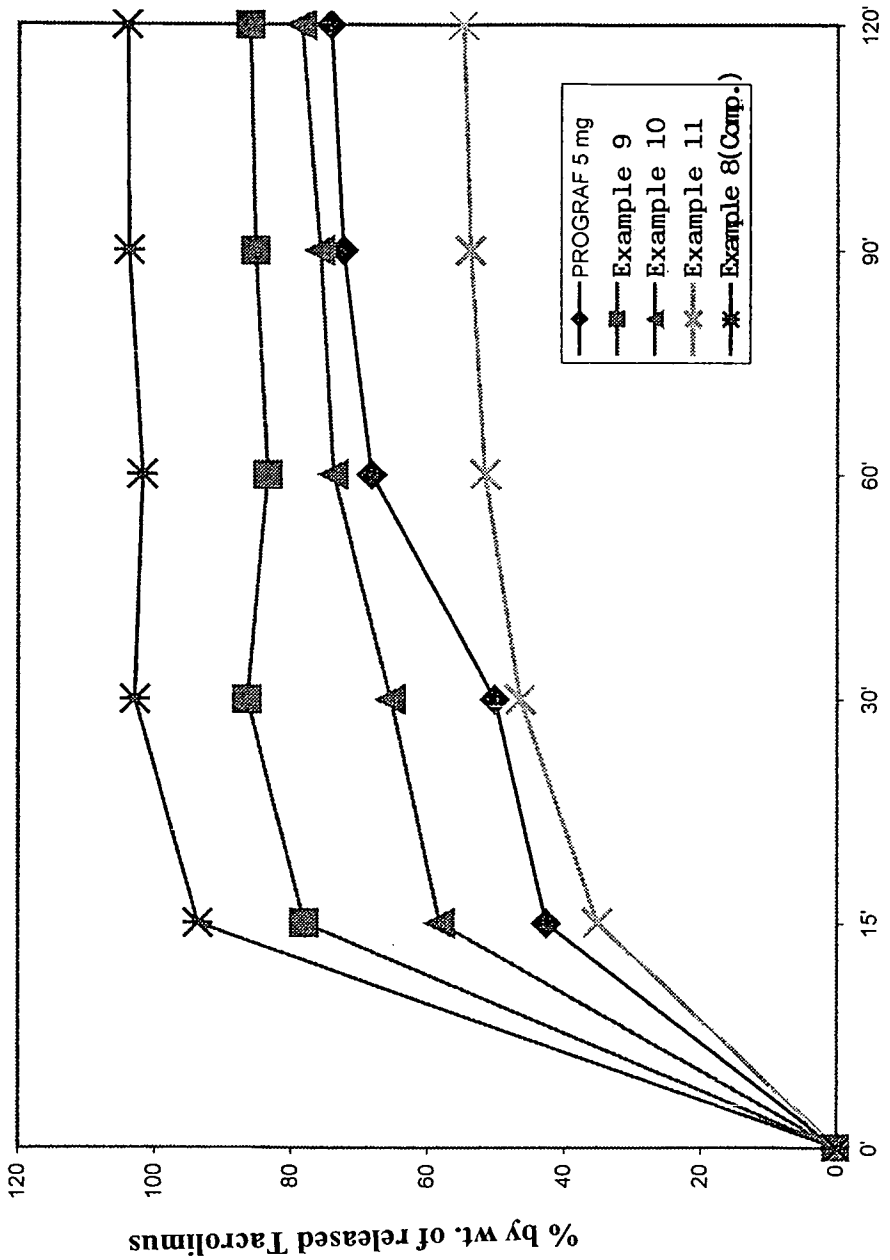

% by wt. of released Tacrolimus
120
100
80
60
40
20
0
0'
15'
30'
60'
90'
120'
time (min.)
PROGRAF 5 mg
Example 9
Example 10
Example 11
Example 8(Comp.)

Figure 3

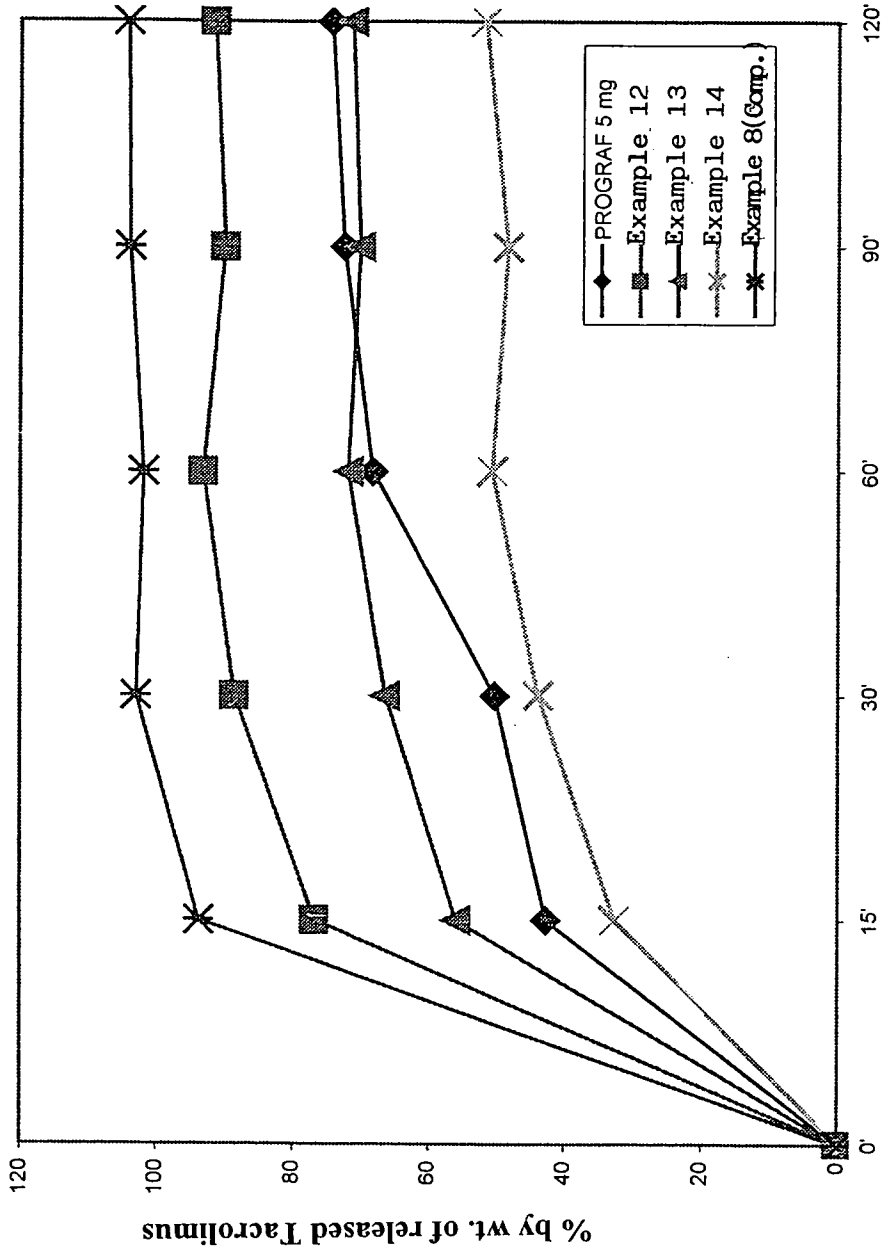

% by wt. of released Tacrolimus
120
100
80
60
40
20
0
0'
15'
30'
60'
90'
120'
time (min.)
PROGRAF 5 mg
Example 12
Example 13
Example 14
Example 8(Comp.)

Figure 4

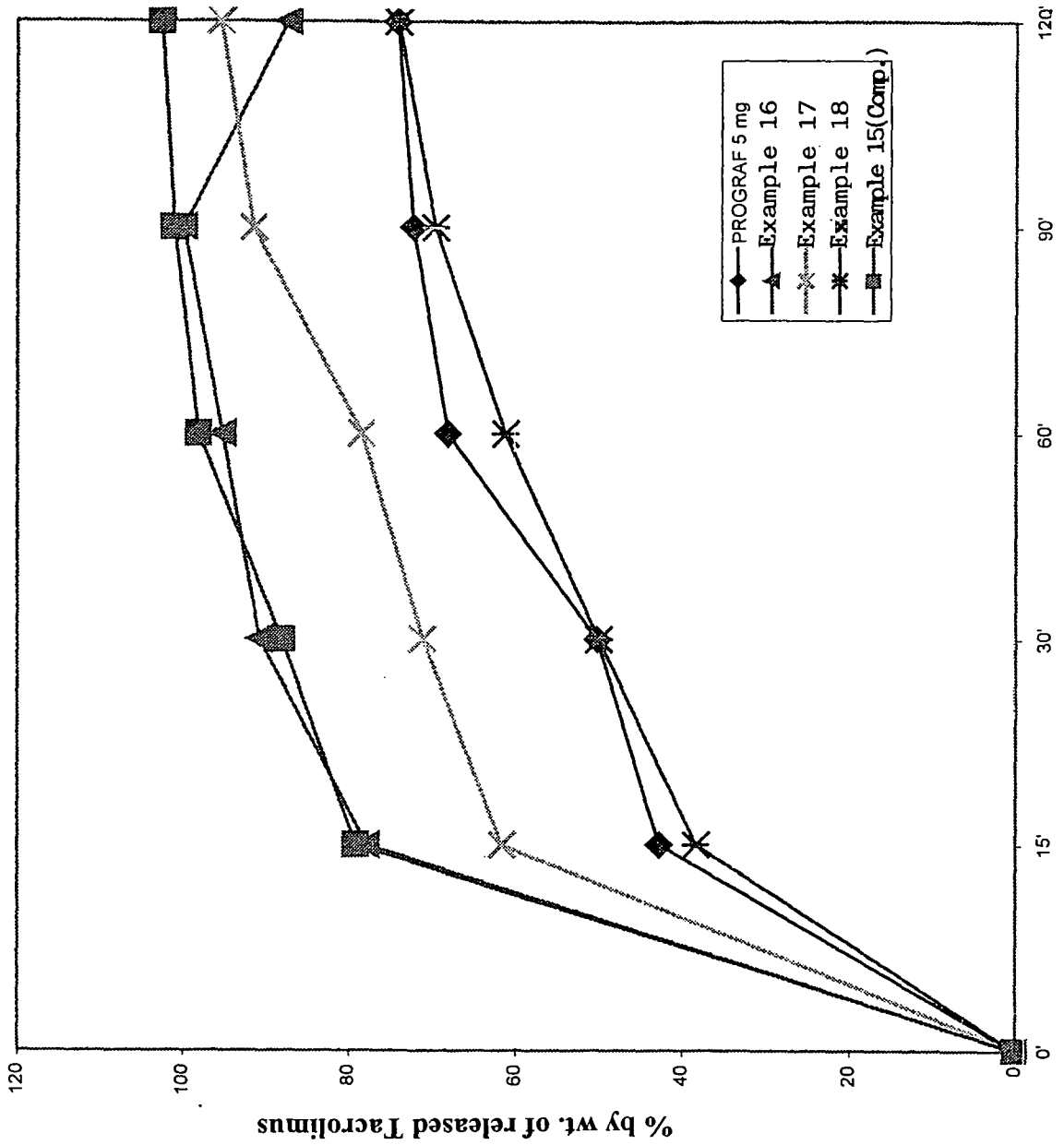

% by wt. of released Tacrolimus
120
100
80
60
40
20
0
0'
15'
30'
60'
90'
120'
time (min.)
PROGRAF 5 mg
Example 16
Example 17
Example 18
Example 15(Comp.)

Figure 5

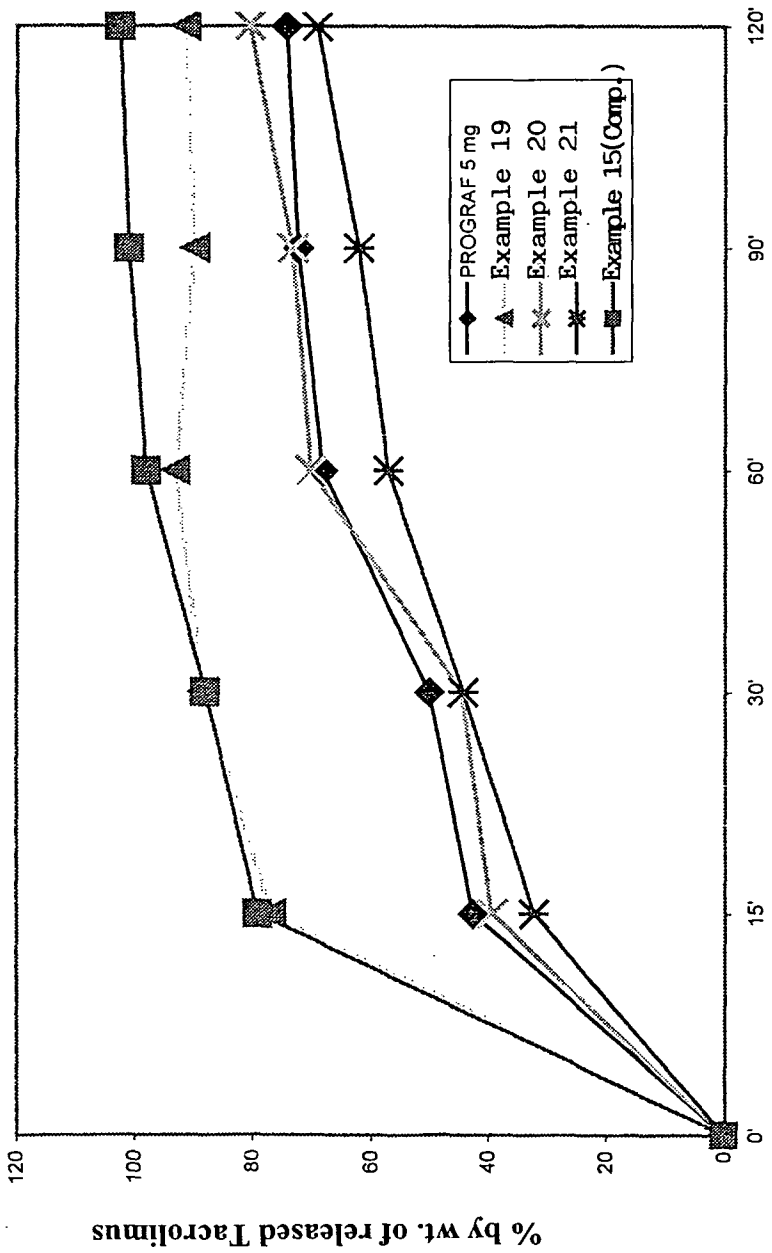
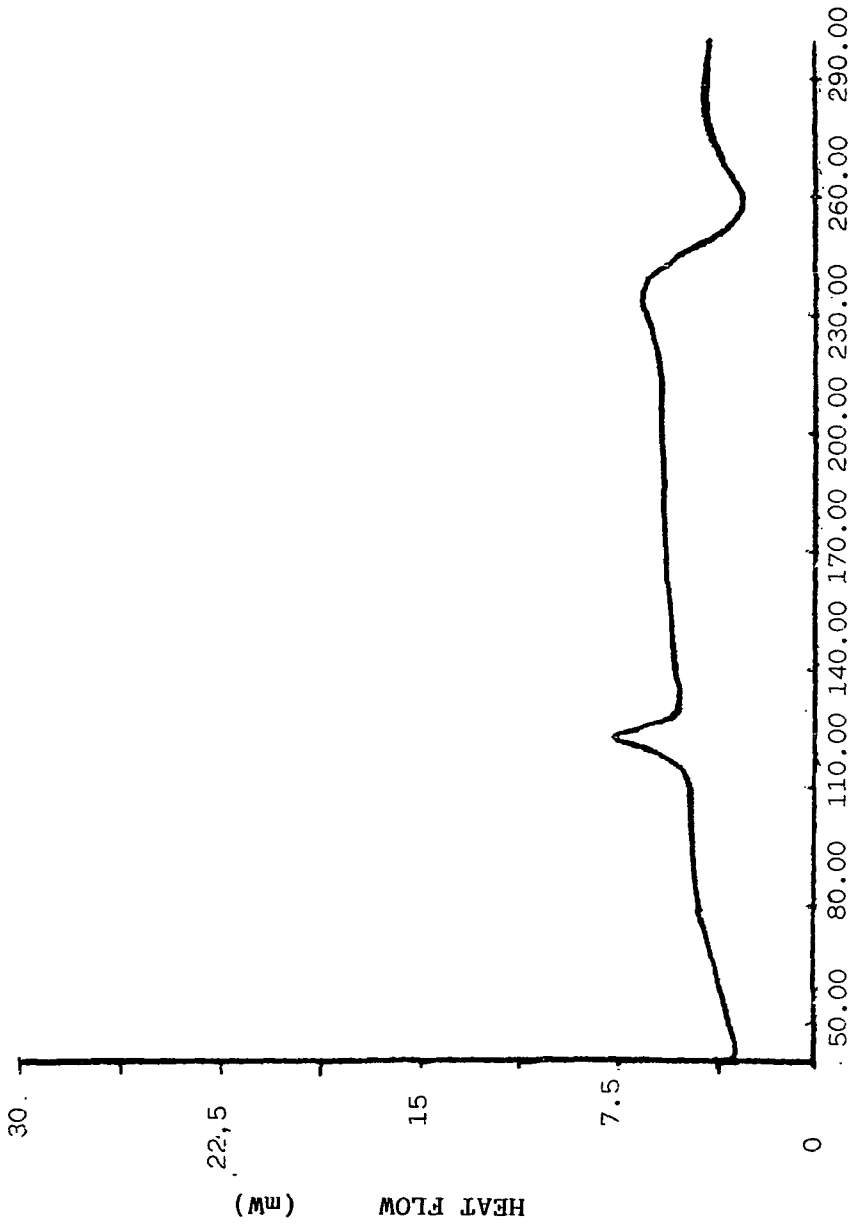

% by wt. of released Tacrolimus
120
100
80
60
40
20
0
0'
15'
30'
60'
90'
120'
time (min.)
PROGRAF 5 mg
Example 19
Example 20
Example 21
Example 15(Comp.)

Figure 6


HEAT FLOW (mW)
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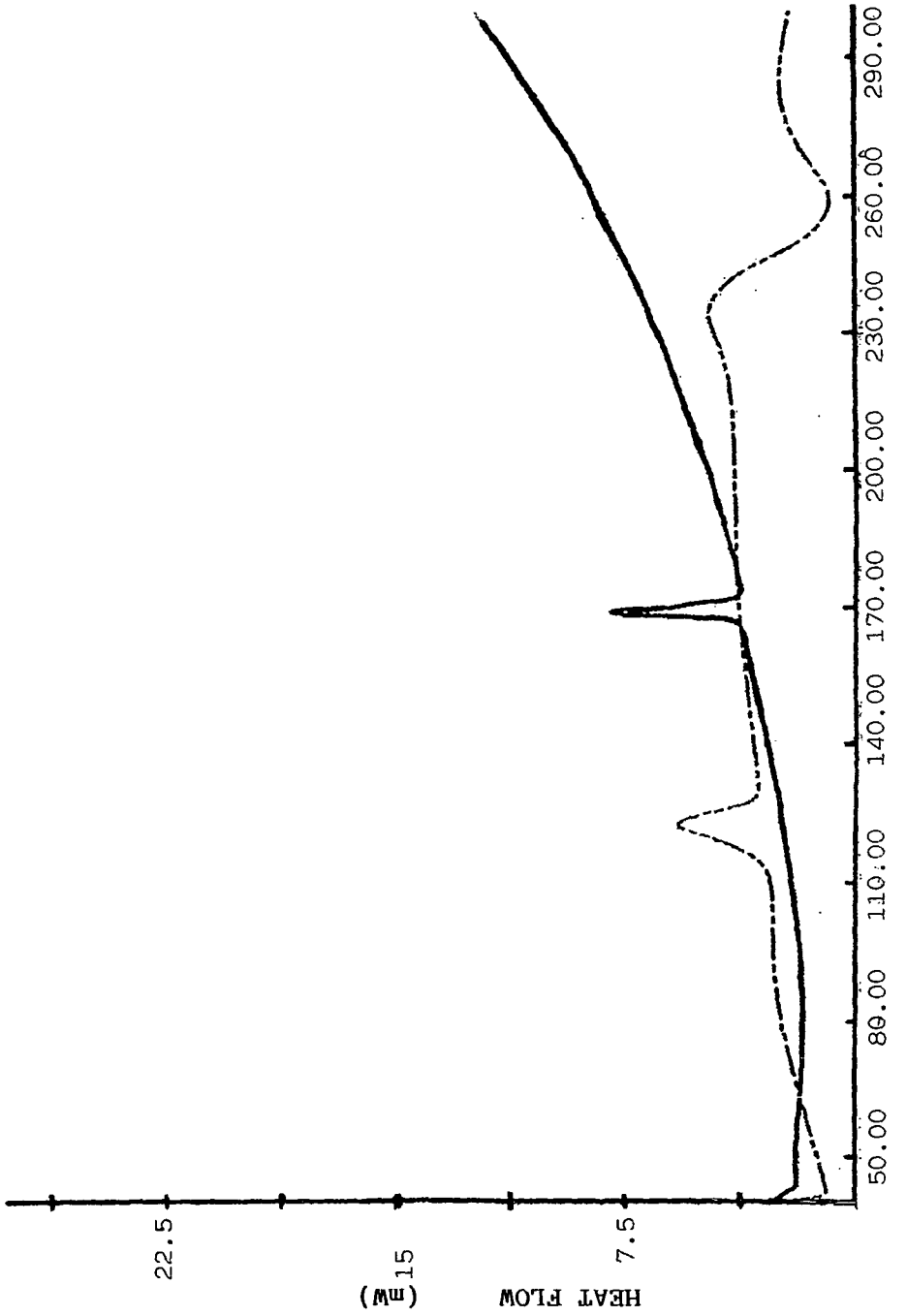
22,5
15
7.5
0
50.00
80.00
110.00
140.00
170.00
200.00
230.00
260.00
290.00
Temperature (C)

Figure 7


HEAT FLOW
(mW)
22.5
15
7.5
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80.00
110.00
140.00
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200.00
230.00
260.00
290.00
Temperature (C)

Figure 8

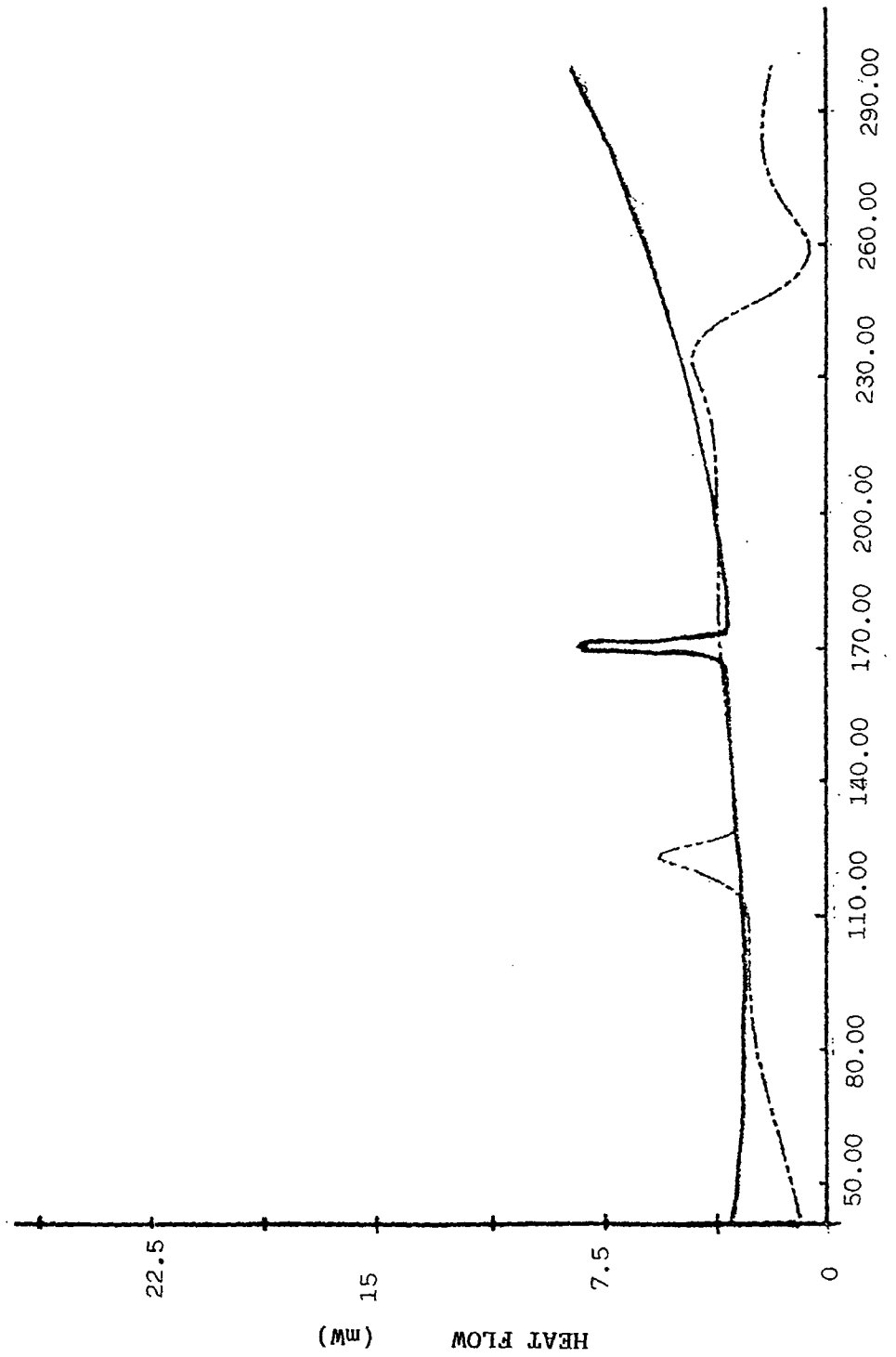

HEAT FLOW
(mW)
22.5
15
7.5
0
50.00
80.00
110.00
140.00
170.00
200.00
230.00
260.00
290.00
Temperature (C)

Figure 9


HEAT FLOW (mW)
22.5
15
7.5
0
50.00
80.00
110.00
140.00
170.00
200.00
230.00
260.00
290.00
Temperature (C)

Figure 10

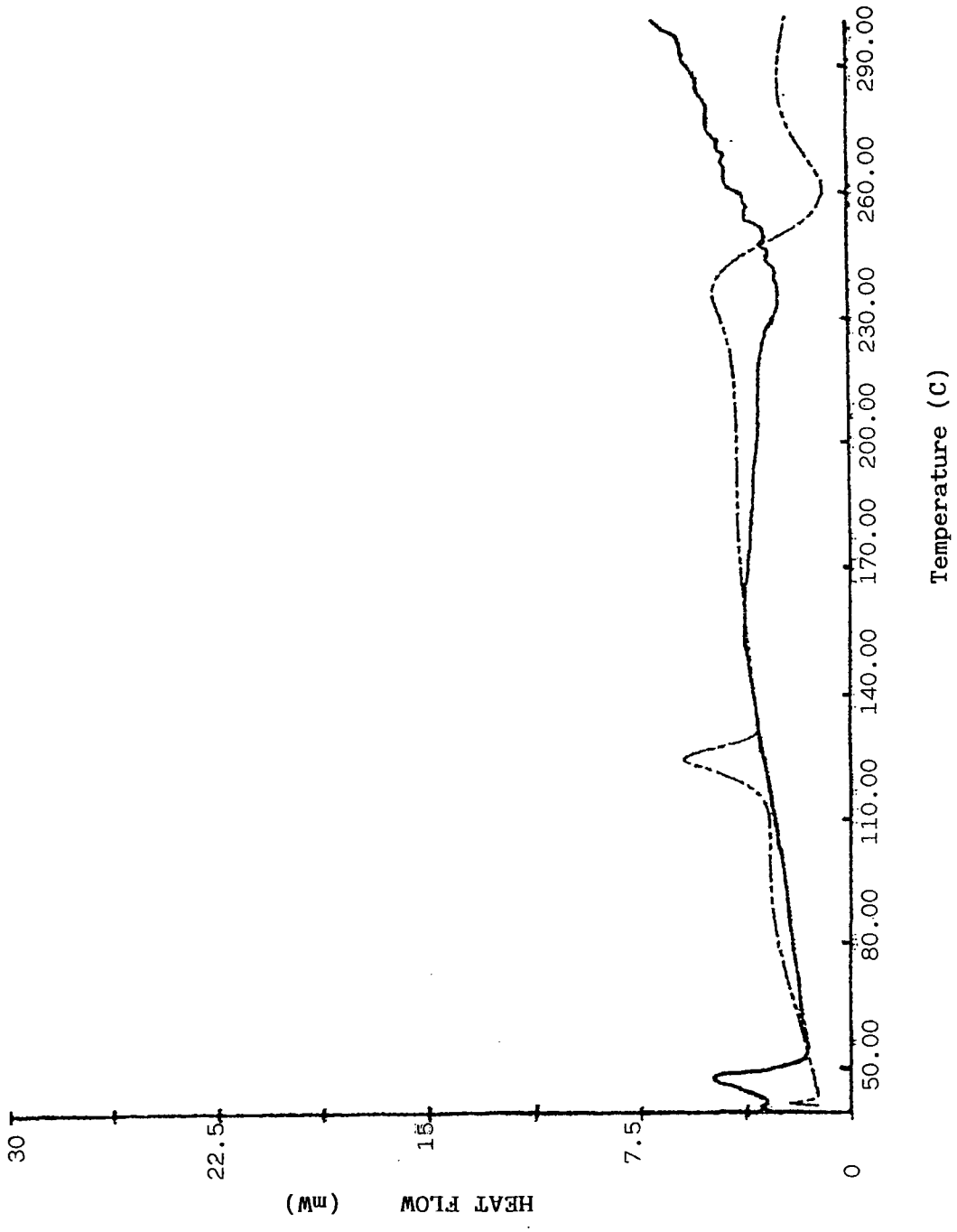
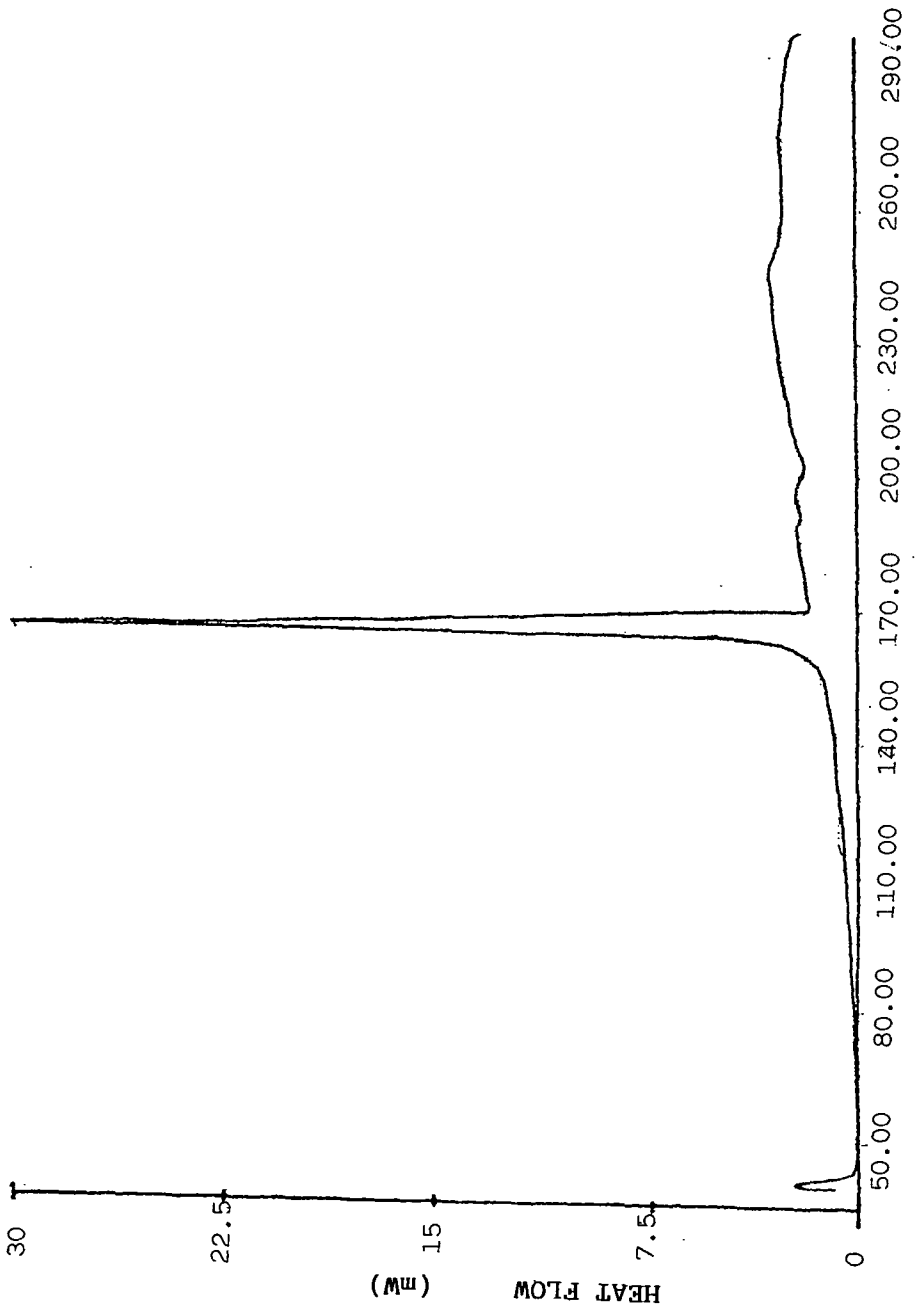

HEAT FLOW (mW)
30
22.5
15
7.5
0
50.00
80.00
110.00
140.00
170.00
200.00
230.00
260.00
290.00
Temperature (C)

Figure 11


HEAT FLOW
(mW)
30
22.5
15
7.5
0
50.00
80.00
110.00
140.00
170.00
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230.00
260.00
290.00
Temperature (C)

Figure 12

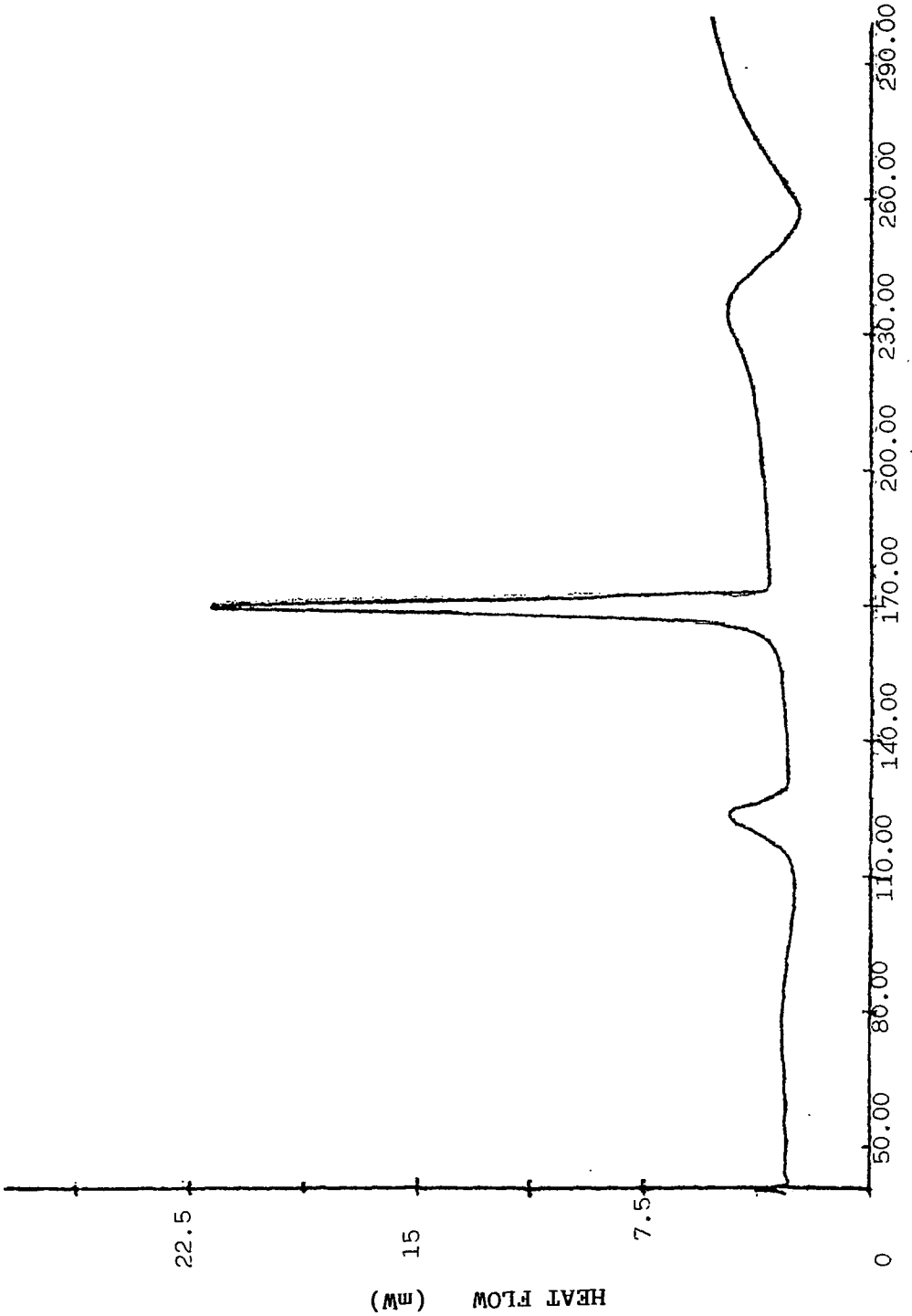
HEAT FLOW (mW)
22.5
15
7.5
0
50.00
80.00
110.00
140.00
170.00
200.00
230.00
260.00
290.00
Temperature (C)

Figure 13