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(54) Titre : PROCEDE DE FABRICATION DU SEL DE CALCIUM AMORPHE, STABLE, D'ACIDE (6S)-N-(5)-METHYL-5,6,7,8-TETRAHYDROFOLIQUE

(54) Title: A PROCESS FOR THE PREPARATION OF THE STABLE, AMORPHOUS CALCIUM SALT OF (6S)-N(5)-METHYL-5, 6, 7, 8-TETRAHYDROFOLIC ACID

(57) Abrégé/Abstract:
The present invention relates to a process for preparing the stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid. The present invention also relates to a process for preparing stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid. The present invention also relates to a process for preparing an aqueous solution of the calcium salt of (6S)-N(5)-methy1-5,6,7,8-tetrahydrofolic acid.

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(54) Title: PROCESS FOR PREPARING THE STABLE, AMORPHOUS CALCIUM SALT OF (6S)-N(5)-METHYL-5,6,7,8-TETRAHYDROFOLIC ACID

(54) Bezeichnung: VERFAHREN ZUR HERSTELLUNG DES STABILEN, AMORPHEN CALCIUMSALZES VON (6S) -N (5) -METHYL-5, 6, 7, 8-TETRAHYDROFOLSÄURE

(57) Abstract: The present invention relates to a process for preparing the stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid. The present invention also relates to a process for preparing stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid. The present invention also relates to a process for preparing an aqueous solution of the calcium salt of (6S)-N(5)-methy1-5,6,7,8-tetrahydrofolic acid.

(57) Zusammenfassung: Die vorliegende Erfindung betrifft ein Verfahren zur Herstellung des stabilen, amorphen Calciumsalzes von (6S) -N (5) -Methyl-5, 6, 7, 8-tetrahydrofolsäure. Die vorliegende Erfindung betrifft auch ein Verfahren zur Herstellung von stabiler, kristalliner (6S) -N (5) -Methyl- 5, 6,7, 8-tetrahydrofolsäure. Die vorliegende Erfindung betrifft auch ein Verfahren zur Herstellung einer wässrigen Lösung des Calciumsalzes von (6S)-N(5)-Methy1-5, 6,7, 8-tetrahydrofolsäure.

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A process for the preparation of the stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid

The present invention is directed to a process for the preparation of the stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula V.

The present invention is also directed to a process for the preparation of stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula IV.

The present invention is also directed to a process for the preparation of an aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula III.

In the present invention for 5,6,7,8-tetrahydrofolic acid is used sometimes the abbreviation THF.

A short summary concerning the pharmacological importance of N(5)-methyl-5,6,7,8-tetrahydrofolic acid - herein sometimes abbreviated with N(5)-methyl-THF - and derivatives thereof is given in the beginning of the description of EP 0 455 013 A1. In the same document is also pointed to the importance of the individual (6S)- and (6R)-diastereoisomers of N(5)-methyl-THF. Herein is also described the prior art concerning the preparation of the pure (6S)- and (6R)-diastereoisomers of N(5)-methyl-THF.

In EP 1 044 975 A1 are described stable crystalline salts of N(5)-methyl-THF. Herein are also de-

scribed crystalline calcium salts of (6S)-N(5)-methyl-THF, whereby these salts have in the respective X-ray powder diffraction diagram well defined 2-Theta-values.

In the preparation process of these salts are used as starting materials either the mixture of the (6RS)-diastereoisomers or the already separated (6S)- or (6R)-diastereoisomers. This process involves a temperature treatment of more than 60°C, preferably of more than 85°C, whereby in the working examples are mentioned temperatures from 90°C to 100°C.

Such a temperature treatment is of course less suitable for an industrial preparation of these salts.

In EP 1 044 975 A1 is not mentioned by what way the individual (6S)- or (6R)-diastereoisomers of N(5)-methyl-THF were obtained.

It is an object of the present invention to provide an industrially applicable process for the preparation of a stable calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula V.

The product obtainable with this process shall have a diastereoisomeric purity of at least 99%.

This process shall be simple and shall especially not involve a thermal treatment.

It is a further object of the present invention to provide a process for the preparation of stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula IV.

It is a further object of the present invention to provide a process for the preparation of an aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula III. With this process shall be obtained an aqueous solution of the calcium salt of N(5)-methyl-THF, in which the (6S)-diastereoisomer is highly enriched (≥ 98%).

These objectives are met with the present invention.

In one aspect, the inventive process for the preparation of an aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,

whereby (6S)-5,6,7,8-tetrahydrofolic acid having a content of the corresponding (6R)-diastereoisomer in the range from 4% by weight to 8% by weight is methylated in water,

is characterized in that:

- to the obtained methylated reaction mixture are added from 0.70 to 0.82 equivalents, referred to the used amount of tetrahydrofolic acid, of calcium chloride,
- from the so obtained aqueous solution the calcium salt of (6RS)-N(5)-methyl-5,6,7,8-tetrahydro-folic acid is crystallized and separated, and
- the aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid having a content of the corresponding (6R)-diastereoisomer of ≤ 2% by weight is obtained.

In one preferred embodiment, the process is characterized in that the calcium chloride is added in solid form or in the form of an aqueous solution.

In another preferred embodiment, the process is characterized in that crystallization of the calcium salt of (6RS)-N(5)-methyl-5,6,7,8-tetra-hydrofolic acid is realized by seeding with previously prepared crystals of the calcium salt of (6RS)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, followed by a lowering of the temperature from room temperature to a temperature in the range from 3°C to 5°C, and that this temperature is kept during 16 to 18 hours.

In another aspect, the invention provides a process for the preparation of stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetra-hydrofolic acid characterized in that:

- to an aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid having a content of the corresponding (6R)-diastereoisomer of ≤ 2% by weight is added either acetic acid or a sulfonic acid in such an amount, until a pH-value of 5.5 is obtained,

- the so obtained solution is warmed to a temperature in the range from 44°C to 46°C,

- to this warmed solution is added either acetic acid or a sulfonic acid in such an amount, until a pH-value in the range from 4.3 to 4.4 is obtained, whereby the crystallization of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid begins, whereby during this crystallization the pH-value is maintained in the range from 4.3 to 4.4 by continuous addition of either acetic acid or of a sulfonic acid, and

- the so obtained crystalline solid is filtered off at a temperature in the range from 44°C to 46°C and is isolated.

In one preferred embodiment, the process is characterized in that the obtained crystalline solid is washed with water having a temperature of about 40°C.

In another preferred embodiment, the process is characterized in that the sulfonic acid is p-toluene sulfonic acid.

In a further preferred embodiment, the process is characterized in that the aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid having a content of the corresponding (6R)-diastereoisomer of ≤ 2% by weight is prepared according to the process as defined above.

In another aspect, the invention provides a process for the preparation of the stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid characterized in that:

- crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, prepared according to the process as mentioned above is suspended in water, whereby the water has a temperature from 35°C to 41°C,
- to this suspension is added a NaOH solution in portions and in such an amount until a pH-value in the range from 6.7 to 6.9 is obtained,
- to the so prepared solution are added 0.90 equivalents of calcium chloride, referred to the used amount of the crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,
- in the so prepared solution the concentration of crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is adjusted either by the addition or by the removal of water to a value in the range from 14% by weight to 16% by weight,

- precipitation of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is started by the addition of a small amount of previously prepared calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,

- the so prepared mixture is kept during 1 hour at a temperature of 40°C,

- then within 2 hours the temperature is lowered from 40°C to a temperature of 23°C,

- said temperature of 23°C is maintained during 16 to 18 hours, and

- the obtained solid is isolated.

In one preferred embodiment, the process is characterized in that the obtained solid is washed with water having a temperature of about 10°C, the washed solid is suspended at room temperature in 94% aqueous ethanol and then the so treated solid is isolated by means of filtration.

In another preferred embodiment the process is characterized in that the calcium chloride is added in solid form or in the form of an aqueous solution.

In another aspect, the invention provides a stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydro-folic acid having a diastereoisomeric purity of at least 99%.

In one preferred embodiment, the stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, is characterized in that it has

- the in Figure 2a shown "differential scanning calorimetric" profile, DSC,

- the in Figure 2b shown thermo gravimetric profile, TGA,
- the in Figure 3a shown X-ray powder diffraction diagram, and
- the in Figure 3b shown 2 Theta-values having an error of about ± 0.2 degree.

In another preferred embodiment the stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, is characterized in that the corresponding X-ray powder diffraction diagram has the following characteristic 2 Theta-values:

15.0

15.6

17.4

18.7

19.9

22.2

24.3 and

29.5.

In a further aspect, the invention concerns the use of the stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid as at least one active component in a dietary supplement or in a medicament

- for the treatment and/or the control of human and/or animal tumors and/or
- for the synergistic exertion of influence of a cancer controlling compound and/or
- for the reduction of the toxicity of a cancer controlling compound and/or

- for the protection of human and/or animal cells.

In still another aspect, the invention provides a stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, obtained as follows:

- crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, prepared according to the process as defined above is suspended in water, whereby the water has a temperature from 35°C to 41°C,

- to this suspension is added a NaOH solution in portions and in such an amount until a pH-value in the range from 6.7 to 6.9 is obtained,

- to the so prepared solution are added 0.90 equivalents of calcium chloride, referred to the used amount of crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,

- in the so prepared solution the concentration of crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is adjusted either by the addition or by the removal of water to a value in the range from 14% by weight to 16% by weight,

- the precipitation of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is started by the addition of a small amount of previously prepared calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,

- the so prepared mixture is kept during 1 hour at a temperature of 40°C,

- then within 2 hours the temperature is lowered from 40°C to a temperature of 23°C,

- said temperature of 23°C is maintained during 16 to 18 hours, and

- the obtained solid is isolated.

In one embodiment, the stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, is characterized in that it has:

- the in Figure 4a shown "differential scanning calorimetric" profile, DSC,
- the in Figure 4b shown thermo gravimetric profile, TGA,
- the in Figure 5 shown X-ray powder diffraction diagram, and
- the in Figure 6 shown Raman spectrum.

In the following part are described possible embodiments of the present invention.

Thereby reference is also made to the Figures.

Figure 1 shows a reaction scheme, starting with the compound of formula I and ending with the compound of formula V.

Figure 2a shows the "differential scanning calorimetric" profile, DSC, of the compound of formula IV. On the abscissa is plotted the temperature in °C, and on the ordinate is plotted the endothermic heat flow in mW.

Figure 2b shows the thermo gravimetric profile, TGA, of the compound of formula IV. On the abscissa is plotted the temperature in °C. On the left ordinate are plotted percents by weight and on the right ordinate is plotted the derivative of these percents by weight against the time t (minutes). The solid line refers to the left ordinate, and the dotted line refers to the right ordinate.

Figure 3a shows the X-ray powder diffraction diagram of the compound of formula IV. On the abscissa are plotted the 2 Theta-values, and on the ordinate is plotted the intensity (counts).

Figure 3b shows the 2 Theta-values having an error of about ± 0.2 degree of the in Figure 3a shown X-ray powder diffraction diagram.

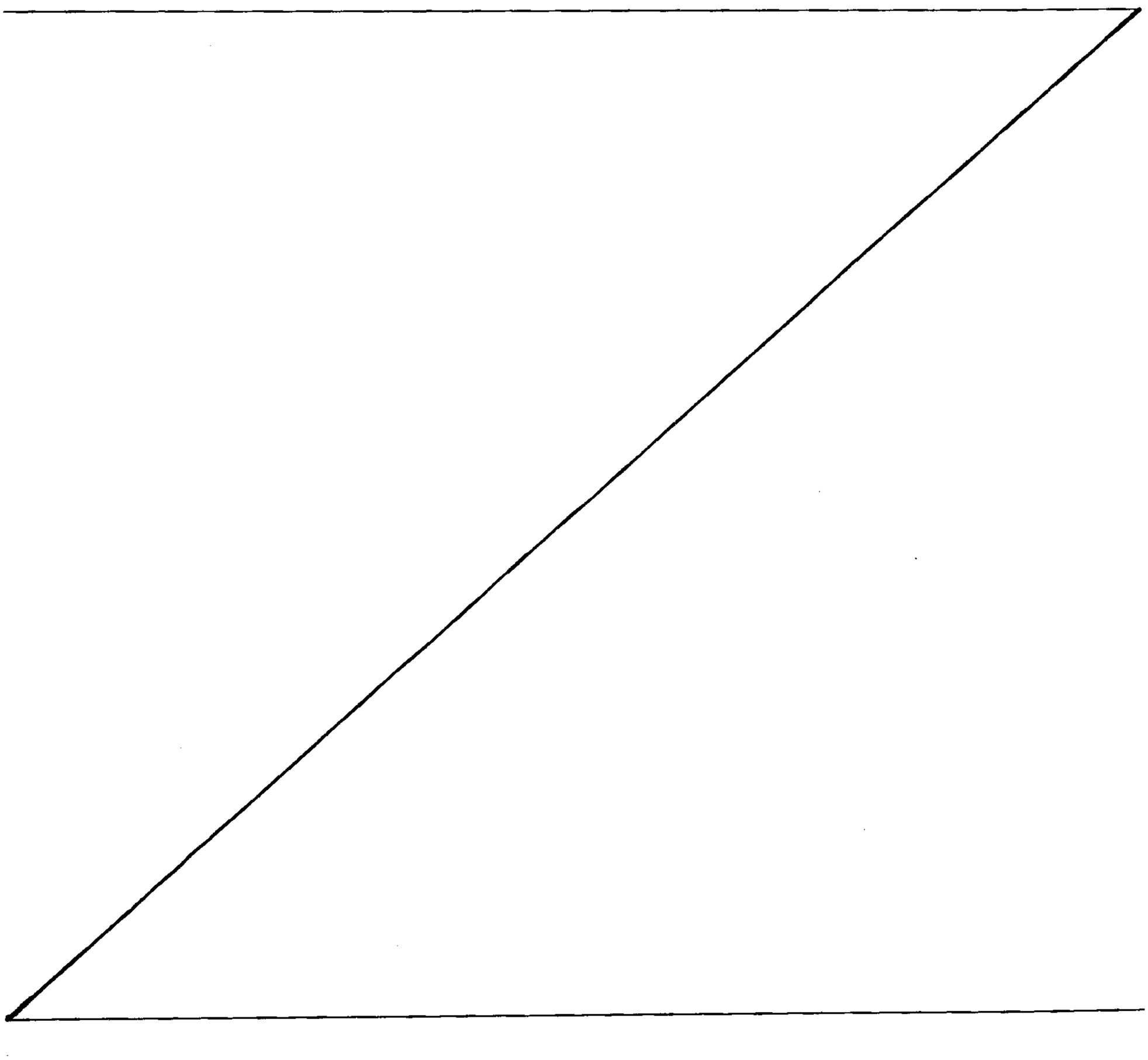


Figure 4a shows the "differential scanning calorimetric" profile, DSC, of the compound of formula V. On the abscissa is plotted the temperature in °C, and on the ordinate is plotted the endothermic heat flow in mW.

Figure 4b shows the thermo gravimetric profile, TGA, of the compound of formula V. On the abscissa is plotted the temperature in °C. On the left ordinate are plotted percents by weight and on the right ordinate is plotted the derivative of these percents by weight against the time t (minutes). The solid line refers to the left ordinate, and the dotted line refers to the right ordinate.

Figure 5 shows the X-ray powder diffraction diagram of the compound of formula V. On the abscissa are plotted the 2 Theta-values, and on the ordinate is plotted the intensity (counts).

Figure 6 shows the Raman spectra of the compound of formula V. On the abscissa is plotted the wave number in cm^{-1}, and on the ordinate is plotted the Raman intensity.

(6S)-5,6,7,8-tetrahydrofolic acid of the formula II having a content of the corresponding (6R)-diastereoisomer in the range from 4% by weight to 8% by weight was prepared according to EP 0 600 460.

It was quite surprising that one could obtain an aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula III with an increased content of the (6S)-isomer by selec-

tively crystallizing out from the solution the 1:1 mixture of the diastereoisomeric salts.

Routinely one could isolate a solution having a content of the (6S)-isomer of at least 98%.

It was also quite surprising to obtain the corresponding stable, crystalline free acid of formula IV from the above described solution: moreover during this step a further increase of the diastereoisomeric purity of at least 99% was achieved.

The stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula IV could be isolated in such a diastereoisomeric purity that this acid may be used as at least one active component in a dietary supplement or in a medicament.

From the acid of the formula IV is prepared the surprisingly stable, amorphous calcium salt of the formula V.

It is obvious from the X-ray powder diffraction diagram of the compound of formula V - see Figure 5 - that this compound is practically amorphous.

The present invention is illustrated by the following examples.

Example 1 (Preparation of the aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula III)

100 g of (6S)-5,6,7,8-tetrahydrofolic acid of the formula II having a diastereoisomeric purity of

(6S):(6R) of 92:8 - prepared according to EP 0 600 460 - were suspended in water having a temperature of 7°C under a nitrogen atmosphere.

The compound of formula II was dissolved by the addition of 65 ml of 20% (w/w) aqueous NaOH solution. The pH-value was then 9.05.

To this solution were added 23.7 g of 36% (w/w) aqueous formaldehyde solution within 5 minutes at a temperature of 8°C under stirring.

After 15 minutes was added an aqueous $NaBH_4$-solution, prepared by dissolving of 21.3 g of $NaBH_4$ in 50 ml of water and 1 ml of 20% (w/w) aqueous NaOH solution, during 1 hour at a temperature of 8°C under stirring.

This mixture was stirred during 30 minutes at a temperature of 8°C and then during 20 minutes at a temperature of 61°C.

The reaction temperature was then lowered within 2 hours to 20°C.

Then were added dropwise 79 ml of 18% (w/w) aqueous HCl solution. The pH-value was then 8.03.

The mixture was cooled to a temperature of 4°C in order to allow the borate precipitation. After 2 hours the borates were removed by filtration.

The pH-value of the resulting solution was adjusted by the addition of 10 ml of 18% (w/w) aqueous HCl solution to a value of 7.03.

This solution was warmed to a temperature of 20°C, and then were added first 1.62 g of di-sodium-EDTA and then 0.82 equivalents (25.44 g) of $CaCl_2.2H_2O$.

Then the pH-value was adjusted to a value of 6.9 by the addition of 2 ml of 20% (w/w) aqueous NaOH solution.

The selective crystallization of the calcium salt of (6RS)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid was realized by seeding the solution with 100 mg of previously prepared crystals of the calcium salt of (6RS)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, followed by a lowering of the temperature from room temperature to a temperature of 4°C within 40 minutes.

The resulting suspension was kept under stirring during 18 hours at a temperature of 4°C.

The obtained crystals were filtered off.

The so obtained aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula III had a content of the corresponding (6R)-diastereoisomer of ≤ 2% by weight, as shown by HPLC on a chiral column.

Example 2 (Preparation of crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula IV)

To the aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula III having a content of the corresponding (6R)-diastereoisomer of ≤ 2% by weight, prepared according to

the above example 1, were added within 20 minutes 6 ml of 100% acetic acid until a pH-value of 5.5 was obtained.

Then the temperature was raised to 45°C.

At this temperature were added consecutively 1.35 g of sodium-dithionite and 1.9 g of di-sodium-EDTA.

To this solution were added 25 ml of 100% acetic acid within 15 minutes.

At a pH-value of 4.5 started the crystallization of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula IV.

The pH-value was kept in the range from 4.3 to 4.4 by continuous addition of 100% acetic acid (10 ml).

This suspension was stirred during 30 minutes and was then filtered off.

The so obtained crystalline solid was washed with water having a temperature of 40°C.

There were obtained 106.9 g of wet crystalline solid.

For the determination of the purity and of the ratio of the diastereoisomers a sample of the compound of formula IV was washed with 94% (v/v) aqueous ethanol and was then dried under reduced pressure. Thereby the following results were obtained:

HPLC purity: 96.45 %

Ratio of (6S)/(6R) = 99.1 : 0.9 (determined by chiral HPLC).

The so obtained product of formula IV had the following stability data (stored at a temperature of 4°C):

Time (months)	HPLC purity (%)
0	96.45
6	96.66

Example 3 (Preparation of the amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula V)

106.9 g of wet crystalline solid, prepared according to the above example 2, were suspended in 400 ml of water having a temperature of 40°C.

To this suspension were added slowly 65 ml of 20% (w/w) aqueous NaOH solution, until a clear solution having a pH-value of 6.8 was obtained.

To this solution were added 0.90 equivalents of $CaCl_2.2H_2O$ (22.7 g, referred to the amount of isolated dry compound of formula IV).

The HPLC determination of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid gave a concentration in solution of c = 14.7 % w/w.

The pH-value was adjusted to a value of 6.8 by the addition of 1 ml of 20% (w/w) aqueous NaOH solution.

The precipitation of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula V was started by the addition of 100 mg of previously prepared compound of formula V.

The so prepared mixture was kept during 1 hour at a temperature of 40°C.

Then within 2 hours the temperature of 40°C was lowered to a temperature of 23°C, and the temperature of 23°C was maintained during 18 hours.

When the concentration of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid of the formula V in the mother liquor was below 7.5 % then the suspension was filtered off.

The obtained solid was washed with 50 ml of water having a temperature of 10°C.

The wet compound of formula V was suspended in 250 ml of 94% (v/v) aqueous ethanol at a temperature of 22°C and was stirred during 30 minutes.

Then this suspension was filtered off and was washed twice with 50 ml 94% (v/v) aqueous ethanol.

There were obtained 95 g of a wet solid which was dried under reduced pressure. There were obtained 47.3 g of the compound of formula V.

Thereby the following analytical data were obtained:

HPLC purity: 98.75 %

Ratio of (6S)/(6R) = 99.75 : 0.25 (determined by chiral HPLC).

The so obtained product of formula V had the following stability data (stored at a temperature of 25°C under vacuum [2 mbar]):

Time (months)	HPLC assay (%)	HPLC purity (%)
0	99.0	98.7
1	98.8	98.7
2	99.8	98.9
3	100.2	98.8
6	99.9	98.5
9	100.7	98.9

WHAT IS CLAIMED IS:

1. A process for the preparation of an aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,

whereby (6S)-5,6,7,8-tetrahydrofolic acid having a content of the corresponding (6R)-diastereoisomer in the range from 4% by weight to 8% by weight is methylated in water,

characterized in that

- to the obtained methylated reaction mixture are added from 0.70 to 0.82 equivalents, referred to the used amount of tetrahydrofolic acid, of calcium chloride,

- from the so obtained aqueous solution the calcium salt of (6RS)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is crystallized and separated, and

- the aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid having a content of the corresponding (6R)-diastereoisomer of ≤ 2% by weight is obtained.

2. The process according to claim 1, characterized in that the calcium chloride is added in solid form or in the form of an aqueous solution.

3. The process according to any one of claims 1 and 2, characterized in that crystallization of the calcium salt of (6RS)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is realized by seeding with previously prepared crystals of the calcium salt

of (6RS)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, followed by a lowering of the temperature from room temperature to a temperature in the range from 3°C to 5°C, and that this temperature is kept during 16 to 18 hours.

4. A process for the preparation of stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, characterized in that:

- to an aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid having a content of the corresponding (6R)-diastereoisomer of ≤ 2% by weight is added either acetic acid or a sulfonic acid in such an amount, until a pH-value of 5.5 is obtained,
- the so obtained solution is warmed to a temperature in the range from 44°C to 46°C,
- to this warmed solution is added either acetic acid or a sulfonic acid in such an amount, until a pH-value in the range from 4.3 to 4.4 is obtained, whereby the crystallization of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid begins, whereby during this crystallization the pH-value is maintained in the range from 4.3 to 4.4 by continuous addition of either acetic acid or of a sulfonic acid, and
- the so obtained crystalline solid is filtered off at a temperature in the range from 44°C to 46°C and is isolated.

5. The process according to claim 4, characterized in that the obtained crystalline solid is washed with water having a temperature of about 40°C.

6. The process according to any one of claims 4 and 5, characterized in that the sulfonic acid is p-toluene sulfonic acid.

7. The process according to any one of claims 4 to 6, characterized in that the aqueous solution of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid having a content of the corresponding (6R)-diastereoisomer of ≤ 2% by weight is prepared according to the process according to any one of claims 1 to 3.

8. Stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid having a diastereoisomeric purity of at least 99%.

9. Stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid according to claim 8, characterized in that it has

- the in Figure 2a shown "differential scanning calorimetric" profile, DSC,
- the in Figure 2b shown thermo gravimetric profile, TGA,
- the in Figure 3a shown X-ray powder diffraction diagram, and
- the in Figure 3b shown 2 Theta-values having an error of about ± 0.2 degree.

10. Stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid according to claim 9, characterized in that the corresponding X-ray powder diffraction diagram has the following characteristic 2 Theta-values:

15.0

15.6

17.4

18.7

19.9

22.2

24.3 and

29.5.

11. Use of the stable, crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid according to any one of claims 8 to 10 as at least one active component in a dietary supplement or in a medicament

- for the treatment and/or the control of human and/or animal tumors and/or
- for the synergistic exertion of influence of a cancer controlling compound and/or
- for the reduction of the toxicity of a cancer controlling compound and/or
- for the protection of human and/or animal cells.

12. A process for the preparation of stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, characterized in that:

- crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, prepared according to the process according to any one of claims 4 to 7, is suspended in water, whereby the water has a temperature from 35°C to 41°C,

- to this suspension is added a NaOH solution in portions and in such an amount until a pH-value in the range from 6.7 to 6.9 is obtained,

- to the so prepared solution are added 0.90 equivalents of calcium chloride, referred to the used amount of the crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,

- in the so prepared solution the concentration of the crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, is adjusted either by the addition or by the removal of water to a value in the range from 14% by weight to 16% by weight,

- precipitation of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is started by the addition of a small amount of previously prepared calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,

- the so prepared mixture is kept during 1 hour at a temperature of 40°C,

- then within 2 hours the temperature is lowered from 40°C to a temperature of 23°C,

- said temperature of 23°C is maintained during 16 to 18 hours, and

- the obtained solid is isolated.

13. The process according to claim 12, characterized in that the obtained solid is washed with water having a temperature of about 10°C, the washed solid is suspended at room temperature in 94% aqueous ethanol and then the so treated solid is isolated by means of filtration.

14. The process according to any one of claim 12 and 13, characterized in that the calcium chloride is added in solid form or in the form of an aqueous solution.

15. Stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, obtained as follows:

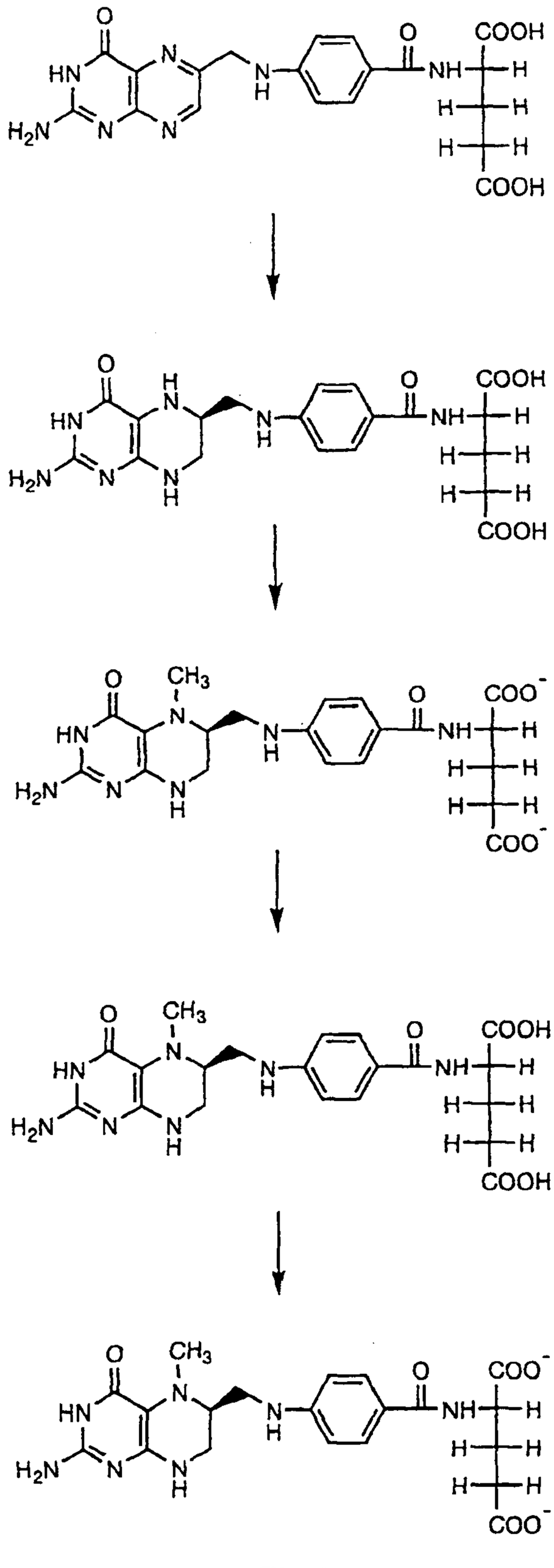
- crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid, prepared according to the process according to any one of claims 4 to 7, is suspended in water, whereby the water has a temperature from 35°C to 41°C,
- to this suspension is added a NaOH solution in portions and in such an amount until a pH-value in the range from 6.7 to 6.9 is obtained,
- to the so prepared solution are added 0.90 equivalents of calcium chloride, referred to the used amount of crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,
- in the so prepared solution the concentration of crystalline (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is adjusted either by the addition or by the removal of water to a value in the range from 14% by weight to 16% by weight,
- the precipitation of the calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid is started by the addition of a small amount of previously prepared calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid,
- the so prepared mixture is kept during 1 hour at a temperature of 40°C,
- then within 2 hours the temperature is lowered from 40°C to a temperature of 23°C,

- said temperature of 23°C is maintained during 16 to 18 hours, and
- the obtained solid is isolated.

16. Stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid according to claim 15, obtained by the process according to any one of claims 13 and 14.

17. Stable, amorphous calcium salt of (6S)-N(5)-methyl-5,6,7,8-tetrahydrofolic acid according to any one of claims 15 and 16, characterized in that it has:

- the in Figure 4a shown "differential scanning calorimetric" profile, DSC,
- the in Figure 4b shown thermo gravimetric profile, TGA,
- the in Figure 5 shown X-ray powder diffraction diagram, and
- the in Figure 6 shown Raman spectrum.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HN
N
N
H
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COOH
NH
H
H
H
H
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COOH
H_2N
N
N
(I)
O
H
N
HN
N
H
O
COOH
NH
H
H
H
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H
H
COOH
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N
N
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(II)
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CH_3
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HN
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COO^-
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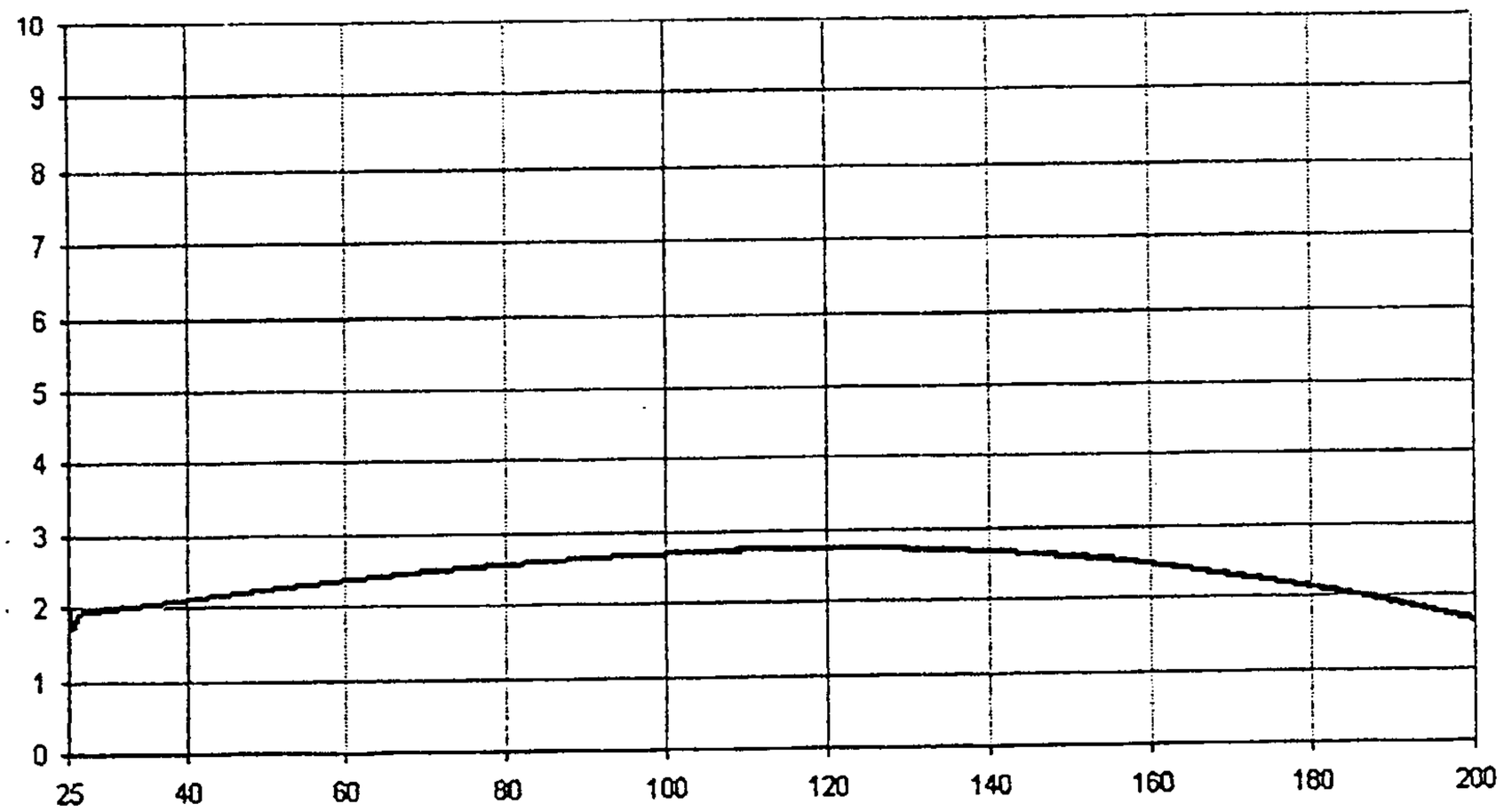
H
(V)

Fig.1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3
2
1
0
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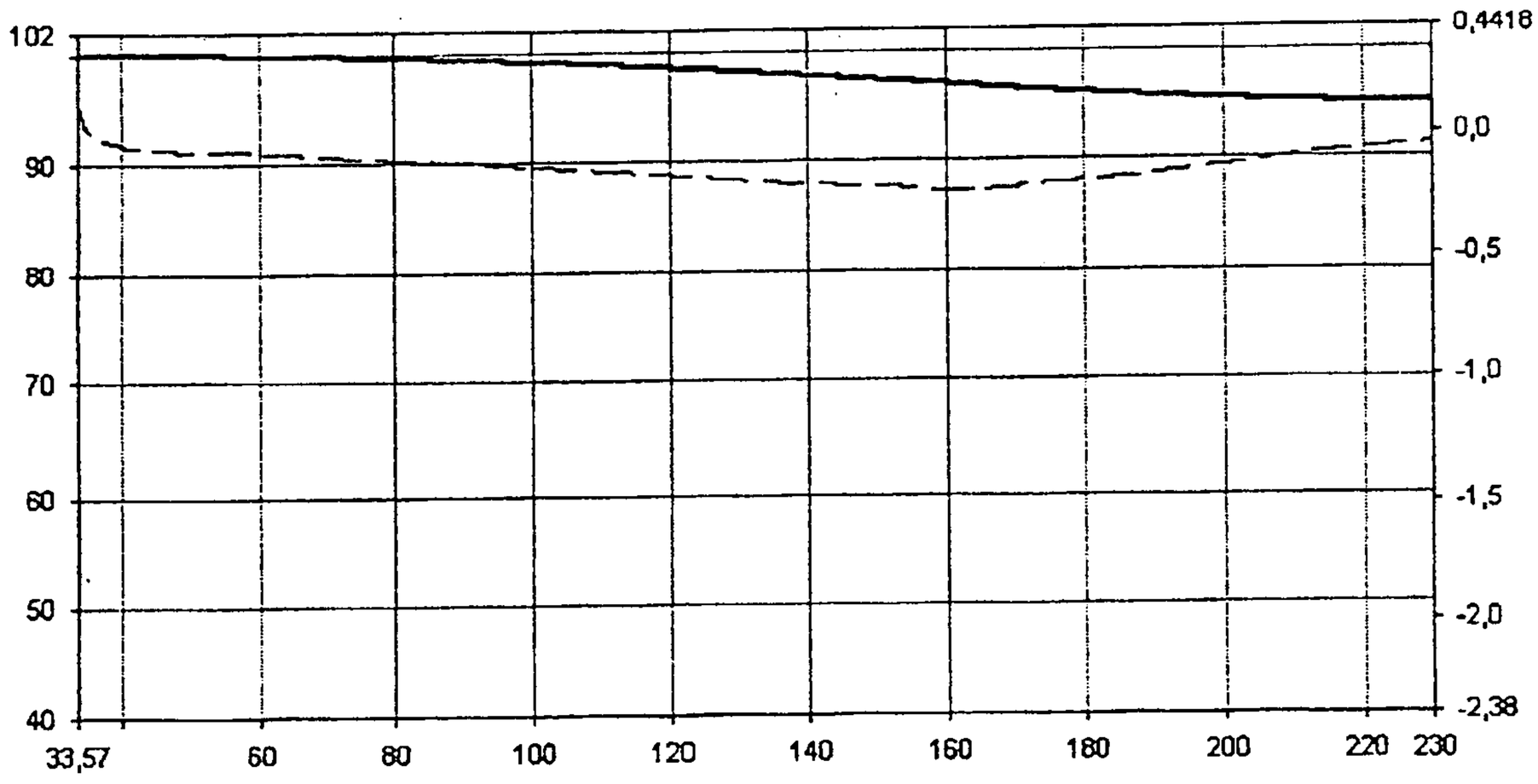
60
80
100
120
140
160
180
200

Fig. 2a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70
60
50
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100
120
140
160
180
200
220
230

Fig. 2b

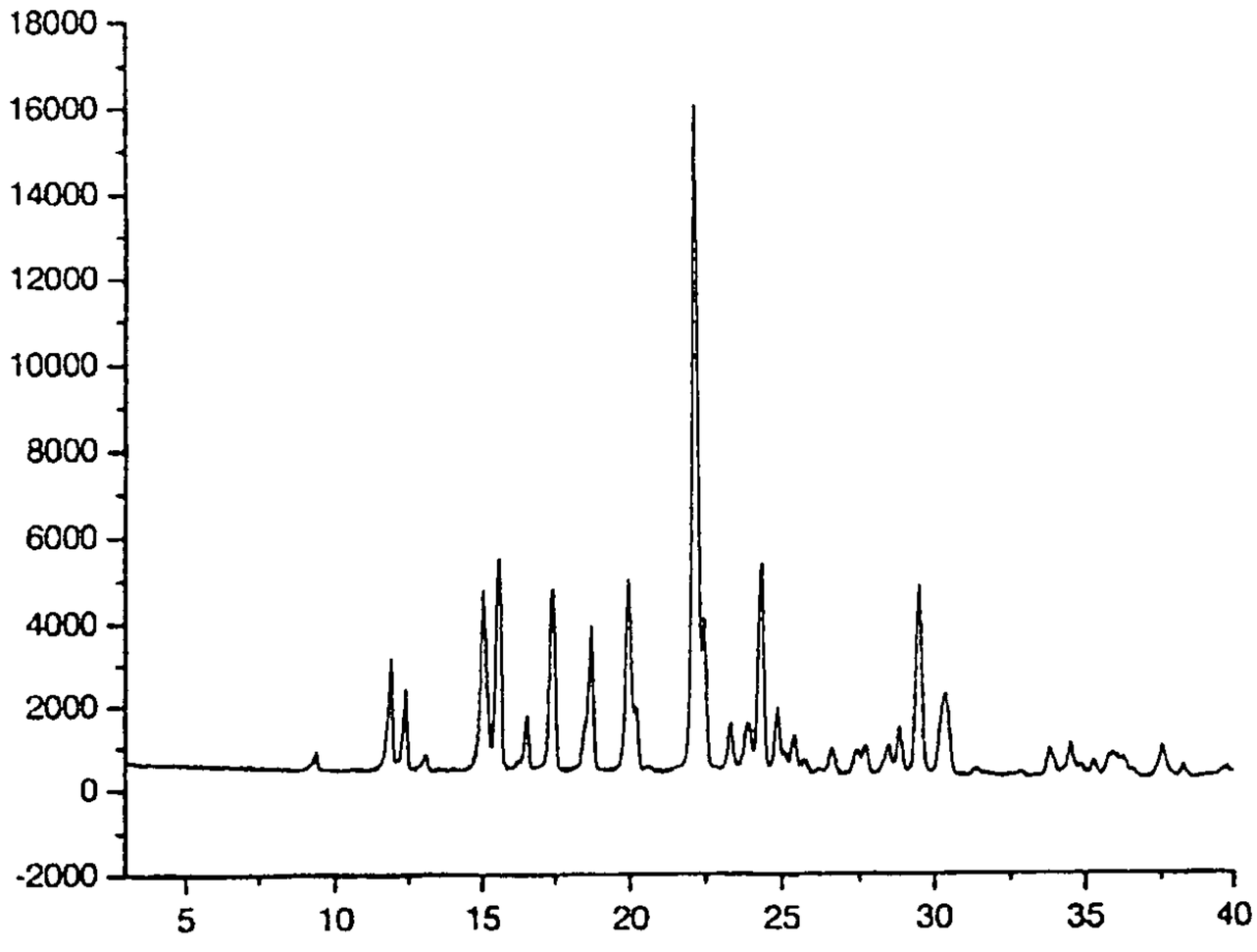
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
-2000
5
10
15
20
25
30
35
40

Fig.3a

Pos.[°2Th.]	Height[cts]	FWHM[°2Th.]	d-spacing[Å]	Rel.Int.[%]
9.3431	359.62	0.0836	9.46587	2.73
11.8681	2647.01	0.1171	7.45705	20.13
12.3773	1821.97	0.1171	7.15137	13.85
13.0412	401.95	0.1171	6.78879	3.06
15.0256	3901.81	0.1673	5.89638	29.67
15.5983	4007.87	0.1506	5.68114	30.47
16.2341	654.91	0.0669	5.46005	4.98
16.5335	1150.34	0.1338	5.36185	8.75
17.4160	4331.05	0.1506	5.09210	32.93
18.4448	951.34	0.1171	4.81033	7.23
18.7080	3064.94	0.1506	4.74324	23.30
19.9280	4004.29	0.1506	4.45553	30.45
20.1856	1379.90	0.1004	4.39924	10.49
20.5606	181.70	0.1004	4.31984	1.38
22.1764	13152.35	0.1673	4.00862	100.00
22.4365	3415.42	0.1004	3.96273	25.97
23.3256	1040.66	0.1171	3.81365	7.91
23.8520	1007.47	0.1224	3.72759	7.66
23.9477	901.13	0.0816	3.72213	6.85
24.3286	4533.85	0.2040	3.65564	34.47
24.8526	1398.77	0.1632	3.57973	10.64
25.0754	450.57	0.1020	3.54842	3.43
25.3878	821.59	0.2244	3.50547	6.25
25.7370	319.21	0.1224	3.45869	2.43
26.6345	568.65	0.1632	3.34414	4.32
27.4247	511.56	0.0816	3.24956	3.89
27.6955	634.16	0.1224	3.21839	4.82
28.4575	553.70	0.2040	3.13392	4.21
28.8157	980.98	0.1836	3.09577	7.46
29.4988	3680.74	0.2448	3.02563	27.99
30.1602	898.39	0.1224	2.96076	6.83
30.4262	1440.45	0.1632	2.93548	10.95
31.3576	124.72	0.1632	2.85038	0.95
32.8840	117.00	0.1632	2.72147	0.89
33.7872	501.16	0.1632	2.65076	3.81
34.5202	607.26	0.1428	2.59614	4.62
34.8769	250.91	0.1224	2.57039	1.91
35.2871	275.14	0.1632	2.54145	2.09
35.8915	432.75	0.3672	2.50003	3.29
36.2611	305.22	0.2040	2.47539	2.32
36.5806	123.96	0.1224	2.45449	0.94
37.5778	586.47	0.2448	2.39162	4.46
38.2761	209.70	0.1428	2.34958	1.59

Fig 3b

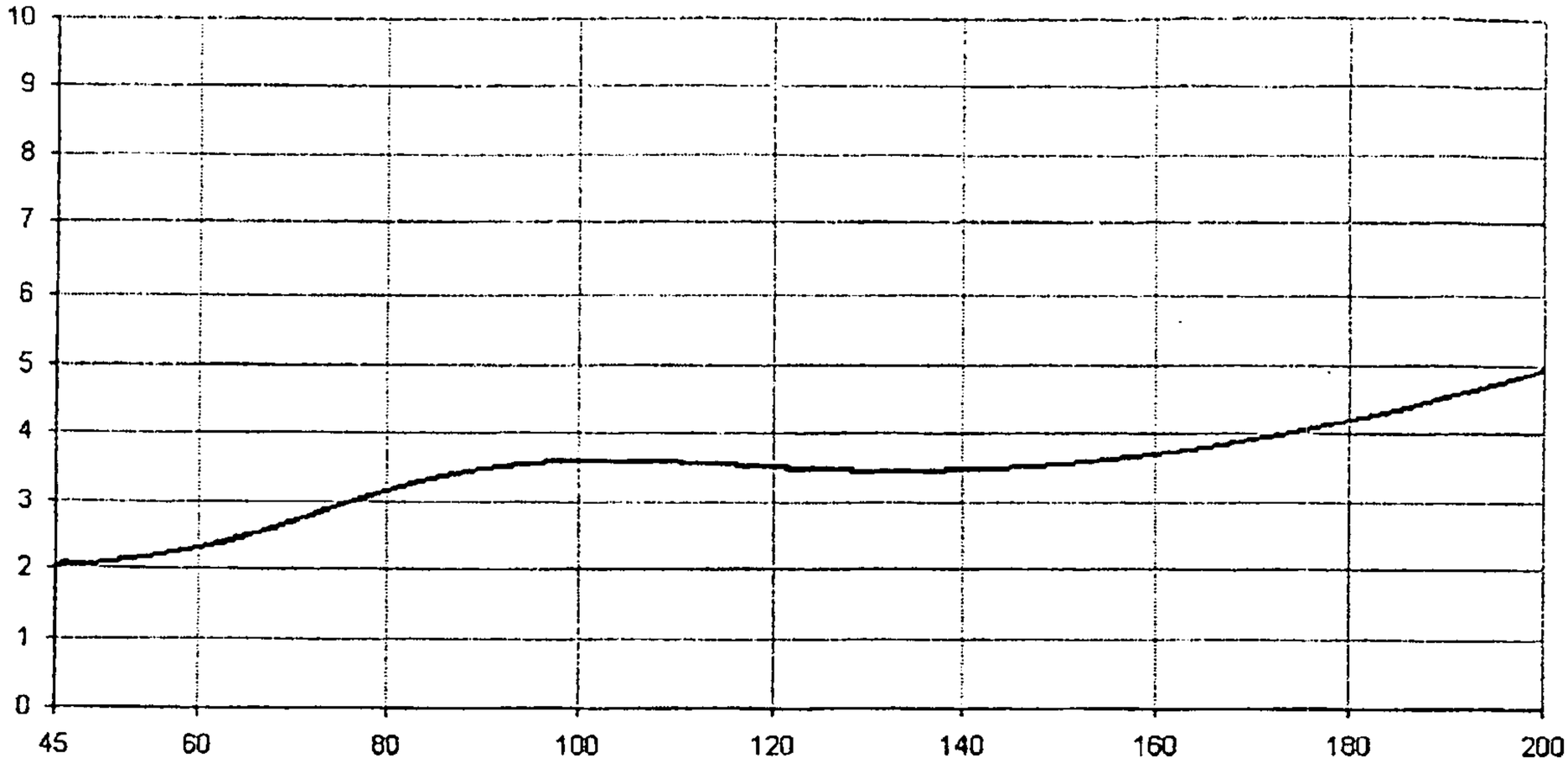
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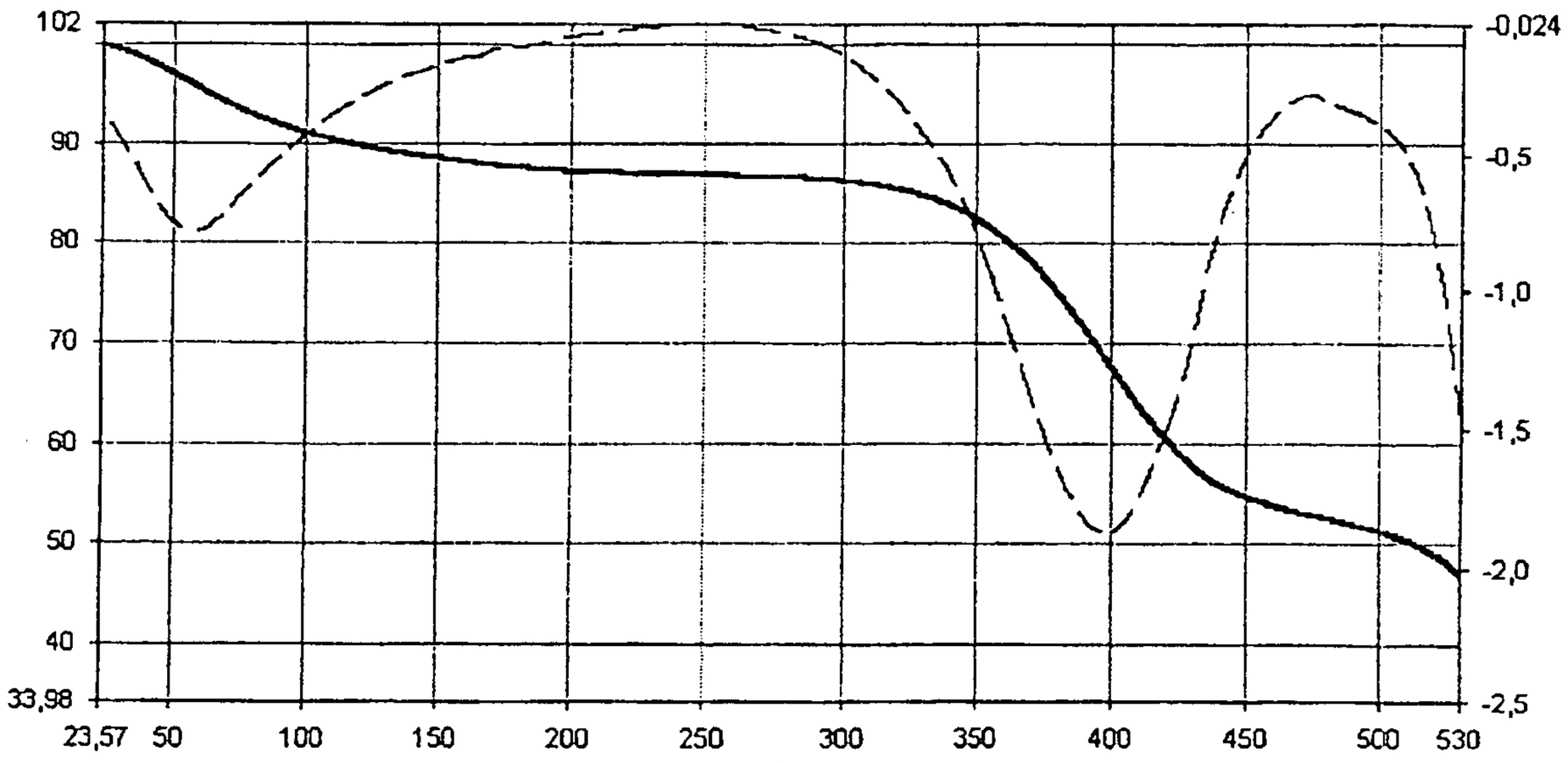
Fig. 4a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40
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23,57
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100
150
200
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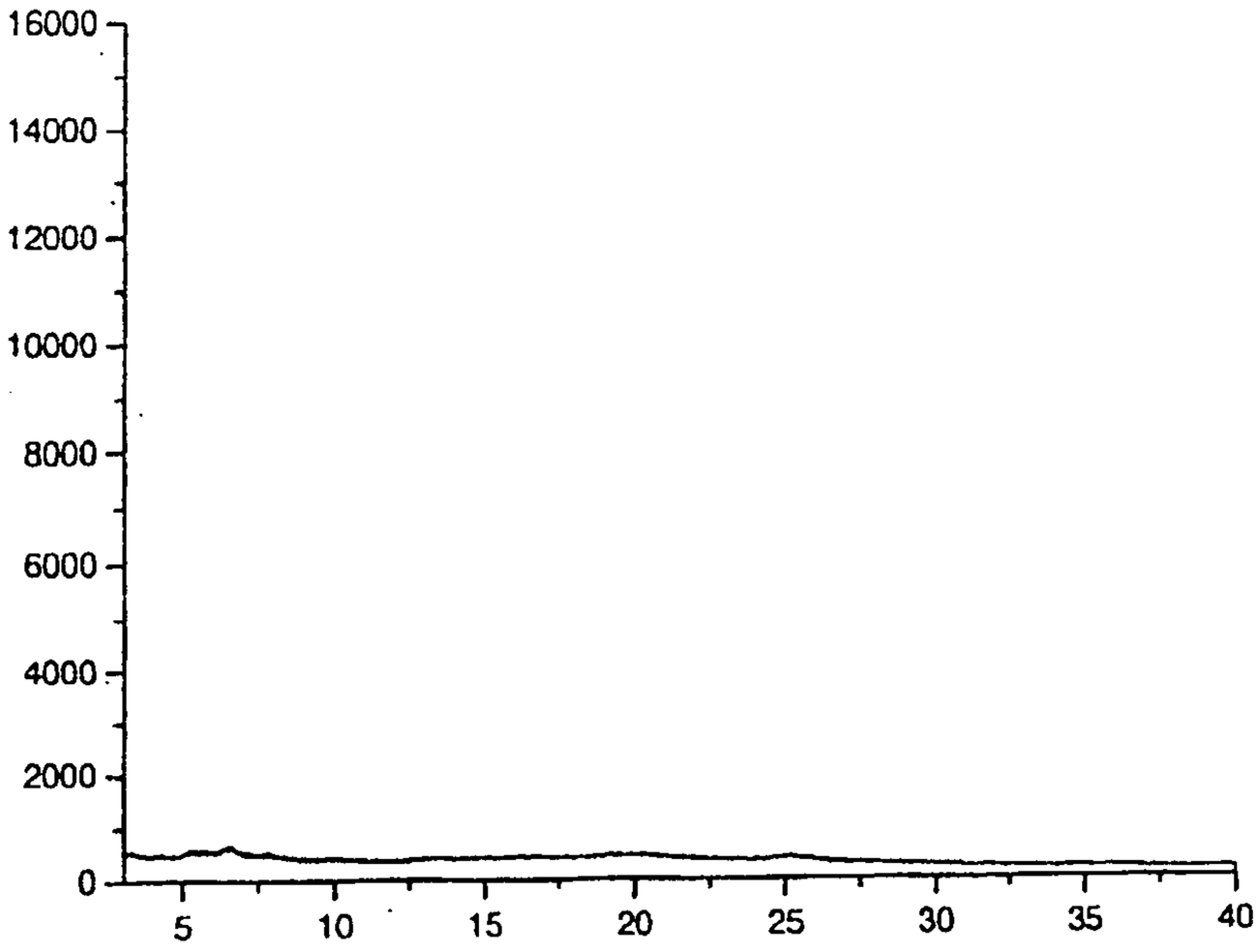
300
350
400
450
500
530

Fig. 4b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35
40

Fig. 5

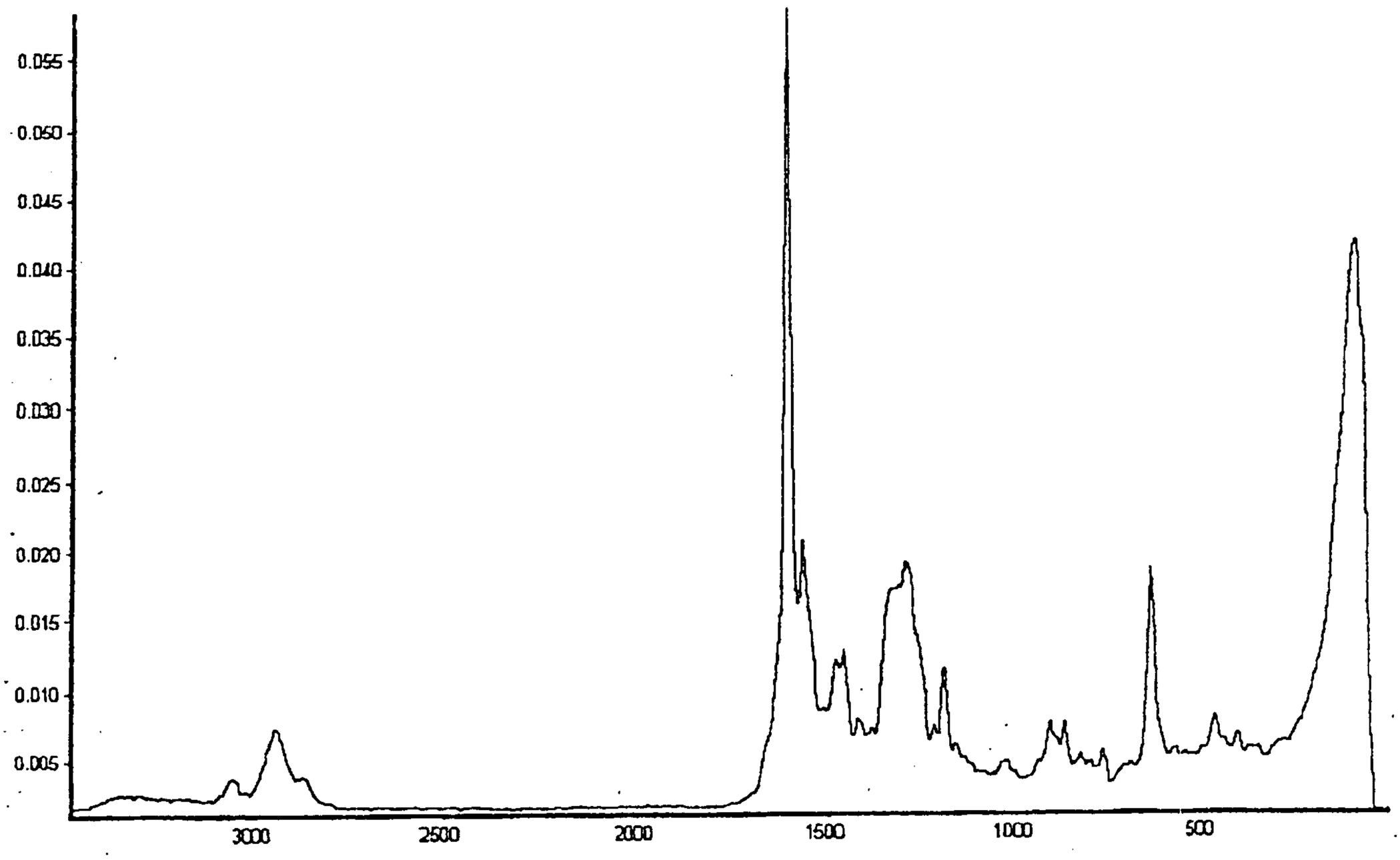
0.055
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005
3000
2500
2000
1500
1000
500

Fig. 6